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EFFECT OF PARTIAL REPLACEMENT OF FISHMEAL WITH DUCKWEED (*LEMNA* SP.*)* MEAL ON THE GROWTH PERFORMANCE OF JUVENILE NILE TILAPIA (*OREOCHROMIS NILOTICUS L.*) IN TANKS

MSC THESIS

BY

AYANTU GETACHEW BEKELE

JUNE, 2023

BISHOFTU, ETHIOPIA

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A Thesis Submitted to the MSc Program of Addis Ababa University, College of Veterinary Medicine and Agriculture in Partial Fulfilment of the Requirements For The Degree of Masters of Science in Animal Production

BY

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JUNE, 2023

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As MSc research advisors, we hereby certify that we have read and evaluated the thesis prepared under my guidance by Ayantu Getachew Bekele entitled: **Effect of Partial Replacement of Fishmeal with Duckweed (*Lemna* Spp.) Meal on The Growth Performance of Juvenile Nile Tilapia (*Oreochromis Niloticus L.)* in Tanks,** we recommend that it can be submitted as fulfilling the MSc thesis requirement.

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DEDICATION

I dedicate this thesis to my father Getachew Bekele, to my beloved mother Melkitu Biru. to my sisters and brothers, also to all my family members who stand beside me throughout my life.

STATEMENT OF AUTHOR

Firstly, I would like to clarify that I am the author of this thesis and that all the sources I used for research have been properly cited. The College of Veterinary Medicine and Agriculture at Addis Ababa University has received this thesis, which is stored in the college library, in partial fulfillment of the requirements for an advanced MSc degree. I hereby solemnly confirm that this thesis is not being submitted to any other institution of learning, anywhere, in order to receive any type of academic degree, diploma, or certificate. The head of the major department or the dean of the college may grant requests for permission for extensive quotations from or reproductions of this work in whole or in part if they believe that the planned use of the material will advance scholarship. Permission must be requested from the author in all other cases, though.

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LIST OF ABBREVIATIONS AND ACRONYMS

AA	Amino Acid
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
CL	Crude Lipid
CRD	Completely Randomized Design
DGR	Daily Growth Rate
DIAAS	Digestible Indispensable Amino Acid
DM	Dry Matter
DO	Dissolved Oxygen
DW	Duckweed
EAA	Essential Amino Acid
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FAOSE	Food and Agriculture Organization for Sub Eastern Africa
FBW	Final Body Weight
FCF	Fulton's Condition Factor
FCR	Feed Conversion Ratio
FL	Final Length
IBW	Initial Body Weight
IC	Incidence Cost
IGF	Insulin- Like Growth Factor
IL	Initial Length
NEAA	Non- Essential Amino Acids
NFALRC	National Fishery and Aquatic Life Research Center
NFE	Nitrogen –free Extract
NRC	National research Council
PI	Profit Index

LIST OF ABREVATIONS (*Continued*)

PPT	Parts Per Thousand
PUFA	Polyunsaturated Fatty Acid
RGR	Relative Growth Rate
SGR	Specific Growth Rate
SR	Survival Rate
TOM	Total Organic Matter
TW	Total Weight
WG	Weight Gain

ABSTRACT

One of the most essential components for optimum productivity in the aquaculture sector is the availability of quality fish feed. Fish meal is one of the most commonly used ingredients as a source of protein for fish feed. However, it is very expensive and is not easily available on the market. The present study therefore aims to determine the impact of partially replacing fishmeal with duckweed (*Lemna* spp.) on the growth performance of juvenile Nile tilapia (*O. niloticus L*.) in tanks. The feeding experiment was conducted from January to April 2023 at the National Fisheries and Other Aquatic Life Research Center (NFALRC), Sebeta, Ethiopia. The growth experiment was run in triplicate tanks in a greenhouse using three treatment diets with different inclusions of duckweed (DW) and one control diet. All four test diets were iso-nitrogenous (34.1–34.9% crude protein), including the control. Duckweed was added to three test diets at rates of 10% DW (T2), 20% DW (T3), and 30% DW (T4), replacing fishmeal, while the control diet (T1) had no duckweed at all. Using a complete randomized design, 360 *O. niloticus* were stocked in four treatments, each in three replicates. Stocked fish were acclimatized in tanks for two weeks before the start of the actual experiment. The results of the study showed that fish fed with 10% DW (T2) and 20% DW (T3) showed a better growth rate than the others. This indicated that increasing the amount of duckweed replacing fishmeal in the diet had a significantly ($P < 0.05$) better growth of juvenile *O. niloticus* up to 20% inclusion of duckweed. The tissue proximate composition of fish fed with 10% DW (T2) and 20% DW (T3) was found to be better than the other groups. From an economic view, 30% DW (T4) showed the lowest incidence cost (77.04) and had a higher profit index (0.57) followed by 20% DW(T3) with (77.82) incidence cost and profit index (0.51). This might be due to the higher inclusion the cheap duckweed replacing the most expensive fishmeal in the diet. In conclusion, a diet up to 20% inclusion rate of DW replacing fishmeal is suitable for juvenile Nile tilapia without affecting the growth of the fish.

***Keywords**: - Aquaculture, duckweed, fish feed, Nile tilapia*

1. INTRODUCTION

Fish is regarded as the primary, economical source of dietary protein for the rapidly growing global population (FAO, 2021). In addition to serving as a source of protein, minerals and fatty acids, fish products provide a significant contribution to food security (FAO, 2016). Globally, fish is currently harvested from both the capture fishery and aquaculture which amounted to 157 million tons in 2020 (FAO, 2022).

Although several fish species are being considered as candidates for aquaculture, tilapia still holds a superior position in freshwater aquaculture (Abebe, 2015). According to Yue *et al*. (2017), Nile tilapia is well- liked in numerous nations due to its cultivable traits, such as fast growth rate, resistance to disease and stress and high production potential in a variety of production systems. In the word of aquaculture, among the finfish, tilapia ranks second next to carp in terms of production, accounting for roughly 2.4% of the finfish aquaculture production in 2020 (FAO, 2022). Fish culture is a relatively recent trend in Ethiopia, having started on a small scale in farmer's ponds by stocking wild fish species, primarily Nile tilapia, collected from lakes (kassaye and Gjoen, 2012). However, the practice of culturing this fish is still in its infancy (Firew *et al.,* 2017). Due to a number of obstacles such as the lack of suitable artificial feeds for small-scale and commercial culture, aquaculture has not advanced in the country despite its enormous water bodies and diverse fish fauna (Aschalew and Zenebe , 2018).

Fishmeal has been the main ingredient of fish feeds among others because it has a balanced amino acid profile (Prabu *et al.,* 2018). Similar to commercial fishmeal, fish offal or discard contains a sufficient amount of crude protein (68.07-72.26%), as well as vitamins, minerals and fatty acids (Ivanovs *et al.,* 2018). Fishmeal has become expensive and its availability is uncertain, thus several countries are now attempting to substitute it with other ingredients (Oke *et al.,* 2016). Various innovations have been developed in response to this steadily rising demand to reduce any potential drawbacks from improving fish farming productivity and sustainability (Irabor *et al.,* 2021). Among these adopted improvements, feed formulation employing inexpensive, readily available plant protein sources that are high in nutrients is required to replace the expensive fish meal (Irabor *et al.*,2021).

Recently, attention has been drawn to small aquatic plants known as" duckweeds" which are macrophytes of the *Lemnaceae* family and valued for their capacity to lower nutrient concentrations in water absorbing nitrogen compounds (Ceschin *et al.,* 2020). The world's smallest flowering plants are duckweeds. *Lemnaceaae* or duckweeds are naturally occurring as freely floating aquatic plants that appear in still or slowly moving waters (Tippery *et al.*, 2021). They have one of the fastest rates of growth in nature, expanding swiftly and broadly and their supply is simple to replenish (Ziegler *et al.*, 2015). It would seem that duckweed has the potential to replace fishmeal and soya bean meal in the formulations of aquaculture feeds due to its high protein content and relatively full spectrum of amino acids. Protein content in dry matter varies greatly depending on the quality of the growth media. Protein content ranges from 7 to 45% (most commonly 20 to 45%) (Hasan and Chakrabarty, 2009). With a high crude protein content, a varied amino acid composition, antioxidants, antibacterial, antifungal and antiviral effects, duckweed (*Lemna minor*) meal is an effective plant protein source (Irabor *et al.*, 2022). Nile tilapias have herbivorous and omnivorous feeding habits and can consume a variety of both artificial and natural feed (Zenebe, 1999; Kariman *et al.*, 2000). The present study is therefore, developed to formulate cost effective fish feed by replacing fishmeal with duckweed feed and evaluate the growth and economic performance of juvenile Nile tilapia (*O. niloticus L.)* in a recirculating tank.

1.1. Statement of the Problem

Generally, the world's population is growing fast whereas the rate of growth is much faster in developing nations. This demands for an increase in the population of food producing sectors. Ethiopia's primary food source is agriculture. However, more food producing industries are still needed to meet the demands of the rapidly growing population in Ethiopia. In this regard, aquaculture has been proposed as one of the potential food producing sectors in Ethiopia. Ethiopia has a number of candidate aquaculture fish species and abundant water resources. However, the aquaculture is still untouched and in its infancy stage. Major inputs required for aquaculture such as artificial dry feeds which are either absent or scare in Ethiopia. Among the main ingredients, fishmeal has been widely used in the preparation of fish feed. Nowadays, it is getting expensive in Ethiopia and elsewhere in the world. Hence, there is a need to find an alternate feed ingredient that can potentially replace fishmeal. These potential feed ingredients should be more available and cost effective.

1.2. Research Questions

- What is the impact of substituting different proportions of fishmeal with duckweed on the growth of juvenile Nile tilapia (*O. niloticus*) in tanks?
- What is the impact of feeding different test diets, replacing fishmeal with duckweed on the proximate chemical composition of the fish fillet?
- What is the economic impact of substituting fishmeal with duckweed on the production of juvenile Nile tilapia?
- What are the effects of the different test feeds fed to the Nile tilapia on the water quality of water in the rearing tanks?

1.3. Objectives

1.3.1 General objective

- To evaluate the impact of substituting different proportions of fishmeal with duckweed on the growth and economic performance of juvenile Nile tilapia *(Oreochromis niloticus L.)* in tanks.

1.3.2. Specific objective

- To evaluate the impact of partial replacement of fish meal with duckweed diets on growth performance of juvenile *O. niloticus* in recirculating tanks.
- To determine the body proximate composition of juvenile Nile tilapia fed on diets partially replacing fishmeal with duckweed.
- To evaluate the effect of substituting fishmeal with duckweed on the economic performance of the fish production.
- To examine the water quality parameters in the fish rearing recirculating tanks.

1.4. Significant of the Study

The finding of this study will have the following significances:

- This study provides details on the dietary requirements for juvenile *O. niloticus*.
- Given the high cost of fishmeal feeds, this study helps fish farmers in making their own fish feed to feed juvenile *O. niloticus* and the farmer can use duckweed partially. The study's findings not only reduce the demand for expensive fish meal but also improve in the production of Nile tilapia juvenile aquaculture meals that are affordable.
- The present study will be essential to increasing productivity in the aquaculture sector.
- Furthermore, this study will be used as a reference by the coming researchers whom wants to conduct further study on this area.

1.5. Limitation of the Study

The following are some of the limitations of the study

- Mixed sex of Juvenile Nile tilapia is used for the study. Sex variation may affect the growth and feed intake.
- Limitation of chromic oxide reagent to run apparent nutrient digestibility.
- In addition, the researcher faced time and financial constraints to fermenting duckweed.

2. LITERATURE REVIEW

2.1. Overview of Fisheries and Aquaculture in Ethiopia

Ethiopia is endowed with inland water bodies that can be utilized to produce fish, which is a low costs source of animal protein. It has numerous lakes and rivers with sizable fish populations. The country's principal lakes including lakes Tana, Ziway, Hawassa, Chamo and Abaya as well as the frequently used Koka and Fincha reservoirs and several rivers, currently provide the majority of the fish consumed in the country. By supplying low-cost, high-quality protein these water bodies serve as a means of livelihood for the poor farmers, fishermen and urban people living near major water bodies (Gebrekidan *et al.*, 2012).

There are some 200 fish species that belong to 75 genera, 31 families and 12 orders in Ethiopia. Of these 194 are native fish species, 40 endemic species and 6 exotic species among the total number of fish species (Golubtsov and Mina, 2003; Redieat, 2012). Some of the introduced species such as *Ctenopharyngodon idella*, *Esoxlucius* and *Hypophthalmichthys molitrix* failed to develop self- sustaining population in natural waters after their introduction. The distribution of fish species and overall diversity within drainage system is extremely uneven (Gashaw and Wolff, 2014). For example, in the Rift Valley fish diversity is highest in the south, lowest in the center and intermediate in the north. A more diverse fish fauna is found in the two southernmost lakes (Abaya and Chamo) as well as the major rivers like the Blue Nile and the Omo (FAO, 2014). The Baro basin has the highest number of fish species in Ethiopia, followed by the Abay, Wabishebele and Omo- Gibe basins. However, Abay and Awash basins appear to have the highest endemicity (EPA, 2005).

The Nile tilapia (*Oreochromis niloticus*), the African catfish (*Clarias gariepinus*), and a few *Cyprinid* species mostly *Labeo barbus* species, Nile perch, (*Lates niloticus*) make up the majority of the fish production. The fish's adaptability to a wide range of environmental conditions and the high demand for the fish among local consumers, Nile tilapia (*Oreochromis niloticus L.*) has been stocked in the majority of cases in several small bodies of water. Even though Ethiopia had a variety of fish species, there was a strong reliance on Nile tilapia. That is Nile tilapia is the benchmark of the country's fish farm production, thus limiting Ethiopia's aquaculture production diversity. Despite the fact that African catfish, along with Nile tilapia make up significant economic component of the country's fisheries product, its farming is not well practiced (Gadisa *et al*., 2017).

Although Ethiopia has favorable physical and hydrographic conditions, which include suitable geographic relief, rich soil quality, a good mean annual rainfall and adequate freshwater availability, the fish production from the aquaculture sector is still negligible in the country. Despite the country's high potential and diverse environmental conditions, aquaculture farming remained small-scale, subsistence-oriented and only few commercial farms are operating today. Ethiopia is estimated to have over 1300 subsistence fish farmers with ponds ranging in size from 100 to 300 square meters (Lemma, 2017). Aquaculture has enormous potential to improve the nutritional status of the human population and alleviate poverty among the country's rural people. The demand for fish has increased in the last decade and as a result, most Ethiopian lakes are overfished. Several national reports indicate that fisheries production in Ethiopia's major lakes is declining at an alarming rate indicating the urgent need to develop aquaculture nationwide (Gadisa *et al.,* 2017).

The development of aquaculture in Ethiopia faced a number of challenges, the most significant of which was a lack of cheap and efficient locally available fish feeds, as well as absence of locally selected and certified fish seeds. Similarly, the country's land ownership policies, over-reliance on capture fisheries, lack of successful integration of aquaculture with other farming activities, absence of small- scale low - cost aquaculture support for rural development, absence of licensed fish seed multiplication centers and a lack of institutional capacity in the areas of training, research and technology transfer are all challenging conditions for aquaculture development. Aside from those difficult conditions, lack of trained labor and weak government attention are also obstacles to the country's aquaculture development (Abriham *et al.,* 2017). Furthermore, absence of hatchery rearing for the development of fish farming particularly among smallholder farmers to larger producers is also a major challenge. To make aquaculture more effective, hatchery rearing and cheap fish feed can play an important role in alleviating fingerling scarcity and intensifying Ethiopia's aquaculture sector (Erkie *et al.,* 2017).

2.2. Nile Tilapia Aquaculture

The term ″tilapia″ refers to a number of cichlid species that are raised on farms all over the world, with a global annual production that has been steadily increasing from 379,169 t in 1990 to 4, 407.2t in 2020 (FAO, 2022). The most widely used species of farmed tilapia is the Nile tilapia (*Oreochromis niloticus*), which is native to northern Africa. After grass carp (*Ctenopharyngodon idella*; 5,791.5t) and silver carp (*Hypophthalmichthys molitrix*; 4,896.6 t), whose global production is expanding at a slower rate than that of Nile tilapia, Nile tilapia is currently the third most abundantly farmed finfish species worldwide (FAO, 2022). Nile tilapia farming is a widespread practice, with commercial production being reported in 74 nations in both hemispheres. According to FAO estimates from 2022, China (1,241,410t) is the world's largest producer, followed by Indonesia (1,172,633 t), Egypt (954,154 t), Brazil (343,596 t) and Thailand (205,971 t).

The main characteristics that differentiate Nile tilapia from other aquaculture species include its excellent feeding ability on external feed, quick growth, tolerance to a wide range of salinity, dissolved oxygen and temperatures, as well as ease of reproduction, omnivorous feeding style and acceptance of exogenous feed immediately following yolk sac absorption (El-Sayed, 2019).

The Nile tilapia typically feeds during the day, which suggests that like trout and salmon, it displays a behavioral response to light as a key factor in feeding activity. But because Nile tilapia reproduces rapidly, overcrowding occurs frequently within the fish populations. The competition for food during the day may lead to night feeding in order to obtain the necessary nutrients. Contrary to popular belief, earlier study showed that gender differences in food intake do not necessarily correlate with differences in fish size between sexes. Because food is converted to body weight more effectively in males than in females, despite the fact that both sexes consume the same amount of food, males tend to grow larger (Toguyeni *et al.,* 1997).

The Nile tilapia can easily be genetically modified to increase fish production and productivity. Hybridization between *O. niloticus* x *O. aureus* occurs frequently, producing mainly male populations that can grow more quickly than their non-hybrid counter parts and regulate widespread reproduction in ponds. Additionally, sex reversals can also be achieved by hormones and temperature (Pandian and Kirankumar, 2003). The creation of the genetically improved farmed tilapia strain is one example of how genetic selection methods are used to increase tilapia productivity because the initial global founder populations were small and unidentified, which led to inbreeding and poor performance (Eknath and Hulata, 2009). In order to keep up their growth developing species-specific and even strain- specific and affordable diets was inevitably important. This is due to the increasing trend of tilapia productivity through genetic programs as well as the change in tilapia aquaculture from extensive to semi-intensive farming practices. This underlines the need of adequate nutrition and feeding management since feeding is typically a significant investment in a modern tilapia farm (Ng and Romano, 2013).

2.3. Nutritional Requirements of Nile Tilapia (*Oreochromis niloticus*)

To meet its nutrient and energy needs, the Nile tilapia, like other finfish and terrestrial animals needs a balanced daily supply of protein, fats, carbohydrates, minerals and vitamins. Age, size, physiological circumstances and water temperature all affect the nutrition and energy needs of tilapia (NRC, 2011).

2.3.1 Protein and amino acid requirements

Proteins are complex biomolecules that are present in all animal and plant cells and tissues and are essential for the development and maintenance of life. Even though there are roughly 300 amino acids (AA) in proteins from natural sources, the majority of proteins only contain 20 amino acids, each of which has a special set of physical and chemical properties. Of these 20 amino acids, 10 are classified as essential (EAA) and must be obtained through diet because animals cannot synthesis them. The remaining 10 amino acids are known as non-essential amino acids (NEAA) since animals can synthesize them. Amino acids are linked into chains by peptide bonds (Lall and Dumas, 2015) and cross-links between chains with sulfhydryl and hydrogen bonds (Molina-Poveda, 2016).

The majority of animal's body is made up of proteins, which account for 65-85% of the weight of fish and shrimp (Jauncey, 1982). The quality of the protein is measured using the digestible indispensable amino acid score (DIAAS) (Wolfe *et al.,* 2016) or the amino acid profile (Jauncey,1982). Compared to terrestrial animal's fish require more protein for growth and protein is regarded as the most important nutrient in fish diets (Wilson, 1994). The ideal range for crude protein in fish feed is 25 to 50 (Lovell, 1989).

The amount of protein needed however, varies greatly depending on the size and age of the fish, the waters temperature, the amount of feed allowed, the amount of non- protein energy in the feed, the type of protein and the availability of feed in the culture environment (NRC, 2011). Tilapia's protein requirement ranges from 40 to 45% CP for brood stock, 40 to 50% CP for fry/fingerlings and 28 to 32% CP for grow out fish (Abdel-Tawwab *et al.,* 2010). As a result, tilapias need for protein reduces as it reaches adulthood. Because of its omnivorous feeding habit, tilapia can feed on diets containing plant and animal components (NRC, 2011).

However, feed ingredients of animal origin are more likely to match the necessary amino acid profile when it comes to EAA requirements (Madalla, 2008). For in situ protein synthesis to occur, all EAA must be present in the diet to promote fish growth and health (Gatlin *et al.,* 2007).

2.3.2 Lipid requirements

In tilapia, fatty acids provide vital metabolic energy for growth, reproduction and movement (Tocher, 2003). Additionally, fatty acids help the body to absorb fat-soluble vitamins including sterol and vitamins A, D, E and K (Lim *et al.,* 2011). These fat-soluble vitamins are essential for several physiological processes, including vision, bone health, immunity and clotting (Reddy and Jialal, 2018). The majority of cellular structure is made up of lipids, primarily phospholipids, which also maintain the flexibility and permeability of membranes (Lim *et al.*, 2011).

The length of fatty acid chains, level of unsaturation (number of double or ethylenic bonds) and location of the ethylenic linkages are used to classify fatty acids (Tocher, 2003). It has been found that tilapia perform well in terms of development and reproduction when given access to plant oils high in C18:2 omega- 6 fatty acids (such as soybean oil, maize oil, sunflower oil and rapeseed oil) (Lim *et al.,*2011). Other research has shown that dietary lipid supplies of linoleic n-3 series fatty acids and omega- 6 fatty acids are crucial for Nile tilapia, blue tilapia (*Oreochromis aureus*) and hybrid tilapia (*Oreochromis aureus* x *O. mossambicus*), as shown by strong growth and reproductive performance of the hybrids.

However, red belly tilapia (*Tilapia zillii*) is known to respond better to omega-6 fatty acids in terms of growth than to omega-3 fatty acids (Lim *et al.,* 2011). Additionally, when plant and fish oil mixes are employed, tilapias have been shown to perform better in terms of growth (El-Tawil *et al.,* 2019). The recommended dietary lipid content for tilapia is 10 to 15% of dry matter (DM), although using lipid levels above 15% may cause growth inhibition and carcass lipid accumulation in young hybrid tilapia (He *et al.*, 2015). The total lipids and fatty acid contents of the Nile tilapia, *O. niloticus* sampled from different lakes in Ethiopia showed significant variations between lakes and seem to vary with the composition of fish diet (Zenebe *et al.,* 1998a; 1998b). All fish studied contain different levels of omega 3 and omega 6 polyunsaturated fatty acids (PUFA) (Zenebe *et al.,* 1998a; 1998b).

2.3.3 Carbohydrates requirements

The primary source of energy in the diets of many animals, including tilapia is carbohydrate (Abro, 2014). Based on the constituent sugars, structure, composition, level of polymerization and glycosidic linkage to non- monomer carbohydrates, they are divided into different categories (Englyst and Hudson, 1996). Carbohydrates are classified as monosacharides and disaccharides (1-2 units; glucose, sucrose, maltose, sorbitol and mannitol), oligosaccharides (3-9 units; dextrins, raffinose and starchylose) and polysaccharides (>9 units; amylose, cellulose, pectins and hydrocolloids) based on the degree of polymerization (monomeric units). Additionally, the modified type of polysaccharide called chitin that is present in invertebrates (such as shrimp and krill) contains nitrogen which is synthesized from units of n-acetyglycosamine (Lall and Dumas, 2015).

For tilapia fish and other animals, carbohydrates are thought to be the cheapest source of dietary energy. The nutritional value of different dietary carbohydrates varies depending on the type of species and the source of carbohydrates (Krogdahl *et al.,* 2005). Fish are known to have a limited capacity for the digestion and metabolism of carbohydrates and an excessive intake of this nutrient may cause nutritional issues such as a decreased growth rate coupled within efficient feed utilization, a rise in disease incidence and a decreased capacity of antioxidant defense (Hemre *et al.*, 2002).

The primary sources of energy for the synthesis of other physiologically important molecules in fish are protein and lipids (Lall and Dumas, 2015). Dietary carbohydrates typically promote growth (Watanabe, 2002), but if they are not delivered in the right amounts, they may have adverse consequences on nutrient uptake, growth, metabolism and health (Abro,2014). In general, herbivorous fish use dietary carbohydrates more effectively than carnivorous and omnivorous fish (Hemre *et al.*, 2002). However, compared to temperate carnivorous fish like salmonids, warm- water omnivore fish like tilapia consume far more dietary carbohydrates (Wilson, 1994). Utilizing foods that provide energy boosts the release and activity of several hormones, namely insulin and glucagon/glucagon- like peptides in fish.

However, environmental elements that impact fish glucose metabolism, such as temperature and photoperiod, also have an impact on growth hormones and insulin-like growth factor (IGF) (Lall and Dumas, 2015). Although the ideal dietary carbohydrate requirements for fish have not been established an ideal dietary carbohydrate supply is crucial for fish growth (Zhou *et al.*, 2013). Increased dietary carbohydrate content enhances tilapia development and metabolism (Azaza *et al.*, 2015). Starch at a dietary inclusion level of 10-40% of dry matter (DM) is said to support tilapias rapid growth (Abro, 2014).

2.3.4 Mineral requirements

All aquatic organisms need inorganic elements or minerals for survival and growth. These vital processes include the development of the skeleton, preservation of colloidal systems, control of the acid- base hormones, enzymes and enzyme activators (Chanda *et al.*, 2015). The skeletons of animals and fish require calcium (Ca) and phosphorus (P) for their development. The equilibrium of the acid-base balance and homeostasis are upheld by the minerals like sodium (Na), potassium (K) and chlorine (Cl), as well as phosphates and bicarbonate. Iodine (I) is necessary for synthesis of thyroid hormones (Chanda *et al.*, 2015).

Nutritionally minerals can be classified as major elements such as calcium (Ca), potassium (K), magnesium (Mg), sodium (Na) and phosphorus (P), minor or trace elements (such as iron (Fe) and iodine (I) or toxic elements (such as mercury (Hg), cadmium (Cd), cobalt (Co)and chromium (Cr) (Das *et al.*, 2018). Like other fish, tilapia obtain minerals from their diet or from the water (Chanda *et al.*, 2015).
Dietary sources of essential elements include diets with animal and terrestrial plant origin, such as potassium- rich weeds, leaves and shrimp, sodium rich prawns and magnesium rich prawns. Aquatic plants and agricultural by products such as mosquito

fern, duckweed and soybean products contain minor mineral elements such as iodine and iron (Hertrampf and Piedad-Pascual, 2000). Limestone and seashells are calcium rich food sources. The amount of minerals that tilapia need depends on various factors including age, physiological state, method of entrance and type of mineral sources (Terech-Majewska,2016).

According to reports, the phosphorus requirements for blue tilapia, Nile tilapia and Hybrid tilapia are 0.3-0.5, 2.1-7.1 and 7.6-7.9 g P kg^{-1} DM, respectively (Furuya *et al.*, 2008). For Nile tilapia and its hybrids, the magnesium requirement ranges from 0.03 to 3.2 g Mg kg^{-1} DM (Lin *et al.*, 2013). For Nile tilapia, the iron requirement ranges from 24.7 to 200 mg Fe kg^{-1} DM, while for hybrid tilapia, it ranges from 85 to 160 mg Fe kg^{-1} (Shiau and Su, 2003).

Therefore, it is necessary to assess the iodine content of the diet in order to meet the minimum required (2.8 mg I kg^{-1} DM) for fish (Watanabe *et al.,* 1997). Having insufficient amounts of vital minerals in fish diets can cause mineral deficiencies, which can result in diseases like anemia, osteoporosis, stunted growth and genetic problems (Bhandari and Banjara, 2015).

2.4. The Nutritive Value of Duckweeds

Studies showed that the nutrient content of duckweed is more influenced by the environment in which it grows than by the species of duckweed (Hassan and Edwards, 1992). Since the duckweed lacks central root system nutrients are taken up directly by the entire plant resulting in the direct assimilation of organic molecules. Therefore, the non-structural but metabolically active tissue is responsible for the rapid development rate (Ice and Couch, 1987). The cost of handling, transportation and drying can be affected by the water content of duckweeds (92 to 95%) (Tegene *et al.,* 2009). Sun drying (Akter *et al.,* 2011), oven drying (Haustein *et al.,* 1994), par-boiling, pressing or forced air drying are some of the drying methods used (Effiong and Sanni, 2009).

The best drying method is air drying, despite its slowness. Additionally, it is crucial to add value to the harvested duckweed plant. The existence of a wax layer on the surface of the plant, which serves as a barrier for fungal growth, has been said to be the reason why sundried duckweed has been observed in storage for 13 years without any trace of fungal growth and nutritional loss. In nutrient poor media, duckweed has a low protein of 9-20%, whereas in nutrient rich conditions, it has a protein content of 24-41% (Mwale and Rumosa Gwaze, 2013).

The nitrogen levels of healthy plant are comparable to those of commercial fertilizers; therefore, the biomass might be used as supplementary fertilizer (Mbagwu and Adeinji, 1988). Its protein has a balanced spectrum of amino acids. In general duckweed contains high levels of leucine, threonine, valine, phenylalanine and lysine (Rusoff *et al.*, 1980). Methionine and lysine are present in greater concentrations in animal proteins than in most plant proteins. The concentrations of methionine and lysine are very similar to those of animal protein (Journey *et al.*, 1991). In addition, duckweed has vitamins and minerals that the body needs for normal function (Men e*t al.*, 1996).

Duckweeds cultivated in nutrient poor water, the lipid content is low (1.8 to 2.5), while it is often higher and varies between 3 and 7% for duckweeds grown in nutrient rich water (Kesaano, 2011). The average amount of fiber in duckweed is around 5% (Kesaano, 2011). However larger amounts have been recorded for duckweeds grown in nutrient rich water. Compared to other aquatic plants, duckweed can withstand high nitrogen stress and seems to be more resilient to pests and diseases (Khang, 2003).

Duckweed has a dry matter content that varies from 3 to 14%. Many macro and microelements including Ca, Cl, K, Na, Si, N, H, C, Fe, Mg, Mn, Al, B, P, Cu and Zn, can be absorbed by duckweed from water (Showqi *et al.*, 2017). This plant's extra benefit is that it includes carotenoids, A, B and E vitamins and amino acids (Showqi *et al.*, 2017).

2.5. Water Quality Parameters and Fish Growth

Water quality is the sum of all physical, biological and chemical properties that affect the growth and survival of fish (Alavaisha *et al.*, 2019). The growth and survival of fish species can be affected by changes in water quality characteristics (Tahar *et al.*, 2018). Important water quality parameters include oxygen, temperature, transparency, pH, salinity, ammonia (Agano *et al.*, 2017).

2.5.1. Temperature

The feed conversion efficiency is greatly influenced by temperature (Samuel *et al.*, 2019). Temperature regime plays a major role in the physiological and metabolic processes of fish, including growth, feed conversion ratio, survival rate and productivity of aquaculture enterprises (Boyd, 1998). However, severe temperatures either too hot or too cold will cause stress and slow growth (Boyd, 1998). Fish's feeding frequency and thus their rate of growth will be impacted by the temperature. This has been documented in a study carried out in controlled environments in aquaria (Zenebe *et al.*, 2003). In a recirculating system's water temperature can be easily controlled and kept at the ideal level (El- Sayed and Kawanna, 2008). Studies showed a direct correlation between growth and temperature with rising temperatures increasing growth rate and feed intakes were observed (Kassaye and Gjoen, 2012). Nile tilapia can survive in water temperatures varying between 10 and 40^{o}C (Watanabe *et al.*, 2002). However, in recirculation systems, the optimal temperature for development and feeding is between 25 and 32^{o}C (Dennis *et al.*, 2009).

2.5.2. pH

Fluctuations in the water pH result in an ionic imbalance which may cause fish kills accidentally. pH is an important component for the development, growth and survival of fish (Abdullah *et al.*, 2017). Fish experience acute physiological disturbances and fatality at pH levels that are both alkaline (8.5-10) and acidic (below 6.0) conditions (Abdullah *et* *al.,* 2017). According to El-Sherif and El-Feky (2009), Nile tilapia culture may benefit from water with a pH of 7-8 for the best growth and survival rates. In recirculating aquaculture systems for Nile tilapia culture, pH is regarded as a crucial parameter that needs to be monitored and managed (Tahar *et al.,* 2018). Thus, tilapia may survive in a pH of 5 to 10 but they thrive in a pH range of 6 to 9 (Popma and Masser, 1999). Like other aquatic creature's tilapia's survival and growth performances are impacted by the pH level of water in a particular rearing system (White *et al.,* 2015). Fish behavior and physiology may be affected when stressed by changes in pH (Dubost *et al.,* 1996). Nile tilapia are said to have strong resilience to progressive acidification however lower pH is said to slow down their growth (Reboucas *et al.,* 2015). Although Nile tilapia can withstand a pH range of 3.7 to 11, optimal growth is recorded between pH 7 to 9 (Ross, 2000) and acidic waters have a negative impact on growth (Webster and Lim, 2002). The pH between 6 and 9 in recirculation systems has no negative effects on feeding rate or growth of tilapia (Reboucas *et al.,* 2015).

2.5.3. Dissolved oxygen

Fish growth, feed utilization and overall health are all influenced by dissolved oxygen (Jobling, 1998). Dissolved oxygen concentration is highly dependent on temperature and its concentration below a certain level lowers fish growth, feed utilization, raises the risk of various diseases or even fish death. Recirculating aquaculture systems boost feeding rates as constant oxygen supplies and continuous water movement in the system removes metabolites (Craig and Helfrich, 2002). Fish have improved growth and survival rates as the amount of dissolved oxygen in the water increases because the food conversion ratio increases up to 90% (Bergheim *et al.,* 2006).

2.5.4. Salinity

Although it is frequently grown in fresh water, the Nile tilapia (*O. niloticus*) can survive a certain amount of salinity. Despite having a high ability to adjust to salinity it is less tolerant than other species of tilapia like *O. aureus* and *O. mossambicus* (Kamal and

Mair, 2005). Different salinity levels have a significant impact on *O. niloticus* growth rate and its tolerance is controlled by its strains, size and the prevailing environmental conditions (Iqbal *et al.,* 2012). While popma and Masser (1999) found better growth of *O. niloticus* at salinities up to 15 ppt and Likongwe *et al.* (1996) at salinities up to 16ppt, with an optimal salinity level of 8 ppt, Azevedo *et al.* (2015) found better growth at salinities up to 7 ppt.

2.5.5. Ammonia

Ammonia is a poisonous substance that can harm fish health and its recommended level is less than 0.05 mg/L (El-Sherif *et al.,* 2008). However, when the concentration is greater than 0.08 mg/L the feeding appetite of fish decreases and their growth is slowed (Popma and Masser, 1999). Body weight also decreases significantly as the ammonia concentration rises (Zeitoun *et al.,* 2016). Fish excrete the nitrogenous chemical ammonia through their gills and feces. Water containing ammonia damages the gills and kidneys, slows growth and raises glutamine levels in the brain (Benli *et al.,* 2008). At concentrations as low as 0.08 mg/L, unionized ammonia tends to inhibit food ingestion. Long term exposure to unionized ammonia concentrations more than 1 mg/L causes losses, particularly in fry and juveniles in water with low DO concentrations (Dubost *et al.,* 1996). In intensive recirculating systems, ammonia and nitrite should be regularly checked since they are poisonous and causes mass fish kill. The quality of the feed, the rate of feeding, size of the fish and the temperature all have an impact on ammonia generation, which are directly tied to fish feeding (Riche and Garling, 2003).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in recirculating tanks of the National Fishery and Aquatic Life Research Center (NFALRC) which is located in Sebeta town, Oromia Regional State. It is located (08° 54'N and 38° 38'E) at an altitude of 2225 m above sea level. The mean air temperature is around 20^{o}C with maximum temperature of 27^{o}C and minimum temperature of 12^{o}C and the average annual total rainfall is about 1350 mm.

3.2. Experimental Setup for the Growth Study in Tanks

The feeding experiment was carried out from January to April 2023 in a greenhouse at the National Fisheries and Other Aquatic Life Research Center (NFALRC), Sebeta, Ethiopia. The growth performance and feed utilization of Juvenile Nile tilapia were studied in twelve plastic recirculating tanks, each holding 1000L of water. The growth performance of juvenile *O. niloticus* was assessed using three experimental diets and a control diet with three replicates for a period of 120 days.

The experimental tanks were carefully cleaned with spring water and detergent prior to starting the actual growth study. A complete random design (CRD) was used to assign the experimental juvenile fish and test feeds to each tank. To remove any leftover feeds at the bottom, all tanks were cleaned every two weeks. After cleaning the tanks were filled with fresh spring water. During cleaning, fish were taken out of tanks and kept in buckets of water to minimize stress on fish. After cleaning was finished, the fish were then carefully put back into their tanks. To raise the water temperature to the desired range (26-30^{o}C), the tanks were fixed inside a greenhouse.

Every day, the fish in the experimental tanks were checked to evaluate their health and mortality of fish.

Figure 1. Experimental tanks used for the growth of juvenile *O. niloticus* in the greenhouse.

3.3. Source of Feed Ingredients and Preparation of Experimental Feeds

The feed ingredients used to make the test diets were purchased at the local market. The fish meal was purchased and processed from Bahir Dar Fisheries and Other Aquatic Life Research Center. Bone and meat were obtained from a slaughterhouse in Addis Ababa.

The duckweed was artificially propagated and processed at Sebeta National Fisheries and Aquatic Life Research Center, First the duckweed was propagated and mass-produced using cow dung manure. Then it was harvested using a hand scoop net and dried for three days under the greenhouse condition. The dried duckweed was then ground using electric mill machine. The Duckweed powder was mixed with other ingredients at different inclusion rate.

Before formulation, oil cakes (made of nug and soybean) were mixed. The National Fisheries and Other Aquatic Life Research Center (NFALRC) provided us all the feed ingredients including fish meal, sorghum, maize, soybean meal, soybean oil, nug meal, premix, L-lysine, wheat middling, meat and bone meal and duckweeds used to prepare the test feed used. Four different percentage replacement levels of fishmeal with duckweeds were used in the four experimental diets, including the control diet (Table 1).

Using Jauncey and Ross (1982) method, the amount of each ingredient used in a compounded diet was largely calculated and measured. The amount of each ingredient calculated was measured using an electronic sensitive balance model LD-0.01 (Table 1). In the three test diets fishmeal was replaced with duckweed at rates of 10% (T2), 20% (T3) and 30%DW (T4), and the control diet had no duckweed at all (T1). Using the feed win software, all of the treatment diets were made to be approximately isonitrogenous and isoenergetic and they were all enriched with lysine, oil, vitamin and mineral premixes (NRC, 2011). Then these ingredients were pelleted using a pelletizer feed machine and dried in the greenhouse. All experimental diets were carefully mixed following the proportion indicated in Table 1.

Table 1. Percentage (%)composition of experimental feeds used in the growth experiment of juvenile Nile tilapia.

Ingredients	**T1 (0% DW)**	**T2 (10%DW)**	**T3 (20%DW)**	**T4 (30%DW)**
Fish meal	5.5	4.9	4.4	3.9
Sorghum	2.0	2.0	2.0	2.0
Maize	20.0	19.0	18.5	18.5
Soyabean cake	45.0	45.0	45.0	45.0
soy bean oil	0.4	0.4	0.4	0.4
Nug cake	7.0	7.0	7.0	7.0
premix	0.5	0.5	0.5	0.5
L-Lysine	0.1	0.1	0.1	0.1
wheat middling	12.0	12.0	12.0	12.0
meat and bone	7.5	8.5	9.0	9.0
duckweed	0.0	0.6	1.1	1.6
CP	34.1%	34.7%	34.9%	34.7%

Total	**100.0**	**100.0**	**100.0**	**100.0**

Note: control (T1 (0% DW) = 0% inclusion of duckweed.

T2 (10% DW) = 10% inclusion of duckweed.

T3 (20% DW) = 20% inclusion of duckweed.

T4 (30% DW) = 30% inclusion of duckweed

The feed was adjusted in accordance with the general NRC (2011) guidelines for water recirculation systems used in Nile tilapia growth. According to the guidelines, the diets were given at 3% their body weight. Two feeding times each day were performed at 10:00 am and 4:00 pm. The recommended 35% crude protein was used in the preparation of the test diets used for the feeding experiment (Riche and Garling, 2003). Pellets were dried and their proximate composition was determined using AOAC techniques (2001).

3.4. Determination of Growth and Feed Utilization

Juvenile *O. niloticus's* growth performance was determined in 12 tanks using three experimental diets and a control diet with three replicates. The experimental tanks were carefully cleaned with spring water and detergent prior to starting the growth experiment. At the NFALRC hatchery, juvenile *O. niloticus*, the experimental fish, were hatched and raised. Each treatment obtained 90 juveniles in total, with 30 fish per tank being the stocking density. Before the experimental fish (360 juveniles) were placed into each tank, their initial weight was measured.

Fish samples were taken on a monthly basis to determine growth performance. Fish samples were measured for overall length and weight. The number of fish initially stocked and ultimately obtained at the end of the study was also noted in order to determine the survival rate. The following variables were used in the experiment to estimate growth performance: specific growth rate (SGR), daily growth rate (DGR), following Ricker (1975).

$$Average\ initial\ length(cm) = \frac{Sum\ of\ individual\ length\ at\ the\ beginning}{Total\ number\ of\ individuals}$$

$$Average\ final\ length\ (cm) = \frac{Sum\ of\ individual\ length\ at\ the\ end}{Total\ number\ of\ individuals}$$

$$Average\ initial\ weight\ (g) = \frac{Sum\ of\ individual\ weight\ at\ the\ beginning}{Total\ number\ of\ individuals}$$

$$Average\ final\ weight\ (g)\ = \frac{Sum\ of\ individual\ weight\ at\ the\ end}{Total\ number\ of\ individuals}$$

$$Survival\ rate\ \ (\%) = \frac{Number\ of\ survivals\ at\ the\ end\ of\ the\ experiment\ * 100\%}{Number\ of Juvnile\ stocked}$$

$$Relative\ growth\ rate\ (\%)\ = \frac{Weight\ gain\ (g)\ \ \ * 100}{Initial\ body\ weight\ (g)}$$

specific growth rate (SGR)=((ln FBW (g))-(ln IBW (g))/number of trial days)x 100 (Ricker, 1975), where, FBW = Final body weight and IBW = Initial body weight

$$Weight\ gain = final\ weight\ (g) -\ intial\ weight\ (g)$$

*Daily growth rate (DGR)=Final weight (g)- initial weight/number of trial period*s

$$Feed\ conversion\ ratio\ (FCR) = Dry\ feed\ fed\ (g)/weight\ gain(g)$$

3.5. Proximate Compositions of Feed Ingredients and Fish Tissue

The proximate analysis of the test feeds and fish tissue was performed in accordance with standard procedures described in AOAC (2001). Samples of fish were taken at the time of harvest for the tissue proximate analysis and samples of diets were also taken from formulated diets.

The standard micro Kjeldahl nitrogen method, described in AOAC (2001), was used to determine and analyze the crude protein (CP) in a triplicate sample. First, a clear solution was produced when one gram of sample was thoroughly digested in concentrated H_2SO_4 using the Behroset InKje M digestion apparatus. The generated clear solution was chilled for 30 minutes after the digestion was finished. The resulting solution was distillated using the automatic Kjeldahl distillation system in an alkaline environment by adding 40 ml of sodium hydroxide (NaOH) (40%) and distilled water. Ammonia-containing distillate was contained in a 30 ml solution of boric acid (H_3BO_3) (2%) for the duration of the experiment. When the receiving flask's volume was between 100 and 150 ml, the distillation operation was stopped. Then the ammonium borate produced was titrated with 0.1 N HCl to determine the nitrogen content. By multiplying the nitrogen content by 6.25, the amount of crude protein was calculated. The result was expressed as a percentage of the original weight of the sample.

$$Kjeldahl\ nitrogen\ \% = (VS - VB)x\ M\ x14.01/Wx10$$

$$Crude\ protein\ \% = \%Kjeldahl\ Nx\ F$$

Where Vs = volume (ml) of standardized acid used to titrate a test; V_B = volume (ml) of standardized acid used to titrate reagent blank; M= molarity of standard HCl; 14.01= atomic weight of N; W= weight (g) of test portion of standard; 10= factor to convert mg/g to percent and F= factor to convert N to protein. The F factors are 5.70 for wheat, 6.38 for dairy products and 6.25 for other feed materials.

Using the Soxhlet extractor and a solvent extraction method, the amount of crude lipid (CL) was determined. First, a porous cellulose extraction thimble that was empty and covered in fat-free cotton was dried in an oven at 105 °C for 30 minutes before being chilled in a desiccator for the same amount of time and weighed. The next step was to put three grams of a ground and dried sample in a thimble. The thimble was placed in an extraction chamber that was suspended above a flask containing diethyl ether solvent and below the condenser. The extraction chamber was filled with boiling chips and a flask containing diethyl ether solvent. The flask was then heated to 60 ^{o}C, enabling the solvent to evaporate and shift up into a condenser, where it was changed into a liquid that trickled into the extraction chamber containing the sample. After the extraction procedure, which usually takes six hours, the thimble containing the extracted sample was oven-dried at 105 °C for 30 minutes, cooled in a desiccator for 30 minutes, and the weight was calculated. The amount of crude fat in the initial sample was calculated as a percentage using the weight of the fat, which was calculated using the difference between the weight of the thimble containing the sample before and after extraction.

Weight of fat = Weight of thimble before extraction – Weight of thimble after extraction

$$\%\ Weight\ of\ fat\ in\ dry\ basis = \frac{Weight\ of\ fat\ x\ 100}{Weight\ of\ sample}$$

Determining each component's water content is essential for balancing the ration. Monitoring the prepared feed's moisture content is also essential because moisture levels above 8% favor the presence of insects and above 14% increase the risk of fungus and bacterial contamination. The method is based on drying a sample in an oven and calculating the moisture content by comparing the weights of the dry and wet materials. Clean crucibles were placed in an oven set at 105°C for an hour, dried there for an additional hour, cooled, and weighed (W1). Then a three-gram sample (W2) was dried at 105°C for 12 hours after being weighed into pre-measured crucibles. It was again weighed (W3) in triplicate toward cooling to room temperature in a desiccator. Using the following equation, the moisture content was then calculated:

$$\text{Moisture content (\%)} = \frac{\text{weight of wet sample (W1)} - \text{weight of dried sample (W3)} \times 100}{\text{Weight of wet sample (W2)}}$$

The incineration process was used to determine the ash content of test diets and carcasses that quantified the total inorganic matter. The crucibles were first cleaned, dried for 30 minutes at 550°C in a muffle furnace, chilled for 30 minutes at room temperature in a desiccator, and weighed (M1). A muffle furnace was used to incinerate 3g of fresh sample with an accuracy of 4 decimal places for 12 hours, obtaining a clean, white output that was then cooled, measured, and recorded as weight (M3). The ash was represented by the resultant increase in the final weight of the crucible following incineration, which was stated as a percentage of the original sample:

$$Ash\ content\ (\%) = weight\ of\ ash\ x\ 100/weight\ of\ sample$$

The total ash content was subtracted from 100 to determine the total organic matter (TOM).

3.6. Measurement of Physico-Chemical Parameters

A portable electronic multiprobe was used to measure the physiochemical parameters of the culture system, including temperature and conductivity (Model, HQ 40d). The amount of dissolved oxygen was measured using a water- resistant electronic dissolved oxygen meter and the average values were taken as the daily value of water quality parameters. The titration method was used to determine the total ammonia concentration once every two weeks (AOAC, 2001).

3.7. Length - Weight Relationship and Fulton's Condition Factor

Fish wellness is indicated by their length - weight relationships and condition factor, which also determines the stress that water pollution, has on the fish. Fish from each tank were obtained out each month to be measured for length and weight. Using a ruler and a digital electric balance, respectively, the total length and weight were determined. Weight and total length were calculated to the nearest gram and centimeter, respectively. The following formulae were used to calculate Fulton's condition factor:

Fulton's condition factor = Total weight / Total length ^{3} *x 100 (Ricker, 1975)*

3.8. Partial Economic Analysis of Experimental Diets

The profit index and incident costs were calculated utilizing (El-Sayed, 1990). The incidence cost (IC), or relative cost per unit weight gain, was determined using the following formulas:

$$Incidence\ cost = cost\ of feed / weight\ of fish\ produced$$

$$Profit\ index = Value\ of fish / Cost\ of feed$$

The value of the fish was calculated based on current market price. The lower the cost of feed ingredients used in the test feed formulation, the more profitable it will be using that particular test feed. The analysis included calculating the profit and variable costs of the experimental diet.

3.9. Data Analysis

The basic growth performance and body composition data were organized and sorted in MS-Excel version 10. Also, MS-Excel version 10 indicated the fundamental descriptive graph and tables of the factors relating to the water quality, growth and feed used. Using one-way analysis of variance (ANOVA) and a significance level of $P<0.05$, the significant differences between each treatment on growth, feed utilization and body composition were examined. Using SPSS version 20.0, all statistical analyses were performed (SPSS, Michiga Avenue, Chicago, IL, USA). Duncan's Multiple Range Tests were used to examine means that were considerably different. The growth parameters of Nile tilapia fed with various feed ingredients were compared using Analysis of Variance (ANOVA). Tukey Multiple Comparison test were used for the means separation at 5% of significance level.

By the model,

Yij = the response variable,

where Yij = μ + ti + eij.

μ = stands for overall mean,

ti for treatment effect, and eij for random error.

4. RESULTS

4.1. Proximate Chemical Composition of Experimental Diets

The test treatment diets used for the experiment, including the control, have the following proximate chemical compositions, which are shown in Table 2. Feed win software was used to formulate the iso-nitrogenous test diets used in the feeding experiment.

Table 2. Proximate chemical composition of experimental diets (g/kg) fed to juvenile Nile tilapia.

	Control (T1) (0% DW)	**T2 (10% DW)**	**T3 (20% DW)**	**T4 (30% DW)**
Crude protein	341	347	349	347
Crude lipid	55	77	72	43.3
Dry Matter	945.6	944.8	944.4	943.9
Ash	67.6	71.4	69.9	69.3
TOM	932.4	928.6	930.1	930.7

Note: % DW indicated the percentage of duckweed replacing fishmeal in the test diets.

4.2. Growth Performance and Feed Utilization

The growth responses of juvenile Nile tilapia fed on experimental diets are presented in Table 3. There were no significant differences (P> 0.05) in initial body weight (IBW) fed on the different experimental diets. The highest final body weight was recorded for the T3 (20%DW) diet (43.82 g) followed by T2 (10%DW) diet (42.39 g), and the growth performance varied significantly (P<0.05) between fish fed on the different test diet. The results of the present study indicated that the growth performance of juvenile *O. niloticus* increased with increasing up to inclusion level of 20% duckweed. However, at 30 % DW replacement level the fish growth declined below the control diet (0% DW). The results of monthly changes in mean body weight and total length of the juvenile *O. niloticus* are given in Figures 1 and 2, respectively. The feed conversion (FCR) values showed significant difference (P < 0.05) between the test diets. However, the percentage daily growth rate was significantly higher (P < 0.05) in fish fed with 20% DW. The highest FCR value was obtained for T4 (30%DW) diet (3.7). Relatively lower FCR was obtained for T3 (20% DW) (3.46) and T2 (10% DW) (3.43). There was no significant difference (P > 0.05) in survival rate of juvenile *O. niloticus* among the experimental diets. Fish body weight showed significant difference (P<0.05) between the test diets. The highest growth rate was observed in T3 (20%DW) and the least in the T4 (30% DW). On the other hand, there was significant (P < 0.05) in the total length of fish fed on the different test diets (Table 3 and Fig.2)

Table 3. Growth performance and feed utilization of juvenile Nile tilapia, *O. niloticus* fed on different experimental diets**.**

Parameters	**T1 Control (0% DW)**	**T2 (10% DW)**	**T3 (20% DW)**	**T4 (30% DW)**
IBW (g)	12.05 ± 0.14^a	11.72 ± 0.25^a	11.84 ± 0.61^a	12.2 ± 0.3^a
FBW (g)	40.17 ± 1.92^a	42.39 ± 0.33^a	43.82 ± 0.93^b	37.62 ± 1.36^c
IL (cm)	8.76 ± 0.04^a	8.55 ± 0.09^a	8.71 ± 0.16^a	8.7 ± 0.2^a
FL (cm)	13.14 ± 0.17^a	13.27 ± 0.05^a	13.48 ± 0.15^b	12.84 ± 0.09^c
SR %	98.89 ± 1.92^a	97.78 ± 3.85^a	92.22 ± 5.09^a	98.89 ± 1.92^a
SGR % /day	3.4 ± 0.07^a	3.48 ± 0.02^a	3.52 ± 0.01^b	3.31 ± 0.05^c
WG	28.11 ± 1.98^a	30.67 ± 0.58^a	31.98 ± 0.33^b	25.42 ± 1.51^c
DGR g/day	0.23 ± 0.01^a	0.25 ± 0.00^a	0.26 ± 0.00^b	0.21 ± 0.02^c
RGR (%)	233.51 ± 0.07^a	262.15 ± 0.02^a	271.26 ± 0.01^b	209 ± 0.05^c
FCR	3.53 ± 0.08^a	3.43 ± 0.14^b	3.46 ± 0.03^a	3.7 ± 0.2^c
FCF	1.77 ± 0.02^a	1.81 ± 0.03^a	1.79 ± 0.02^a	1.76 ± 0.03^a

The values are means ± SE, and the values in the same row with different letters are significantly different (P $\leq$ 0.05). The following terms are used in this statement: IBW (initial body weight), FBW (final body weight), WG (weight gain), SGR (specific growth rate), RGR (relative growth rate), DWG (Daily weight gain), FCR (feed conversion ratio), and FCF (Fulton's condition factor).

Note: 0%-------------------Control T1(0% DW)

10%------------------T2(10% DW)

20%-------------------T3(20% DW)

30%--------------------T4(30% DW)

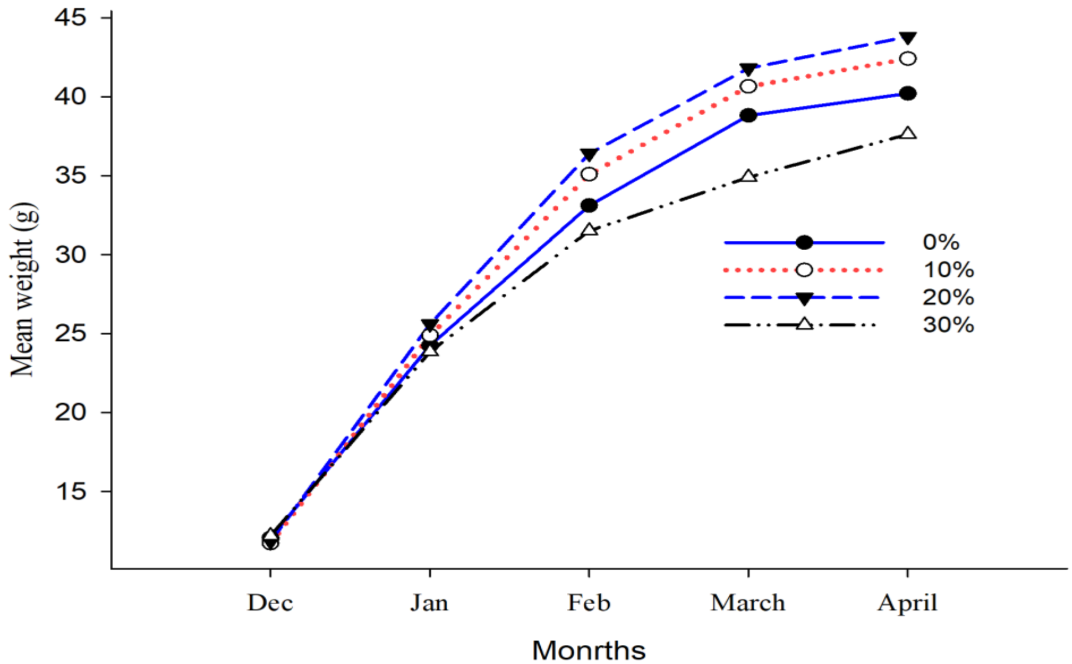

Figure 2 : Monthly growth performance of juvenile *O. niloticus* fed on experimental diets.

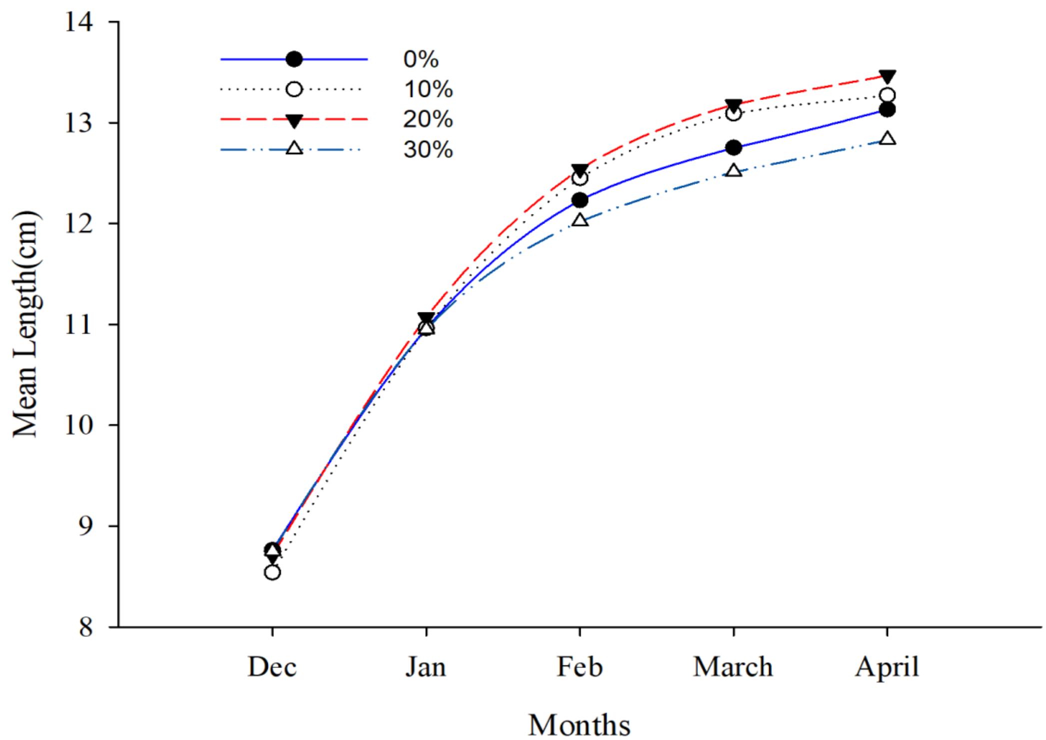

Figure 3. Monthly growth in mean length of juvenile *O. niloticus* fed on the different experimental diets.

4.3. Body Proximate Composition

The proximate chemical composition of fish tissue fed on the various test diets is presented in Table 4. Fish fed on T4 (30% DW) had significantly lower crude lipid and crude protein content among the test diets. The crude protein contents of fish tissue were significantly ($P < 0.05$) affected by the inclusion level of duckweed in juvenile tilapia diets. The highest CP was recorded in fish fed T2 (10% DW) diet (63.5% CP) and the least in T4 (30% DW) diet (55.1 %CP). Ash contents were not significantly ($P>0.05$) different from the control diet.

Table 4: Whole body tissue proximate composition (% dry weight) of juvenile Nile tilapia (*O. niloticus*) fed on different experimental diets.

Components	**Control (0% DW)**	**T2 (10% DW)**	**T3 (20% DW)**	**T4 (30% DW)**
Moisture	71.58±0.31^{b}	72.59± 0.92^{c}	71.86± 0.27^{a}	71.95± 0.23^{a}
Crude protein	59.2±2.43^{a}	63.49±1.96^{b}	60.38±0.55^{a}	55.11±1.14^{c}
Crude lipid	4.67±0.00^{a}	5.33±0.67^{b}	3.66±0.00^{a}	2.83±0.16^{c}
Ash	8.42± 0.52^{a}	9.75± 0.69^{a}	8.17± 0.96^{a}	12.34±1.59^{a}

The results are the mean ± SE of three replications, and values in the same row with different letters are significantly different ($P \leq 0.05$)

4.4. Physicochemical Parameters

Data on the physicochemical parameters of the experimental tanks are summarized in Table 5. The values are shown as mean ± SE. Throughout the experiment, the water's temperature varied between 23.01 °C and 25.35 °C, the DO concentration varied between 3.79 mgL^{-1} and 6.07 mgL^{-1}, and NH3 concentration varied from 0.06 mgL^{-1} to 0.07 mL^{-1} and all showed no significant ($P>0.05$) differences between treatments. The concentration of DO record in the afternoon showed significant ($P<0.05$) difference between the test diets.

Table 5: Physicochemical parameters (mean ± SE) of water in recirculating tanks maintained under different experimental diets.

Parameters	Time	T1 Control (0% DW)	T2 (10% DW)	T3 (20% DW)	T4 (30% DW)
Temperature(°C)	10:am	23.01 ± 0.16^{a}	23.38 ± 0.17^{a}	23.07 ± 0.13^{a}	23.15 ± 0.15^{a}
	4:pm	24.9 ± 0.2^{a}	25.35 ± 0.23^{a}	25.07 ± 0.21^{a}	25.02 ± 0.22^{a}
Conductivity(µS/m)	10:am	177.08 ± 1.31^{a}	178.39 ± 1.27^{a}	176.74 ± 1.08^{a}	177.65 ± 1.16^{a}
	4:00pm	176.02 ± 0.95^{a}	177.78 ± 1.1^{a}	175.87 ± 0.87^{a}	176.79 ± 0.96^{a}
DO (mgL^{-1})	10:am	4.21 ± 0.12^{a}	4.25 ± 0.14^{a}	4.21 ± 0.13^{a}	3.79 ± 0.13^{a}
	4:00pm	5.89 ± 0.15^{a}	5.92 ± 0.16^{a}	6.07 ± 0.14^{b}	5.42 ± 0.17^{c}
NH3 ($mg\ L^{-1}$)		0.07 ± 0.01^{a}	0.06 ± 0.01^{a}	0.07 ± 0.01^{a}	0.06 ± 0.01^{a}

The results are the mean ± SE of three replications; values within the same row with the same superscripts are not significantly different ($P > 0.05$), while values within the same row with different superscripts are significantly different ($P \leq 0.05$). where DO, dissolved oxygen.

4.5. Length weight relationship and Fulton's condition factor

Results of the length-weight relationship of juvenile *O. niloticus* is shown in Figure 5. The regression coefficient (b) calculated for juvenile O. *niloticus* fed on various proportions of fish meal and duckweed. found in the present study ranged from 2.83 to 2.91. All treatment diets showed from slightly negative allometric growth to values close to isometric growth. Similarly, the calculated Fulton's condition factor (FCF) was not significantly ($P > 0.05$) different for fish fed on the various test diets (3).

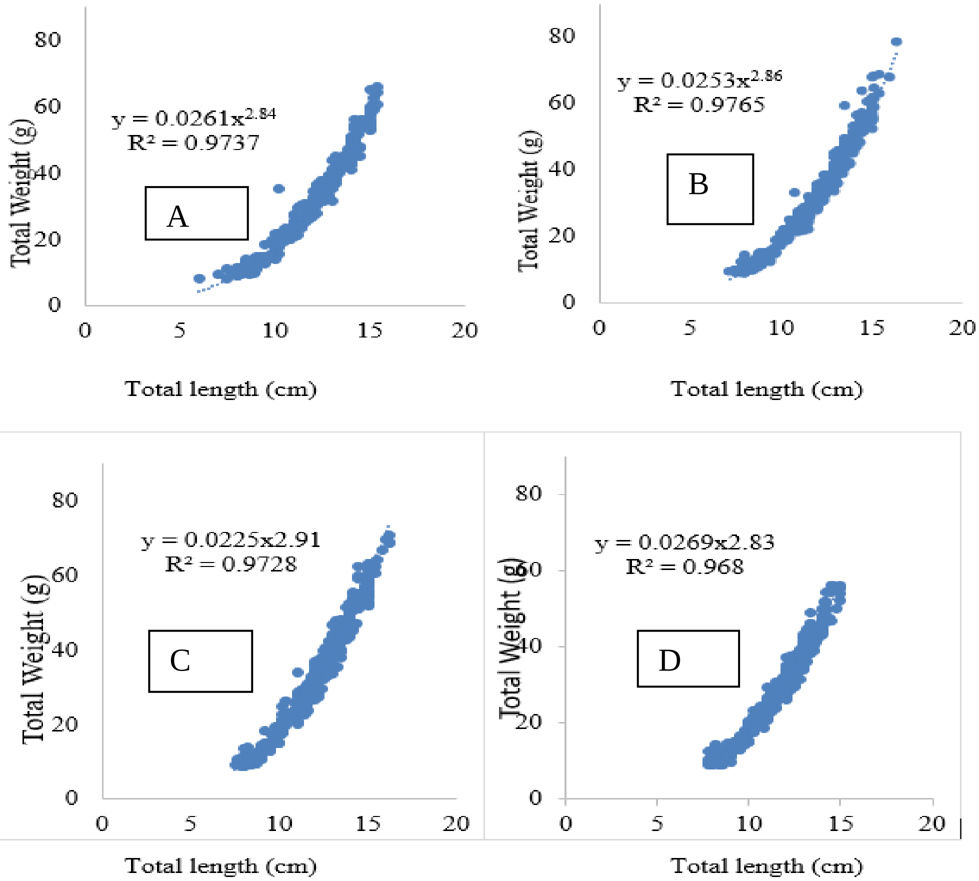

Figure 4: The length weight relationships of juvenile *O. niloticus* fed on the different treatment diets: A, Control diet (T1 0 %), B, T2 (10% DW), C, T3 (20% DW) and D, T4 (30% DW).

4.6. Partial Economic Analysis of Experimental Diets

The most expensive ingredient was fish meal (55 Birr kg^{-1}), which was followed by soybean cake (38 Birr kg $^{-1}$). The least expensive ingredients were duckweed and wheat middling, which had prices of 10 and 12.5 Birr per kilogram, respectively. Table 6 shows the cost analysis of experimental diets. The price of the diets as a whole ranged from 33 to 31.6 Birr per kilogram, with 0% DW being the most expensive option. The total feed given to the experimental fish per treatment varied from 8.29 to 9.3 kg. The overall cost of the experimental diets used during the experiment was highest for the T3 (20% DW) (295.74 Birr) and the lowest for the T4 (30% DW) (261.96 Birr).

However, T3 (20% DW) and T2 (10% DW) showed substantially higher overall fish weights (Table 6). The price of whole fish Nile tilapia on the local market is currently about 150 Birr per kilogram near Addis Ababa. The incidence cost (IC) and profit index (PI) economic efficiency parameters ranged from 80.3 to 77.04 and from 0.51 to 0.57, respectively. When compared to the other experimental diets, T4 (30% DW) had the lowest incidence cost (Table 6).

Table 6: Partial economic analysis of experimental diets fed to juvenile *O. niloticus*.

Parameters	**T1 Control (0% DW)**	**T2 (10% DW)**	**T3 (20% DW)**	**T4 (30% DW)**
Cost of feed (Birr kg $^{-1}$)	33	32.13	31.8	31.6
Total feed used (Kg)	8.76	9.1	9.3	8.29
Cost of total feed used (Birr kg $^{-1}$)	289.08	292.38	295.74	261.96
Produced fish weight (Kg)	3.6	3.7	3.8	3.4
Estimated local market value (Birr kg $^{-1}$)	150	150	150	150
Incidence cost	80.3	79.02	77.82	77.04
Profit index	0.51	0.51	0.51	0.57

5. DISCUSION

5.1. Growth Performance and Feed Utilization

The study indicated that duckweed inclusion levels more than 20% in the diets of juvenile *O. niloticus* caused a significant (P <0.05) decline in growth performance of the fish. It is evident that the increased level of duckweed inclusion showed progressive growth rate until 20% replacement of duckweed and then dropped at 30% duckweed inclusion in the diet. The T3 diet that included 20% duckweed showed the highest final body weight. This indicated that juvenile Nile tilapia can take and assimilate feeds with a limit not more than 20 percent. It could be due to the negative effect of anti-nutritional factor occurring in duckweeds over 20% DW inclusion rate which hindered the growth of juvenile in the present study. Similar to the findings of the current study, According to Mohapatra *et al.* (2013), duckweed plant (*Lemna minor*) substituted for fishmeal at percentages of 0% and 15% resulted in the best growth rate and weight increase for common carp (*Cyprinus carpio*) fingerlings, however duckweed substituted at 45% resulted in a lower rate of growth and weight gain. This is in agreement with the findings of Asimi *et al.* (2018), who 15% duckweed was used and showed better growth and weight gain results than more inclusion levels (30% and 45%DW). In addition, when commercial fish meal substitution for 20% duckweed meal in common carp (*Cyprinus carpio*), Yilmaz *et al.* (2004) reported no weight difference in the fish. The growth depression that was reported at 30% fermented *Lemna* leaf meal addition to *Labeo rohita's* diet was where the maximum effectiveness was seen. Vinogradskaya and Kasumyan (2019) suggest that the *Lemna* may contain nutrients that are causing damage to fish performance.

Duckweed can be used in alternative to fishmeal, according to other experiments on Nile tilapia. According to Fasakin *et al.* (2001), the Nile Tilapia (*Oreochromis niloticus*) used the duckweed *Spirodellla polyrrhiza* and grew more quickly than fish fed diets containing mainly of water fern meals. On the other hand, Hutabarat *et al.* (2019) showed increased growth in *O. niloticus* as the amounts of duckweed in the diets increased. The decrease in growth performance of the fish was observed when the inclusion level of the test ingredient reached 75% fermented DW and beyond. This was related to the inadequate utilization of nutrients by the fish. This supported the findings of Tavares *et al.* (2008) who observed that *O. niloticus* growth decreased as the amount of duckweed in the diet increased more than 50%. The same result was observed by Flores-Miranda *et al.* (2015), who noted that *L. vannamei* growth performance suddenly declined when the test component (fermented duckweed) was raised by more than 50% DW in the diets. The growth decline was noted at 30% DW substitution rate which was in contradiction to the findings reported by Tavares *et al.* (2008), who reported growth depression in Nile tilapia given exclusively of duckweed diet. Poor feed utilization, which was seen at these substitution levels, can be attributed to the high levels of antinutritional factors. In another study, dietary levels of 15, 25, 35, and 45% were used to assess the effects of fermented duckweed (*Lemna minor*) on juvenile barramundi (*Lates calcarifer*). They found that barramundi fed up to 35% fermented *L. minor* meal had performance characteristics similar to control fish, with barramundi fed 25% fermented duckweed meal even marginally outperforming control fish (Mustofa and Wahidah, 2022).

However, in similar to the present study, it was also shown that *O. niloticus'* growth rate decreased as duckweed levels increased. The growth depression that was noticed at 30% fermented *Lemna* leaf meal included in the diet of *Labeo rohita* (Bairagi *et al.*, 2002). Fish productivity would decline if duckweed were completely substituted for fish meal (Yilmaz *et al.*, 2004). This is supported by Tavares *et al.* (2008), who found that 100% inclusion of duckweed did not improve Nile tilapia growth performance.

There was a significant difference ($P < 0.05$) between treatment diets in the specific growth rate (SGR), which is the percentage increase in fish weight per day (Table 3). Weight gain followed a similar pattern, being comparatively higher in fish given the T3 (20% DW) and T2 (10% DW) diets than fish fed all other diets. The SGR was higher or similar to that found by other authors in studies with juvenile Nile tilapia of similar sizes (Tiengtam *et al.*, 2015), showing that the fish were raised in good conditions and their health was not affected.

The SGR recorded in the present study ranged from 3.31 to 3.52%. This agrees with Luiz *et al.* (2021) who reported similar SGR values ranging from 3.34 to 3.44% for *O. niloticus* in controlled environment. The SGR was higher or similar to that obtained by other authors in studies conducted with Nile tilapia juveniles of similar sizes (Tiengtam *et al.,* 2015), indicating that the fish were in good rearing conditions and their health was in good condition. This *Lemna* supplementation result is remarkable and can be considered as an achievement because it showed that *Lemna* can be used as a dietary supplement to improve fish welfare. According to Vinogradskaya and Kasumyan (2019), there may be antinutritional elements in *Lemna* sp. that can make fish less productive. Specific growth rate recorded in the present study is higher as compared to numerous reports done on *O. niloticus* fed supplemental and compound feeds (Yong *et al.,* 2018). Moreover, SGR ranging from 2.27 to 2.96% and 4.90 to 4.98% have been reported by Mary *et al.* (2020) and Rahman *et al.* (2022), respectively. Lower results were reported by Sharda *et al.* (2017) for *O. niloticus* in a controlled environment, with SGR values ranging from 0.51 to 0.92%. The addition of lysine to the meals in the present study appears to have improved the growth performance of the Nile tilapia.

In this study, daily growth rates significantly ($p < 0.05$) (0.21 g/day) to (0.26 g/day) were observed. Both the T2 (10% DW) and T3 (20% DW) diets had higher relative daily growth rates of 0.25 and 0.26 respectively. Similar to our results, a lower daily growth rate was reported (0.28 g/day - 0.40 g/day) for *O. niloticus* reared in ponds given commercial feed and farm-produced feeds (Zenebe and Abelneh, 2021).

The relative lower growth rates of fish reported in previous studies were due to the use of a mixture of juvenile males and females, which reduced fish growth due to early maturation and nutrients invested in reproduction instead of growth. In this study, egg was also found in some mouth of fish and tanks. The higher fish growth rates were attributed to the quality of the dietary supplements provided and the use of all-male transsexual tilapia, where energy and nutrients from the feed were used primarily for fish growth. Therefore, the relatively lower daily growth rate of fish observed in the present study could be due to the combined effect of the poor-quality feed ingredients used and the stocking of mixed juvenile.

The amount of feed required to produce one gram of fish is known as the feed conversion ratio, and it was significantly different between diets tested in the present study. In this study, the FCR values calculated varied from 3.43 to 3.7 (Table 4). The T2 (10% DW) and T3 (20% DW) diets both had relatively lower FCR values of 3.43 and 3.46, respectively. According to Hassan and Edwards (1992), tilapia fed on fresh duckweed had a feed conversion ratio that varied from 1.6 to 3.3, which was different from the results reported in the present study. Lower feed conversion ratios suggested stronger feed conversion efficiency, which lead to improved growth of fish (Ugwumba and Ugwumba's, 2007). Additionally, in a controlled context, ideally low FCRs for Nile tilapia were reported by Do Espirito Santo *et al.* (2015), Alofa and Abou (2021), and ranged from 1.2 to 1.5. The higher inclusion of duckweed of plant origin might affect the digestive efficiency of the juvenile tilapia and likely resulted in the higher FCR reported for T4 (30% DW) in this study. The concept is consistent with Yakubu *et al*. (2013) study that increased FCR implies poor diet acceptance or utilization.

Regarding the survival rate, few fish died in relation to the impact of experimental diets. The higher survival rate seen across all experimental diets was caused by fish death during the unusual high temperature observed in the green house. Fish fed on the various test diets ranged in percentage survival rate from 92.2% to 98.9%, although there was not a significant difference ($P > 0.05$). Similar mortality was reported for *C. gariepinus* fingerlings raised in tanks ranging from 80% to 96%, (Marimuthu *et al.*, 2011). Previous study conducted in the ponds on the Nile tilapia reported relatively lower survival rate ranging from 80.5 to 92% survival rate (Zenebe and Abelneh 2021). Similarly, several authors also reported comparatively lower survival rates than the present study. For example, Hossain *et al.* (2017) and Liu *et al.* (2012) found survival rates ranging from 88.4% to 92.8% and 73.3% to 93.5%, respectively. In contrast to the present study, Sulawesty *et al.* (2014) found that carp (*Cyprinus carpio L*.) that were not fed with *L. minor* had a lower survival rate of 60%.

5.2. Proximate Compositions of Fish Tissue

When assessing a fish's nutritional and edible qualities, which indicate the fish's quality, information on the fish's proximate body composition is necessary (Anani and Agebo, 2018). The moisture content of juvenile *O. niloticus* fed different experimental diets showed significant difference (P < 0.05) and ranged from 71.58 to 72.59 (Table 4). According to Adewumi *et al.* (2014), the percentage moisture content of the juvenile *O. niloticus* in the present study is within the acceptable range reported for fish (60%-80%). which is similar to the findings of Akter (2015) who found 73.91 and 79.62 moisture in cultured and wild koi (*Anabastestudineus*), respectively. Different values were determined by Garduno-Lugo (2003) and El-Hawarry (2012), who reported 79.1% and 79.09% of moisture content for *O. niloticus*, respectively. The moisture content was also reported to be 79% by Hirut *et al.* (2020). Additionally, Akewake, (2015) stated that Nile tilapia fed with mixtures of various inclusion levels of different plant proteins had moisture contents that ranged from 75.45% to 77.81% and 76.4% to 77.48%, respectively.

In this study, the percentage of crude protein (CP) in juvenile *O. niloticus* fed on different inclusion levels of duckweed diets ranged from 55.11 to 63.49% (Table 4) and showed significant difference (P < 0.05) among treatment diets. Juvenile *O. niloticus* fed on T4 (30% DW) showed a relatively lower proportion of crude protein. *Lemna* has a high protein and amino acid content (Chakrabarti *et al.,* 2018), but contains relatively low levels of essential amino acids for tilapia, specifically lysine, methionine, and tryptophan (Goopy and Murray, 2003). Therefore, decrease in body protein content that was observed may be related to the higher levels of duckweed inclusion in the diet. This may cause a high fiber content and low nutrient digestion and bioavailability for the fish. This was in agreement with Jim *et al.* (2017), who noted that a fish's ability to assimilate and absorb the essential nutrients from its diet may have an impact on the chemical composition of the fish's body. Additionally, the proportion of crude protein in the current study agreed with the percentage of crude protein in controlled conditions reported by other authors. Similarly, Tsegay (2020), who has discovered the crude protein content of *O. niloticus* range from 56.96 to 57.35% in Nile tilapia fed on lupin and grass pea diets. *O. niloticus* fed on a diet including a combination of various plant and animal proteins had CP ranging from 66.11 to 68.58%, Soltan *et al.* (2008). Additionally, Opiyo *et al.* (2014) clearly indicated that the protein content of feed directly influences the protein content of fish fillet.

Percentage crude lipid content (CL) varied significantly (P < 0.05) from 2.83% to 5.33% in the *O. niloticus* fish tissue (Table 4). Fish fed with T4 (30% DW), had a rather low tissue CL and this might be the high proportion of duckweed rich in crude fiber which lowers digestibility of nutrients in fish diets. Contrary to our study, Zenebe (2010), reported high fatty acid content from *O. niloticus* from Lake Hashengie. Tsegay *et al.* (2016) also confirmed that *O. niloticus* in Lake Hashengie had a greater crude lipid content. Additionally, the percent crude fat content of the present study was higher than the crude fat (2.07) reported for *O. niloticus* by Garduno-Lugo (2003).

The high moisture content of the fish tissue in the present study may be the reason for the low lipid reported. According to Zmijewski *et al.* (2006), fish species typically show a reverse relationship between their fat and water content. The higher the water temperature, the lower the crude fat content will be, hence the lower crude fat content in the present study may also be the effect of the rearing temperature and agrees with (Tsegay *et al.*, 2016) finding. The higher dietary protein level used in this study may potentially be the cause of the lower crude fat content. This is due to the direct relationship between diet protein content and fillet protein content of the fish (Opiyo *et al.*, 2014). Fish fillets with high protein content may have lower levels of some other nutrients, such as fat. This is in agreement with the finding of Al Hafedh (1999).

Additionally, the large proportion of duckweed in the diet may be the reason for the lower CL of fish fed on T4 (30% DW) diet. According to Zenebe (2010), *O. niloticus* from Lakes Babogaya, Hora, Haiq, and Tana had lower fat contents than Lake Hashengie. This was because the diet of *O. niloticus* from the former lakes was primarily made up of phytoplankton, whereas the diet of the other lake had a higher concentration of zooplankton.

The percentage ash content of the fish fillet in the present study ranged from 8.17 to 12.34% dry weight. When compared to the T3 (20% DW) diet, the control was found to have a significantly higher ash content. In comparison with the other diets, fish fed the control diet showed significantly ($P < 0.05$) lower ash content. In a related finding, *O. niloticus* fed Moringa seed meal showed a lower ash content (5.85 to 10.7%) (Hashem *et al.*, 2017). Contrary to our study, Muin *et al.* (2017), reported ash contents of *O. niloticus* ranging from 14.8 to 16.51% in diets fed on different levels of the black soldier fly, *Hermetia illucens*.

5.3. Length Weight Relationship and Fulton's Condition Factor

The regression coefficient "b" obtained in the present study varied from 2.82 to 2.9. Thus, the calculated values of the regression coefficient indicated slight negative allometric growth of the fish in all treatments, which causes the fish to become slim as its weight grows. This is supported by Shingleton (2010) where length-weight relationships indicate an isometric growth when $b=3$, *i.e.*, the relative growth of both variables is equal (Quinn II and Deriso, 1999). While $b>3$ showed a positive allometric growth and is known as hyper allometry, $b<3$ has been shown to have a negative allometric growth and is known as hypo allometry. The fact that the values calculated in this study appears to be close to the isometric value of "b," which is 3, indicates that the strains' growth was rather near to isometric growth. The obtained "b" value in the present study was similar to previous studies conducted on the same species in a controlled environment and published by various authors. For instance, Abelneh *et al.* (2015) reported regression coefficients ranging from 2.76 to 2.86 for Nile tilapia grown in pond culture systems. Fiyor (2022) also obtained "b" values for tank cultured Nile tilapia that ranged from 2.61 to 2.77. Similarly, different values of "b" have been reported for Nile tilapia collected from. For instance, Nile tilapia in Lake Tana has a regression coefficient value of 2.74 (Zenebe, 1997). Million *et al.* (2021) obtained a regression coefficient value of 2.91 for Nile tilapia in Lake Chamo.

The Fulton's condition factor (FCF), a measurement of fish health showed no significant difference ($P > 0.05$) between the test diets used in the present study (Table 3). The calculated FCF of juvenile *O. niloticus* varied from 1.76 to 1.81. The T2 (10% DW) diet showed a slightly higher but statistically insignificant condition factor (1.81) than the other treatment groups. Additionally, the condition factors in each treatment diet were within the acceptable range for fish in good condition.

Furthermore, the Fulton condition factor obtained in this study agreed well with condition factors previously reported by different investigators. For instance, *O. niloticus* in Lake Ziway had an FCF value of 1.89 (Zenebe, 1988). This is consistent with the recommendation made by Shahabuddin *et al.* (2015) for a condition factor greater than 1 indicates the state of healthy fish. Additionally, FCF values of 1.87, 1.81, and 1.84 have been reported for *O. niloticus* in Lakes Koka, Ziway, and Langeno, respectively (Gashaw and Zenebe, 2008). In a controlled environment, Mary *et al*. (2020) also found FCF values for Nile tilapia that ranged from 1.74 to 1.81. The greater breeding rate seen during the experiment may be the cause of the difference in the FCF levels between the current study and a previously reported finding. According to Flipos *et al.* (2013), variations in condition factor may be caused by a number of factors including the quality of fish diet, sex and gonad development. Similar arguments were given by Zenebe (1997) regarding the poor condition of *O. niloticus's* observed during the peak breeding season.

5.4. Partial Economic Analysis of Experimental Diets

The partial economic analysis of the different experimental diets used in this study are presented in Table 6. The lowest incidence cost (IC), which shows the cost of feed needed to produce 1 kg of fish, was obtained in T4 (30% DW) (77.04), followed by T3 (20% DW) (77.82), T2 (10% DW) (79.02) and control T1 (0% DW) (80.3) feed. This shows that it is economically more feasible to use T4 (30% DW) diet from the perspective of the economy. Abu *et al.* (2010) suggested that the more cost-effective a diet is, the lower the IC value which is in agreement with our result obtained. However, comparatively, a higher profit index (0.57) was obtained for the T4 (30% DW) diet despite the lower growth rate of fish recoded. The lowest incidence cost of 30% DW (T4) diet might be due to the higher inclusion the cheap duckweed replacing the most expensive fishmeal in the diet. This might be due to the high inclusion (30% DW) of the cheap duckweed replacing the more expensive fishmeal makes it more cost effective than the other diets. The findings of the present study also agreed with the results reported by Fiyor (2022), who found that replacing animal and plant protein sources in the diets of Nile tilapia with 78% plant protein and 22% animal protein resulted in a lower incidence cost (48.7) than the ABP65-based diet, (68.8) which was formulated with 35% plant protein and 65% animal protein.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the results obtained in this study, it can be concluded that the essentially cheap duckweed (*Lemna* spp.) could be used at inclusion levels of 20%, as a replacement for fishmeal, which is extremely expensive, in the diet of juvenile Nile tilapia. This will reduce cost of production and there by further enhance aquaculture development in Ethiopia.

- Increasing the amount of duckweed in a juvenile *O. niloticus'* diet over 20% had negative effects on the growth performance and feed utilization of the fish. In fact, the diet that included duckweed meal at rates of 20% performed better on all growth and feed utilization parameters. Additionally, lysine supplementation could help to improve the amino acid profile in the fish diet.
- The proximate body compositions of the fish fed on different experimental diets showed significant differences ($P < 0.05$) in terms of major nutrients including crude protein and crude lipids. The results of the study showed that the lowest crude protein and low-fat contents of the fish body were obtained in juvenile *O. niloticus* fed a 30% DW (T4) diet. However, the 10% DW (T2) showed the highest crude protein content of the fish tissue.
- The economic analysis of the current study showed that feeding fish a diet with T4 (30% DW) duckweed inclusion produced higher economic returns. Moreover, T4 (30% DW) diet showed the lowest incidence cost (77.04) and had high profit index (0.57), followed by T3 (20% DW) incidence cost (77.82) and profit index (0.51) demonstrating its viability from an economic perspective. This can be attributed to the high inclusion rate of the cheap duckweed in the diet.

6.2. Recommendations

- Based on the conclusion reached in this study, it is recommended that 20% DW can be included in the diet of juvenile fish.
- Based on the results, it is recommended that duckweed can be cultivated for a small- or large-scale Nile tilapia fish production.
- We also suggest to conduct similar experiments on grown- out Nile tilapia and other culture fish species.
- The Nile tilapia used in the present growth experiment was composed of mixed sexes. This likely slows the growth of the fish. Therefore, additional research is recommended on monosex Nile tilapia in the future.

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8. APPENDICES

Appendix 1. Picture showing planted duckweed **A**, Duckweed harvesting B, Juvenile during feeding **C**, Duckweed in powder form **D**

Appendix 2. A picture showing pellet preparation and drying of pelleted feeds.

Appendix 3. Picture showing juvenile collection and selection to run the experiment.

Appendix 4. Photo showing protein proximate analysis in the laboratory

Appendix 5. Picture showing ash determination in the furnace and analysis of crude lipid proximate chemical composition by Soxhlet extractor analysis in the NFALRC laboratory

Appendix 6. Picture showing ammonia analysis in the NFALRC laboratory.

Appendix 7. Picture showing measurement of body length and measurement of body weight.

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Length	Between group	4855.554	4	1213.888	1058.717	.000
	Within treatments	2045.474	1784	1.147		
	Total	6901.027	1788			
Weight	Between group	205010.548	4	51252.637	679.777	.000
	Within treatments	134506.950	1784	75.396		
	Total	339517.498	1788			

Appendix 8. Analysis of the variance of feed

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
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Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/34/04/15/2023

Name and affiliation of applicant: **Ayantu Getachew (BSc, MSc student)**
Department of Animal Production Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Effect of partial replacement of fishmeal with duckweed (lemna sp) meal on the growth performance of juvenile Nile tilapia (Oreochromis niloticus L.) in tanks*

Date of application:	**December, 2022**
Nature of the project:	**Experimental feeding trial**
Target animal species:	**Fish**
Number of animals involved:	**360**
Study area:	**National Fishery and other aquatic life research center, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/15/022, 02/02/2023**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Ayantu Getachew.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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