



Partial replacement of fish meal by a mixture of lupin (*Lupinus albus* L.) and grass pea (*Lathyrus sativus* L.) meal in the diets of Nile tilapia (*Oreochromis niloticus* L.) fingerlings in a recirculating aquaculture system

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By

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GRADUATE PROGRAMS

This is to certify that the thesis prepared by Tsegay Fisseha, entitled: Partial replacement of fish meal by a mixture of lupin (*Lupinus albus* L.) and grass pea (*Lathyrus sativus* L.) meal in the diets of Nile tilapia (*Oreochromis niloticus* L.) fingerlings in a recirculating aquaculture system and submitted in partial fulfillment of the requirements for the Degree of Master of Sciences (Aquatic Ecosystems and Environmental Management) complies with the regulations of the university and meets the accepted standards regarding originality and quality.

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LIST OF ABBREVIATIONS

ANFs	Antinutritional Factors
AOAC	Association of Official Analytical Chemists
CF	Crude Fiber Content
CL	Crude Lipid Content
CMC	Carboxyl Methyl Cellulose
CP	Crude Protein Content
DO	Dissolved Oxygen
EIAR	Ethiopian Institute of Agricultural Research
EPHI	Ethiopian Public Health Institute
FAO	Food and Agriculture Organization
FBW	Final Body weight
FCF	Fulton's Condition Factor
FCR	Feed Conversion Ratio
FI	Feed Intake
GE	Gross Energy
Hb	Hemoglobin
Hct	Hematocrit
HP	Horse Power
HSI	Hepatosomatic Index
IBW	Initial Body Weight
IC	Incidence Cost
LGM	Lupin and Grass pea Mixture Meals

MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
NFALRC	National Fisheries and other Aquatic Life Research Center
NFE	Nitrogen Free Extract
PI	Profit Index
RBC	Red Blood Cell
SBO	Soybean Oil
SGR	Specific Growth Rate
SPSS	Statistical Package for Social Sciences
SR	Survival Rate
VMP	Vitamin and Mineral Premix
WBCs	White Blood Cell Counts
WG	Weight Gain

ABSTRACT

Aquaculture is one of the fastest-growing animal production sectors in the world producing near half (47%) of the world total fish production. However, it depends heavily on the expensive fish meal which is a limiting factor for the formulation of optimal fish diet in aquaculture systems. Although several investigators have tried to replace the fish meal with different locally available sources of plant proteins, similar studies on incorporating a mixture of lupin and grass pea meal are non-existent. This study evaluated the effects of fish meal replacement by a mixture of lupin and grass pea meal at inclusion levels of 0, 25, 50, and 75% as potential plant protein sources in the diets of Nile tilapia fingerlings on growth performance, feed utilization, carcass composition, and hematological parameters. The study was carried out in the Center for Aquaponics and Recirculating Aquaculture System, in the College of Natural and Computational Sciences, Addis Ababa University from October, 2019 to December, 2019 for 10 weeks. Experimental diets were formulated to be iso-nitrogenous (36g 100 g⁻¹), iso-lipidic (10g 100 g⁻¹) and iso-energetic (18 KJ g⁻¹), and fed at 6-10% of their body weight day⁻¹. A total of 276 Nile tilapia fingerlings were stocked into four treatments, each in three replicates using a completely randomized design where different dietary treatments were randomly assigned to the experimental aquaria. At the end of feeding trials, results revealed that replacement of 25, 50, and 75% of fish meal by lupin and grass pea mixture meals did not significantly ($P > 0.05$) affect most of growth and feed utilization parameters. However, fish fed with 25% lupin and grass pea mixture meal revealed enhanced final body weight and improved feed intake. This enhanced growth performance of fingerlings might be attributed to the compensatory effects of mixing plant proteins that improved the amino acid profile and bioavailability. Compared to the control diet, increasing lupin and grass pea mixture meals in the diets significantly increased hepatosomatic index. This might have been associated with higher feed intake and the high energy content in the feed that resulted in increased storage of glycogen or lipid in the liver. Replacement of fish meal by lupin and grass pea mixture meal has also resulted in comparable carcass composition and hematological parameters. These comparable and promising results might have been because of the improved amino acid profile due to the effect of mixing feed ingredients, and reduced antinutritional factors in the experimental diets due to preprocessing of lupin and grass pea by soaking, dehulling, and roasting. However, Hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and lymphocytes had shown significant increase at higher fish meal replacement levels (75%). From the economic point of view, lowest incidence cost (44.31) and higher profit index (1.58) was obtained in the 75% replaced diet, whereas highest incidence cost (72.5) and lowest profit index (0.96) was recorded from the control diet. In general, there are promising nutritional and economic justifications for replacing fish meal by a mixture of lupin and grass pea protein sources in the diets of Nile tilapia considering growth parameters, feed utilization, carcass composition, hematological parameters, and economic analysis up to 75% level.

Keywords: *Carcass composition, Feed utilization, Fish feeds, Hematology, Lupinus albus*

1. INTRODUCTION

1.1. Background and Justification

Aquaculture is among the fastest-growing food production systems globally, producing nearly half (47%) of the total world fish production (FAO, 2018) which depends heavily on aquafeed input. It has become responsible for the continuing impressive growth in the supply of fish in contrast to the relatively static growth of capture fishery. The rapid growth of the aquaculture sector results from the progressive intensification of using quality aquafeeds and production systems (FAO, 2006). However, aquafeed is the major determinant factor that accounts for up to 70% of the total aquaculture production costs in semi-intensive and intensive types of systems (El-Sayed, 2004; Dorothy *et al.*, 2018). As a predominant source of protein, fish meal is a highly preferred ingredient for formulating aquafeeds though feed production depends on many protein and energy ingredient sources (El-Saidy and Gaber, 2003; Hasan *et al.*, 2012).

Fish meal is an excellent protein source in aquafeeds because of its high digestibility and palatability, high protein content, balanced amino acid, rich in omega-3 fatty acids and having physical properties that improve the preparation of nutritious feeds (Kirimi *et al.*, 2016). However, the increase in the cost of fish meal poses real problems for cost-effective feed formulation (El-Saidy and Gaber, 2003; El-Sayed, 2004), and its supply is subjected to overfishing and not readily available. Nowadays, there is a growing concern for reducing the inclusion rate of fish meal in aquafeeds and searching other economically viable, abundant, and environmentally friendly options for replacement of fish meal and fish oil. Commercially formulated diets lack the extent of accessibility and acceptability by fish farmers to adopt sustainable commercial fish production (El-Sayed, 2004; Jackson, 2012). Thus, aquaculture researches should be focused on the production of high-quality aquafeeds using locally available feedstuffs which can effectively replace fish meal and help reduce production costs.

In the replacement of fish meal, protein components of animal origins like poultry by-products and their combinations, meat and bone meal, feather meal and blood meal, fish-offal, agro-industrial by-products and other local fishery by-products are among the reasonable alternatives (Mondal *et al.*, 2012; Zenebe Tadesse *et al.*, 2012). But, there are adverse health hazards with using animal by-products due to disease outbreaks like bird flu, swine fever virus, and mad cow diseases (Wang *et al.*, 2008). Besides that, the non-conventional feedstuffs of animal origin are unavailable in large commercial quantities to sustain the aquaculture industry. Conversely, plant

proteins are not only less risky, but they are also abundant offering a more suitable option as alternative protein sources in fish feeds (Naylor *et al.*, 2009; Alayu Yalew *et al.*, 2019) although they often contain antinutritional factors (ANFs), which can affect growth performance and fish health.

Several published scientific reports have indicated the inclusion or/and replacement of fish meal and potential protein sources of different plant-based aquafeeds. Among these protein sources include soybean meal, cottonseed meal, linseed meal, niger seed, cereals, and their byproducts, wheat bran, sunflower meal, jatropha kernel meal, and cornflour were the few among many and reported to have a good potential in tilapia diets (Zenebe Tadesse *et al.*, 2012; Kassaye Balkew *et al.*, 2013; Madalla *et al.*, 2013; Akewake Geremew, 2015).

Ethiopia is endowed with diverse agroecological diversity favoring for farming of the different pulses and crops, supplying a huge potential for many feed ingredients and alternative foodstuffs comfortable for incorporating as aquafeeds. Among these huge potentials, the leguminous food ingredients of lupin (*Lupinus albus*) and grass pea (*Lathyrus sativus*) are highly produced in Ethiopia. Lupin and grass pea have a nutritional quality comparable with most legumes as they contain similar nutritional quality in the proportion of protein, fat, minerals, and vitamins (Caruso, 2015; Lambein *et al.*, 2019). However, their potential suitability as aquafeeds is not well studied and reported particularly in Ethiopia. Utilization of these resources to fulfill the natural resource base promises a considerable potential for success and, thus it is imperative to address the role of locally available feed resources in minimizing production cost and finally make an attractive aquaculture production system to farmers in Ethiopia. However, the replacement of fish meal with plant origin feedstuff has been limited by the presence of ANFs. These include phytate, lectins, protease inhibitors, haemagglutinins, saponins, alkaloids, glycosides, oxalic acids and lathyragens to mention a few despite their nutritional values and low-cost implications (Viola *et al.*, 1988; Francis *et al.*, 2001).

Several researchers have studied the inclusion of different locally available animal and plant protein sources into aquafeeds in different fish species including Nile tilapia. For instance, Zenebe Tadesse *et al.* (2012) evaluated niger seed cake, brewery waste and wheat bran as supplementary feed ingredients, Kasaye Balkew *et al.* (2014) evaluated jatropha curcas kernel meal as a protein source, Akewake Geremew (2015) evaluated some Ethiopian oilseed cakes as protein sources, and Mohammed Oumer *et al.* (2016) evaluated feeds of plant and animal origins

as supplementary feed on the growth performance of Nile tilapia. Similarly, utilization of poultry manures (Firew Admasu *et al.*, 2017), and inclusions of silkworm feces in diets of Nile tilapia (Mastewal Asfaw *et al.*, 2018) have been studied. These are few among others of the reported scientific studies, and all revealed promising results on growth performance, survival rate, feed conversion ratio (FCR), and cost-effective production. However, none of the studies have looked into the replacement of fish meal with a mixture of lupin and grass pea-meal (LGM) on the growth performance, feed utilization, body proximate composition, and hematology of Nile tilapia fingerlings.

1.2. Statement of the Problem

Intensification of Nile tilapia culture necessitates the development of biologically sound, cost-effective, acceptable, and palatable feeds for complete and supplementary feeding (Viola *et al.*, 1988; Akewake Geremew, 2015; Aschalew Lakew *et al.*, 2018). Aquafeed is one of the major determinant factors affecting the progressive intensification and development of aquaculture industries since it depends predominantly on fish meal and its combination. But still, fish meal is expensive and inaccessible for small scale aquaculture producers (El-Saidy and Gaber, 2003; Soltan *et al.*, 2008). Lack of cheap and efficient locally available quality fish feeds is a bottleneck for expanding fish farming activity by small-scale fish farmers in most developing countries like Ethiopia (Gadisa Natea, 2019). Similarly, commercial fish feeds are not available in Ethiopia and the existing aquaculture practices depend on feeds produced on-farm from the locally available feed ingredients of both plant and animal origin although their regular supply is not reliable (Mulugeta Wakjira *et al.*, 2013). As a result, there is a constant search for alternative protein sources of aquafeeds.

Since proteins of plant origin are abundant and less expensive compared to animal protein, they are considered the most viable alternatives to replace fish meal and maximize fish production (Naylor *et al.*, 2009; Hossain *et al.*, 2018). Among the locally available plant protein sources legumes such as soybean, faba bean, bean, chickpea, cowpea, and garden pea; lupin and grass pea have a great potential to be used as ingredients for the replacement of fish meal in aquafeeds (Zhang *et al.*, 2012; Caruso, 2015). Several studies have aimed at complete or partial replacement of fish meal at higher levels with individual plant proteins and have generally resulted in a decrease in fish growth performance due to ANFs and low amino acid profile (Glencross *et al.*, 2004; Sklan *et al.*, 2004). But, some studies have stressed that a mixture of

plant protein sources is a more appropriate strategy to obtain an adequate amino acid profile and reduce exposure to individual ANFs compared to incorporating a single plant protein source (Regost *et al.*, 1999; Borgeson, 2005; Zhang *et al.*, 2012). Comparative studies were conducted in Nile tilapia, rainbow trout, turbot, sea bass, and sea bream to substitute fish meal by a mixture of plant proteins (Fournier *et al.*, 2004; Soltan *et al.*, 2008; Khan *et al.*, 2013). The results of the studies revealed that it is possible to replace fish meal by a mixture of plant proteins without adverse effects on feed utilization and growth performances of fish. Therefore, to ensure the sustainability and growth of aquaculture in Ethiopia there is a need for developing alternative and cost-effective feed formulations and feeding strategies. Though numerous researchers have individually or with other ingredients evaluated the legumes on Nile tilapia (Viola *et al.*, 1988), juvenile turbot (Fournier *et al.*, 2004), rainbow trout (Zhang *et al.*, 2012), and catfish (Alayu Yalew *et al.*, 2019), the effects of mixtures of both legumes have not been studied. The present study evaluated the effects of replacing fish meal by a mixture of lupin and grass pea meal in the diets of Nile tilapia fingerlings on the growth performance, feed utilization, carcass composition, hematology parameters, and cost-effectiveness.

1.3. Research Questions

This study was mainly conducted to answer the following questions:

- Does the nutritional composition of a mixture of lupin and grass pea meal have the potential to replace fish meal in the diets of Nile tilapia fingerlings at higher levels?
- Does a mixture of lupin and grass pea meal-based diet affect the growth performance, feed utilization, carcass composition, and hepatosomatic index of Nile tilapia fingerlings as compared to a fish meal-based diet?
- Does replacing fish meal by a mixture of lupin and grass pea meal have changes on hematological parameters and cost-effectiveness of Nile tilapia fingerlings?

1.4. Objectives

1.4.1. General Objective

- To evaluate the suitability of a mixture of lupin and grass pea meal in the diets of Nile tilapia and contribute to developing sustainable aquaculture feed resources in Ethiopia

1.4.2. Specific Objectives

- To determine the appropriate incorporation levels of a mixture of lupin and grass pea meal in the diets of Nile tilapia fingerlings
- To assess the effects of incorporation of a mixture of lupin and grass pea meal on the growth performance, feed utilization, carcass composition and hepatosomatic index of Nile tilapia fingerlings
- To determine effects of replacement of fish meal by a mixture of lupin and grass pea meal on hematological and cost-effectiveness parameters

1.5. Research Hypothesis

This study hypothesized that replacing fish meal with a mixture of lupin and grass pea meal has no known effect on the growth performance, feed utilization, carcass composition, and hepatosomatic index of Nile tilapia fingerlings. The study also hypothesized that replacing fish meal by a mixture of lupin and grass pea meal has no negative effect on hematological parameters in the diets of Nile tilapia fingerlings.

1.6. Significance of the Study

Basic information on growth performance, feed utilization, hematological parameters and cost-effectiveness of using mixtures of two locally available plant feed ingredients (lupin and grass pea) in the diets of Nile tilapia was generated. The study suggested the appropriate inclusion level of the mixtures of the two plant feed ingredients that should be used in the diets of Nile tilapia for better intensification of aquaculture production.

2. LITERATURE REVIEW

2.1. The State of Aquaculture in the World

Aquaculture in simple terms is the farming of aquatic organisms under a controlled environment, including fish, mollusks, crustaceans and aquatic plants with some intervention in the rearing process to enhance production, such as regular stocking, feeding, and protection from predators (Hasan and New, 2013; Olatunji *et al.*, 2017). It covers all forms of farming of aquatic animals and plants in freshwater, brackish, and saltwater mainly used for food and non-food uses. Aquaculture is one among the best alternatives for the over-exploited natural aquatic resources, therefore aquaculture can help to scale back pressure and maintain the ecological balance of the natural ecosystems. It is probably one of the most promising answers to world population growth in providing additional food security and employment.

Food fish is a high-quality animal protein that contains rich in nutritional profile and higher than all terrestrial meat, and an excellent source of high-quality animal protein, highly digestible energy and a rich source of omega-3 polyunsaturated fatty acids, fat-soluble vitamins and water-soluble vitamins and minerals (FAO, 2006). Nowadays, aquaculture is the fastest-growing sector of alternative food production and contributes great potential as a sustainable solution to the world food security. From the total world fisheries production, China and the rest of Asian countries remain the dominant global aquaculture producers all over the world (FAO, 2017). In 2016, the total global aquaculture production (including aquatic plants) was reported at 110.2 million tonnes by volume and the first-sale value estimated was USD 243.5 billion (FAO, 2018). Aquaculture is growing at an average annual growth rate of 6.6% since 1995 (FAO, 2016), but recent reports have indicated a declined annual growth of 5.8% between 2001 and 2016. At the continent level, African aquaculture growth during 2001-2015 averaged at 10.4%, followed by Asia (6%) and the Americas (5.7%) whereas, in Oceania and Europe it has grown by 2.9% and 2.5%, respectively, in the last 15 years (FAO, 2017).

The total world aquaculture production includes 80.0 million tonnes of food fish (USD 231.6 billion), 30.1 million tonnes of aquatic plants (USD 11.7 billion), and 37, 900 tonnes of non-food products (USD 214.6 million) mostly for the production of fish meal. According to the FAO (2018) report from 171 million tonnes of total fish produced in 2016, about 88% (over 151 million tonnes) was utilized for direct human consumption. The greatest part of the total fish

product used for non-food (about 20 million tonnes) is reduced to fish meal and fish oil (FAO, 2017). Fish meal production peaked in 1994 at 30 million tonnes (live weight equivalent) and has followed a fluctuating but there has been an overall declining trend since then. A growing share of fish meal is being produced from fish by-products, which previously were often wasted. It is estimated that by-products account for about 25 to 35% of the total volume of fish meal and fish oil produced (FAO, 2017). Fish meal and fish oil are still considered the most nutritious and most digestible ingredients for farmed fish feeds, but their inclusion rates in compound feed for aquaculture have shown a clear downward trend as they are used more selectively and expensive (El-Saidy and Gaber, 2003; Kassaye Balkew *et al.*, 2014).

The contribution of aquaculture to the total global fisheries production has risen continuously, reaching 47% in 2016, up from 25.7% in 2000 (FAO, 2018). The aquaculture sector is expected to play a greater role in contributing to food security, poverty alleviation, and economic development of the poor. However, the global population is increasing, and to maintain at least the current level of per-capita consumption of aquatic foods (19.7 kg) (FAO, 2017); the world will require an additional more than 23 million tonnes thereof by now (2020) and in the next years. This additional supply will need to come from aquaculture. Food fish from aquaculture depends largely on the availability of quality feeds at affordable prices in the requisite quantities, and aquaculture is now the largest user of fish meal. Even though using fish meal in aquafeeds seems more prevalent for higher trophic level finfishes and crustaceans (marine shrimps, marine fishes, salmons, freshwater crustaceans, trout, eels, etc.), lower trophic level finfish species such as Nile tilapia is also fed with these products. Over recent decades, considerable efforts have been made by the aquaculture industry to reduce the levels of inclusion of fish meal. There has been a gradual reduction of fish meal utilization in aquafeeds for decades, although the discussion on the availability and use of aquafeed ingredients often focuses on fish meal and fish oil resources. The aquaculture sector should, therefore, strive to make sure sustainable supplies of terrestrial and plant feed ingredients.

2.2. Aquaculture in Africa

Historically, Africa's fisheries output is dominated by capture fisheries, but the contribution of aquaculture to the entire fish produced within the region has grown at a gentle pace over the past decade (FAO, 2017). In the East African countries comprising Burundi, Ethiopia, Kenya, Rwanda, Uganda, and Tanzania fish are produced from freshwater inland and marine capture fisheries and aquaculture (Obiero *et al.*, 2019). Capture fishery products have been declining, due to multiple anthropogenic pressures, including climate change, over-fishing, habitat destruction, and expansion of invasive species (FAO, 2016). Generally, aquaculture production of the Eastern region of Africa is relatively low as compared to the main producers in Africa, Egypt, and Nigeria that produce about 1.3 million tonnes and 306,727 tonnes, respectively (FAO, 2018).

Aquaculture is introduced to Africa lately, especially to sub-Saharan Africa and Ethiopia (Zenebe Tadesse *et al.*, 2012), and it is growing faster than any other part of the world, though contributing the least fish produced, consumed, and traded globally (Pauly and Zeller, 2017). In 2015, the contribution of African to the global aquaculture production from all inland, marine, and coastal aquaculture was 1.772 million tons (FAO, 2017). This accounts for only 2.3% of the world's total aquaculture production. Of this, 1.54% is contributed by Egypt, the largest contributor to world aquaculture from Africa followed by Nigeria (0.42%). The whole Sub-Saharan Africa region, excluding Nigeria, contributes 0.33% to world aquaculture. From 2005 to 2016, the contribution of African aquaculture to the world aquaculture production increased from 0.6462 million to 1.772 million tons (FAO, 2017).

The current supply trends combined with the ever-increasing population, the per capita consumption of fish in Africa is stagnating (Kassahun Asaminew, 2012). To change this condition and boost the production of fish, aquaculture remains the most feasible option that can sustain adequate fish supply in Africa. Although Africa is intensifying the aquaculture and having a faster growth of aquaculture (with the annual growth rate of 11.7% since the new millennium), the per capita fish supply was the lowest in the world with 9.7 Kg year⁻¹ (FAO, 2014). Nowadays, the fisheries and aquaculture sector in Africa is increasingly contributing to food and nutrition security, currency exchange, employment, and livelihood support services predominantly within the chief producing countries like Egypt and Nigeria (De Graaf and Garibaldi, 2019). Egypt is the largest contributor to African aquaculture (84.5%) followed by

Nigeria (7.9%) and as a whole; the Sub-Saharan Africa region contributed 14.6% to African aquaculture output (Asfaw Alemayehu, 2011).

2.3. Aquaculture in Ethiopia

The beginning of conducting researches on aquatic resources of Ethiopia dates to the early 1940s with the short-term expedition of European travelers and short-term residents, whereas the involvement of Ethiopian scientists became realized in the 1970s (Aschalew Lakew *et al.*, 2018). The studies were mainly focused on the description of the water bodies and limnological features, the biology of the few fish species, fish diversity and ecology, and estimation of fish production potentials. Ethiopia has a high potential for practicing fish farming both in terms of land/water and in its climatic regime with a rich biological diversity of native fish species (Shibru Tedla, 2016).

According to the geographic information system-based site suitability analysis reported by Eshete Dejen and Zemenu Mintesnote (2012), about 1.4, 55.49 and 43.1% of the country are highly suitable, moderately suitable and marginally suitable, respectively for commercial production of Nile tilapia under pond culture system. In the same authors, the country has an array of fish ranging from cold water to warm water species that can be farmed in the central highlands which present favorable temperature characteristics. Furthermore, the lowlands representing about 33 percent of the total area could also be suitable for the cultivation of tilapia species and other warm-water fish species. Despite the country's favorable environment and socioeconomic conditions, aquaculture in Ethiopia has not yet attained its full potential (Adem Mohammed and Assefa Tessema, 2017).

Ethiopian fisheries provide direct and indirect economic support to about half a million people and serve as a source of affordable protein for many households, and the country has an estimated mean annual potential yield of 94,500 tonnes distributed as 73,100 tonnes (lentic) and 21,400 tonnes (for lotic) ecosystems (Gashaw Tesfaye and Wolff, 2014). This value is higher by about 83% than previous estimates due to the large number of water bodies considered in the study, including the newly constructed reservoirs for irrigation development and hydropower generation. However, the fisheries product for some of the lakes have already reached the level of none rewarding to the fishermen due to very small size catch of fish species like Nile perch in Lakes Abaya and Chamo and Nile tilapia in Lake Ziway that can probably lead to extinction of species in these lakes as the result of pre-mature harvesting (Gadisa Natea, 2019).

Fisheries and aquaculture research was recognized as one of the research commodities in the Ethiopian agricultural research system in 1997 under the Ethiopian Institute of Agricultural Research (EIAR) through the Proc. No. 79/1997 (Aschalew Lakew *et al.*, 2018). The center was renamed as National Fisheries and Other Aquatic Life Research Center (NFALRC) and mandated to conduct, coordinate and support fishery and aquaculture research nationwide. In the past eighteen years, the center has undertaken enormous improvement in terms of research coverage, scientific information generated, and adaptation and upscaling fishery and aquaculture technologies, development of human resources and research capacity, and strengthened local and international collaboration.

Besides to its research activities, NFALRC continued culturing commercially important fish species, namely tilapia, common carp, and catfish to stock and restock water bodies in the country. Similarly, Ziway and Bahr Dar Fisheries Resource Research Centers are also institutes that engaged in research and training activities at their respective regional states. Besides stocking the above fish species into different water bodies, NFLARC performs breeding and dissemination of ornamental fish and goldfish (*Carassius auratus*) to users. NFALRC has conducted tremendous research trials aiming at different culture systems (e.g. mono and mixed-sex, polyculture, cage culture, integrated aquaculture), fish feeds, characterization of Nile tilapia from geographically isolated water bodies, growth and survival of cultured fish species, semi-artificial and artificial fish breeding techniques (Kassahun Asaminew *et al.*, 2012; Zenebe Tadesse *et al.*, 2012).

Currently, aquaculture is one of the priority thematic research areas for both federal and regional fisheries and aquaculture research centers. Besides establishing culture-based capture fishery in reservoirs and dams, the research centers are trying to develop small scale backyard aquaculture. However, lack of input suppliers (inexpensive and efficient locally available quality fish feeds, seeds) and high initial investment requiring nature of aquaculture are the major bottlenecks in its development (Kasahun Asaminew, 2012; Gadisa Natea, 2019; Aschalew Lakew *et al.*, 2018).

2.4. The Need for Aquaculture in Ethiopia

In Ethiopia, fishery production exclusively comes from inland water bodies and it is mainly both artisanal and subsistence, though the country has a high fish diversity and fishery potentials (Gashaw Tesfaye and Wolff, 2014). In recent years, capture fishery is in constant danger due to illegal and overfishing problems, and the Ethiopian population is increasing, pressuring food

security and irrigated agriculture and water harvesting in dams. However, aquaculture has emerged as the best possibility that can improve the food production system of the country (Lemma Abera, 2017). Aquaculture in Ethiopia, like most countries of the continent, remains more potential than actual practice. This country is endowed with abundant aquatic resources but could not realize substantial production from aquaculture due to a lack of inputs such as quality feeds, seeds, and educated labor and technology in all aspects. Aquaculture has great potential in the struggle for the improvement of the nutritional consumption of the human population and the eradication of poverty of rural people, particularly in developing countries and employment (Kassahun Asaminew, 2012; FAO, 2017).

The demand for fish has increased in the last decades; most of the Ethiopian lakes are overexploited and fisheries production of the major lakes of Ethiopia is declining at an alarming rate (Kassaye Balkew *et al.*, 2014). The fisheries in some of the Ethiopian lakes have reached the level of nonproductive to the fishermen due to the small size of the catch. The production of small-sized fish (Nile perch in Lakes Abaya and Chamo and Nile tilapia in Lake Ziway) probably could lead to the extinction of the species in these lakes as the result of pre-mature harvesting (Kassahun Asaminew, 2012; Gadisa Natea, 2019).

In Ethiopia, fishery products from the natural water bodies have failed to meet the ever-increasing demand for fish. As a result of high population growth in the country, there is high competition to be engaged in fisheries activities around the lake region, so, intensifying sustainable aquaculture is the best alternative to tackle this problem. Although Ethiopia has a total estimated production potential of about 94,500 tonnes per annum of the natural lakes, reservoirs and rivers (Gashaw Tesfaye and Wolff, 2014), the annual yield is not over 30% of its total estimated potential (Alemu Yitayal *et al.*, 2017). Based on this amount of fishery products, the per capita fish production is less than 240g (FAO, 2006). This implies there is an imbalance between the demand and the supply of fisheries products in Ethiopia. Meanwhile, tremendous reports have shown the stock decline and over-exploitation of the natural lakes, reservoirs, and rivers for their fisheries potential. Thus, this reminds of the expanding and intensifying of aquaculture development in Ethiopia as it is an indispensable way to ensure and contribute to the conservation of biodiversity and boosting economic development. It can also create employment, solving the stagnation of market demand for fish protein, and satisfy and make hunger-free people.

2.5. Nile tilapia as the Best Candidate for Aquaculture

The aquaculture capabilities of several species have been studied and still being explored by different researchers. Meanwhile, Nile tilapia is a tropical fish species predominantly farmed globally and remains the most successful aquaculture candidate fish appropriate for any aquaculture development for many reasons. This is because Nile tilapia easily adapts to wide range of environmental conditions (e.g. temperature, salinity, low dissolved oxygen, etc.), most important in production volume, having higher consumer preference and more promising about the market viewpoint as compared to other fish species such as catfish, and thus socio-economically the most important fish species in Ethiopia (Mulugeta Wakjira *et al.*, 2013; Gashaw Tesfaye and Wolff, 2014). Similarly, its fast growth, resistance to stress and disease, ability to reproduce in captivity, short generation time, feeding at low trophic levels (Zenebe Tadesse, 1999; Tadesse Fetahi, 2010; Workiyie Worie and Abebe Getahun, 2015) and acceptance of artificial feeds immediately after yolk-sac absorption (El-Sayed, 2004) makes ideal candidate for aquaculture.

Tilapia farming has been performed in 135 countries globally and Asia represents about 70% of the production with China as the major producer contributing about half of the global tilapia production (FAO, 2017). The rapid increase of tilapia production is due to developing more efficient cultivation techniques with better environmental and disease management, improved feed nutrients which generates higher profits. Nile tilapia is one of the most important commercial fish in Africa and other tropical regions and the most dominant and economically valuable fish in Ethiopia contributing to over 60% of the total annual landings (Alemu Yitayal *et al.*, 2017). Therefore, conducting aquaculture researches about Nile tilapia supports for its intensification and maximizes aquaculture products through incorporating environmentally available fish feeds.

2.6. Nutritional Requirements of Nile tilapia

In aquaculture, the provision of high-quality aquafeeds that satisfy the nutritional requirements of cultured species and improving growth is a prerequisite for increasing yields, lowering production costs, and improving economic returns for the farmers. Better management and control of feed quality and feed management strategies in fish farming are of critical importance in maintaining a cost-effective and environmentally sustainable industry. The food and feeding habits of Nile tilapia consist of a wide range of aquatic organisms depending on availability.

Although the proportion varies with stages in their life cycles, Nile tilapia feeds on a wide variety of natural feeds in natural water bodies, including plankton, some aquatic macrophytes, planktonic and benthic aquatic invertebrates, detritus and organic matter (Zenebe Tadesse, 1999; Tadesse Fetahi, 2010; Workiyie Worie and Abebe Getahun, 2015). In semi-natural pond-cultured tilapia, these natural food organisms account for 30-50% of overall growth (Popma and Masser, 1999). In intensive aquaculture systems, however, fish mainly depend on artificial feeds than the natural fish foods as the latter do not grow in enough quantity in such systems. The quality of fish feed is mainly characterized based on different parameters. Among these include the level of protein, lipid, energy, palatability, and digestibility. In intensive systems, tilapia species have the advantage that they can feed on a prepared diet that includes a high percentage of plant protein.

Aquafeed contains essential nutrients and energy sources that are indispensable in maintaining their normal growth rate and health status. Nutritionally complete diets are used in systems that can provide no dependable nutrition. Nile tilapia exhibits best growth response when fed a balanced diet that provides a proper mix of protein, carbohydrates, lipids, vitamins, minerals, and fiber. However, deficiencies or excess of the substances can reduce their growth rate increase disease susceptibility. For instance, deficiency of essential amino acid and essential fatty acids results in loss of appetite, poor feed utilization efficiency, and retarded growth in common, and deficiencies of essential fatty acids also cause swollen pale and fatty livers in Nile tilapia (Dabrowska *et al.*, 1989).

Protein is a major dietary nutrient that affects Nile tilapia growth (Lovell and Limsuwan, 1982) by providing essential amino acids for maintenance. Amino acids are essential because the fish cannot synthesize them in adequate amounts. Therefore, they must be provided by the diet for producing muscle, assist with enzymatic functions, and supply energy to the fish which helps their growth. The ability of tilapia to utilize dietary protein for growth is related to both dietary protein levels and the availability of non-protein energy sources (Anani and Nortey, 2017). If non-protein energy is insufficient or if the protein is of poor quality, the protein will be deaminated in the body to supply energy for metabolism (Sans *et al.*, 2000). The correct level of protein required for the optimum growth of Nile tilapia is controversial. Zenebe Tadesse *et al.* (2012) have reported that fish supplied with feed containing a higher protein level grew better than fish groups supplied with feed containing a relatively lower percentage of crude protein.

Similar results have been also reported by several authors (Jauncey, 1998 and Choudhary *et al.*, 2017) where Nile tilapia resulted in fast growth and higher net fish yields when supplied with feed containing a higher percentage of crude protein. However, higher protein levels may not always result in better growth. According to Bahnasawy *et al.* (2009), Nile tilapia fingerlings fed on a 30% protein diet attained growth higher than fingerlings fed on a 35% protein diet. This is because each fish size has a certain protein limit after which excess protein level could not be utilized efficiently (Daudpota *et al.*, 2014). It has also been reported that dietary protein requirements of Nile tilapia change as the maturity level changes (Craig, 2009), where mature fish require less protein to support growth than fry, fingerling and juvenile (Choudhary *et al.*, 2017).

In feed, dietary lipids serve as a source of fatty acids, phospholipids, sterols, and fat-soluble vitamins necessary for the proper functioning of physiological processes, maintenance of biological structure and function of cell membranes (Ayisi *et al.*, 2017). Lipids are high in energy that supplies approximately twice the energy contained by carbohydrates or proteins (Craig, 2009). Exceeding dietary lipids, however, can cause a decrease in feed consumption and reduce the utilization of other nutrients, leading to reduced growth rates and increased fat deposition (El-Kasheif *et al.*, 2011). Some uncertainty regarding the optimum lipid requirements of Nile tilapia are apparent between the life stages. El-Kasheif *et al.* (2011) reported that Nile tilapia fingerlings fed on a diet containing 9% supplemented lipid attained maximum final weight and showed better feed utilization efficiency. In another experiment, Jauncey (2000) has suggested that to maximize protein utilization, dietary lipid concentration should be between 8% and 12% for tilapia up to 25g, and (6 to 8%) for larger fish. This is within the range reported by FAO (2016) where dietary crude lipid requirement of Nile tilapia ranges between 6-13% for fry and fingerling and 4-12% for adults.

Carbohydrate is the cheapest source of energy available in abundant quantities at a low price and has a protein-sparing effect in some lower protein diets and for binding other ingredients that may complicate the level of its digestibility (Stone *et al.*, 2003; Yones *et al.*, 2019). Carbohydrate digestibility differs in different fish species dependent on the complexity of the carbohydrate. According to Krogdahl *et al.* (2005), the dietary content of simple carbohydrates is more easily digestible while the digestibility of carbohydrates generally decreases as dietary content increases. According to Hancz (2011) fishes are expected to have lower dietary energy

requirements because they need not maintain constant body temperature (homeostasis), use less energy in protein waste excretion and less energy requirement to maintain a position in space because of their neutral buoyancy in the medium where they live. However, several researchers reported that an increase in dietary carbohydrate content improves metabolism and growth in tilapia (Azaza *et al.*, 2013). The study done by Wang *et al.* (2017) has also reported that about 20 percent of dietary starch is appropriate for the normal growth of Nile tilapia. FAO (2016) recommends greater than 25% carbohydrate concentration for all stages of Nile tilapia, however, feeding excessive dietary carbohydrates for fish may cause harmful effects on growth, feed efficiency, physiological dysfunction, and fat deposition by stimulating lipogenic enzymes (Tian *et al.*, 2012). Even though there is a lack of well-documented data on the nutritional requirements of Nile tilapia for all life stages in different feed types, it is expected that early stages (fries, fingerlings, and juveniles) would require a diet containing high protein, lipid, vitamin and minerals, a lower composition of carbohydrate. In contrary, fish with sizes of 10-25g requires more energy from lipids and carbohydrate for metabolism and lower composition of protein for their growth and adults greater than 25g require food composed of lower dietary protein for their growth and they can also utilize higher levels of carbohydrates as a source of energy (Lim and Webster, 2006).

Table 1: Summary of the nutritional requirement of Nile tilapia

Nutrients	Weight (g)	Requirements (%)	Sources
	First feeding larvae	45-50	
Protein	Fries (0.02-1.0)	40	(Ng and Chong, 2004; Hu <i>et al.</i> , 2006; Lim and Webster, 2006; NRC, 2011; FAO, 2016)
	Fingerlings (1.0-10)	35-40	
	Juveniles (10-25)	30-35	
	Adults (25-200)	30-32	
	Broodstock	40-45	
Carbohydrate		Maximum (35-40)	
Crude fiber		Maximum (8-10)	
Crude lipid		Maximum (10-15)	

2.7. Water Quality Requirements of Nile tilapia

Water quality is the sum of biological, physical, and chemical parameters that affect the general condition of cultured organisms. According to Begum *et al.* (2014), water quality parameters are the key factors affecting the growth and health of the animals in aquaculture practice. These parameters mainly include water temperature, pH, dissolved oxygen, NH_3 , NO_2^- and NO_3^- . Water temperature is the degree or intensity of heat present in water and it is the main factor affecting growth performance, physiology, reproduction, feed consumption, survival rate, and metabolism of Nile tilapia (Adem Mohammed and Assefa Tessema, 2017). Nile tilapia tolerates a wide range of water temperature variation depending on the culture system and the size of the fish since smaller fish are more sensitive to cold water compared to larger fish. Popma and Masser (1999) reported that the lethal temperature levels for Nile tilapia are evident in below 12°C and above 42°C . In the same fashion, better growth performance in larval stages of Nile tilapia have been reported in the temperature range of 27.5-30.6 (Boussou *et al.*, 2017) and their growth rates were increased with increasing temperature. However, as the experimental temperature reaches the upper limit of the tolerance range, growth performance also decreases and physiological processes associated with digestion and nitrogen retention function become less efficient. Besides that temperature has an inverse relationship with dissolved oxygen and its negative impact is catastrophic to the fish stock.

Dissolved oxygen (DO) is one of the most important and critical gases, which are found in dissolved condition naturally in water. Maintaining good levels of DO in fish aquaria is an essential issue for successful aquaculture production since it has a direct influence on feed intake, disease resistance, metabolism, and general condition of Nile tilapia (Begum *et al.*, 2014). A concentration of DO lower than the critical level results in reduced fish growth, affect feed utilization efficiencies and increases the risk of potential diseases and fish death (Sultana *et al.*, 2017). Generally, the growth performance of Nile tilapia is highly affected by the concentration of DO and the metabolic demand for DO in aquatic animals doubles or triples with every 10°C increase within the range of temperature that the animal can tolerate (Begum *et al.*, 2014).

The pH of the water is a measure of a hydrogen ion that causes acidity and alkalinity on a scale of 0-14 with 7 as the neutral state and it is used universally to illustrate the intensity of the acid or alkaline condition of a solution. It is a crucial factor for the growth and survival of fish where its fluctuation results in ionic imbalance and could lead to death (Abdullah *et al.*, 2017). Both

alkaline (pH 8.5-10) and acidic (pH below 6.0) water cause an acute physiological disturbance in fish, affecting the normal growth rate and finally can cause a potential death. Besides, to DO water temperature and pH; ammonia, nitrite, alkalinity, and salinity are also key parameters in the growth and survival of Nile tilapia. Ammonia is the initial product formed in a decomposition of nitrogenous organic wastes and respiration. Ammonia is present in water and it is highly toxic when present in the un-ionized NH_3 form and in the non-toxic ionized NH_4^+ and their chemical equilibrium is driven by temperature and pH (Hegazi, 2011). Ammonia causes gill and kidney damage, reduced growth rates, and decreased ATPase level depresses food consumption at concentrations as low as 0.08 mg/L and it can cause massive losses of fries especially in water with low DO (Benli *et al.*, 2008).

Similarly, nitrite is a by-product of oxidized NH_3 or NH_4^+ , or an intermediary in the conversion of ammonia or ammonium into Nitrate through nitrification, which is done by the highly aerobic, gram-negative, chemoautotrophic bacteria found naturally in the system (Lawson, 1995). This conversion process is fast, thus high NO_2^- concentration rarely is found, but when it occurs in high concentration it can cause hypoxia by deactivation of hemoglobin in fish blood a condition known as the brown blood disease (Popma and Masser, 1999).

Table 2: Summary of the water quality parameter requirements for Nile tilapia

Water quality parameters	Recommended levels	Sources
Dissolved oxygen (mg L^{-1})	Above 5	
Water temperature ($^{\circ}\text{C}$)	25-32	
pH	6.5-9.0	(Lawson, 1995; El-Sherif and El-Feky, 2009; Begum <i>et al.</i> , 2014)
Ammonia (mg L^{-1})	Less than 0.1	
Nitrite (mg L^{-1})	Less than 0.5	
Salinity (ppt)	Less than 25	
Alkalinity (mg L^{-1})	20-200	

2.8. Fish meal as a Protein Source in Aquaculture Feeds

Fish meal is a nutritionally rich and highly digestible, palatable with balanced amino acids and rich in omega-3 fatty acids which help improve the growth and health of fish. It is an attractive material to include in a wide range of pelleted aquafeed products. However, the production of fishmeal is declining in recent years. For instance, in 2014 the global fisheries production excluding aquatic plants was reported about 167.2 million tons and 21 million tons (22.4% of the total catches) was destined for non-food products and 21 million tons, 76% (15.8 million tons) was used for fishmeal and fish oil production (Pauly and Zeller, 2017). This figure of 21 million tons has fallen from 34.2 million tons in 1994 and the reason for this declining range is the increased use of human consumption, decreased in dedicated fishing for feed production (due to tighter quota setting), increased use of fish residues and products, and increasingly replacing whole fish for fish meal production (FAO, 2016). Thus, the supply of fish meal is reducing globally and its cost is increasing, and to offset the increased price of fish meal, as feed demand increases, the fish meal used in compound feeds for aquaculture has shown a clear downward trend.

According to the reports of FAO (2016) globally, nearly 20 million tons of raw material is used annually for the production of fish meal and fish oil. Of this, around 14 million tons comes from the whole fish and, total of 3.7million tons of by-products comes from the processing of wild caught fish and 1.9 million tons comes from aquaculture by-products. In line with, the recent fish meal production is 4.445 million tons (in 2016) which has shown a clear indication of declining in its production from the total fishmeal produced in 2007 of about (5.697 million tons) by about 1.252 million tons of fish meal (Jackson, 2012). With this reduced potential of fish meal production and inaccessible resource, intensification of the aquaculture industry becomes a big challenge in most of the developing countries including Ethiopia. The scarcity of fish meal is one of the main obstacles to run aquaculture farms and in the intensification and development of aquaculture in Ethiopia. Thus, searching for locally available and low-cost alternative feed ingredients that can be used as protein sources is highly sought for Ethiopia.

2.9. Replacement of Fish meal by Plant-based Protein Sources in the Diets of Nile tilapia

The belief of incorporating plant-protein constituents in fish feed has increased because of its low cost, availability and offering suitable and sustainable option to obtain adequate balanced amino acids through simple or complex mixtures (Naylor *et al.*, 2009; Prusinski, 2017). Incorporation of plant-based protein sources and their byproducts have been broadly assessed for the replacement of fish meal in aquafeeds. For instance, plant protein sources and other by-products such as oilseed cake meals, maize bran, bagasse mix, brewery wastes, fish pellets and legumes produced from farm mainly or as byproducts from various industries have been studied as aquafeeds (Zenebe Tadesse *et al.*, 2012; Khan *et al.*, 2013; Akewake Geremew, 2015; Dorothy *et al.*, 2018). These resources are easily available at a low cost and promising results have been reported.

Several published reports have indicated that the inclusion and replacement of fish meal in the diets of Nile tilapia with different plant proteins such as alfalfa and dehulled barley (Lech and Reigh, 2012), rice polish and mustard oil cake (Khan *et al.*, 2013), soybeans (Ajani *et al.*, 2016), lupin and pea mixtures (Zhang *et al.*, 2012) were performed in different parts of the world. In a similar trend, in Africa replacement of fish meal with a large number of plant products has been evaluated as potential protein sources in the diets of Nile tilapia. Among these include cottonseed meal, sunflower meal and corn meal, soybean meal, cassava leaf meal, sweet potato leaf meal, groundnut cake, pea, and rapeseed plant protein concentrate are few among many trials reported (El-Sayed, 2004; Lech and Reigh, 2012). Generally, studies on the effects of replacement of fish meal by leguminous feed ingredients on growth performance and body proximate composition of Nile tilapia are limited. But, there are different studies conducted on the replacement of fish meal by other locally available resources. Among these, solvent extracted cottonseed meal and cottonseed cake have been tried as feed for Nile tilapia in Egypt (Tacon, 1997) and successful growth performance and feed utilization parameters were reported. Similarly, several studies have evaluated various alternative animal protein sources (EL-Sayed, 1998; Fasakin *et al.*, 2005; Kiriimi *et al.*, 2016) and plant protein sources for fish feed (El-Saidy and Gaber, 2003; Soltan *et al.*, 2008; Hassaan *et al.*, 2018; Yones *et al.*, 2019). This has yielded quite a considerable success in establishing alternative strategies for protein supply in different African countries.

In line with the above facts, partial replacement of fish meal by locally available feedstuffs has been also studied in Ethiopia. For instance; Abeneh Yimer and Yared Tigabu (2016) studied the growth performance of Nile tilapia in cage culture by using brewery waste, Noug cake, and wheat bran, and reported sustained growth performance and better FCR in the species. Similarly, Kassaye Balkew *et al.* (2014) studied the replacement of fish meal by a mixture of soybean, bone meal, groundnut, maize bran, coffee husk, wheat bran, brewery waste, and potato scrub at different inclusion levels and promising results have reported. Besides, a feeding trial conducted in the Ziway Aquaculture System that included silkworm feces (Mastewal Asfaw *et al.*, 2018), oilseed cakes (Akewake Geremew, 2015) in the diets of Nile tilapia reared in a recirculating aquaculture system resulted in comparable growth performance to that of standard tilapia control diets throughout the experimental period. Therefore, conducting similar researches on other potential feed ingredients became encouraging since locally available proteinaceous plants play an indispensable role in developing semi-intensive and intensive fish farming practices.

2.9.1. Lupin and Grass pea as Protein Sources in Aquaculture

The preference of plant protein sources to animal proteins in replacing fish meal in the formulation of fish feeds led to an intensification of aquaculture yield maximization and meets the ever-increasing food demand of the population. Among the widely known leguminous seeds which are believed to be highly proteinaceous and locally available, lupins and grass pea are widely produced and abundant in Ethiopia (Mulugeta Atnaf *et al.*, 2015). Grass pea has a high amount of protein content which is comparable to that of soybeans and some lupin species, and it is rich in mineral concentrations such as magnesium, phosphorus, and calcium (Urga Kelbessa, 2005). Both legumes contain a large number of essential proteins, carbohydrates, vitamins, and less fat content. Lupin also consists of essential nutrients which in turn have high importance in health aspects. For instance; feeding diets with high lupin proteins help to protect colorectal cancer, cardiovascular diseases maintain insulin resistance, decrease serum cholesterol and hypertension, and lupin oil has desirable ratios of omega-6 to omega-3 acids for consumption purposes (Khan *et al.*, 2013; Prusinski, 2017).

Similarly, grass pea is the only known dietary source of L-homoarginine amino acid, which provides essential benefits in treatments of cardiovascular disease and the hypoxia at tissue level

associated with cancer tumor development (Lambein *et al.*, 2019). Therefore, these locally available legume seeds are enriched with nutraceutical (nutritional and pharmaceutical) potentials. The protein content of lupin seed ranges from 30 to 40% (Sujak *et al.*, 2006; Yones, 2010) depending on the species and agroecological natures. Grass pea is also appreciated for its high protein content (26-32%), high adaptability to extreme environmental conditions, disease resistance, and low agricultural input prerequisite for its cultivation (Urga Kelbessa, 2005). The amino acid composition of grass pea is similar to those of many grain legumes, and it is rich in lysine but deficient in methionine, cysteine, and tryptophan (Prusinski, 2017). Grass pea is highly suitable to use as a feed ingredient because 58% of the fatty acids are polyunsaturated (Lambein *et al.*, 2019). However, these grains are also enriched with some ANFs, including phytic acid, lectins, trypsin inhibitor, tannins, protease inhibitors, oligosaccharides, alkaloids, and saponins.

The nutritional components and ANFs of grass pea and lupin have been extensively studied in many parts of the world. The nutritional components and ANFs of grass pea (Yan *et al.*, 2006) and lupin (Arnoldi and Greco, 2011; Prusinski, 2017) have extensively been studied. Besides to the commonly found ANFs, seeds of grass pea contain a neurotoxin Oxalyl diamino propionic acid, which can cause paralysis of the lower limbs (lathyrism) (Hanbury *et al.*, 2000). Like many other ANFs found in lupin, alkaloids are also predominantly present and affect palatability and can be minimized using different techniques. Dehulling and soaking are the best methods to increase the nutritive value of lupin seeds by reducing the fiber content, increasing the crude protein content, and decreasing the number of carbohydrates without affecting the lipid level (Glencross *et al.*, 2004; Zhang *et al.*, 2012). The way these ANFs affect also varies, i.e., some affect palatability, others digestion and some interfere with the metabolic rate. The protein content of lupin is higher in the processed meal than raw seeds since the ANFs and their effect are minimized. Thus, to replace the fish meal with a mixture of grass pea and lupin, the ANFs found in these seeds and their effects must be minimized by applying physical, chemical, and biological processing techniques.

Previous studies conducted by Hanbury *et al.* (2000) and Phuc *et al.* (2000) suggested that using legumes as sources of protein in the animal feed industry is expected to increase further soon. Because the amino acid requirement of feeds could not be provided with a single source of plant protein and the exact amino acids required for all animals varies with species and their

accessibilities. It is, therefore, preferable to include the proteinaceous leguminous crops of grass pea and lupin in the formulations of fish feed by complementing with other ingredients.

In Ethiopia, though there is limited information about the potentials of lupin and grass pea seeds, particularly concerned with their nutritional and ANFs, there have been published reports by different researchers in different aspects. For instance, Urga Kelbessa (2005) evaluated proximate composition of *Lathyrus sativus* cultivated in Ethiopia; Mulugeta Atnaf *et al.* (2015): the importance of legumes in Ethiopia; Habtamu Fekadu and Negussie Ratta (2014): nutritional and antinutritional components of grass pea; Likawent Yeheyis *et al.* (2012): on the adaptability of lupins in different agroecological zones of Ethiopia; and Zerihun Nigussie (2012): on the contribution of white lupin for food and feed production. All the researchers found that both crops are endowed with enough crude protein. However, scientific reports which indicate the suitability and possibility of these legumes to support the replacement of fish meal in aquaculture have not been available in Ethiopia.

Replacement of fish meal by lupin species in aquaculture had been possible due to their high protein content (30-40%), availability, and low price (Zhang *et al.*, 2012; Alayu Yalew *et al.*, 2019). They are also suitable because of their quality of dietary protein sources depending on their digestibility and amino acid profile. According to the study done by Higuera *et al.* (1988), lupin seed meal is a good alternative plant protein of high nutritive quality when used at levels up to 30 - 40% in rainbow trout diets. Similarly, Smith (2002) also studied the effect of replacing fish meal by lupin kernel in the diets of *Penaeus monodon*. These studies revealed that lupin meal is efficient to substitute fish meal up to 40% without adversely affecting the weight gain by the primary protein source and it has digestibility higher than the full-fat soybean meal. In the same author, dehulled lupin seed meal at 40% inclusion levels in 44% CP diets for juvenile rainbow trout resulted that 49% of the fish meal could be replaced without affecting the growth performance and nutrient utilization of the fish. Besides, Burel *et al.* (2000) also reported that it is possible to include (50-70%) of extracted lupin (*Lupinus albus*) in the diets containing 40 and 44% crude protein diets to replace over 50% and 76% of the fish meal, respectively.

Viola *et al.* (1988) evaluated the effect of the whole lupin (CP 32.7%) in the diets of common carp at inclusion levels of 30% and 45% for 44 days of two studies and revealed considerably better growth. They reported a better weight gain (171.3g) when common carp was fed 30%

lupin mixture diets as opposed to those fed the soybean meal (133.5g gain) and a better feed utilization parameters were also reported. Replacement of fish meal using the nutritional composition of grass pea has been also reported. For instance, Ramachandran and Kumar (2008) evaluated the replacement of fish meal by the grass pea-meal in the diets of rohu (*Labeo rohita*) fingerlings and found that processed grass pea-meal could be incorporated at 30% level with no adverse effects. In contrast with the aforementioned facts, other studies indicated replacing fish meal with lupin at higher levels affects growth performance, feed utilization and carcass composition in red hybrid tilapia (Yones, 2010). Besides, Alayu Yalew *et al.* (2019) evaluated replacement of fish meal by lupin meal at higher inclusion levels in the diets of juvenile catfish and resulted in decreased growth performance and feed utilization parameters. However, other scholars suggested that mixing plant protein ingredients with different nutritional characteristics can improve dietary nutritional balance and overcome adverse effects on growth parameters and feed utilization efficiency (Borgeson, 2005; Zhang *et al.*, 2012).

3. MATERIALS AND METHODS

3.1. Experimental Design and Culture Facility

The study was carried out in the Center for Aquaponics and Recirculating Aquaculture System of the Department of Zoological Sciences in the College of Natural and Computational Sciences, Addis Ababa University. The study was performed for 10 weeks using twelve rectangular glass aquaria having a total volume of 0.23m³ (45cm x 50cm x 100cm) and an actual water level of 0.1 m³ (25cm x 45cm x 100cm). The experimental aquaria were connected to recirculating water between filtration units and aquaria helped by a pump, plumbing system that supplies water to aquaria, and a plumbing system that takes water from aquaria to the filtration units (Plate 1). The system water temperature was maintained at a constant range helped by submerged crystal heaters in the sump tank (the tank where the pump is connected to) (Plate 2) capable of supplying 300W potentials for efficiently regulating water temperature between 16-32 °C of 200L. The recirculating aquaculture system was also equipped with aerators (aquarium air pump having 4.2W power) to keep stable DO levels for the whole experimental period. The study was conducted with three experimental diets and a control diet, fed in triplicates using a completely randomized design where different dietary treatments were randomly assigned to the experimental aquaria. The aquaria were cleaned every day by siphoning early in the morning, before feeding, using a long draining tube without disturbing the fish.



Plates 1: Filtration components of recirculating aquaculture system, (biological filtration-A), (mechanical filtration-B) and twelve experimental aquaria (C)

3.2. Experimental System for Growth and Feeding Trials

In the recirculating aquaculture system each aquarium fitted with inlets had a mean water flow of 2 liter minute⁻¹ with a spray fashion into the experimental aquaria to enhance aeration. Water from all experimental aquaria drained through open gutters to the settling/biological and mechanical filtration tanks (set two tanks for each filtration unit) containing fine-mesh nets, which filtered wastewater (Plate 1 A and B). The mechanical filtration was supported with sponges and bush- like chips, whereas the biological filtration was filled with a simulation of bio-balls set to help bacteria as attaching site to facilitate the nitrification process of toxic nitrogen products, and support in filtering the system water and let it to the sump tank. The sump tank fitted to receive draining water from the mechanical and biological filtration units was attached internally to receive water from another tank (the tank in which dechlorinated water is stored). It was essentially fitted to maintain the same water level, and heated, well-aerated, and filtered water was pumped to each aquarium in a recirculating style. Meanwhile, replacement of a little water from the recirculatory aquaculture system was continually done to avoid an accumulation of excretory products. The mechanical filtration units were washed during every sampling week to get rid of an accumulated sludge resulting from feces and uneaten feed. In doing so, the physicochemical parameters mainly DO, pH and temperature were monitored daily (Plate 2B) whereas NH₃ and NO₃⁻ were monitored weekly.



Plates 2: Submerged heaters and aerators in the sump tank (A) and collecting physicochemical parameters (pH, DO and temperature) (B)

3.3. Experimental Fish and Broodstock Management

Conditioned male and female Nile tilapia broodstock were kept in a breeding hapa placed in fiberglass green pond at a 3:1 female to male ratio at a density of 4 fish m⁻² inside a greenhouse. The pond was provided with continuous aeration to maintain oxygen supply and submersible heaters to attain an average water temperature of 27.5 °C (Plate 3A). Eighteen females and six males were selected, weighed, and stocked in a green pond spawning hapa. Broodstock was fed with a diet containing 36% crude protein formulated and prepared from fish meal, soybean, wheat flour, cornflour, vitamins and soybean oil, which was used in this study at a feeding rate of 2% of their body weight with a feeding frequency of two times a day at 9.00 am and 4.0 pm. After careful follow up of the spawning activity of broodstock for two weeks, eggs from the female mouth were removed, incubated, hatched artificially in a recirculating system, and maintained in a hatchery system until the yolk sac was absorbed (Plate 3B) and the fries become ready for external feeding. The resulting swim-up fries were maintained for one week in a rectangular tank (Plate 3B) provided with a continuous flow of dechlorinated filtered freshwater and aeration. The Nile tilapia fries were transferred and reared in a small fiberglass green pond by being fed with a powdered fish meal as supplementary feed until they get ready for growth trial. The swim-up fries were hand-fed four times (8:00, 11:00, 14:00, and 17:00) per day.





Plates 3: Spawning fiberglass green pond (A) and hatchery system (B)

3.3.1. Acclimatization, Stocking and Weighing Procedures

One week before the start of the experiment fingerlings were transferred into the experimental glass aquaria from the hapas in a green fiberglass pond for acclimatization. To reduce variability in weight of fish fingerlings within each aquarium, fish were graded into similar sizes of $\pm 1\text{g}$ before stocking randomly at a density of 23 fingerlings per aquarium in triplicates of treatments. Before any experimental procedure, all fish were anesthetized using clove oil/ethanol (1:9, v/v) prepared and mixed with 20 liters of aerated clean water. For initial and final samples all fingerlings were individually weighed and measured their total length under anesthesia. Fish were netted, drained of water, and gently blotted on a soft paper towel (to reduce errors of fish weights recorded due to water adhering to each fish) before an individual weighing to the nearest 0.01g on an electronic digital balance. Their lengths were also measured to the nearest 0.1cm using a fish measuring board. Clean aerated water was used to recover the fish before being returned to the experimental aquaria. Measurements of fish weight were made at the beginning and throughout growth experiments at weekly interval. For all intermediate weight measurements, fish were bulk weighed, without anesthesia, weekly. All fish in each aquarium were netted, using a fine mesh handnet and excess water was then removed from the fish by blotting the net with a soft paper towel (Plate 4A). Fish were then transferred to a tarred, water-filled, container, and weighed collectively to the nearest 0.01g (Plate 4A). Fingerlings were fed at 10% of their body weight for the first four weeks and reduced to 6% for next six weeks as an adjustment to the increase in fish weight following their feeding rate. Feeding rates were adjusted every week following the weekly weight gained. Each ration was dispensed over 10-15

minutes in small portions to minimize feed wastage. The weekly mean weights of fingerlings were used for subsequent determination of daily food ration and feed utilization for the following week.



Plates 4: Weekly data collection [weighing (A); and measuring total length (B) at the start and end of the experiment] on fish growth

3.4. Diet Formulation and Preparation

The feed ingredients of grass pea (*Lathyrus sativus*) and lupin (*Lupinus albus*) (Plate 5A) were bought from the local market in Addis Ababa (Mesalemia) whereas fish meal was prepared from whole fish purchased from the market with respect to the price presented in Table 5. Freshly collected whole fish was purchased from Lake Ziway, Ethiopia. The freshly collected whole fish was minced using an electrical meat mincer and then dried in an oven for 24 hours at 72 °C. The dried fish carcass was ground into a fine fish powder using an electrical smashing machine (Plate 5C), sieved (0.5mm mesh size sieve), and then stored in a plastic bag at -18 °C in a deep freeze.

Generally, four iso-nitrogenous (36% protein), iso-lipidic (10% lipids), and iso-energetic (18 KJ g⁻¹) diets were formulated for the experiment (Table 3). These levels were based on the requirements for Nile tilapia. The control diet was formulated with a fish meal protein and this was replaced at different levels (25, 50, and 75%) with a mixture of lupin and grass pea-meal (LGM) (mixed 1:1 ratio). The diets were formulated on a fed basis, and fish meal was a main dietary protein source, and wheat and corn grains were used as a main carbohydrate source in the experiment. A poultry grade vitamin/mineral premix (AMINOVIT) at 34.4 g Kg⁻¹ and binder carboxymethyl cellulose, high viscosity at 20 g Kg⁻¹ were added (Table 3). The vitamin/mineral premix was purchased from the local market in Addis Ababa (Merkato) and soybean oil was also used as a source of lipid in the diets.

Table 3: Composition of feed ingredients in the diets (g Kg^{-1} as-fed) fed to Nile tilapia fingerlings in the replacement of fish meal with a mixture of lupin and grass pea-meal

Feed ingredients	Control diet	LGM25	LGM50	LGM75
Fish meal	323.00	242.25	161.50	80.75
Wheat grain	120.00	120.00	120.00	120.00
Corn grain	109.80	109.80	109.80	109.80
Soybean grain	362.80	362.80	362.80	362.80
LGM	0	80.75	161.50	242.25
CMC	20.00	20.00	20.00	20.00
VMP	34.40	34.40	34.40	34.40
SBO	30.00	30.00	30.00	30.00

Note: LGM- lupin and grass pea mixture meal, SBO- Soybean oil

LGM25 = 25% of fish meal replaced by lupin and grass pea mixture meal

LGM50 = 50% of fish meal replaced by lupin and grass pea mixture meal

LGM75= 75% of fish meal replaced by lupin and grass pea mixture meal

CMC = high viscosity carboxymethyl cellulose used as a binder

VPM- (AMINOVIT): is a mineral and vitamin premix that helps to improve performance in weight gain, growth rate, normal metabolism, resistance to infections, and alleviate stress. It is an important ingredient that supports vitamin and nutritional supplements for the general upkeep of health and productivity. It consists of different essential vitamins and minerals as presented in Table 4.

Table 4: Composition of a poultry grade vitamin/mineral premix (AMINOVIT) used in the experimental diets

Content	Amount (mg 100 g ⁻¹)
Vitamin-A	500 000 I.U
Vitamin-D3	29000 I.U
Vitamin-E	200
Vitamin-K3	40
Vitamin-B2	300
Vitamin-B6	60
Niacin amide	1320
Calcium-pantothenate	440
Choline chloride	150
Folic acid	10
Vitamin-B12	0.4
L-Tryptophan	20
L-Lysine	1000
DL-Methionine	2000

The proportion of the feed ingredients in the experimental diets was prepared using WinFeed version 2.8 feed formulation packages after proximate analysis of each ingredient was done as presented in Table 6. Lupin and grass pea grains were soaked for 48 hours with a continual water change, dried, roasted, dehulled, ground, sieved and then mixed in a 1:1 ratio before being incorporated into the feed formulation (Plate 5A, B and C).



Plates 5: Soaked seeds of grass pea and lupin (A); dehulling and air separation (B); grinding, sieving and mixing of feed ingredients (C)

Diets were prepared by wet extrusion using a meat mincer (TJ22 model). All ingredients were finely ground and sieved through a 350 μ m sieve to obtain a homogenous mixture. The dry ingredients were then weighed out according to the formulation, placed in an aluminum bowl and mixed until uniformly blended (Plate 6A). The resulting homogenate was moistened after adding water (20-30%) slowly with continuous stirring until the dough was formed before passing through an electrical meat mincer. The expeller like strand (Plate 6B) made with the meat mincer was dried in an oven with a convector fan at 40 °C for 24 hours. It was then crushed into crumbles and sieved with a 1mm mesh size sieve. The resulting pellets were packed in polyethylene bags (Plate 6C) and stored in a deep freeze at -18 °C to minimize microbial growth. Feed ingredients and prepared experimental diets were separately analyzed for a proximate composition to check the nutritional quality.



Plates 6: Mixing of feed ingredients (A) pelleting of experimental diets through an electrical meat mincer (B) and packing in a polyethylene bag (C)

3.5. Methods for Proximate Composition Analysis and Energy Determination

Chemical analysis of the feed ingredients, experimental diets, and the carcass of fish was carried out in the laboratory for determining crude protein, crude fiber, crude lipid, carbohydrate, ash, and dry matter content as in AOAC (2001).

3.5.1. Moisture Content

Moisture content of different feed ingredients, experimental diets and fish carcass was determined by drying the samples in an oven at 105 °C for 3 hours till constant weight is achieved. It is a gravimetric measurement of water in the feed ingredients, diets, and carcass expressed as a percentage of the initial sample weight. In doing so, clean and dried crucibles at 105 °C for 1 hour, cooled in a desiccator and weighed (W_1). Then, five grams of sample (W_2) was weighed into pre-measured crucibles and dried at 105 °C for 3 hours. After cooling in a desiccator to room temperature, it was again weighed (W_3) in triplicates. Thus, the moisture content was determined using the following equation:

$$\text{Moisture content (\%)} = \left(\frac{W_2 - W_3}{W_2 - W_1} \right) * 100 \quad (1)$$

3.5.2. Ash Content

The ash content of feed ingredients, diets and carcass that measure the total inorganic matter was done with incineration procedure. First, crucibles were cleaned and dried in a muffle furnace for 30 minutes at 550 °C, cooled in a desiccator for 30 minutes at room temperature, and weighed (M_1). Then 2.5g of fresh sample to an accuracy of 4 decimal places in the dish was weighed (M_2)

into a pre-weighed crucible and incinerated for 5 hours at 550 °C using a muffle furnace and finally resulted in clean and white in appearance, cooled, weighed and recorded as weight (M_3). The resulted increase in the final weight of crucible after incineration represented the ash and was expressed as a percentage of the original sample:

$$\text{Total Ash (\%)} = \left(\frac{M_3 - M_1}{M_2 - M_1} \right) * 100 \quad (2)$$

where: ($M_2 - M_1$) is sampled mass in gram on dry base and ($M_3 - M_1$) is the mass of Ash in grams.

3.5.3. Crude Protein

Crude protein (CP) was determined by the standard micro-Kjeldahl Nitrogen method as described in AOAC (2001) analyzed in triplicate. First, five grams of sample was digested in concentrated H_2SO_4 using Behroset InKje M digestion apparatus in the presence of one gram catalyst copper sulfate and potassium sulfate until a clear solution was formed. After digestion was completed, the formed clear solution was cooled for 30 minutes. The resulting solution was distilled under alkaline condition using the Behr S1 steam distillation apparatus by adding 50 ml sodium hydroxide (40%) and 70 ml distilled water. The distillate containing ammonia was trapped in a 25 ml boric acid (2%) solution. The distillation process was terminated when the volume of the receiving flask reached between 100 to 150 ml. Then, nitrogen content was estimated by titration of the formed ammonium borate with 0.1N HCl. Crude protein was estimated by multiplying the nitrogen content by a factor of 6.25. The result was expressed as a percentage of the original weight of the sample.

$$N (\%) = \frac{\text{Normality of HCl [the volume of HCl titrates sample - volume of HCl titrates blank]}}{\text{Weight of sample}} \times \frac{14g}{\text{mole}} \times 100 \quad (3)$$

3.5.4. Crude Lipid

Crude lipid (CL) was determined by the method of solvent extraction using Soxhlet extractor for 4 hours. First, empty porous cellulose extraction thimble covered with fat-free cotton was oven-dried at 105 °C for 1 hour, cooled in a desiccator for 30 minutes and weighed. Next, two grams of a dried and ground sample was placed in a thimble. The thimble was placed in an extraction chamber suspended above a flask containing diethyl ether solvent and below the condenser. A flask containing diethyl ether solvent in the presence of boiling chips was placed inside the extraction chamber then heated at 55 °C and the solvent evaporates moves up into a condenser where it's converted into a liquid that trickles into the extraction chamber containing the sample.

At the end of the extraction process, which typically lasts for 4 hours, the thimble containing the extracted sample was oven-dried at 105 °C for 1 hour, cooled in a desiccator for 30 minutes and weight was determined. Weight of fat was calculated from the difference in weight of the thimble containing the sample, before and after extraction and the crude fat in the initial sample was then calculated as a percentage value. Weight of fat = Weight of thimble before extraction - Weight of thimble after extraction.

$$\text{CL (\%)} \text{ on a dry basis} = \frac{\text{Weight of Fat}}{\text{Weight of sample}} \times 100 \quad (4)$$

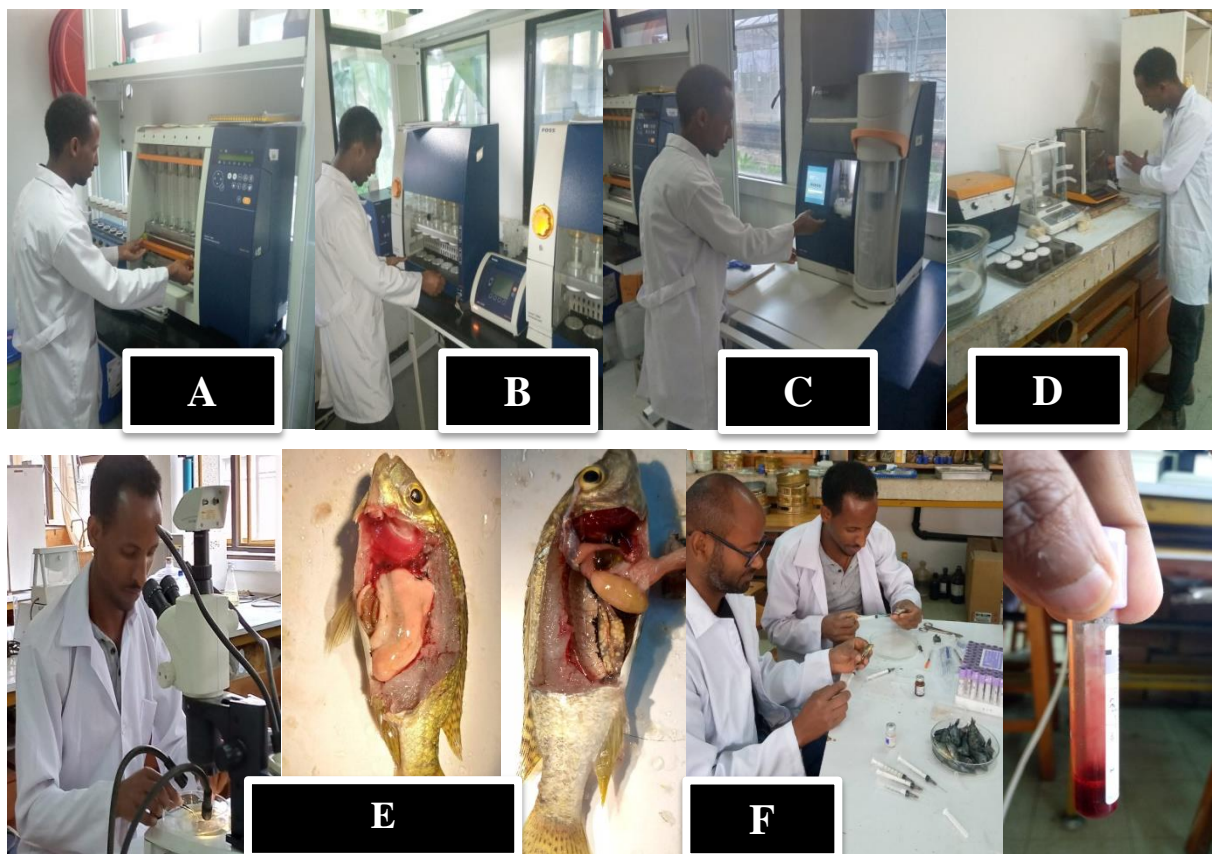
$$\text{CL (\%)} \text{ on a wet basis} = \frac{\text{Crude fat (\%)} \text{ in dry weight (100 - Moisture content)}}{100}$$

3.5.5. Crude Fiber

Since crude fiber content (CF) of animal origin feedstuff is considerably low, analysis was done for plant protein ingredients, but not for fish meal in the present study. In doing so, one gram of defatted sample was boiled in a standard solution of 3.13% H₂SO₄ for 10-15 minutes. The remaining sample was rinsed with hot water followed by boiling in 3.13% NaOH for another 10-15 minutes. Thereafter the remaining sample was rinsed repeatedly with hot water followed by acetone. The residue was oven-dried at 60 °C for 4 hours, cooled in a desiccator, weighed, and residue was ashed at 600 °C in a muffle furnace overnight. Then, crude fiber was quantified by expressing the loss in weight after ashing as a percentage of the original weight of the sample.

3.5.6. Gross Energy

Gross energy (GE) value (KJ 100g⁻¹) was calculated by the overall addition of the (carbohydrate content x 4.2), (protein content x 5.65) and (fat content x 9.45) using Atwater's conversion factors. The result was expressed as KJ per 100 grams. Nitrogen free extract (NFE) was also calculated by subtracting the total of moisture content, crude protein, crude lipid, ash content, and crude fiber from 100. NFE = [100 - (moisture content + protein content + fat content + ash content + crude fiber)].



Plates 7 (A) Fiber determination by FiberTM 8000, auto fiber analysis system; (B) Fat determination by Soxtec 8000 technology; (C) Protein determination; (D) weighing of crucibles for ash and moisture determination, (E) Dissecting, weighing and determination of HSI, (F) Blood collection for hematological parameters.

3.6. Analysis of Experimental Data

3.6.1. Estimation of Growth Performance and Production Parameters

Experimental data gathered during the growth trial and results from the analysis of diets were used to determine various biological parameters, namely: growth and feed utilization parameters such as percentage weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), survival rate (SR) and Fulton's condition factor (FCF) following the procedures described by Cho and Kaushik (1985):

$$\text{Average initial weight (IBW, g)} = \frac{\text{Sum of individual weight at the beginning}}{\text{Total number of individuals}} \quad (5)$$

$$\text{Average final weight (FBW, g)} = \frac{\text{Sum of individual weight at the end}}{\text{Total number of individuals}} \quad (6)$$

$$\text{Weight gain (WG, \%)} = \frac{(\text{FBW}-\text{IBW})}{\text{IBW}} \times 100 \quad (7)$$

$$\text{Specific growth rate (SGR, \% per day)} = \frac{\ln(\text{FBW})-\ln(\text{IBW})}{\text{Culturing days}} \times 100 \quad (8)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total weight of dry feed given}}{\text{Total live weight gain by fish}} \quad (9)$$

$$\text{Survival rate (SR, \%)} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100 \quad (10)$$

$$\text{Fulton's condition factor (FCF)} = (\text{Wt}/\text{L}^3) \times 100 \quad (11)$$

Where Wt = body weight and L= length of the fish. Feed intake (FI) was determined by adding the total amount of feed consumed by the fingerlings in each treatment for the whole experimental period. It was extrapolated by using the weekly feed ration adjustment of each treatment where fingerlings were fed at 10% of their body weight for the first four weeks and then reduced to 6% of their body weight based on weekly weight gain.

3.6.2. Body Proximate Composition of Fish

At the end of the experiment, whole body proximate and energy content, and hepatosomatic index (HSI) were determined. The proximate analysis was done following the methods described in AOAC (2001), and the parameters such as dry matter (moisture), crude protein, crude lipid, and dry ash were analyzed and expressed as a percentage of dry weight basis. Similarly, fish samples from each treatment were collected, including the control treatment, and euthanized by using concentrated clove oil to kill the fish ethically, dissected and livers were removed, weighed (plate 7-E) and used to estimate HSI (El-Saidy, 1990).

$$\text{HSI} = \frac{\text{Liver weight}}{\text{Bodyweight}} \times 100\% \quad (12)$$

3.6.3. Hematological Parameters

At the end of the feeding experiment, analysis of hematological parameters was done at clinical Chemistry laboratory of the Ethiopian Public Health Institute, Addis Ababa, Ethiopia. Fish were anesthetized by immersing in water containing 3ml clove-oil mixed in 10ml of ethanol which was diluted in one liter of water. Whole blood sample was taken aided by three ml syringes (27 gauge needles) from the caudal vein of a set of ten randomly chosen Nile tilapia fingerlings from each treatment. Then, the collected blood sample was dispensed into 4ml lavender top vacuum

ethylene diamino tetra acetic acid K3 tube (Hensso medical, China) as an anticoagulant until the determination of hematological parameters done following the procedures used in Kefas *et al.* (2015). Hematocrit (Hct) and hemoglobin (Hb) concentrations were determined as soon as the blood sample was collected within two hours using the microhaematocrit technique, and Drabkin's reagent as absorbance at 540nm respectively. The red blood cell (RBC) and white blood cell (WBC) counts were made by Neubauer's improved haemocytometer using hyem's and Turk's solution, and WBC differential counts, including lymphocytes, monocytes, neutrophils, and eosinophils was also done using Giesma staining method and expressed as percentages (Stoskopf, 1993). The hematological indices of mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated using RBCs count, Hb and Hct following the procedures described by Dacie and Lewis (1977):

$$\text{MCH (pg)} = [\text{Hb (g dL}^{-1})/\text{RBCs} \times (10^6 \text{ ml}^{-1})] \times 10 \quad (13)$$

$$\text{MCHC (g dL}^{-1}) = [\text{Hb (g dL}^{-1})/\text{Hct (\%)}] \times 100 \quad (14)$$

$$\text{MCV (dL)} = [\text{Hct (\%)/RBCs} \times (10^6 \text{ ml}^{-1})] \times 10 \quad (15)$$

3.7. Cost Analysis of Experimental Diets

A simple economic analysis was conducted to assess the cost-effectiveness of experimental diets used in the feeding trial. Since all other operating costs remained constant, only the cost of feed ingredients was used in the calculations. Costs of feed were calculated using market prices of feed ingredients found in the local market in Addis Ababa, Ethiopia in 2019 where feed were bought. In calculating the economic performance of experimental diets, parameters such as incidence cost (IC) which is determined by unit cost of the feed and its apparent FCR, and profit index (PI) was applied (El-Sayed, 1990). IC is expressed as the cost of feed used to produce a Kg of fish, i.e. as relative cost per unit weight gain and the lower the value, it is the more profitable using that feed (Nwanna, 2003). It was calculated as the cost of the feed per weight of fish produced. The feed that resulted in lower IC value is more profitable. Besides, PI was also calculated as an additional indicator of economic analysis. In calculating the PI the harvested biomass of each treatment was multiplied by the local market value of a Kg of Nile tilapia and then divided by the cost of total feed used.

$$\text{Incidence cost (IC)} = \frac{\text{Cost of Feed used}}{\text{Weight of fish produced}} \quad (16)$$

$$\text{Profit index (PI)} = \frac{\text{Local market value of fish produced}}{\text{Cost of feed used}} \quad (17)$$

3.8. Statistical Analysis

The experimental design used in this study was a completely randomized design where the different dietary treatments were randomly assigned to the experimental aquaria. The results were expressed as Mean \pm SE by using SPSS version 20, and figures were produced using Sigma plot version 11.0. The differences among treatments were analyzed using one way Analysis of Variance (ANOVA) and significant mean differences were compared using Tukey's Multiple Comparison Test. The statistical significance was set at $P < 0.05$, and SPSS for Windows version 20 (SPSS, Michigan Avenue, Chicago, IL, USA) was used.

4. RESULTS

4.1. Physicochemical Parameters

The physicochemical parameters such as temperature, pH, DO, NH₃, and NO₃⁻ recorded in the culture system are shown in Table 5. During the whole experimental period water temperature ranged from 25.4-29.8 °C, DO from 5.24-6.23 mg L⁻¹, pH from 7.22-8.16, and NH₃ and NO₃⁻ exhibited considerably low concentrations as stated in Table 5. There were no significant differences in physicochemical parameters among treatments during the whole experimental period, indicating that the experimental diets did not have a detrimental effect on the surrounding water quality where the cultured Nile tilapia fingerlings had been stocked. These physicochemical parameters are expressed as mean values ± SE as presented in Table 5. Thus, the growth performance and feed utilization of Nile tilapia fingerlings were not significantly affected by the physicochemical parameters of the culture system and all fish were in normal condition.

Table 5: Some representative water quality parameters of recirculating aquaculture system

Parameters	Treatments			
	Control	LGM25	LGM50	LGM75
Temperature (°C)	27.9±0.140 ^a	27.7±0.127 ^a	27.7±0.12 ^a	27.8±0.108 ^a
DO (mg L ⁻¹)	5.83±0.072 ^a	5.80±0.081 ^a	5.77±0.079 ^a	5.71±0.083 ^a
pH	7.81±0.084 ^a	7.89±0.072 ^a	7.83±0.077 ^a	7.87±0.096 ^a
NH ₃ (mg L ⁻¹)	0.02±0.002 ^a	0.02±0.002 ^a	0.02±0.002 ^a	0.02±0.002 ^a
NO ₃ ⁻ (mg L ⁻¹)	0.390±0.031 ^a	0.410±0.031 ^a	0.380±0.021 ^a	0.384±0.016 ^a

The values here in the table are mean ± SE of three replicates, and values within the same row with the same superscripts are not significantly different (P < 0.05).

4.2. Proximate Composition and Energy Contents of Feed Ingredients

As stated in Table 6 lupin and grass pea meals contained good protein content (38.08% and 25.58%, respectively), high-fat content (12.29.% for lupin), and allowable ash content, while nitrogen-free extract recorded were with moderate to higher value (39.73% to 60.33%). The prices of individual feed ingredients used in the study are shown in Table 6. From the economic

viewpoint, fish meal was the most expensive ingredient (60 Birr Kg⁻¹) as the cost for it is directly converted from the cost of fresh whole catfish (20 Birr Kg⁻¹) and 3 Kg of fresh whole catfish dried in an oven can make approximately 1Kg of dried fish meal. Soybean grain was the most expensive ingredient next to fish meal, about double the price (23 Birr Kg⁻¹) of the rest four ingredients in Table 6.

Table 6: Proximate composition (g Kg⁻¹), energy content (KJ g⁻¹) and prices (Birr Kg⁻¹) of individual feed ingredients used in the study

Ingredients	DM	CP	CL	CF	Ash	NFE	GE	Prices
Lupin	94.90	38.08	12.29	2.76	2.04	39.73	20.84	6.5
Grass pea	95.34	25.58	3.77	2.83	2.83	60.33	18.20	10
Wheat grain	89.10	11.28	1.74	5.57	1.46	69.05	15.55	10.5
Corn grain	88.67	8.93	4.09	2.63	1.40	71.62	16.37	8.5
Soybean grain	90.70	38.87	7.83	5.90	6.50	31.60	17.90	23
Fish meal	95.21	60.57	15.93	0	20.8	0	20.70	60

Where: DM- dry matter content, CP- crude protein content, CL- crude lipid content, CF- crude fiber content, NFE- nitrogen-free extract, and GE- gross energy.

4.3. Proximate Composition and Energy Contents of Experimental Diets

The experimental diets were generally formulated and prepared to be almost iso-nitrogenous (36% protein), iso-lipidic (10% lipids), and iso-energetic (18 KJ g⁻¹). Any differences in the performances of the cultured Nile tilapia fingerlings that received such diets could be attributed to the quality of the feed, feeding value of the experimental ingredients and level of replacement. The proximate composition and energy contents of the experimental diets including the control diet are presented in Table 6. The CP contents of diets ranged (358.3- 376.0 g Kg⁻¹), CL (109.6-120.1 g Kg⁻¹), CF (46.5-79.5 g Kg⁻¹), ash (44.7-47.4 g Kg⁻¹), NFE (308.5-325.4 g Kg⁻¹). The GE content of experimental diets varied little within a narrow range (18.71-19.14 KJ g⁻¹). The CF content in diet 3 (LGM75) (79.5g Kg⁻¹) was higher almost double to the CF content of control

diet (46.5 g Kg⁻¹) and it was the highest CF content found among the rest of experimental diets from the nutritional viewpoint. All experimental diets were accepted readily by the stocked Nile tilapia fingerlings. Feed was given in hand moistened expeller-like strands to improve palatability and fingerlings were fed actively in all experimental groups and within approximately 15-20 minutes, all feeds had been consumed from the aquaria. No abnormal Nile tilapia or disease symptoms were observed during the experimental feeding trial. No health-related behavioral issues were observed in response to the experimental diets.

Table 7: Proximate composition and energy contents of experimental diets fed to Nile tilapia

	Control	LGM25	LGM50	LGM75
Components (g Kg⁻¹)				
DM	905.8	902.6	903.7	910.7
CP	376.0	366.4	362.6	358.3
CL	117.3	109.6	120.1	113.3
CF	46.5	53.8	67.5	79.5
Ash	46.4	47.4	45.0	44.7
NFE	319.6	325.4	308.5	314.9
GE (KJ g ⁻¹)	19.14	18.71	18.74	18.48

Note: LGM25 = 25% of fish meal replaced by lupin and grass pea mixture meal

LGM50 = 50% of fish meal replaced by lupin and grass pea mixture meal

LGM75 = 75% of fish meal replaced by lupin and grass pea mixture meal

4.4. Growth Performance and Production Parameters

The growth and feed utilization responses of Nile tilapia fingerlings fed lupin and grass pea mixture meal at different fish meal replacement levels are presented in Table 8. The highest final weight gain was recorded in LGM25, and improved feed intake was recorded in all experimental diets as compared to the control diet. However, there were no significant differences ($P > 0.05$) in IBW, %WG, SGR, SR, FCR, and FCF. No significant feed-related mortality was observed during the whole experimental period. However, the survival rate of the fish that fed on control and LGM50 resulted in a little lower than the fish groups fed on the rest of the experimental diets. The recorded mortality of fish was clear since continuous signs of sexual competition behavior for courtship were observed at the end of the ninth and tenth weeks. The results of the present study revealed that all experimental diets were well accepted by tilapia fingerlings. This implies that the different experimental feed ingredients did not negatively affect the palatability of the diets, and diets were actively eaten by fingerlings.

Table 8: Growth performance and feed utilization of Nile tilapia fingerlings fed different experimental diets.

Parameters	Experimental diets			
	Control	LGM25	LGM50	LGM75
IBW (g)	1.34±0.03 ^a	1.41±0.10 ^a	1.35±0.04 ^a	1.37±0.08 ^a
FBW (g)	9.03±0.045 ^a	9.49±0.052 ^b	9.11±0.129 ^a	8.92±0.035 ^a
WG (%)	573.38±18.76 ^a	583.85±42.21 ^a	576.24±27.52 ^a	551.09±47.61 ^a
SGR (%)	2.72±0.04 ^a	2.73±0.10 ^a	2.73±0.06 ^a	2.62±0.09 ^a
SR (%)	99.52±0.48 ^a	100±0.00 ^a	99.52±0.48 ^a	100±0.00 ^a
FCR	2.30±0.17 ^a	2.37±0.23 ^a	2.33±0.19 ^a	2.50±0.26 ^a
FI (g fish ⁻¹)	17.65±0.20 ^a	19.15±0.16 ^b	18.07± 0.38 ^{ab}	18.87±0.31 ^b
FCF	2.69±0.012 ^a	2.73±0.029 ^a	2.62±0.038 ^a	2.66±0.012 ^a

The values here in the table are mean \pm SE of three replicates, and values within the same row with different letters are significantly different ($P < 0.05$). Where: IBW = initial body weight (g), FBW = final body weight (g), WG = % weight gain, SGR = specific growth rate, SR = survival rate (%), FCR = feed conversion ratio, FI = feed intake (g fish^{-1}), and FCF = Fulton's condition factor.

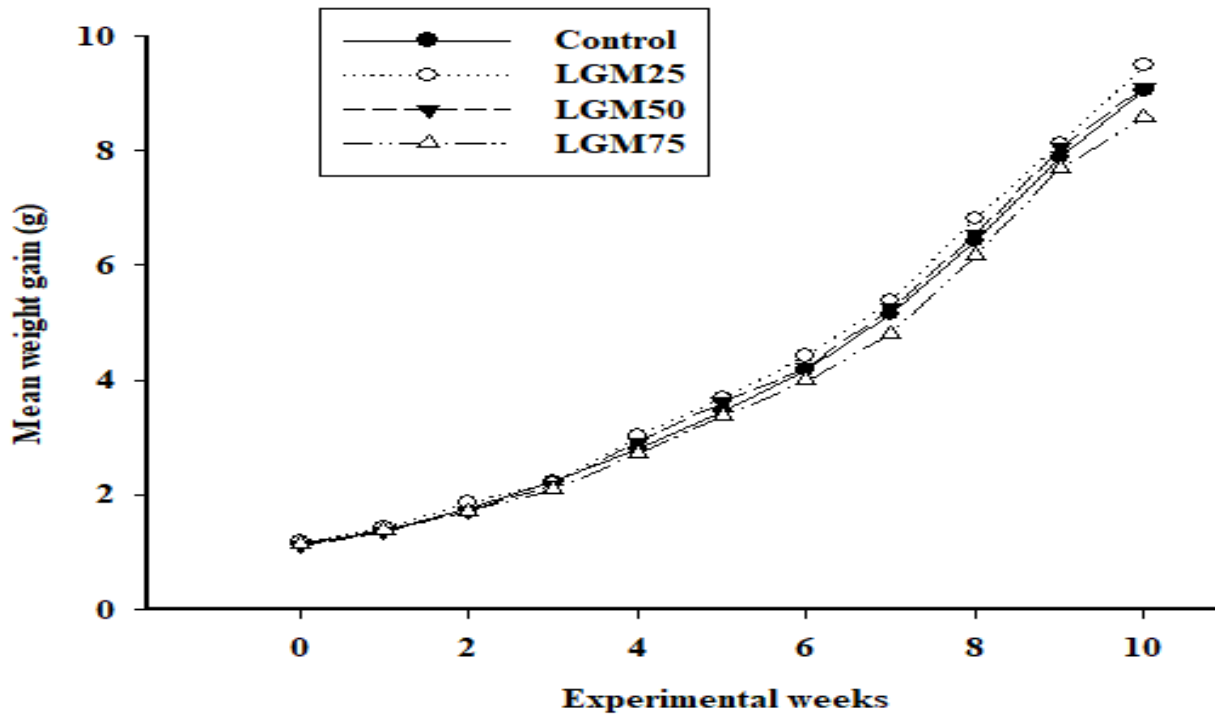


Figure 1: Growth response of Nile tilapia fingerlings fed LGM diets for 10 weeks

4.5. Body Proximate Composition

The whole-body proximate composition of all experimental fish samples including a control group is presented in Table 9. The moisture content of carcasses increased little with the increased replacement of fish meal at higher levels (LGM75) protein, however, fish fed the control diet, LGM25 and LGM50 had not shown significant differences ($P > 0.05$) on moisture content. The crude lipid and gross energy contents of whole-body proximate were significantly affected ($P < 0.05$) by the replacement of lupin and grass pea mixture meal in Nile tilapia fingerlings fed LGM75 diet as compared to fish fed on a control diet. The crude protein and ash contents of all whole-body proximate were not significantly different. Highest HSI value was recorded from fish fed with LGM50 and LGM75 diets.

Table 9: Whole body proximate composition (% dry weight), gross energy and HSI of Nile tilapia fingerlings fed the different experimental diets

Components	Experimental Diets			
	Control	LGM25	LGM50	LGM75
MC	21.63±0.18 ^a	21.76±0.12 ^a	21.87±0.25 ^a	23.43±0.34 ^b
CP	57.33±0.13 ^a	57.35±0.05 ^a	57.13±0.23 ^a	56.95±0.02 ^a
CL	18.42±0.08 ^a	18.49±0.10 ^a	18.40±0.09 ^a	17.30±0.08 ^b
Ash	13.12±0.07 ^a	12.96±0.09 ^a	13.05±0.15 ^a	13.13±0.09 ^a
GE	20.91±0.04 ^a	20.93±0.04 ^a	20.86±0.06 ^a	20.38±0.03 ^b
HSI	2.70±0.08 ^a	2.88±0.09 ^{ab}	3.16±0.17 ^b	3.23±0.09 ^b

The values here in the table are mean ± SE of three replicates, and values within the same row with different superscripts are significantly different ($P < 0.05$). Where: MC = moisture content, GE = gross energy (KJ 100g⁻¹), HSI = hepatosomatic index

4.6. Hematological Parameters

The mean hematological parameter indices of Nile tilapia fingerlings fed on different replacement levels of fish meal by lupin and grass pea mixture meal diets are presented in Table 10. The hematological parameters ranged as Hct (27.43-28.20%), RBCs (1.48-1.54 x 10⁶ mm⁻³), Hb (8.08-975 g dL⁻¹), MCH (53.70-64.66 pg), MCHC (28.91-35.52 g dL⁻¹), MCV (181.54-191.42 dL), and WBCs (66.66-72.16 x 10³ mm⁻³). The results of the present study revealed that no significant ($P > 0.05$) differences were recorded among all treatments in RBCs, MCV, WBCs, monocytes, and eosinophils. However, Hb, MCH, MCHC (Table 10), lymphocytes, and neutrophils (Figure 2) had shown significant differences among treatments.

Table 10: Hematological parameters of Nile tilapia fingerlings fed experimental diets for 10 weeks

Hematological parameters	Experimental diets			
	Control	LGM25%	LGM50%	LGM75%
Hct (%)	28.20±0.78 ^a	27.94±0.56 ^a	27.80±0.46 ^a	27.43±0.46 ^a
RBCs (x 10 ⁶ mm ⁻³)	1.48±0.05 ^a	1.51±0.06 ^a	1.54±0.04 ^a	1.51±0.04 ^a
Hb (g dL ⁻¹)	8.37±0.26 ^a	8.08±0.25 ^a	8.28±0.22 ^a	9.75±0.46 ^b
MCH (pg)	56.93±2.67 ^{ab}	53.70±1.56 ^a	54.29±2.65 ^{ab}	64.66±3.54 ^b
MCHC (g dL ⁻¹)	29.78±1.07 ^a	28.91±0.47 ^a	29.87±1.11 ^a	35.52±1.43 ^b
MCV (dL)	191.42±6.95 ^a	185.64±3.65 ^a	181.54±4.04 ^a	181.94±5.89 ^a
WBCs (x10 ³ mm ⁻³)	72.16±1.92 ^a	68.38±4.34 ^a	66.66±3.23 ^a	68.78±4.07 ^a

The values here in the table are mean ± SE of three replicates, and values within the same row with different letters are significantly different (P < 0.05). Where: Hct = hematocrit, RBCs = red blood cell, Hb = hemoglobin, MCH = mean corpuscular hemoglobin, MCHC = mean cell hemoglobin concentration, and MCV = mean corpuscular volume.

The percentage composition of WBC differential counts is dominated by lymphocytes ranged as (55.88-59.35% mm⁻³), monocytes (4.85-5.12% mm⁻³), neutrophils (14.48-17.68% mm⁻³), and eosinophils (20.83-21.62% mm⁻³) as presented in Figure 2.

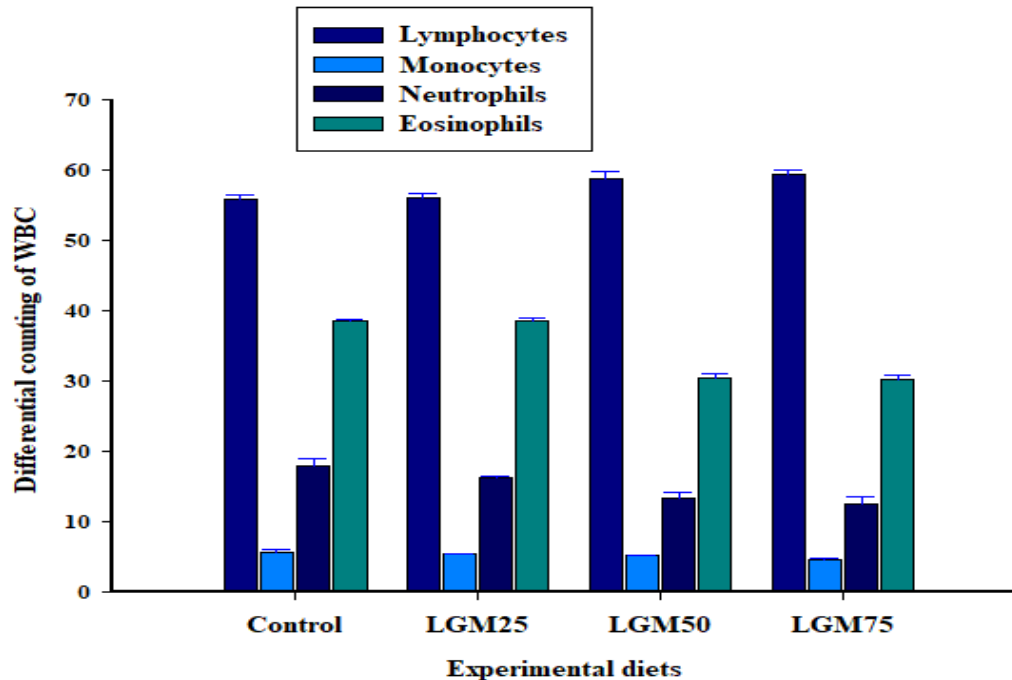


Figure 2: Differential white blood cell counts of Nile tilapia fingerlings fed LGM protein for 10 weeks

4.7. Preliminary Cost Analysis and Feasibility of Lupin and Grass pea Mixture Diets

All other costs were the same for all treatments until the end of the experiment. Feed provided for experimental fishes was narrowly ranged between 0.460 to 0.502 Kg per aquarium. The treatment with a higher proportion of fish meal (100%) incurred significantly more cost than treatments with lower levels; the cost was higher in the control group (Table 11). The feed consumed by each treatment was almost similar to the control group, but the cost was still higher for the control though each treatment received similar number of Nile tilapia fingerlings (n=23). The estimated average local market value of (whole fish) Nile tilapia at around Addis Ababa was 70 Birr Kg⁻¹. The economic efficiency parameters of incidence cost (IC) and profit index (PI) were ranging from 44.31 to 72.5, and 0.96 to 1.58, respectively.

Table 11: Cost-effectiveness of all experimental diets fed to Nile tilapia fingerlings

Parameters	Treatments			
	Control	LGM25	LGM50	LGM75
Cost of feed (Birr Kg ⁻¹)	31.52	27.35	23.16	18.98
Total feed used (Kg)	0.479	0.502	0.486	0.460
Cost of total feed used (Birr Kg ⁻¹)	15.09	13.73	11.25	8.73
Produced fish weight (Kg)	0.208	0.218	0.209	0.197
Estimated local market value (Birr Kg ⁻¹)	70	70	70	70
Incidence cost	72.5	62.98	53.8	44.31
Profit Index	0.96	1.11	1.30	1.58

Note: Total feed used is computed by adding each weekly consumed feed ration of total fish per aquarium, and the total weight of fish produced in each treatment was computed using the average final body weight of fish in triplicates multiplied by the number of individuals in each treatment. Similarly, the cost of total feed used is calculated in accordance to amount of feed consumed by each treatment and the cost of a Kg of treatment diet. Whereas, the estimated local market value (Birr Kg⁻¹) of fingerlings was calculated using the average market price for unprocessed Nile tilapia (whole fish) at the local market, Piassa, Ethiopia.

5. DISCUSSION

5.1. Growth Performance and Production Parameters

The result of the present study indicated the potential of lupin and grass pea mixture meal (LGM) as a major protein source replacing fish meal in the diets of Nile tilapia fed at higher levels with success. Fish fed with mixtures of lupin and grass pea meal resulted in enhanced body weight gain (Figure 1). This might be associated to reduced exposure to ANFs in the diet responsible for suppressing feed intake, palatability and digestibility resulted from the combined preprocessing techniques in the feed preparation. Similarly, combining several plant protein sources with inherent ANFs can reduce the exposure of fish to those ANFs due to lower inclusion levels of each of the individual protein sources in the combined mixture (Borgeson, 2005). This is in agreement with Francis *et al.* (2001) who reported that mixing plant proteins may also lead to interactions between various ANFs or with other components in the diets resulting in the reduction of their deleterious effects. For instance, Fish and Thompson (1991) reported that interaction between tannins and lectins removed the inhibitory action of tannins on digestive enzyme amylase, and interactions between tannins and cyanogenic glycosides reduced the deleterious effects of cyanogenic glycosides (Goldstein and Spencer, 1985).

Besides, result of the present study agrees with the findings of Yones (2010) who reported replacement of fish meal with a mixture of extruded lupin kernel meal (*Lupinus albus*) at 25, 50, and 60% inclusion levels in diets of red hybrid tilapia that found best growth performance. Similarly, the results of the present study confirmed the previous work of Zhang *et al.* (2012) who reported a better growth performance of Nile tilapia fed on a mixture of lupin and soy protein concentrate than fish groups fed on a fish meal-based diet. The current finding also agrees with the study reported by Fournier *et al.* (2004) who found that replacing fish meal up to 50% by plant protein mixtures of lupin, wheat and corn gluten meal in the diets of juvenile turbot (*Psetta Maxima*) had not significantly affected growth responses. Similarly, a study conducted by El-Saidy and Gaber (2003) found that partial or complete replacement of fish meal by mixtures of plant protein sources such as soybean, cottonseed, sunflower and linseed meals (all in equal proportion of 25%) in the diets of Nile tilapia did not exhibit significant differences in growth performances as compared to a control diet. Furthermore, Al-Thobaiti *et al.* (2018) evaluated the replacement of fish meal by mixtures of corn gluten meal, wheat gluten meal, and

bagasse kenna meal on growth performance of Nile tilapia fingerlings. They found that fish groups fed with 20% of fish meal replaced diet by plant protein mixtures revealed enhanced growth performance and improved feed utilization efficiency as compared to 100% fish meal based diet.

The Specific growth rate (SGR) which is described as the percentage increase in weight of fish per day resulted in no significant variation among treatments (Table 8) and a similar trend was observed with the other growth and production parameters. The growth parameter results of the present study revealed enhanced SGR values as compared to several reports done on Nile tilapia fed with mixture and individual feed ingredients. This might be attributed to the compensatory effects of mixing plant proteins that improve the amino acid profile, bioavailability, and palatability of diets. This is supported by the findings of Akewake Geremew (2015) who reported better growth performance of Nile tilapia fingerlings fed with mixtures of different oil seed cakes (niger seed cake and linseed cake) than fish fed with individual feed ingredients. Moreover, the improved growth performance recorded in the present study might be associated with the preprocessing techniques applied to remove ANFs and their effects on LGM feed ingredients. For instance, dehulling of legume grains reduce saponins and tannin content and positively affects the appearance, digestibility and palatability of diets. It also improves the crude protein content of feed ingredients by (10-23%), decrease crude fiber content by approximately (76-83%), and increases mineral bioavailability (Brenes *et al.*, 2003; Khan *et al.*, 2013; Hendek and Bektas, 2018). Similarly, soaking and roasting of LGM feed ingredients remove alkaloids, water-soluble phytates, trypsin inhibitors, and thermo-labile ANFs such as haemagglutinins (lectins) thus maximizing their potential utilization as aquafeed ingredients (Akande and Fabiyi, 2010). Furthermore, the enhanced growth responses could be attributed to a high concentration of L-homoarginine amino acid in the LGM diets that could be a substrate for arginase, since *Lathyrus* seeds are the only dietary source of this natural amino acid (Petyala and Rao, 1999; Lambien *et al.*, 2019). Besides that, this improved growth could be attributed to the controlled and optimum physicochemical parameters of culture system for a healthy growth of Nile tilapia (El-Sherif and El-Feky, 2009; Begum *et al.*, 2014).

The SGR result of the present study agrees with the findings of Soltan *et al.* (2008), Khan *et al.* (2013) and Akewake Geremew (2015) who reported SGR values ranging from 2.39 to 2.73, 2.60 to 2.73 and 2.08 to 2.76 for Nile tilapia in a controlled environment in the replacement and inclusion of a mixture of cottonseed, sunflower, canola, sesame; linseed meal, rice polish, and oilseed cakes into the basal diet, respectively. However, the result of the present study is in contrast with the findings of Sudaryono *et al.* (1999) where replacement of lupin kernel meal in the diets of *P. monodon* containing 40% CP replaced at (25, 50, 75, and 100%) of fish meal protein with an equivalent amount of lupin protein resulted in a progressive decline of weight performance for those replaced over 25% of fish meal.

Unlike, to the present study, Fournier *et al.* (2004) reported that replacing fish meal by plant protein mixtures of lupin, wheat and corn gluten meal in the diets of juvenile turbot (*Psetta maxima*) at highest replacement levels (75 and 100%) significantly reduced growth performance. Besides, Goda *et al.* (2007) also reported lower SGR ranged from 1.73 to 1.97 in the diets of Nile tilapia fed with mixtures of different plant proteins. Furthermore, Alayu Yalew *et al.* (2019) reported very low SGR ranged from 1.68 to 2.02 in the replacement of fish meal by sweet lupin meal at inclusion levels of 75% and 100% in the diets of juvenile catfish. The contradiction between the current and the aforementioned results could be attributed to identified or unidentified ANFs in the experimental diets which could reduce FI and adverse FCR (Sklan *et al.*, 2004; Soltan *et al.*, 2008). The ANFs that are dominantly abundant in lupin and grass pea grains such as phytic acid, lectins, trypsin inhibitors, protease inhibitors, alkaloids, saponins, and oligosaccharides could increase the delay of diet retention in stomach affecting FI through feedback on satiety signals (Khan *et al.*, 2013). Aquafeeds made of lupin, grass pea and other pulses contain phenolic compounds such as tannin that may reduce palatability and reduce protein digestibility. Besides, phytic acid in both grains negatively affect the utilization of minerals which can be seen by its ability to bind up 75% of all phosphorus, chelating di and trivalent metals such as Ca^{2+} , Mg^{2+} , and Fe^{3+} into compounds not easily absorbed in the intestine and inhibiting digestive enzymes (NRC, 1998).

Feed conversion ratio (FCR) that is commonly used as indicator of quality of feed was not significantly different among treatments (Table 8). The FCR values of the present study ranging from 2.30 to 2.50 indicated that 2.3 to 2.5g of feed was used to produce one gram fish flesh.

According to Stickney (1979), 1.5g of feed is recommended to produce one gram of live weight in aquaculture. This high FCR value of the present study might be associated to the quality of experimental diets, dietary protein and energy content (Elham *et al.*, 2012; Khan *et al.*, 2013) and relatively higher CF content that affect feed utilization efficiency and adverse FCR (Soltan *et al.*, 2008). Similarly, the increased FCR values of the present study could be attributed to variations in fish strain (Guimaraes *et al.*, 2008), size and life stage of the stock (Ridha, 2006). Besides that, the stocking density of the present experiment could affect the FCR values since culturing at a confined environment results in increased energy expenditure because of increased agonistic behavior (Diana *et al.*, 2004). However, the FCR value of the present study agrees with the findings of El-Saidy and Gaber (2003) 2.03 to 2.17, Khan *et al.* (2013) 2.23 to 2.31, Agbo *et al.* (2015), 2.07 to 3.17, Al-Thobaiti *et al.* (2018) 2.33 to 3.0 in the diets of Nile tilapia fed with mixtures of different plant protein sources. Besides, the result of present study is in agreement with the study done by Yones (2010) who reported that the FCR values of hybrid Nile tilapia fed with a replaced fish meal at (25 and 50%) of basal diet by lupin kernel meal had not shown significant variation as compared to the control diet. Unlike the present study, high FCR values (low feed conversion efficiencies) have been also reported by other studies. For instance, Alayu Yalew *et al.* (2019) reported very high FCR values ranging from 2.70 to 8.92 for catfish fingerlings that fed sweet lupin meal in the replacement of fish meal at 0, 50, 75, and 100% inclusion levels in a controlled condition. Moreover, Kassaye Balkew *et al.* (2014) also reported FCR values ranging from 3.23 to 3.38 in the diets of Nile tilapia fingerlings fed with mixtures of different plant proteins. This variation could be associated with the preprocessing techniques used in the present study that may efficiently reduce the ANFs like saponins, phytic acids, tannin content, trypsin inhibitors and alkaloids which can affect the digestibility, palatability, and improve feed utilization efficiency (Brenes *et al.*, 2003; Hendek and Bektas, 2018).

The feed intake (FI) results of the present study ranged from 17.65 to 19.15 g fish⁻¹. Fish groups fed on all experimental diets revealed improved FI values as compared to control group. The slight tendency towards a higher FI as dietary LGM content increased can be explained as a compensatory intake to meet demands for protein to maintain maximum growth. This is supported by the previous findings reported by Higuera *et al.*, (1988) in rainbow trout, Yones (2005) in sea bream, and Espe *et al.* (2007) in Atlantic salmon, which had shown a similar trend in feed utilization. Interestingly, feeding with lupin and grass pea mixture meal led to higher FI

compared to the control diet, and this might be also attributed to the actual nutritional composition of feed ingredients, and the size and life stage of stock affect FI values (Abdel-Tawwab *et al.*, 2010). Moreover, the energy content of the control diet in the present study is higher than others, thus fish fed on it might not consume a high amount of feed as compared to others that resulted in reduced FI values.

The result of the present study is consistent with the findings of Elham *et al.* (2012) who reported higher FI in rainbow trout as compared to the control diet, which might be due to the mixture of feed ingredients used (lupin, mango, and stinging nettle) had better nutritional composition. The result of the present study confirmed the feed intake ranging from 38.1 to 39.3 reported by Zhang *et al.* (2012) where an increasing tendency with increasing fish meal replacement by mixtures of lupin and pea proteins in the diets of rainbow trout at different inclusion levels. The FI results of the present study varied from the results reported by several authors who found a decreasing trend of FI with increasing the replacement levels of fish meal by mixtures of different plant protein in the diets of Nile tilapia. For instance, Soltan *et al.* (2008) reported a decreased FI trend ranged from 36.12 to 41.50 g fish⁻¹ in the diets of Nile tilapia fingerlings where a fish meal was replaced by a mixture of cottonseed, sunflower, canola, sesame, and linseed meals up to 75%. Similarly, the results of the current study deviate from the findings of El-Saidy and Gaber (2003) who reported FI ranging from 30.2 to 38.4 g fish⁻¹ in the diets of Nile tilapia fingerlings fed on fish meal replaced by mixtures of 25% for each (soybean, cottonseed, and sunflower meals) at 25, 50, 75, and 100% inclusion levels. In contrast to the aforementioned FI reports, the results of the present study showed a slight tendency toward higher FI as dietary LGM content increased. Besides, Borgeson (2005) reported improved feed utilization and enhanced growth response of Nile tilapia fed with a mixture of soybean meal, canola, whole peas and whole flax meal at inclusion levels of 0, 33, 67, and 100%.

In the case of survival, no mortality concerning the effect of experimental diets was recorded. The relatively lower survival rate of 99.52% was recorded in fish groups fed on control and LGM50 and the survival rate of LGM25 and LGM75 was 100%. However, there was no significant ($P > 0.05$) difference in survival rate among treatments. The insignificant reduction in survival rate obtained was due to the mortality rate recorded in both groups during the ninth and tenth weeks of the experimental period and some observed that cultured fish were showing

sexual competition for courtship. As a result, lot of eggs were found while samples were taken and dissected during hepatosomatic index analysis in the laboratory as indicated in Plate 7-E. Generally, the survival rate was successful which implies LGM meal can be incorporated into the diets of Nile tilapia by replacing fishmeal up to 75% without posing any survival problem.

5.2. Whole body Proximate Composition

The moisture content among treatments of Nile tilapia fingerlings in the present study presented in Table 9. The percentage range of moisture content in the present study is within the acceptable moisture level required for fish commonly reported to about 60-80% by different authors. For instance, the moisture content of the current study is close to the results of El-Saidy and Gaber (2003) and Akewake Geremew (2015) who reported moisture content ranging from 75.45 ± 1.10 to 77.81 ± 0.90 and 76.4 ± 0.10 to 77.48 ± 0.18 for Nile tilapia fed with mixtures of different plant proteins, respectively. Moisture content of the present study closely agrees with the findings of Gebremedhin Gebreanenia (2018) who reported moisture content ranging from 77.8 ± 0.23 to 78.1 ± 0.06 in the growth performance and proximate composition of three Nile tilapia strains in a controlled condition.

Percentage crude protein (CP) content of Nile tilapia fingerlings fed on LGM protein in the present study ranged from 56.96 to 57.35% (Table 9) and no significant variation was obtained in all treatments. The percentage CP content of the present study is in the range of permissible protein limit in dry weight basis for fish fillets and fisheries products. This might be associated to the dietary protein levels of feed ingredients and experimental diets. This is supported by the findings of Al-Hafedh (1999) who pointed out that carcass composition of fish is significantly affected by the dietary protein level where fish fed on a high-protein diet resulted in a higher percentage of protein than fish fed on low protein diets. The percentage CP of the present study agrees with the CP content reported by Montoya-Mejia *et al.* (2017) ranging from 57.5 ± 0.1 to 60.0 ± 0.8 for Nile tilapia fed on experimental diet prepared from mixtures of extruded bean, extruded chickpea meal, coconut paste, jatropha curcas meal, and chickpea meal in a controlled environment. Similarly, the CP content of the present study closely agrees with the previous works done by other different researchers. For instance, Elham *et al.* (2012) and Mzengereza (2015) have reported CP content ranging from 56.28 ± 1.68 to 62.45 ± 6.74 and 61.88 ± 0.25 to 63.21 ± 0.03 for rainbow trout and tilapia rendalli fed with mixtures of mango, lupin, stinging

nettle; sweet potato, cassava, banana, and maize proteins as dietary supplements, and as main protein sources, respectively.

However, the CP content of the present study is lower than reports of others. For instance, Soltan *et al.* (2008) reported CP ranged from 66.11 to 68.58% in the diets of Nile tilapia fed with diets prepared from a mixture of plant proteins in the replacement of fish meal at inclusion levels of 15 to 100% of the basal diet in a controlled environment. Hassaan *et al.* (2018) have also reported CP content ranging from 61.75 ± 1.17 to 65.31 ± 1.12 for Nile tilapia grown in controlled condition. The variation in the CP content of the present study and formerly reported findings could be attributed to differences in the quality of experimental diets and protein content of feed ingredients used that affect directly the protein content of fish fillet. This is supported by the findings of Glencross *et al.* (2004) who reported similar results where rainbow trout fed with a mixture of plant proteins consisted of 12.5% yellow lupin meal resulted in increased CP content as compared to other diets, and body proximate composition of fish depends on the composition of the feed since it affects directly the protein content of fish fillet.

The crude lipid (CL) content of cultured fish recorded in the present study ranged from 17.30 ± 0.08 to 18.49 ± 0.10 . Fish group fed LGM75 protein had a relatively lower percentage of CL content as compared to the control diet, and there was a significant difference ($P < 0.05$) in CL content among fish groups fed with the different experimental diets. This lower CL content of fish groups fed on LGM75 might be attributed to high moisture content of fish carcass and composition of experimental diets. Yildirim *et al.* (2003) have reported an inverse correlation between fat and moisture content and it is common among fish species. Similarly, FAO (2010) has pointed out an inverse relation between moisture content and CL contents in fish fillet, and feed composition significantly affects CL and fatty acid profiles of fish flesh (Zenebe Tadesse, 2010). The CL content obtained in the present study is in the range of CL content of 17.50 ± 0.23 to 17.65 ± 0.47 and 15.5 ± 0.00 to 22.1 ± 0.6 reported for Nile tilapia fed with fermented sunflower meal, and chickpea meal, chickpea, and *Jatropha curcas* meal (Mzengereza, 2015; Montoya-Mejia *et al.*, 2017) in the replacement of fish meal. Furthermore, the percentage of CL of the present study is closely in agreement with CL contents ranged from 22.69 to 27.2% reported by Hassaan *et al.* (2018) in the diets of tilapia *rendalli* fed with a mixture of different plant proteins using glass aquaria.

However, CL content of the present study is lower than the reports of Elham *et al.* (2012) who reported higher CL contents of rainbow trout fed with plant protein mixture of lupin, mango and stinging nettle as dietary supplement ranging from 27.77 ± 2.11 to 35.28 ± 0.43 . Unlike results of the present study, Akewake Geremew (2015) and Yones *et al.* (2019) have also reported low CL contents ranging from 5.13 to 7.81% for juvenile Nile tilapia in the replacement of fish meal by mixtures of different plant proteins at different inclusion levels. The variation in the CL content of the present study and previous works might be attributed to the lower moisture content of the carcasses, and the inclusion of higher dietary protein content in the experimental diet (Al-Hafedh, 1999).

The percentage of ash content of the present study is presented in Table 9. The increased ash content in the present study might be due to increased dietary levels of plant proteins, indicating sufficient amounts of dietary minerals in diets and its absorption. This increased ash content of carcasses is supported by the reports of Zhang *et al.* (2012) and Hassaan *et al.* (2018) that showed the same trend of ash contents in fish carcasses. Furthermore, the increased ash content of the present study might be attributed to processing techniques applied to remove different ANFs that otherwise reduces the availability of several minerals such as calcium, magnesium, zinc, iron, and phosphorus in the fish diets and severely affect the liver function.

Ash content of the present study follows the ash (11.23 to 15.12%) content reported by Soltan *et al.* (2008) for Nile tilapia fingerlings cultured in glass aquaria in the replacement of fish meal with a mixture of cottonseed, sunflower, canola, linseed and sesame meals at inclusion levels of 15 to 100%. Besides, several authors have reported similar ash content of fish carcasses. For example, Hassaan *et al.* (2018) have reported ash content ranged from 15.05 ± 0.56 to 16.05 ± 0.52 for Nile tilapia in a controlled environment. Azaza *et al.* (2009) have also pointed out similar ash content ranging from 14.80 ± 0.11 to 16.51 ± 0.49 for Nile tilapia in the replacement of fish meal by a mixture of plant proteins and black soldier fly maggot meal at 0, 25, 50, 75, and 100% inclusion levels. However, Zhang *et al.* (2012) have reported higher ash content ranging from 20 to 23.3% for rainbow trout fed with a mixture of lupin and pea protein concentrates in a controlled environment. The obtained ash content of the present study is higher than the ash content reported by other authors. Akewake Geremew (2015) have reported ash content ranging from 4.17 ± 0.07 to 4.28 ± 0.11 and 4.09 ± 0.08 to 4.23 ± 0.09 in dietary inclusions of linseed cake and Niger seed cake meals on growth performance and feed utilization of juvenile Nile

tilapia, respectively. Furthermore, Goda *et al.* (2007) and Yones *et al.* (2019) have also found lower ash content values ranging from 5.0 ± 0.2 to 6.73 ± 0.53 in the inclusion of mixtures of wheat bran, corn and sorghum and full-fat soybean meal in the diets of Nile tilapia fingerlings.

The HSI result of the current study ranged from 2.7 ± 0.08 to 3.23 ± 0.09 . In the present study fish groups fed LGM75 and LGM50 had shown higher HSI as compared to fish groups fed on control and LGM25 diets and an increasing trend was observed with increasing the replacement of fish meal by LGM protein at higher levels (Table 9). This increasing tendency might be associated with the storage of glycogen or lipid and is most likely associated with the higher feed intake and high energy content in the experimental diets (Zhang *et al.*, 2012). Hemre *et al.* (2002) pointed out that excessive digestible carbohydrate in fish diet may produce negative effects in glycogen accumulation, liver morphology and function that indicate the nutritional and physiological status of fish. These effects may have a great influence on suppressing the immune system and increase susceptibility to infectious diseases (Abarra *et al.*, 2017). However, the HSI result of the present study agrees with the reports of several researchers and it was within the acceptable range for healthy Nile tilapia. For instance, Akewake Geremew (2015) reported HSI values ranged from 2.26 ± 0.76 to 3.23 ± 0.25 in the diets of Nile tilapia fingerlings fed with Nigerseed cake meal at inclusion levels of (0, 20 and 40%) of a basal diet. Similarly, Velasquez *et al.* (2016) reported HSI ranged from 1.92 ± 0.22 to 3.13 ± 0.13 . Furthermore, Montoya-Mejia *et al.* (2017) have also reported HSI values ranged from 2.1 ± 0.4 to 2.9 ± 0.9 .

5.3. Hematological Parameters

The results of the present study revealed there were no significant differences ($P > 0.05$) in the values of Hct, RBCs, WBCs, and MCV of Nile tilapia fingerlings fed at all levels of LGM diets. However, the results of Hb, MCH, and MCHC had shown an increasing trend with increasing replacement levels of fish meal by LGM75 (Table 10). Hematocrit value of Nile tilapia fingerlings narrowly ranged from 27.43 to 28.20%. A normal Hct value of healthy fish and other organisms usually fall within the range of 20-35% and are rarely greater than 50% unusually in fish (Ayandiran *et al.*, 2010), and hence, the Hct value of the current study is within the acceptable range and fish were healthy and active throughout the experimental period. The Hct value recorded in the present study agrees with the normal Hct value (25.5 to 31.20%) reported by Montoya-Mejia *et al.* (2017) in healthy juvenile Nile tilapia fed with mixtures of extruded

bean and chickpea meal, coconut paste, *Jatropha curcas* meal, and chickpea meal. Similarly, the result of the present study agrees with the previous works of Yones (2010) who reported Hct values ranging from 29.20 to 30.00% in hybrid red tilapia fingerlings fed with the diets containing mixtures of extruded *Lupinus albus* meal in the replacement of fish meal at 0, 25, 50, and 60% and no significant variation was observed. However, the current results are higher than the Hct value that ranged from 12.50 to 19.00% reported by Adeyemi *et al.* (2015). The Hct value of the present study is lower than the Hct value ranging from 32.25 to 33.78% where Nile tilapia fingerlings were fed a mixture of wheat bran, corn, and sorghum at different inclusion levels in a controlled system (Yones *et al.*, 2019). This deviation of Hct value could be attributed to the binding effects of a remained phytic acid (phytate) to minerals (iron or/and amine group of amino acids) causing their low availabilities in the body and increase in erythrocyte fragility (Akinleye *et al.*, 2011) since the exact ANFs remained in the processed feed ingredients was not quantified, and phytase was not added to prevent the problem.

Red blood cell (RBC) value of the present study is ranging from 1.48 to 1.54 x 10⁶ mm⁻³ and no significant differences were observed at all treatments including the control fish groups. The observed results are closely similar to the RBCs of Nile tilapia fingerlings of 1.84 ± 0.02 to 1.89 ± 0.03 x 10⁶ mm⁻³ reported by Yones *et al.* (2019). Similarly, the results of the present study confirmed the earlier work of Jimoh *et al.* (2012) who reported that erythrocyte count greater than 1 x 10⁶ mm⁻³ is considered high and is an indication of high oxygen-carrying capacity of the blood, which is characteristic of fishes capable of aerial respiration and with high activity. However, reduced erythrocyte parameters less than the level are indications of haemolysis or macrocytic anemia emanating from increased destruction and effects from accumulated toxic substances which results in enhanced erythropoiesis (Barad and Kulkani, 2010).

The hematological parameters of Hb and Hct are also taken as indicators of the rate of Hb synthesis of RBCs formation and erythrocyte fragility (Barraza *et al.*, 1991). Hb is an indicator of physiological health, and it is crucial to the survival of fish, being directly related to the oxygen-binding capacity of blood (Kefas *et al.*, 2015). The Hb value of the present study ranged between 8.08 ± 0.25 to 9.75 ± 0.46 g dL⁻¹ (Table 10). Although Hb result of present study was not negatively affected, there was an increasing tendency with increasing replacement levels of LGM proteins, and the highest Hb value was recorded from fish groups fed with LGM75 protein.

This might be attributed to effects of physical techniques used to remove toxic ANFs like phytates, alkaloids, saponins, lectins and protease inhibitors in the feeds that can deter the properties of Hb by decreasing their affinity towards oxygen-binding capacity rendering the erythrocytes more fragile and permeable for tilapia. This is supported by the reports of Hassaan *et al.* (2018) where Nile tilapia fed unprocessed plant protein mixture diets resulted in lower Hb values as compared to fish fed fermented experimental diets. Concomitantly, the increased number of RBC multiplies the concentration of Hb that ultimately results in a higher capacity for oxygen-carrying in fish. The Hb values recorded in the present study were all within the normal range of hemoglobin standards for a healthy Nile tilapia ($10.52 \pm 3.09 \text{ g dL}^{-1}$) stated by Bittencourt *et al.* (2003) cultured in a semi-intensive system purposefully done to investigate the reference ranges for hematological and biochemical values for Nile tilapia. Similarly, the current results closely agree with the findings of Yones *et al.* (2019) who evaluated the effects of using a mixture of wheat, corn, and sorghum in the diets of Nile tilapia fingerlings for hematological changes and reported Hb values ranging from 7.49 ± 0.40 to $7.70 \pm 0.42 \text{ g dL}^{-1}$. Moreover, the result of the present study agrees with the report of Hassaan *et al.* (2018) who reported Hb values ranging from 10.95 ± 0.65 to $11.15 \pm 0.71 \text{ g dL}^{-1}$ in the diets of Nile tilapia fed with soybean meal at inclusion levels of 0, 25, 50, and 75%.

White blood cells play a major role in the defense mechanism in fish. The results of the present study for WBCs revealed there were no significant differences ($P > 0.05$) in the diets of Nile tilapia fingerlings fed at all levels of LGM proteins as compared to control diets. The present result of WBCs ($\times 10^3 \text{ mm}^{-3}$) ranged from 66.66 ± 3.23 to 72.16 ± 1.92 . This is higher than the WBCs of Nile tilapia that ranged from 35.67 ± 7.55 to 50.65 ± 8.15 (Osman *et al.*, 2018). However, higher WBCs numbers have been reported by other researchers. For instance, Yones *et al.* (2019) evaluated the effects of different dietary carbohydrate sources in the diets of juvenile Nile tilapia, and WBCs ($\times 10^3 \text{ mm}^{-3}$) values ranging from 81.45 ± 0.65 to 82.27 ± 0.47 were reported. Adeyemi *et al.* (2015) have reported WBC counts ranging between 106.50 ± 7.78 to 130.50 ± 14.85 in the diets of Nile tilapia fingerlings fed with a mixture of watermelon seed meal in the replacement of soybean meal. The value of WBC count in fish is generally variable even among similar individuals and similar conditions, and it depends on several environmental and nutritional components (Ighwelu *et al.*, 2012). The hematological indices of MCH, MCHC, and MCV are also presented in Table 10. MCH and MCHC values have shown a decreasing trend in

fish groups fed diets of LGM25 and LGM50 as compared to the others. The MCH and MCHC values of the present study agree with the findings of Akinleye *et al.* (2011), Adeyemi *et al.* (2015) and Bittencourt *et al.* (2003) who reported the normal and healthy standard range of MCH (40.74 ± 34.19 pg) and MCHC (35.24 ± 14.92 mg dL⁻¹) in Nile tilapia. Unlike the results of MCH and MCHC, MCV has not shown a significant difference in the replacement of fish meal by higher LGM proteins levels. This agrees with the results of Bittencourt *et al.* (2003) and Adeyemi *et al.* (2015) who found MCV (dL) ranged from 114.66 ± 9.26 to 166.27 ± 35.98 in the replacement of soybean meal with a watermelon seed meal at different inclusion levels and no significant variation was observed.

Changes in differential WBCs count are also recognized as a sensitive indicator of environmental stress. The differential WBC count of Nile tilapia fingerlings of the present study was characterized by a predominance of lymphocytes (% mm⁻³) that ranged from 55.88 ± 0.62 to 59.35 ± 0.67 (Figure 2). Generally, four types of leukocytes, namely lymphocytes, monocytes, neutrophils, and eosinophils were identified in circulating blood of Nile tilapia fingerlings where the basophils were not counted. The lymphocytes are reported to be responsible for immune response producing antibodies and chemical substances serving as a defense against infection and an increase in its percentage in the blood may be a compensatory response of lymphoid tissues to the destruction of circulating lymphocytes (Singh and Tandon, 2009). The higher proportion of lymphocytes in the WBCs of Nile tilapia fingerlings confirmed the former works of Ighwela *et al.* (2012) and Osman *et al.* (2018). The authors have reported a dominance of lymphocytes in WBCs that ranged from 51.66 to 59.60 % mm⁻³ in Nile tilapia. Neutrophils, monocytes, and eosinophils are also important components of WBCs that play a key role in protecting the body through their phagocytic activities and inflammation against bacterial infection in damaged cells (Singh and Tandon, 2009). Their percentage generally decreases after exposure to pollutants and other toxic substances.

The percentage of monocytes and eosinophils in fish groups fed with all experimental diets were not significantly different ($P > 0.05$) as compared to the control diet, and the results were within a normal range for Nile tilapia. Similar findings have reported by several researchers, and monocytes comprise less than 10% of the total WBCs production in animals of all species (Kelly, 1979). Moreover, the present result agrees with Osman *et al.* (2018) who reported

percentage monocytes of juvenile Nile tilapia ranging from 4.78 ± 1.66 to 14.47 ± 4.06 . Generally, the results obtained in the present study revealed that the replacement of fish meal protein at levels of up to 75 % by LGM proteins in the diets of Nile tilapia fingerlings caused no adverse effects on the growth performance, feed utilization, body carcass composition, and hematology. Meanwhile, the statistical analysis also indicated that replacing fish meal with LGM up to 75% levels of dietary protein is promising for exemplified an increased growth performance, improved feed utilization efficiency, and enhanced overall condition of Nile tilapia fingerlings.

5.4. Cost Analysis

As stated in Table 11, it is cost-effective to culture Nile tilapia fingerlings with LGM75 diet which contained 75% replaced fish meal by LGM meal as a protein source with IC (44.31). However, the control diet, which consists of the highest proportion of fish meal as a source of protein had the highest IC (72.5), implying that it is more costly to use fish meal as a dietary protein source in formulating fish feeds. The less IC, the higher the PI with using LGM75 meal resulted in the highest PI (1.58) followed by diets LGM50 (1.30), LGM25 (1.11), and the lowest PI recorded from the control diet (0.96). These results follow the reports of Hossain *et al.* (2018) where sunflower cake meal used as a protein source in the diets of Nile tilapia and the diet prepared from sunflower meal was the less costly with IC (45.45) as compared to the fish meal based formulated control diet observed to have (64.83) incidence cost.

The economic analysis of this study revealed that there is a promising potential for higher economic returns when LGM diets are used in the diets of Nile tilapia to replace 75% fish meal inclusions in the fish feeds. The study also suggested that replacing fish meal by LGM diets and reducing the inclusion levels of fish meal in fish feeds could cause increased economic return. It is also evident that fish meal is not only expensive, but it is also becoming less available in the market globally (El-Saidy and Gaber, 2003).

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

In Ethiopia, fishery products exclusively come from inland water bodies and the fishery is mainly both artisanal and subsistence, though the country has huge aquaculture potentials. However, the contribution of aquaculture is far below its potential due to different problems including lack of quality feeds. Searching and formulating quality feeds using locally available low-cost feed ingredients that can be used as protein sources in fish diets is promising for developing aquaculture in Ethiopia. This experiment was conducted to evaluate the effectiveness of a mixture of lupin and grass pea meal (LGM) as a potential feed ingredient in the diets of Nile tilapia fingerlings. These feed ingredients were selected based on their local availability, abundance, higher nutritional composition and potential cost-effectiveness.

- ❖ The results of the current experiment demonstrated that Nile tilapia diets formulated with variable levels of LGM meal as a replacement for a more expensive fish meal protein had no discernable negative effects on all evaluated growth performance and feed utilization parameters and no feed-related mortality was observed. There were no significant differences in growth performance in response to the experimental diets. This might be due to a compensatory-effect of both grains which could lead to reducing ANFs and improved palatability and amino acid profile because of mixing feed ingredients.
- ❖ Evaluation of nutritional composition of LGM to replace fish meal at inclusion levels of 0, 25, 50, and 75% in the diets of Nile tilapia fingerlings indicated that it is possible to be used up to 75% with no adverse effect on the whole-body proximate composition.
- ❖ The results of the present study also suggested that replacing fish meal at inclusion levels of 0, 25, 50, and 75% with LGM meal diet as a protein source had no adverse effect on the HSI and hematological parameters of Nile tilapia fingerlings. However, an increasing tendency of HSI in the increased replacement of fish meal at LGM50 and LGM75 was recorded, and interestingly enhanced final body weight and improved feed utilization efficiency of tilapia fingerlings fed on LGM25 was obtained. This increased HSI might have been associated with higher FI and the high energy content in the feed that resulted in increased storage of glycogen or lipid in the liver. Besides, the recorded comparable and promising results of hematological parameters might have been because of the

improved amino acid profile of the diets due to the effect of mixing feed ingredients, reduced ANFs in the experimental diets due to preprocessing of LGM ingredients by soaking, dehulling, and roasting, and mixing plant proteins may also lead to interactions between various ANFs or with other components resulting in the reduction of their deleterious effects.

- ❖ Due to the low price of LGM and uncompromised growth performance trends, all experimental diets (LGM25, LGM50, and LGM75) generated economic returns higher than the 100% fish meal-based diet in ascending order, respectively. The highest PI (1.58) and lowest IC (44.31) was recorded from LGM75, whereas, the lowest PI (0.96) and highest IC (72.5) were found from the control diet.

6.2. Recommendations

- This study provided information on the suitability potentials of LGM meal in the diets of Nile tilapia fingerlings. Further research is recommended to see the effect of complete replacement of the expensive and less available fish meal protein in the diets of Nile tilapia on the digestibility, supplement of prebiotic, immune response, intestinal histology, and stress-related factors may also be the focus for further succeeding studies and assure its best potential as a replacement for fish meal.
- Since the experimental diets used in the present study were formulated and prepared based on the proximate composition of feed ingredients, studies focused on evaluating Nile tilapia diets formulated based on the amino acid and fatty acid profiles of mixtures of lupin and grass pea meal are recommended.
- There are different methods of reducing the ANFs found in lupin and grass pea grains. Among them, physical techniques such as soaking, dehulling, roasting, and air separation are economical and commonly used techniques. Similarly, biological and chemical methods like germination, fermentation, and genetic-based improvements are also used. However, no techniques efficiently remove the ANFs to the levels where the diets could be used without any effect. Therefore, exploring sophisticated methods that reduce ANFs maximize the nutritional profile and increased bioavailability of nutrients may further step-forward the success of complete fish meal replacement with LGM diets.

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