EFFECT OF FEED SUPPLEMENTATION WITH SEAWEED AND USE OF NOVEL MEMBRANE BIOREACTOR WASTEWATER TREATMENT METHOD ON THE QUALITY OF FARMED NILE TILAPIA

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JOMO KENYATTA UNIVERSITY
OF
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2024

Effect of Feed Supplementation with Seaweed and Use of Novel Membrane Bioreactor Wastewater Treatment Method on the Quality of Farmed Nile Tilapia

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Science and Nutrition of the Jomo Kenyatta University of Agriculture and Technology

2024

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as the University Supervisors

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DEDICATION

I dedicate this thesis to God Almighty for giving me the strength to do this work. To my brother Kinyua R. for the encouragement and guidance and to my late parents Mr. and Mrs. Daniel and Gladys Kubai for empowering me for life. To my entire family for all their support and prayers.

ACKNOWLEDGMENT

I thank the Lord Almighty for guiding me through my studies and His faithfulness for this far he has enabled me. I am grateful to DAAD and RUFORUM for funding my PhD studies and my research work, without their support this work would not have been possible.

This research was possible with the effort of many dedicated experts and professionals. With heartfelt gratitude I would like to acknowledge and express my sincere appreciations to my supervisors: Dr. Michael Wawire and Dr. Peter Kahenya for their guidance, support, suggestions and comments during the study. I would also like to thank the Department of Agriculture Livestock and Fisheries, Kisumu East specifically the fisheries officers Mr. E. Oyoo and Mrs S. Adhiambo; the ViclnAqua site staff Vincent, Gilbert and Peter for their help at the project site; Many thanks to Kenya Marine and Fisheries Research Institute (KEMFRI), Sangoro for their technical input and in the sourcing of the vitamin and mineral premixes; staff at the Kisumu Water and Sanitation Company (KIWASCO), Nyalenda ponds site led by Odhiambo; Wahome and Wanja from JKUAT for helping in the mathematical modelling . The support and technical assistant of Mr. D. Abuga, Ms. J. Oruka, Mr. Votha and Mr. Kamathii in the Food Biochemistry Laboratory (JKUAT) is greatly appreciated. To my brother Kinyua, family and friends who asked “How is the PhD going” and offered words of encouragement and prayers.

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ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ALA	Alpha Linolenic Acid
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ARA	Arachidonic Acid
BOD	Biological Oxygen Demand
CF	Condition Factor
CHD	Coronary Heart Disease
COD	Chemical Oxygen Demand
DHA	Docosahexaenoic Acid
DO	Dissolved Oxygen
DWB	Dry Weight Basis
EPA	Eicosapentaenoic Acid
FAME	Fatty acid Methyl Esters
FAO	Food and Agriculture Organization
GC	Gas Chromatograph
GDP	Gross Domestic Product
FCR	Feed Conversion Ratio

HUFA	Highly Unsaturated Fatty Acids
IMTA	Integrated Multi-Trophic Aquaculture
ISO	International Organization for Standardization
KNBS	Kenya National Bureau of Statistics
LA	Linolenic Acid
LBDA	Lake Basin Development Authority
LC-PUFA	Long Chain-Polyunsaturated Fatty Acid
LG	Length Gain
LSD	Least Significant Difference
LVBC	Lake Victoria Basin Commission
MBR	Membrane Bioreactor
MP	Maturation Pond
MUFA	Monounsaturated Fatty Acid
MWCO	Molecular Weight Cut-off
NARDTC	National Aquaculture Research Development & Training Center
ND	Not Detected
NEMA	National Environment Management Authority
NFE	Nitrogen Free Extract
NSP	Non-Starch Polysaccharides

PES	Polyethersulfone
PUFA	Polyunsaturated Fatty Acids
RMSE	Root Mean Square Error
SDA	Stearidonic Acid
SDG	Sustainable Development Goals
SE	Standard Error
SFA	Saturated Fatty Acid
SGR	Specific Growth Rate
SR	Survival Rate
TVC	Total Viable Count
TW	Tap Water
WG	Weight Gain
WHO	World Health Organization

ABSTRACT

The Kenyan aquaculture sector has a great potential of expansion and with a growing population, it can play an important role towards achievement of food and nutrition security and promoting sustainable aquaculture production in Kenya. There has been an increase in the demand for fish and fish products given the fact that fish is a rich source of protein and essential fatty acids. With the growth in aquaculture, there has been an increase in the need for quality feeds to produce farmed fish of high nutritional value and also access to adequate water resource due to competition from other water uses. In order to achieve improved output from fish farming, a seaweed-based fish feed was formulated and municipal wastewater was treated using the self-cleaning membrane bioreactor (MBR) and used in rearing the Nile tilapia (*Oreochromis niloticus*). The brown seaweed *Sargassum portieranum* was identified, based on literature review, and used in supplementation of tilapia fish feeds to improve the fatty acid profile of the fish. For the feed supplementation, three experimental diets were prepared at 0% (control), 5% and 10% (dry weight basis) inclusion levels of the seaweed. One hundred and eighty Nile tilapia were randomly distributed into three groups in triplicate and fed the experimental diets for 12 weeks. In a separate experiment, to assess the effect of water quality on fish growth and muscle quality, three water treatments were used; a) MBR treated water, b) maturation pond water and c) tap water (control). Weight and length gain were measured after every two weeks throughout the experimental period. The biochemical composition was determined using standard methods of the Association of Official Analytical Chemists (AOAC) while all microbial safety analysis was done according to standardized ISO methods. The concentration of minerals and heavy metals in the water and fish muscles were analyzed using atomic absorption spectroscopy (AAS) while the fatty acid profile was determined using Gas chromatography–mass spectrometry (GC-MS). In addition, the physicochemical properties (pH, dissolved oxygen, conductivity, biological oxygen demand, chemical oxygen demand, nitrates and ammonia) of the water were determined. Analysis of variance (ANOVA) was used to establish significant difference in the data using R, version 4.0.2 Software. Based on the review on the nutritional properties of seaweeds, the brown seaweed species was identified to contain the highest content of total lipids compared to the red and green seaweed species. After feeding the tilapia fish for 12 weeks, the final body weight differed significantly ($P < 0.05$) between the fish that was fed on seaweed diet supplemented at 10% (66.12 ± 2.24 g) and un-supplemented diet (59.19 ± 1.03 g). The weight gain and length gain in the fish that was supplemented with seaweed at 5% and 10%, ranged between 28.08 to 35.00 g and 3.13-3.87 cm respectively. The highest total lipid content was in the fish diet that was supplemented at 10% (0.93%). The fatty acids palmitic acid (20.33-21.91%), linoleic acid (24.65-37.34%) and docosahexaenoic acid (19.51-26.16%) were the predominant saturated, omega-6 and omega-3 fatty acids respectively in the fish muscle. Although the biological oxygen demand, chemical oxygen demand, pH and nitrates values of the maturation pond water were significantly higher than those of the MBR water, both water qualities met the recommended standards for water for use in aquaculture as set by Food and Agriculture Organization (FAO). However, the heavy metal content (Cu, Pb and Cr) in the maturation pond and MBR water exceeded the permissible levels for discharge in the environment as per the National Environment Management Authority (NEMA) standards (fifth schedule for effluent discharge in the environment). Besides, the survival rate of the fish was significantly affected (P = 0.001) by the water quality; with the control at 95.56%, MBR, 86.67% and maturation pond, 76.67%. Likewise, the crude protein, fibre and lipid varied significantly in the three treatments. The heavy metal content in the fish reared in the maturation pond and MBR were above the safe levels for human consumption. The findings of this study also demonstrated that apart from the water used in rearing fish, feeds can also be a source of contaminants in fish. In conclusion, the present study revealed that the brown seaweed has the potential to improve the fatty acid profile of fish as an alternative supplemental lipid source. At low inclusion levels, the brown seaweed is beneficial to fish growth and overall nutritional quality of final product. In addition, the present study provided evidence that MBR wastewater is a promising technology in treating wastewater for alternative uses. It effectively treats to meet most of the standards for water used in aquaculture. It is recommended that, an addition step like reverse osmosis be added to upgrade the water quality to remove heavy metals.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Global fish production has increased over the years with aquaculture boosting the production. Wild capture production was relatively static in the 1980's but increased in the subsequent years (FAO, 2020). This growth in aquaculture is attributed to advances in technologies in fish production like hybridization, genetic engineering, formulated diets, and biofloc technology that is used in ponds, cages, tanks, and recirculation systems (FAO, 2014). Total global fisheries and aquaculture production (aquatic animals and algae) in 2020 was 214 million tonnes, of which 122.6 million tonnes was from aquaculture. Production of aquatic animals from aquaculture in the same year amounted to 87.5 million tonnes (FAO, 2022a). Asia, led by top producers such as China and India, was the leading aquaculture producer in 2020, accounting for more than 90% of the total production.

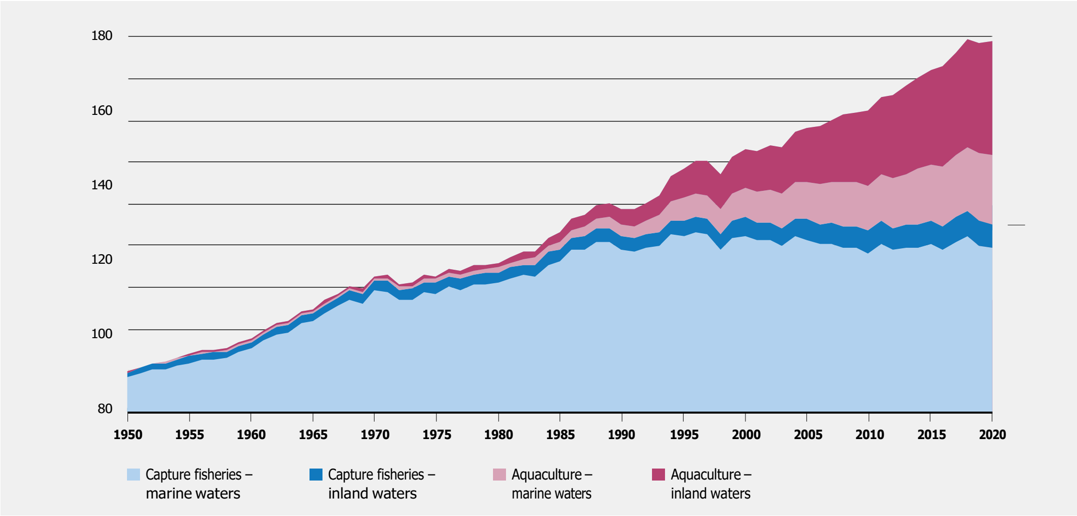

Figure 1.1: World Capture Fisheries and Aquaculture Production from 1950-2020

Source: (FAO, 2022a)

The fisheries and aquaculture sector in Kenya had a total production of 142, 833 tonnes in 2020 with 83.3 percent from inland capture fisheries. Lake Victoria accounted for 90 percent of the total inland captures (FAO, 2022b; KNBS, 2022). This production contributed to about 0.8 percent to the country's GDP (KNBS, 2022). Natural inland water bodies like Lake Victoria, which are the main sources of fish, have had many challenges over the years. These include dwindling catches due to overfishing, illegal, unreported and unregulated fishing, pollution and reduction of water levels that compromise fisheries (LVBC, 2014). In addition, environmental pollution, ecosystem degradation and climate change have also contributed to decline in capture fisheries (FAO, 2018; Ogello *et al*., 2013). With escalating human population growth to unsustainable levels and increasing food insecurity and dwindling capture fisheries, aquaculture is therefore the best option to bridge the gap of supply and demand for fish (FAO, (2016). The current supply of fish from aquaculture is about 20,000 tonnes and needs to reach 150, 000 tonnes to meet the growing demand for fish, that is driven by population growth and increasing incomes, increased awareness of the health benefit of fish consumption and changes in consumer preferences and taste (Obiero *et al*., 2019; Munguti *et al*., 2021).

There has been a noted improvement in aquaculture in Kenya after the introduction of the Economic Stimulus Program in the year 2009 by the government. The aim of the program was to improve nutrition, create over 120,000 employment opportunities and income generation and increase production of farmed fish from 4000 metric tonnes to over 20,000 metric tonnes in the short term and over 100,000 metric tonnes in the long term (Charo-Karisa & Gichuri, 2010). Implementation of the program led to an increase in fish production from about 962 metric tonnes in 2002 to 19,584 metric tonnes in 2011 (Munguti *et al.*, 2014). Kenya has great potential for fish farming due to favorable climatic conditions, geographic location, and a well distributed network of rivers, streams, dams, and wetlands. Export markets for aquaculture products such as the European Union also opened up to Kenya in 2016. However, of the 1.4 million ha potential aquaculture sites, only 0.014% is being exploited (Munguti *et al.*, 2014).

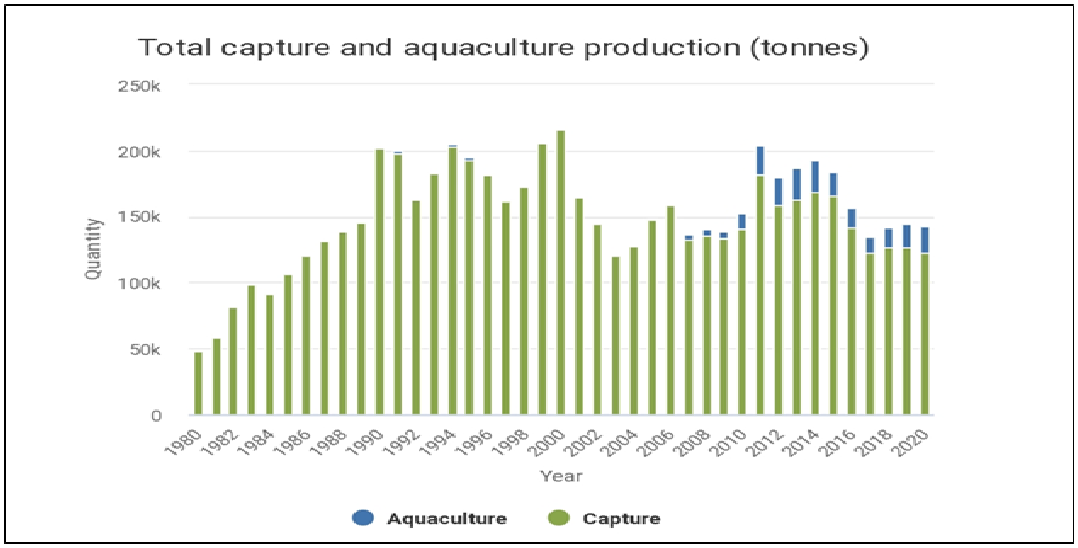

Figure 1.2: Total Fishery Production in Kenya (1980-2020)

Source: (FAO, 2022b)

Fish feed is a key component in fish rearing and providing farmers with well-balanced feeds at cost effective prices is crucial for profitable production. The feeding needs of fish are specific to the fish species and stages in life (Rana & Hasan, 2013). Improving the quality and preparation of these feeds can therefore greatly improve productivity. Water quality is also an important factor in fish farming since it determines the quality of the fish products, the health and growth of the fish. Water properties that are considered important for fish rearing include; temperature, oxygen concentration, pH, salinity and hardness. Different fish species require specific water quality for survival, growth and reproduction (Ng & Romano, 2013).

Seaweeds (macroalgae), are marine plants that are important for both human and animal nutrition. They are broadly classified as green (Chlorophyta), red (Rhodophyta) and brown (Phaeophyta) based on their surface pigmentation. Globally, the estimated number of species is about 12 000 (Guiry & Guiry, 2022). Of these, only 34 species are intensively farmed (FAO, 2013). Over the years, seaweeds have been used as fodder for animals like sheep, cattle and pigs to enhance growth and stimulate uptake of feed (Rajauria, 2015).

However, the practical use as feed ingredient in aquafeed has not been widely studied. Decline in fish meal and oil for aquafeed production has necessitated the need for other alternative feed ingredients to supply the lipids and protein in the feed. Seaweed is a great alternative as they are rich in minerals, essential fatty acids, protein and fibre with trace amounts of vitamins (MacArtain *et al*., 2007; Radulovich *et al.,* 2015). In addition, seaweeds are available throughout the year and are easy to harvest (Rajapakse & Kim, 2011). Furthermore, seaweeds are already in use as a natural food to fish in the wild (Mouritsen, 2013). With these promising characteristics, seaweeds may be harnessed to promote fish growth and sustainability of aquaculture.

Fish is an important source of animal protein in human diet and it provides valuable nutrients such as the long-chain omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (FAO & WHO, 2011). Fish is also a good source of micronutrients especially when consumed whole (small fish); with heads and bones, such as iodine, selenium, zinc, iron, calcium, phosphorus, potassium, and vitamins such as A, D and B (Roos *et al.,* 2007). The Omega-3 fatty acids are important in promoting the development of the nervous system and brain in foetus and infants and also reduce the risk of cardiovascular diseases (Bonham *et al.*, 2009, Obiero *et al*, 2019).

1.2 Problem Statement

Fish can be sourced from wild marine, freshwater stocks or produced through fish farming/aquaculture activity. However, there are claims that farmed fish is less nutritious (nutritional quality of fillet and other fish products) than wild-caught fish. At times, claims are made regarding the feed, quality of water, or the alleged misuse of veterinary drugs (Little *et al*., 2012). Essential fatty acids such as EPA and DHA in farmed fish come from fish oils in the diet; and in wild, they come from the naturally occurring algae they feed on (Toppe, 2012; 2013). The aquaculture sector currently consumes about 75 percent of global fish-oil production as feed ingredients. This percentage is declining owing to the increasing demand for fish oil for supplements and other food purposes, but there are no good alternative sources of EPA and DHA for feeding cultured fish at present (FAO, 2014). There is also the need to establish the qualitative and quantitative relationship between the impact of supplementation and farm- made feeds on the nutrient cycling and retention in the farmed fish. Developing a better understanding of these dynamics is important in optimizing aquaculture and improving nutrition security. The need to feed a growing global population, and to address a growing demand for fish, puts pressure on natural resources and challenges the sustainability of marine and inland fisheries (HLPE, 2014). Availability of water is a factor constraining aquaculture growth in Kenya due to competition from other uses and climate change (Munguti *et al*., 2014). There is need to produce good quality fish, fit for human consumption, and in adequate quantities, sustainably. To this end, development of a low-cost technology to treat household waste water that can be used in a recirculating aquaculture system, can go a long way in meeting these needs.

1.3 Justification

Aquaculture is increasing challenged by demand to be more sustainable. Its therefore important for all inputs involved in production such as feed and water to be also sustainable (Aarset *et al*., 2020). Therefore, there is need for these resources to be better integrated in order to have novel solutions that meet the demand for fish and fish product without depleting the natural resources. Aquaculture is a diverse activity globally and its growth has impact on food nutrition and human well-being (Gephart *et al*., 2020). The demand for fish and fish products has increased with an increasing human population worldwide. This growing demand for fish can be mainly met by increased production from aquaculture since capture fisheries production has levelled off (FAO, 2014). Aquaculture is also one way of meeting the sustainable development goal 2 (SDG 2), “End hunger, achieve food security and improved nutrition and promote sustainable agriculture”. Farmed fish quality and nutritional value can be monitored and controlled in a farming system. By controlling the composition of aquaculture feeds, water and other inputs, healthy fish and fish products with optimal nutritional composition can be produced (Rana & Hasan, 2013). Therefore, aquaculture products can constitute a larger share of the market in future. In addition, consumption of fish is important as it is a source of the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA) that are required for optimal brain and neural system development in children and lowers the risk of coronary heart disease (CHD) mortality.

1.4 Objectives

1.4.1 Main Objective

To evaluate the effect of dietary seaweed supplementation and using wastewater treated with a membrane bio-reactor on tilapia fish rearing

1.4.2 Specific Objective

1. To profile the nutritional properties of seaweed species in Kenya based on literature.
2. To determine the nutritional and physical properties of *Sargassum portieranum*-based feeds and assess their influence on growth performance and nutritional quality of farmed tilapia.
3. To evaluate the effectiveness of treating waste water using the membrane bio-reactor and the impact of the treated waste water on the biochemical composition and microbial safety of Nile tilapia
4. To develop models for Nile tilapia weight prediction using empirical knowledge

1.5 Hypothesis

1. There is no difference in the nutritional properties of seaweed species in Kenya
2. The nutritional properties and physical characteristics of *Sargassum portieranum*-based fish feed diets have no effect on the growth performance and nutritional quality of tilapia.
3. Use of membrane bio-reactor in wastewater treatment has no effect on the biochemical composition and microbial safety of tilapia.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fish Production

2.1.1 Trends in Fish Production

2.1.1.1 World Fish Production

The global fish production in 2022 was 177.8 million tonnes. The total fish capture production in 2020 was 90.3 million tonnes, with 78.8 million tonnes from marine waters and 11.5 million tonnes from inland waters. In the same year, fish harvested from aquaculture amounted to 87.5 million tonnes, which was 49.2% of total fish production (FAO, 2022a). During the past two decades, global aquaculture has increased significantly and widely. This was marked by an increase from 52.5 million tonnes in 2008 to 82.5 million tonnes in 2018, representing a 36.3% increase. Aquaculture, which is the cultivation of aquatic organisms in controlled aquatic environment, is growing rapidly in the world. This is attributed to the increase in domestication of new aquatic species and species improvements by hybridization, chromosome manipulation and traditional breeding (FAO, 2014).

China is the main fish producer and largest exporter of fish and fishery products accounting for 35% of total fish produced, globally. India comes in second followed by Indonesia (FAO, 2022b). Almost all fish produced from aquaculture are destined for human consumption, although by-products may be used for non-food purposes. About 580 species and/or species groups are farmed around the world. They include finfishes (including hybrids), frogs and reptiles, mollusks, crustaceans, aquatic invertebrates, and aquatic plants. Global food fish (finfish, crustaceans, mollusks and other aquatic invertebrates excluding aquatic mammals and aquatic plants) supply has grown steadily in the last two decades, at an average rate of 3.2 % outpacing the world population growth at 1.6%. Global annual per capita consumption of fish from 17.0 kg in the 2000s to 19.6

kg in the 2010s, with a record high of 20.5 kg in 2019 (FAO, 2022a). A huge difference exists in fish consumption per capita among countries. China is the leading consumer leading with 40.1 kg per capita consumption in 2019.

2.1.1.2 Fish Production in Kenya

Kenya is well endowed with numerous aquatic resources with aquaculture potential. It has highly varied climatic and geographic regions, covering part of the Indian Ocean coastline, a portion of the largest freshwater lake in Africa (Lake Victoria), lake Turkana, lake Naivasha and several large rivers, swamps, and other wetlands. These aquatic environments range from marine and brackish waters to cold and warm fresh waters, and many can sustainably contribute to the operation of ponds for fish production. Despite this enormous potential for aquaculture in Kenya, the sector has been characterized by low levels of production that have stagnated over the last decade due to various challenges facing the industry (Obiero *et al*., 2019). The fisheries and aquaculture sector contribute to approximately 0.8 percent to the country’s GDP. Total fishery and aquaculture production in 2020 totaled to 142, 833 tonnes in, with 83.3 percent coming from inland capture fisheries and Lake Victoria contributing about 90 percent of the inland capture. In the same year, about 220,000 people derived their livelihood from fishing and fish farming and approximately 5.7% protein required was attained (KNBS, 2022). As a result of increased awareness on the health benefits of consumption of fish, the demand for fish has raised to about 500,000 tonnes annually (Munguti *et al*., 2021).

With an increasing population, and increasing food insecurity, Kenya’s dwindling capture fisheries are unable to adequately provide cheap protein for the growing population. Natural water bodies, which are the main sources of fish, have had many challenges that include dwindling catches, pollution and reduction of water levels that compromise fisheries (LVBC, 2014).

The development of fish farming is one of the core activities in the Ministry of Agriculture, Livestock and Fisheries because aquaculture has the potential to reduce fishing pressure on oceans, lakes and rivers and improve food security, create employment and wealth, and promote healthy living (Munguti *et al*., 2021). There are a variety of challenges and constraints facing Kenya in fully exploiting its potential in aquaculture. These including: inadequate and/or inappropriate legal and regulatory framework, reduced effectiveness of and inadequate capacity in extension services, inadequate infrastructure and facilities, low adoption of modern technology, limited capital and access to affordable credit, inadequate government funding, high cost and/or low quality of key inputs such as seed and feed, pre- and/or post-harvest losses and marketing infrastructure, insufficient water and increasing incidence of various diseases that result in loss of productive labor and human capital (GOK, 2010).

2.1.1.3 Development of Aquaculture in Kenya

In Kenya, fish farming development started in the 1920s with the arrival of European settlers through introduction of trout in rivers for sport fishing while fish culture (tilapia, common carp, and catfish) as a source of protein for rural indigenous population began in the late 1950s and early 1960s (Maar *et al.,* 1966; Ngugi *et al.,* 2007). Fish campaigns were also introduced by the government in the late 1960s to accelerate the interest in rural fish farming. Mariculture was introduced in the late 1970s with the establishment of the Ngomeni Prawn Pilot Project (Nyonje *et al*., 2011) but it is not fully exploited to date. This is mainly due to accessibility problems, conflicts over land ownership and lack of clear policies. Fish farming in Kenya picked up in 2009 with the introduction of the Economic Stimulus Program. Despite the enormous potential for fish farming in Kenya, aquaculture has been characterized by low levels of production that have stagnated at less than 1% of the country's protein needs over the past decade (Nyonje *et al.,* 2011).

The primary cultured fish species in Kenya today are Nile tilapia (75%) and African catfish (15%) and are mostly cultivated under intensive earthen pond (closed) systems (Fisheries annual statistical bulletin, 2016), but efforts to introduce other indigenous fish, such as *Labeo victorianus,* in aquaculture have not been widely adopted by farmers.

Mariculture remains under-developed, although there are research initiatives to promote seaweed culture, milkfish, and shrimp (Munguti *et al.*, 2014).

In a bid to promote and develop aquaculture, the Kenyan government has set up several aquaculture facilities to serve as research centers, training facilities, and sources of fingerlings and feed for fish farmers. They include the National Aquaculture Research Development & Training Center (NARDTC) in Sagana, Kisii fish farm training center, Kiganjo trout farm, Ndaragua trout farm, Chwele fish farm, Lake Basin Development Authority (LBDA) in Kisumu, Wakhungu fish farm in Busia, Sangoro research station, Kegati research station, and Kabonyo and Ngomeni fish farms (Charo-Karisa & Gichuri, 2010). However, most of these centers lack sufficient basic laboratory equipment and human capacities to spur significant aquaculture development in their respective spheres of influence. Recognizing aquaculture as one of the viable options for revamping the country's food sector, the Kenyan government initiated intensive aquaculture through the Economic Stimulus Program in 2009 to stimulate economic development, improve the food security, foster economic recovery, alleviate poverty, and spur regional development. The Kenyan aquaculture industry growth had been slow for decades until the year 2009 when the government-funded Economic Stimulus Program increased fish farming nationwide (ESP, 2009; Munguti *et al*., 2014)

To achieve the goals set in the stimulus programme, 200 fish ponds were constructed in each of the selected constituencies (Western Kenya, Nyanza, parts of Rift Valley, Eastern, Central Kenya and Coast regions) at an estimated cost of Kshs. 8 million per constituency (Munguti *et al.*, 2014). The State Department of fisheries also trained fish farmers, implementing officers and stakeholders on fish farming practices, conducted a national aquaculture suitability appraisal, developed fish breeding structures with a holding capacity of over 200,000 brood-stock, developed fish feed specifications for tilapia, catfish and trout and related supply chain (Fisheries annual statistical bulletin, 2016). The implementation of this program led to an increase in fish production from about 962 metric tons in the year 2002 to over 19,584 metric tons in the year 2011. Food security improved and poverty levels reduced and there was also an increase in commercialization of Nile tilapia, (*O. niloticus*) and the African catfish (*C. gariepinus*) (Obiero *et al*., 2019).

2.1.2 Tilapia Production

2.1.2.1 Description/Scientific Classification

Globally, tilapia is the second most farmed fish after carp due to its many characteristics such as tolerance to crowding, relative ease of captive spawning year-round, high resistance to disease, high marketability, success with polyculture and ability to accept low-cost diets from terrestrial based ingredients (Nelson, 2004; Ng & Romano, 2013). World production of tilapia exceeded 6 million Tonnes in 2014 with 88% being from aquaculture up from 79% in 2008 (FAO, 2016). Tilapia is a freshwater cichlid native to Africa, with about hundred identified species. They were later introduced to the rest of the world during the second half of the twentieth century either deliberately or accidentally (Beveridge & McAndrew, 2000; Eknath & Hulata, 2009). There are relatively few commercially important tilapias and they are divided based on their reproduction characteristics into three major taxonomic groups: Tilapia *spp*; guard the developing eggs and fry in the nests (substrate spawners); Oreochromis *spp;* female incubate eggs and fry orally (maternal mouthbrooders) and Sarotherodon *spp;* male and/or females incubate eggs and fry orally (maternal/paternal mouth brooders). (Nelson, 2004; Trewavas, 1983). Ten commonly cultured commercial tilapia include; Nile tilapia (*Oreochromis niloticus*), Mozambique tilapia (*O. mossambica*), Blue tilapia (*O. aureus*), Mango tilapia (*Sarotherodon galilaeus),* Blackchin tilapia (*S. melanotheron*), Longfin tilapia (*O. macrochir*), Redbelly tilapia (*Tilapia zilli*), Redbreast tilapia (*Tilapia rendalli*), Sabaki tilapia (*O. spirulus)* and the spotted tilapia (*O. andersonii*).

Nile tilapia (*Oreochromis niloticus)* is one of the most important tilapia species in aquaculture. It is commonly preferred due to its fast-growing rate (grow to length of 60 cm and can weigh of 3.6 kg), adaptability and tolerance to various culture conditions (resistance to poor water quality and disease) and high consumer acceptability (Shelton, 2002). Tilapia can grow from fingerling to market size in three months and due to fast reproduction, they reach sexual maturity before reaching marketable size. Females then start spawning asynchronously and very frequently, which can lead to overpopulation and stunting. Use of genetic manipulation to improve productivity of tilapia is common practice especially hybridization of O. *niloticus* × O. *aureus* to yield mostly male population that are capable of growing faster than non-hybrid counterparts and in the process also control widespread reproduction within the ponds (Ng & Romano, 2013).

2.1.2.2 Fish Feeds and Feeding Management

With the growth in aquaculture, there has been an expansion in feed production. In addition, a large portion of the total production cost in tilapia farming is mainly on feeds (Ng *et al.*, 2013) and therefore understanding the feeding and nutrition management is important in ensuring efficiency. Most fish farmers in Kenya rely primarily on farm-made feeds, as commercial (pelleted) feeds are too expensive (Munguti *et al.*, 2021). Most of the farm-made feeds in Kenya use oilseed cakes (cotton, soybean or sunflower), freshwater shrimp and/or fishmeal as protein sources; rice, corn and wheat bran as energy sources, kitchen wastes and/or vegetables. The feed ingredients are mixed at predetermined ratios by hand or with the aid of mechanical mixers. The resulting feed dough is processed by a simple device or a pelletizing machine that makes moist strands that are dried and broken up into suitable pellet sizes (Munguti *et al.*, 2006).

Commercial feeds are prepared either complete or supplemental. Complete diets feeds supply all the required nutrients (protein (18-50%), lipids (10-25%), carbohydrates (15-20%), ash (<8.5 %), phosphorus (<1.5%), water (<10%) and trace amounts of vitamins for optimal growth. Commercial tilapia feeds are commonly grouped as pre-starter, starter, grower or finisher feeds. Supplemental or incomplete feeds are intended for supporting the natural food (insects, algae, small fish) and do not contain a full complement of vitamins or minerals. They are also used to fortify the naturally available diet with extra protein, carbohydrates or lipids (Jauncey, 2000; Bhujel *et al.*, 2001). Fishmeal is commonly used as the major component of commercial feed formulations. This is because it’s a rich source of quality protein, polyunsaturated fatty acids (PUFA), vitamins, minerals and attractants (Naylor *et al.,* 2009). PUFAs, which include docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) are important in the development of structure, neural visual systems and the function of cell membrane that is key for the growth, survival and stress resistance in fish (Tocher, 2010; Rajkumar, 2006).

The size of the fish is greatly influenced by feeding practices including feeding frequencies and rates. Based on the size, fish maturity can be classified as yolk sac larvae (3 days), weaning larval (40 days), fry (0.1-2.0 g), fingerlings (10-20 g) and broodstock or grow-out (300 g-1 kg). The yolk sac larvae depend on their egg yolk sac for nutrition for the initial three days after which weaning on artificial diets is started. Weaning larva and fry (0.1-2.0 g) need to be fed a high protein diet frequently and usually in excess because they have relatively fast growth rates. A high protein diet of between 40–50%, with frequencies of six times per day at 30–45% body weight or to satiation is recommended (Creswell, 2005; Ng *et al.,* 2013). The fry are often fed with crumble, while the fingerlings and throughout the grow-out period, various feed types such as sinking pellets, moist pellets and extruded feeds are used.

Broodfish require lower feeding rates and frequencies of about three times per day. Using a lower feeding rate for broodfish provides a variety of benefits including lower feeding costs and minimizing nitrogenous waste production, which is particularly beneficial to reduce fouling in hapa systems used for tilapia breeding (El-Sayed *et al*., 2013). Nutritional status of broodfish affects the offspring quality. In case of excess feeding, the growth rate of broodstock is higher and this may be undesirable since fish over 250 g are sometimes discarded due to the belief that they are more susceptible to disease and sub-optimal environmental conditions in addition to having higher feed intake rates, and more difficult to handle when removing eggs for subsequent incubation (Matsiko *et al*., 2010).

2.1.2.3 Fish Nutrient Requirements and Supplementation

Ensuring good nutrition in fish farming is important in production of quality fish for human consumption. It is therefore important to have feeds that are balanced to promote optimal fish growth and health. There many factors that affect the specific nutrient requirement of fish. They include the species, sex, intake of feed, presence of toxins in the diet, nutrient interactions and balance, expected level of performance, digestibility, environmental factors and desired carcass composition (Jauncey, 2000). Cultured/farmed tilapia has different nutrient requirement in their diet depending on their life stages. Early juvenile fish (0.02-10.0 g) require a diet higher in protein, lipids, vitamins and minerals and lower in carbohydrates. Sub-adult fish (10-25 g) require more energy from carbohydrates and lipids for metabolism and a lower proportion of protein for growth. Adult fish (above 25 g) require less dietary protein for growth and utilizes higher levels of carbohydrates (Abdel-Tawwab *et al.,* 2010; National Research Council, 2011; Ng & Romano, 2013).

Protein requirement for tilapia is influenced by factors such as the species, digestibility and amino acid profile of the source and life stage status. For optimal performance, fingerlings and the grow out stages require about 20-30% while the fry and spawning females require between 30-40% (Sweilum *et al*., 2005; Abdel-Tawwab *et al.,* 2010). There are ten essential amino acids required for tilapia nutrition and they include lysine, methionine, histidine, tryptophan, arginine, phenylalanine, leucine, valine, isoleucine and threonine (Furuya *et al.,* 2004; Nguyen & Davis, 2009). Cysteine and tyrosine are considered semi-essential. Fishmeal is considered the best protein source due to its high digestibility, complete amino acid profile and residual lipids content with beneficial fatty acids. Alternative sources of protein are being used due to the rising cost and decreasing availability of fishmeal. Most of these alternative sources are deficient in one or more limiting amino acids and therefore supplementation with synthetic amino acids is necessary to achieve optimal growth (Ng *et al.,* 2013; Gaye-Siessegger *et al.,* 2007).

Lipids in fish diet are important as they play a role as component of the cellular membranes, precursors to hormones and aid in absorption of lipid soluble vitamins (Ng & Chong, 2004). Including appropriate levels of lipids in foods helps in preventing stunted growth or excessively fatty fish in case of deficiencies or excesses respectively. They are used as a concentrated and highly digestible source of energy. An increase in lipid content in the diet is key in ensuring that protein is not broken down (sparing protein) as an energy source (Gao *et al.,* 2011). For hybrid tilapias the optimal levels are about 12% of dietary lipid (Hajizadeh *et al.,* 2008). In general, fish do not have specific requirement for carbohydrates. Carbohydrates in the feeds are used as a cheaper source of energy, sparing proteins and hence promoting growth (El-Sayed, 2006). They also help in improving the pellet binding properties. Based on growth performance and feeding efficiencies of tilapia, complex sugars like starch and disaccharides are utilized more effectively than glucose (Hsieh & Shiau, 2000; Lin *et al.*, 2000). Complexity of the starch and presence of intestinal bacterial determines the ability of tilapia to digest and utilize the starch feed (Leenhouwers *et al.,* 2007). The Nile Tilapia is capable of fermenting all carbohydrates and this ability helps in increasing energy production and creates an environment unfriendly to pathogens (Leenhouwers *et al.,* 2008). Vitamins are necessary for optimal growth and health of tilapia in intensive culture systems due to limited natural foods. Tilapia is able to absorb minerals from the culture water. Despite the ability to absorb the minerals and the presence of minerals in the feed ingredients, tilapia feeds should contain supplemental mineral pre-mixes to prevent deficiencies that can arise from reduced bioavailability. The mineral added to the diet is dependent on the source of the element (Ng & Romano, 2013).

2.1.2.4 Fish Growth Conditions

Tilapia are more tolerant to harsh environmental factors such as low dissolved oxygen, high water temperature, salinity and high ammonia concentration but limited by sensitivity to low water temperature. Water temperature of between 29 ^{o}C–31 ^{o}C provides a conducive environment for optimal growth. Stress induced disease and mortality are experienced when the temperature exceeds 37 ^{o}C while at temperatures below 17 ^{o}C, resulting in stress induced trauma, feeding ceases and reproduction hindered. Tilapia tolerates a diverse range of salinities with no adverse effects on growth (Khan *et al*., 2017).

2.1.3 Fish and Fish Products

Fish is normally distributed in the live form or processed into various products that are destined for food or non-food uses. As a food, fish can be processed into a wide array of products such as chilled, frozen, heat-treated, fermented, dried, smoked, salted, pickled, boiled, fried, freeze-dried, minced, powdered or canned, or as a combination of two or more of these forms (Sampels, 2015). Fish by-products serve a wide range of purposes. Heads, frames and fillet cut-offs are used directly as food or turned into products for human consumption such as fish sausages, sauces, gelatin and cakes. Small fish bones, with a minimum amount of meat, are also consumed as snacks. Other by-products are used in the production of feed, fertilizers, biodiesel/biogas, pharmaceuticals, natural pigments, dietetic products (chitosan), cosmetics (collagen) and in other industrial processes. Fish viscera and frames are a source of protein hydrolysate, which is a potential source of bioactive peptides that is used in the pet-food and fish feed industries (Kim & Mendis, 2006; Olsen *et al.,* 2014). Internal organs of fish are an excellent source of specialized enzymes such as pepsin, trypsin, chymotrypsin and collagenases as well as lipase enzymes. Fish bones are an excellent source of calcium and other minerals such as phosphorus that can be used in food, feed or as supplements (Kim & Mendis, 2006).

2.1.4 Fish Handling and Storage

Fish is highly perishable and it can degrade more rapidly under the ambient conditions of the tropics leading to high post-harvest losses, either in quantity or quality. The post-harvest losses occur from handling during transport, storage and processing. This is attributed to poor handling infrastructure, lack of proper cold storage facilities and inadequate packaging (Gustavsson *et al.* 2011). The spoilage can also result from microbial growth, chemical changes and breakdown by endogenous enzymes. Loss in nutritional value can lead to substantial economic losses as the value of fish decreases with quality loss. Specific requirements and preservation techniques are needed in order to preserve fish's nutritional quality, extend its shelf-life, minimize the activity of spoilage bacteria and avoid losses caused by poor handling (Adams & Moss, 2008). The rate of degradation can be slowed down by application of preservation and processing techniques such as heat treatment (canning, smoking and boiling), lowering of the temperature (freezing and chilling), reduction in water activity (salting, drying and smoking) and changing the storage environment (packaging and refrigeration). Spoilage and wastage are experienced all along the fish food chain (Gustavsson *et al*., 2011). Harvested fish can either be sold to local markets and enter short value chains as fresh whole fish with little or no transformation and processing, or after the traditional forms of transformation such as drying, smoking or salting. To get to distant markets without deteriorating in quality, fish require either cold chain or processing such as canning (Samples, 2015).

2.1.5 Fish Health and Safety Concerns

The increasing focus on the benefits of fish consumption has brought corresponding and increasing concern about fishery products as a source of contaminants. Consumption of fish, as with any food, may lead to ingestion of harmful inorganic and organic compounds such as heavy metals, dioxins, poisonous micro-organisms, pesticides and residues of veterinary medicines (Mozaffarian & Rimm, 2006; Hoekstra *et al.,* 2013). Heavy metals such as methylmercury, cadmium, lead and organic tin represent the most significant health hazards (STAP, 2012). Therefore, introduction of a cheap and safe water treatment technique would not only improve fish quality but also lead to control of the product for the market and control final food quality. Heavy metals affect the peripheral nervous system in adults and the central nervous system in children. The fetal brain is especially vulnerable, and increased concentrations may result in impaired cognitive and motor skills (Grandjean *et al.,* 2004).

With the expansion in the consumption and commercialization of fish products, there has been growing interest in food quality and safety, nutritional aspects, and reduction in wastage. Bacteria in fish products can come from the listeria, camphylobacter, yersinia, shigella and salmonella species. Their occurrence is often due to lack of hygiene practices during processing operations. Salmonella is the most significant cause of infection in humans, and is a challenge in all food types, including fish. To lower bacterial exposure, stringent hygiene and good processing practices must be adhered to. Hygiene and processing method also affect the presence of viruses, especially hepatitis A and norovirus (Doyle & Buchanan, 2013). To overcome shortcomings in sanitary and unhealthy conditions in fish farming, there has been an increase in use of therapeutic and prophylactic antibiotics/antimicrobial agents including those important in human therapeutics. The unrestricted use of the antibiotics is detrimental to fish, human health and environment, and therefore the need to prevent development and spread of antibiotic/antimicrobial resistance in aquaculture to reduce the risk to human health (Serrano, 2005; Heuer *et al.*, 2009; Cabello *et al.*, 2013). In the effort to ensure food safety and consumer protection, increasingly stringent hygiene and regulation measures have been adopted at national and international trade levels.

2.1.6 Importance of Fish in Human Nutrition

Fish is a good animal source of both macronutrients and micronutrients that are important for human growth, development and wellbeing. Fish is particularly rich in numerous micronutrients that are often missing in diets, particularly those of the poor. These include essential nutrients such as iodine, vitamin A, B and D, calcium, iron, and zinc. Fish is also rich in high quality proteins and healthy fats, including a unique source of essential long-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (FAO & WHO, 2011; Kawarazuka & Béné, 2011; Bonham *et al.*, 2009). Therefore, a diet with fish can help reduce the risks of both malnutrition and of non-communicable diseases such as cardiovascular disease, which may co-occur when a high intake of energy is combined with a lack of balanced nutrition (Larsen *et al.*, 2011; Rangel-Huerta *et al.*, 2012). With its valuable nutritional properties, fish can play a major role in correcting unbalanced diets and, through substitution, in countering obesity. The omega-3 fatty acids in fish improves the neurodevelopment of foetus and infants and is therefore important for women of childbearing age, pregnant women and nursing mothers. (FAO & WHO, 2011)

Digestibility of protein from fish is approximately 5–15 percent higher than that from plant-sources and therefore can play an important role in plant-based diets by increasing the bioavailability (Tacon & Metian, 2013). The presence of essential amino acids lysine and methionine in fish also help in meeting the need in plant-based diets that lack the amino acids. Fish species consumed whole with bones, heads, and viscera play a critical role in micronutrient intakes as these parts are where most micronutrients are concentrated. Multiple micronutrient deficiencies can therefore be easily addressed by intake of fish in the diet even in small quantities (Kawarazuka and Béné, 2011; Thilsted, 2012).

2.1.7 Food Security Situation in Kenya

Kenya has experienced high population growth the last fifty years. Over these fifty years, the production of most basic food crops did not keep pace with population growth. The basic crops (potatoes, sweet potatoes, rice and beans) did so through area increase than through yield increase. In the 1960s, basic food crop production improved both in terms of area harvested and in terms of yield and its population was food sufficient based on WHO requirements at the time. After 1970, the situation began to deteriorate as a result of diminishing government support for agriculture and rural development (Fernando, 2013) and deepening socio-economic divides (Nyanjom, 2013). Crop production areas expanded somewhat in the 1970s but yields dropped, partly due to severe droughts. During the 1980s the harvested area of cereals, roots and tubers stabilized and that of pulses more than doubled, and yields recovered, for roots and tubers to their highest levels ever. In the 1990s yield levels deteriorated for all basic food crops and the harvested area of pulses declined again. In the last decade improvements were seen, until 2006. Kenya produced 3.9 million tons of cereals, 0.7 million tons of pulses and 3.8 million tons of roots and tubers in 2006. Its total basic food production could have potentially fed 96% of its population that year, up from 68% in 2000 (assuming that staple foods cover 65% of energy requirements) (GoK, 2010).

At the end of 2007, the political situation altered the trend as many farmers had to seek refuge in camps or with relatives elsewhere and had to abandon their fields. The harvested cereal area went down by about 15%. In 2008, there was a further reduction in the area under cultivation but yields fell back to only 1420 kg/ha and in 2009 the cereal production levels were average. Kenya’s basic food production in 2009 reached low levels and the country could only potentially feed 72% of its population of 39 million at WHO food requirement levels. By 2009, total food energy in Kenya had dropped by 19% compared to 2006 and this was partly associated to the 2007/2008 post- election violence. After 2009, the agricultural situation started to normalize and in 2011 Kenya could feed 88% of its population based on its own agricultural production (assuming staple food covers 65% of all dietary requirements) (Munguti *et al.,* 2014).

2.2 Seaweed Production

2.2.1 Nutritional Properties of Seaweeds

2.2.1.1 Polysaccharides

Two forms of polysaccharides are found in seaweeds; storage and cell wall polysaccharides. Storage polysaccharide mainly serve as a source of energy to the algae and include laminaran in the brown macroalgae, floridean starch in red macroalgae and starch in green macroalgae (Usov, 2011; Busi *et al.,* 2014; Barsanti & Gualtieri, 2014). The most abundant cell wall or non-starch polysaccharides (NSP) are sulfated galactan (carrageenan and agar) in red seaweeds and sulfated fucans and alginates in brown algae. Others occurring in small quantities include fucoidans (brown seaweeds), xylans (red and green seaweeds) and Ulvans (green seaweeds). In addition, cellulose is found in all the seaweeds in varying levels (Rioux & Turgeon, 2015; Delattre *et al.,* 2011; Craigie, 2010). Most of these polysaccharides found in seaweeds are not digestible in the guts of humans, while in some animals they are digested at a slow rate and therefore classified as dietary fibre. Total carbohydrate content in most seaweeds range between 20 to 76% of dry weight (Holdt & Kraan, 2011). Their content is influenced by several factors such as the season of harvest, species, environmental conditions (water salinity, temperature, tides) and the geographical location (Murata & Nakazoe, 2001). NSP also known as dietary fibre are the most abundant of the carbohydrates (Rajapakse & Kim, 2011) accounting for about of 33-62% dry matter. Moreover, these levels are higher than those found in higher plants (Dawczynski *et al.*, 2007).

Apart from being a good source of dietary fibre and a source of energy to fish, polysaccharides also have antioxidant activity that can promote the health and immunity of fish (Jung *et al.*, 2012). Tilapia fish has no specific requirement (in terms of quantity) for carbohydrates (Ng & Romano, 2013). As earlier established, seaweeds are rich in NSP which are relatively indigestible by the fish due to lack of the necessary enzymes (β-glucanases and xylanases) that are required for NSP digestion. This, in turn affects mineral and water absorption in the fish gut resulting in increased digesta viscosity (Leenhouwers *et al*., 2007) and at high levels of about 9.7% inclusion they reduce protein and lipid digestibility (Hossain *et al*., 2003). However, studies have shown that pre-treatment (with enzymes, heat and acid) of feed ingredients containing NSP can improve their digestibility and tilapia growth (Li *et al*., 2009b; Belal, 2008).

2.2.1.2 Protein

Protein content in seaweeds varies greatly among the species and is influenced by a number of factors such as the growth environment and season of harvest (Lourenço *et al*., 2002; McDermid & Stuercke, 2003). Protein can account for 5 to 47% dry weight of seaweed (Černá, 2011). All the ten essential amino acids required for growth in tilapia nutrition are present in seaweeds: threonine, tryptophan, phenylalanine, methionine, histidine, arginine, valine, lysine, isoleucine and leucine (Santiago & Lovell, 1988; Rajapakse & Kim, 2011), with the aspartic and glutamic acid being the most abundant. In a study carried out by Dawczynski et al. (2007), using 34 varieties of seaweed (17 brown and 17 red), they reported higher levels of crude protein in red seaweeds (30.9-31.4 g/100 g semi-dry weight) as compared to the brown seaweeds (7.5-19.8 g/100 g semi-dry weight). The same range results were reported by Murata and Nakazoe (2001); 30-40%, 15% and 30% of dry matter in the red, brown and green seaweeds respectively. The red seaweeds typically have higher protein content than the brown and green (Kim, 2011) and this is attributed to the occurrence of the functional proteinic pigments phycobiliproteins (phycoerythrin and phycocyanin) in the red seaweeds (Harnedy & FitzGerald, 2011). As regards to seasons, the protein content is highest during the cold seasons and lowest in the summer as result of heat destruction of the phycobiliproteins (Pangestuti & Kim, 2015).

Protein requirement in tilapia is dependent on a number of factors such as protein source, fish body weight, and stage of maturity. Based on the stage of maturity, the recommended protein content in the diet for spawning females and fry is about 30-40% while for the fingerlings and grow- out is 20-30% (Abdel-Tawwab *et al*., 2010). Soy bean protein content which is most used plant-based protein source in aqua feeds is comparable to that of seaweed (40-44%). Studies on soy bean meal digestibility in tilapia have reported high digestibility of the meal especially when fermented (Guimarães *et al*., 2008; Zhou & Yue, 2012).

2.2.1.3 Lipids

The main classes of lipid found in seaweeds are glycolipids and phospholipids. The composition of the classes in seaweeds is dependent on species type, season of harvest and environmental factors like water temperature, salinity and light (Marinho-Soriano *et al.*, 2006; Sanchez-Machado *et al*., 2004). Total lipid content of seaweeds is low, ranging from about 1-5 g/100 g dry weight (Terasaki *et al.,* 2009; Li *et al.*, 2002; Vaskovsky *et al*., 1996). However, some studies have shown that the total lipid content in brown seaweeds of the sargassum can rise to 15% dry weight with 40% of the total fatty acids being the omega 3 polyunsaturated fatty acids (PUFA) (Nomura *et al*., 2013). This difference in the contents of lipid could be due to species and seasonal variations;

production of lipids increases in the hot seasons and the poly unsaturated fatty acids in cold seasons (Narayan *et al.*, 2005).

Seaweeds lipids comprise both the saturated and unsaturated fatty acids. The saturated fatty acids include the lauric acid (C12:0), myristic (C14:0), pentadecylic (C15:0), palmitic (C16:0), margaric (C17:0), stearic (C18:0) and arachidic (C20:0) acids while the unsaturated fatty acid include the monounsaturated (C12:1-C18:1) and polyunsaturated long chain (omega-3 and omega-6) fatty acids (Hamid *et al*., 2015; Ragonese *et al.,* 2014; Sánchez-Machado *et al*., 2004). Moreover, seaweeds contain significantly higher levels of polyunsaturated fatty acids compared to terrestrial vegetables (Mendis & Kim, 2011). Omega 3 PUFAs in seaweeds include the α-linolenic acid, ALA (C18:3n-3), stearidonic acid, SDA (C18:4n-3) and eicosapentaenoic acid, EPA (C20:5n-3) while the omega 6 PUFA is the arachidonic acid (C20:4n-6) (Miyashita *et al*., 2013; Terasaki *et al*., 2009). In most seaweeds the EPA accounts for almost half of the total fatty acids (Dawczynski *et al*., 2007). Lipids in fish feeds are important source of energy for growth and survival of the fish. Tilapia fish need 5-12% lipid inclusion in their diet for optimal growth (Chou & Shiau, 1996). Seaweeds can meet the lipid requirement for tilapia although it would require the supplementation with linoleic acid, LA (C18:2n-6) which is an essential fatty acid for tilapia diet.

2.2.1.4 Vitamins and Minerals

Seaweeds contain a wide array of minerals both macro-elements and trace-elements like iodine, calcium, sodium, selenium, iron, zinc, potassium and phosphorus (Holdt & Kraan, 2011) that they draw mostly from the marine waters. They have high sorbent capacity for minerals than terrestrial plants and can account for about 36% of dry matter (Rajapakse & Kim, 2011). Higher mineral contents are recorded in brown seaweeds (30.1-39.3%) than in the red seaweeds (20.6-21.1%) (Rupérez, 2002). This was attributed to their physiological differences. Seaweeds contain both the hydro-soluble vitamins, C and B group as well as the fat-soluble A and E (MacArtain *et al*., 2007). The vitamin concentrations and profiles are affected by a number of factors including species, stage of maturity, season, geographical location, temperature and salinity (Škrovánková, 2011). Vitamin B12 which is generally present in animal products is also found in seaweeds and can reach to highs of 134 µg/100 g dry weight in the red seaweed Porphyra sp. (Miyamoto *et al*., 2009). This could be attributed to the microorganisms especially the bacteria living on the surface of waters, that serve as a source of the vitamin (Baweja *et al*., 2016).

Most aqua feeds are supplemented with mineral and vitamin premixes to meet the needs of tilapia. Fish can accumulate some minerals from their culture environment and diet. Seaweeds in a tilapia diet can provide most of the required minerals at varying percentages (Ca, Na, K, P, Zn & Fe). However, the interaction of the dietary mineral with carbohydrates and proteins in the diet may affect its bioavailability and therefore the need for supplementation (Ng & Romano, 2013). Bioavailability of the minerals in seaweeds has to be considered since some of the minerals are linked to polysaccharides that are not easily digested (Gómez-Ordóñez *et al*., 2010). However, the bioavailability can be improved by altering the physical-chemical properties of the seaweed through pretreatments that are physical, chemical or biological in nature (Wan *et al*., 2019).

Table 2.1: Nutritional Composition of Some Commercially Important Seaweeds (% Dry Weight)

Species	Carbohydrates	Protein	Lipid	Ash	Fibre	References
Rhodophyta						
Porphyra umbilicalis	43.0	29-39	0.3	12	29-35	Holdt & Kraan, 2011, Morais *et al*., 2020
Gracilaria cerviconis	57.71-68.29	14.29-22.70	0.33-0.51	8.07-13.11	4.87-7.67	Marinho-Soriano *et al*., 2006
Eucheuma denticulatum	-	4.9	2.2	43.6	-	McDermid & Stuercke, 2003
Phaeophyta						
Laminaria digitata	48	8-15	1.0	38	37	Rajauria *et al*., 2015
Sargassum vulgare	52.62-68.54	9.19-19.94	0.15-0.79	13.07-30.35	4.80-10.51	Marinho-Soriano *et al*., 2006
Undaria pinnatifida	-	19.8	4.5	-	45.9	Dawczynski *et al*., 2007
Chlorophyta						
Ulva clathrata	-	21.9-25.9	2.5-3.5	44.8-49.6	24.8-26.3	Peña-Rodríguez *et al*., 2011
Codium fragile	39-67	8-11	0.5-1.5	21-39	5.1	Holdt & Kraan, 2011; Morais *et al*., 2020
Caulerpa lentilifera	-	9.7	7.2	46.4	-	McDermid & Stuercke, 2003

2.2.2 Other Components of Interest

2.2.2.1 Pigments

Fucoxanthin is the major carotenoid found in brown seaweeds and contributes to more than 10% of carotenoid produced in nature (Rodríguez-Bernaldo *et al.*, 2010). The characteristic green color in green seaweeds is due to the presence of chlorophyll a and b, β-carotene and xanthophylls (yellowish) while the proteinic pigments, phycoerythrin and phycocyanin in the red seaweeds, is responsible for their red color (O'Sullivan *et al*., 2010; Hamid *et al.*, 2015). Pigments play an important role in fish nutrition and in the overall health (Rodriguez-Amaya, 2016). They contribute and enhance to the skin and flesh color of some fish like salmon, tilapia and seabream (Gomes *et al.*, 2002; Araújo *et al*., 2016). Color of fish skin and flesh influences consumer choice. In promoting organic aquaculture, use of seaweeds in aquafeeds is great natural alternative to artificial colorants in feeds.

2.2.2.2 Toxins

With use of macroalgae as fish feed there is need to assess the presence of toxins such as heavy metal and pesticide residues that may accumulate over time due to pollution from anthropogenic sources such as industries, agricultural water runoff, oil spillage and mining activities in the sea (Sudharsan *et al*., 2012). Furthermore, fish and other sea foods are known to build up these toxins in the fat tissues, thus accumulating them in the food chain as the fish feed on each other. Due to this ability, they have been used worldwide as biomonitors for metal pollution in coastal waters (Melville & Pulkownik, 2006). The level of contamination with pollutants in seaweeds is not only affected by the bioavailability of the pollutant but also by the environmental conditions such as temperature, light, oxygen and salinity and the seaweed uptake ability (Żbikowski *et al.*, 2006; Sánchez-Rodrıguez *et al*., 2001).

Heavy metals such as mercury, cadmium, arsenic, lead and tin have been found in seaweeds and pose a threat to both human and animal health. Arsenic is a major pollutant in seaweed and contributes to about 50% of dietary source of the pollutant (Scoop, 2004). A study by Van Netten *et al.,* (2000) showed that commercial seaweeds can have arsenics to levels between 17 to 88 μg/g (dwb) with the brown species having higher concentrations than the other species. Brown macroalgae have a higher metal binding capacity than the green and red species. The EU regulation for the minimum levels permitted in food for lead and cadmium is less than 3 ppm per dry weight and less than 0.1 ppm dry weight for mercury (EU, 2008). These standards could also apply for fish feeds since the fish is finally consumed as food. In addition, seaweeds need to be tested for heavy metal (organic & inorganic) contamination before any feed formulation to avoid contamination in the food chain.

2.2.2.3 Use in Aquaculture

Macroalgae have been used in the past years as livestock feeds for chicken, pig, sheep, cattle and studies have shown that they improve growth, reduce stress and enhance gastrointestinal health, increase egg, meat and milk quality when included in feed (Archer, 2005; Leonard *et al.,* 2011; Rajauria, 2015). The most common seaweeds used in livestock feed include the Laminaria sp., Ulva sp., Enteromorpha sp., Sargassum sp. and Gracilaria sp. (Rajauria *et al*., 2015). Application of macroalgae as feed ingredient in aqua feeds is an option of ensuring sustainability of fish meal and oil whose production is on the decline. It is a novel aquaculture feedstuff that can supply protein, lipids and minerals to farmed fish (FAO, 2018). The practical use of seaweeds as feed in cultured tilapia is relatively low (Fiogbé *et al.,* 2004). This is because seaweed has high moisture content (64.9%-94.0%) and therefore larger quantities of fresh seaweed biomass would be needed to produce the same amount of dry matter compared to terrestrial flora (Wan *et al.,* 2019). However, scientific studies have been done to evaluate the effects of seaweed supplementation and inclusion at different doses in experimental diets for fish and beneficial effect have been identified. Most of the feeding experiment focus on assessing the quality of the seaweed based on palatability, digestibility, utilization, immunological effect, functionality and the effects on growth of the fish (Glencross, 2020). Typically, more than one of these aspects is assessed in studies to get an understanding of the ingredient quality as summarized in Table 2.2.

Table 2.2.: Effects on Characteristics of Various Cultured Aquatic Animals Fed on Seaweed-Enhanced Diet

Seaweeds studied	Studied species	Doses of inclusion	Study period	Findings	References
Gracilaria spp. Ulva spp. Fucus spp.	European seabass (*Dicentrarchus labrax*)	2.5% & 7.5%, Mixed (2.5% each)	84 days	Growth performance-(0); Digestive capacity- (+); Antioxidant response (+)	Peixoto *et al*., 2016
Ulva spp.	Nile tilapia (*Oreochromis niloticus*)	10, 15 & 20%	63 days	Growth performance was higher in 10% than in 15 & 20%; Highest Lipid content at 20%	Marinho *et al*., 2013
Gracilaria bursa-pastoris, GP Ulva rigida, UR Gracilaria cornea, GC	European seabass (*Dicentrarchus labrax*)	5 & 10%	10 weeks	Growth performance (+) in all diets except in GC-10% (-); Nutrient utilization (+) in all diets except in GC-10% (-); Muscle composition (0)	Valente *et al*., 2006
Gracilaria spp. Porphyra spp. Ulva spp.	Nile tilapia (*Oreochromis niloticus*)	10%	84 days	Growth performance and feed intake in Gracilaria spp. is (-), while in the other seaweeds (+); Body composition (0)	Silva *et al*., 2015
Ulva rigida Ulva lactuca	Nile tilapia (*Oreochromis niloticus*)	5 & 10%	68 days	Sensory attributes (0); Carotenoid deposition on skin (+); Lysosome and peroxidase activity (0); Alternative complement activity (ACH50) (+)	Valente *et al*., 2016
Ulva rigida	Nile tilapia (*Oreochromis niloticus*)	5%	16 weeks	Growth performance (+); Nutrient utilization (+); Muscle composition (+)	Ergün *et al*., 2008
Gracilaria vermiculophylla	Rainbow trout (*Oncorhynchus mykiss*)	5 & 10%	91 days	Carotenoid deposition on skin (+); Innate Immunity response (+)	Araújo *et al*., 2016
Ulva sp.	Red tilapia (*Oreochromis sp.*)	5, 10, 15, 20 & 25%	9 weeks	Growth performance (+) up to 15%. No additional effect from 15% to 25%; Muscle lipid content (+) up to 10%. No additional effect from 10%;	El-Tawil, 2010

Seaweeds studied	**Studied species**	**Doses of inclusion**	**Study period**	**Findings**	**References**
				Muscle protein content (+)	
Ecklonia cava	Olive flounder (*Paralichthys olivaceus*)	2, 4 & 6%	6 weeks	Non-specific immunity (+)	Kim & Lee, 2008
Schizochytrium sp.	channel catfish (*Ictalurus punctatus*)	0.5, 1.0, 1.5 & 2.0%	9 weeks	Growth performance (+) from 1% inclusion to 2%; Filet protein, moisture & fat concentration (no effect); Long chain polyunsaturated fatty acid composition (+)	Li *et al.*, 2009a
Porphyra dioica	Rainbow trout (*Oncorhynchus mykiss*)	5, 10 & 15%	12.5 weeks	Growth performance (0); Protein content (+); Flesh pigmentation (+)	Soler-Vila *et al.*, 2009
Ulva lactuca	African catfish (*Clarias gariepinus*)	10, 20, & 30%	10 weeks	Growth performance (-) at 20 & 30% inclusion while (+) at 10%; Feed utilization (-) at 20 & 30% inclusion	Abdel-Warith *et al.*, 2016

As shown in Table 2.2, the inclusion of seaweeds in the diets of fish has diverse effects on its overall performance and quality varying among the species (both of fish and seaweed). Incorporating seaweeds in feeds at low levels (< 15%) enhances growth performance (weight gain, feed conversion ratio, survival) while an increment in inclusion above this level results in detrimental effects (Marinho *et al*., 2013; El-Tawil, 2010; Valente *et al.,* 2006). This suggests that small quantities of seaweeds in fish diet are adequate to promote their growth. Kamunde *et al*., (2019), evaluated the effect of supplementing the diet of Atlantic salmon with a brown seaweed meal (Laminaria sp.) on growth, antioxidant activity and resistance to temperature stress. The study showed enhanced growth and antioxidant activity while reducing the stress effect of acute temperature rise on mitochondrial respiration when the meal was included in the diet at 3% and 10%.

2.2.3 Feasibility and Sustainability of Using Seaweeds as Feed Ingredients

The highest volume of seaweeds produced globally is cultivated, contributing to about 97% of the global production, of which more than 90% is used in the hydrocolloid industries (FAO, 2020). A relatively small group of seaweed species are cultivated due to their commercial importance. Despite this loss of diversity in cultivation of seaweeds, the focus on aquaculture as the major source of seaweed creates the space to conserve the species in the wild (open waters) especially from dredgers that destroy the natural habitat of aquatic animals and plants (Buschmann *et al*., 2017). A promising strategy in sustainability of using seaweed as a feed ingredient is using the biorefinery approach, where waste from the hydrocolloid extraction can be redirected into production of feed additives (Wan *et al*., 2019). Multiple products such as functional additives, meal can be generated from the waste after hydrocolloid extraction. This could reduce the need to expand the cultivation of seaweeds as well as the cost of feed additives.

Another sustainable approach to meeting the demand for seaweed as a feed ingredient is the use of Integrated multi-Trophic Aquaculture concept (IMTA). IMTA systems are practiced in controlled environment where seaweeds are cultivated together or in proximity to aquatic animals such as fish at different trophic levels (Troell *et al*., 2009). This system allows for waste from aquaculture to be reduced while at the same time providing feed for the fish. In addition, the cultivation of seaweeds can be augmented (to meet the demand for feeds) without competing with food crops for land since most of the farming is carried out offshore using nets, floating lines or rafts (Radulovich *et al*., 2015). Sustainable production of seaweeds has diversified the livelihoods of rural, poor, coastal communities (Largo *et al*., 2020). A study by Mirera *et al.* (2020) in the south coast of Kenya showed that seaweed farming has a high return in investment while contributing to development infrastructure and production of value-added products such as fish feed. The study also indicted that women participation as seaweed farmer was highest (75.2%) compared to men. This translated to empowering them in decision making in the family and community. Similar studies in Asia demonstrated that seaweed cultivation benefitted the local communities by improving the infrastructure (Beveridge *et al*., 2010). With proper management of seaweed farms, negative effects such as introduction of pathogens, invasive species in IMTA can be mitigated to cushion the environment and society (Skjermo *et al*., 2014).

2.2.4 Future Prospects

Seaweeds are an important marine resource gaining diverse use. The increase in global production over the decades is an indication that they have great potential for uses in diverse areas, undoubtedly aquaculture being among the core areas. Seaweeds have a great potential for exploitation in aquaculture as a feed ingredient due to its unique nutritional profile. It is rich in protein, minerals and PUFA, in addition to other functional compounds like pigments and polysaccharides that are important for fish nutrition. Use of seaweeds in aquaculture is an interesting prospect because, besides having nutritional benefits, it also helps improve growth performance in fish and boosted their immunity. When grown in an integrated multi-trophic aquaculture system, they can serve as both feed to the fish and help in cleaning the water by removing nutrients from the water.

With careful selection of seaweeds based on the target nutrient in the feed and understanding optimum conditions for production of the specific nutrients will help in achieving the desired results in the fish. Therefore, more research focused on strains and species of seaweed tailored for feeds as has been the case in hydrocolloid industry. The high mineral content in seaweeds make them an excellent resource for the manufacture of natural mineral supplements for use in feeds. In addition, its natural pigments are a great alternative to artificial colorants in feeds. The total lipids in the brown seaweed species, sargassum can rise up to 15% dry matter and therefore can be used in aquafeeds to reduce dependence on fish oil as a source of lipid. Use of seaweed as source of feed in aquaculture is important in developing countries like Kenya, as it will free up important fish feed like soya, to be used for human food. Currently, the feed industry takes up 80% of food crops in aquafeeds, therefore seaweeds can be a great replacer and reduce the competition. The growth in global seaweed production expected to continue to be on the rise, the uses and demand will also increase and aspects of sustainable production and use should be addressed. New production systems like the integrated multi-trophic aquaculture systems should be adapted.

2.2.5 Conclusion

In conclusion, seaweeds, are widely used as food and applied in other food allied industries, pharmaceuticals, cosmetics and agrochemical industries. Their production has increased with advancement in identification and cultivation of different seaweed species. Over the years, seaweeds have been explored as a food due to their nutrition value and bioactive compounds that are beneficial to human nutrition and health. With this principle, seaweeds can also be used as feed ingredient in aqua feeds especially due to the fact that it is a source of omega-3 and hence can be used as an alternative to fish oil whose supply has declined. Studies have shown that polyunsaturated fatty acids which are important in fish nutrition can account for about 50% of total fatty acids in seaweeds. In addition to being a good source of polyunsaturated fatty acids, seaweeds provide protein, minerals, and vitamins. They are also characterized with high levels of protein rich in all the amino acids relative to some higher plant-based protein crops like soya bean.

2.3 Water Treatment Technologies

Wastewater contains a variety of pollutants that includes inorganic, organic, and biological materials, depending on wastewater releasing activities (industrial, agricultural and municipal). The most common inorganic water pollutants are heavy metals, which are highly toxic and carcinogenic in nature, nitrates, sulphates, phosphates, fluorides, chlorides and oxalates. Toxic organic pollutants are from pesticides, phenols, biphenyls, detergents, oils, greases, lignin and pharmaceuticals. These water pollutants remain either in solvated, colloidal or in suspended form (Kabra *et al.,* 2004; Chong *et al.,* 2010). Water treatment technologies are classified as primary, secondary and tertiary treatment. In a complete water treatment plant, all these three processes are combined together. Primary treatment includes preliminary purification processes of a chemical and physical nature while secondary treatment involves biological treatment of the wastewater (Gupta *et al.,* 2012). In tertiary treatment processes, up to 99% of the pollutants are removed and the water is converted into good and safe quality for a specific use. The tertiary treatment is normally applied when the wastewater has undergone primary and secondary treatment (Chong *et al.,* 2010).

Conventional primary wastewater treatment methods aims to remove large settleable organic and inorganic solids by filtration using mechanical screens, sedimentation and skimming the floating components (scum). Heavy metals, organic phosphorous and organic nitrogen associated with solids are also removed in sedimentation but colloidal and dissolved constituents are not affected (Hashem & Qi, 2021). To increase treatment capacity, the primary processes can be enhanced using coagulants and flocculants chemicals (metal salts and /or polymers in the form of organic polyelectrolyte) to remove suspended solids, organic carbon and nutrients from wastewater and concentrate it in sludge (Dong *et al*., 2019). Depending on the wastewater characteristics and types of coagulants and/or flocculants used, the chemically enhanced primary treatment can remove between 70.00-99.50% suspended solids and 40.00-99.30% phosphate but nitrogen removal is limited (Shewa & Dagnew,2020). In addition, the elimination efficiencies of heavy metals like chromium, nickel, copper and zinc is increased in the process (Johnson *et al*.,2008).

Secondary treatment is the further treatment of effluent from primary treatment to remove suspended solids and residual organics. The biodegradable colloidal and dissolved organic matter are removed through aerobic biological treatment processes such as trickling filters, rotating biological contractor, aerobic granulation, activated sludge or anaerobic processes such as constructed wetlands and aerated lagoon. The processes aims to reduce the biological oxygen demand (BOD), chemical oxygen demand (COD) and suspended solids (Hashem & Qi, 2021; Shukla & Ahammad, 2023). COD represent the quantity of oxygen required to stabilize carbonaceous organic matter using strong oxidants such as potassium permanganate ($KMNO_4$) or potassium dichromate ($K_2Cr_2O_7$) (Von Sperling, 2007). On the other hand, BOD is the amount of dissolved oxygen used to oxidize organic matter by aerobic microbes in water at a certain temperature over a specific period, usually five days (Dionisi, 2017).

Membrane bioreactor (MBR) is a tertiary treatment technology that applies two water treatment steps. It is a combination of the conventional biological sludge process, a wastewater treatment process characterized by a suspended growth of biomass, with a micro- or ultrafiltration membrane system (Judd, 2011). The biological unit is responsible for the biodegradation of the waste compounds and the membrane module is responsible for the physical separation of the treated water from the mixed liquor. The pore sizes are between 0.01-0.1µm and therefore capable of removing bacteria and large colloids; precipitates and coagulates during microfiltration and viruses, high molecular weight protein and organics at ultrafiltration (Hoinkis, *et al.*, 2012). MBR is preferred due to its high efficiency in degradation of organic compounds and hence higher product water quality and low footprint. This makes it a suitable wastewater treatment technology for municipal and industrial wastewater treatment and process water recycling.

Membranes are highly efficient, flexible and easy to scale up (Galiano *et al.*, 2015). However, one main drawback of MBR is its membrane fouling tendency caused by accumulation of organic or inorganic substances on the membrane surface. Fouling causes pore clogging and scaling, leading to rapid decline in membrane performance and durability (Gukelberger *et al.*, 2019) and water is hindered from passing through the membrane. To overcome this problem, membrane surfaces can be modified using different techniques to develop membranes with intrinsic antifouling properties (Rana *et al.,* 2010). One strategy that has been demonstrated to be effective is the use of polymerizable coating on polyethersulfone membranes (Galiano *et al*., 2017). The study was carried at laboratory scale and showed reduced fouling properties as verified by higher affinity for water (lower contact angle), reduced surface roughness and higher antimicrobial activity (Gukelberger *et al*., 2019). The present study will apply the use of polyethersulfone membranes and another in parallel coated in Polymerizable bicontinuous microemulsion for the treatment of wastewater for reuse in a recirculating aquaculture system.

CHAPTER THREE

DIETARY SUPPLEMENTATION OF NILE TILAPIA FEED WITH SEAWEED AND ITS INFLUENCE ON GROWTH AND NUTRITIONAL QUALITY OF THE FISH

Abstract

Feed is a major component of production costs in aquaculture, accounting for about 80% of the production costs. High-quality aquafeeds are a prerequisite to healthy and nutritious fish. Aquafeeds are expensive owing to the fact that fish oil and fish meals are the main sources of lipid and protein components, respectively. Having alternative, cheap sources of lipids in the feeds is therefore important. The brown seaweed (*Sargassum portieranum*) that is locally available on the Kenyan coast is known to be rich in omega-3 fatty acids. The objective of this study was therefore to determine the suitability of brown seaweed dietary supplementation and its effect on the nutritional quality and growth performance of Nile tilapia (*Oreochromis niloticus*). A total of 180 male Nile tilapia fingerlings were divided into three experimental groups in triplicate. The fish were assigned to one of the three treatment diets: 0% (control diet), 5%, or 10% inclusion of the brown seaweed, and fed for 12 weeks. The weight and length (from head to tail) of the fish were measured every two weeks to determine the growth performance. At the end of the experiment, the fish muscle protein, lipid, and mineral content were determined using AOAC methods. Seaweed supplementation significantly ($P < 0.05$) improved the body weight, length, survival, and specific growth rate of the fish, with the 10% inclusion showing higher performance than the 5%. The protein, mineral, and lipid contents of the fish muscles were also significantly affected by the seaweed supplementation. Fish fed on the 10% diet had the highest total lipid content in the muscle, at 0.93%, compared to 0.78% in the fish fed on the control diet. The protein content in the fish muscle was not significantly affected ($P < 0.05$) by the inclusion of seaweed in the feed. Overall, the results showed that supplementing the feed with 5% or 10% brown seaweed improved the growth performance and nutritional quality of tilapia fish. Thus, including brown seaweed

meal in the diet of tilapia fish could offer an effective means to boost production in aquaculture.

3.1 Introduction

Fish and fish products play an important role in the human diet and health. They are sources of high-quality protein, omega-3 polyunsaturated fatty acids (*n*-3, PUFA), and micronutrients such as vitamin D, selenium, calcium, iodine, and iron (Kawarazuka & Béné, 2011; Weichselbaum *et al*., 2013). Regular consumption of fish is associated with several health benefits, such as improved neural development in infants and a reduced risk of cardiovascular inflammatory disease and insulin resistance (Chowdhury *et al*., 2012; Corella & Ordovas, 2012).

With the increase in human population and the emergence of a large number of people with greater purchasing power and a preference for animal protein over plant protein, demand for fish is increasing (Jennings *et al*., 2016; Kharas, 2010). Fish is supplied from two main sources:1) wild-capture fisheries and 2) aquaculture. In 2018, aquaculture contributed 46% (82.1 million tonnes) of the global fish production, of which 52% was used as food for human consumption (FAO, 2020). In the same year, the total fisheries production in Kenya was at 147,000 metric tons, with a per capita consumption of about 5 kg compared to the global consumption of 20 kg per capita (KNBS, 2020). Kenya’s fish production is a major factor influencing its fish consumption; that is, an increase (or decrease) in domestic fish production tends to increase (or decrease) per capita fish consumption (Obiero *et al*., 2019). Aquaculture has great potential for growth to meet the growing demand for fish. To ensure sustainability and optimize aquaculture, all dynamics involved in production, such as feed ingredients and quality, nutrient cycling, and retention in the fish, need to be researched and understood.

Aquafeeds play an important role in aquaculture production. They account for about 50% of the variable production costs (Rana *et al*., 2009). Due to the high inclusion rate of nutrients like protein (up to 40%) in feeds for fingerlings, the cost of feeds is relatively high (Cho *et al.*, 2003). In addition, fish oil is the major source of polyunsaturated fatty acids (PUFA) in aquafeeds but is expensive (Klinger & Naylor, 2012). Approximately 75% of annual fish-oil production is used as a feed ingredient in aquafeeds (Auchterlonie, 2018). This in turn reduces the amount of fish oil available for human consumption, and this is exacerbated by the stagnation in the production of fish oil (Shepherd & Jackson, 2013). These factors combined, necessitate the need for sustainable and cheap alternative sources of PUFA to use in aquafeeds. Seaweeds are a promising feed ingredient since they are a source of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (FAO, 2020). Omega-3 fatty acids are important in fish for cellular metabolism and maintenance of cell membrane structure and integrity (Miller *et al.*, 2008; Tocher, 2010).

Seaweeds are a rich source of carbohydrates, protein (with a high content of essential amino acids), and minerals like magnesium, calcium, iodine, and sodium (Bocanegra *et al.*, 2009; Fleurence, 1999; Miyashita *et al.*, 2013). The brown seaweeds (*Phaeophyta*) have been reported to have the highest lipid content among the seaweeds, with predominantly long-chain PUFA, eicosapentaenoic acid (EPA, C20:5 n-3), and arachidonic acid (ARA, C20:4 n-6) (Wan *et al.*, 2019). Although seaweeds may not have as much lipid content as other plant sources, the proportion and profile of their fatty acids are high in polyunsaturated fatty acids (PUFA). In some seaweed species, PUFA can account for up to 40% of total fatty acids (Nomura *et al.*, 2013). Several studies have demonstrated that dietary supplementation with seaweeds in a fish diet can greatly improve the muscle lipid profile, especially the omega-3 fatty acids (Garcia-Vaquero & Hayes, 2016; Güroy *et al.*, 2013). A study by Dantagnan *et al*. (2009) reported an increase in muscle total PUFA and omega-3 PUFA of up to 73% and 64%, respectively, in rainbow trout when fed on the brown seaweed, *Macrocystis pyrifera*. Furthermore, the inclusion of plant-based ingredients at various levels in feeds affects the final product quality (lipid, amino acid, color, and texture) (De Francesco *et al*., 2004). In assessing the suitability of a feed ingredient for use in aquafeed, determining its nutrient utilisation is one key step (Glencross, 2020). This involves feeding trials and then assessing the growth responses. Therefore, the objective of this study was to evaluate the effect of supplementation with brown seaweed (*Sargassum portieranum*) on the growth performance, muscle biochemical composition, and lipid profile of Nile tilapia (*Oreochromis niloticus*).

3.2 Materials and Methods

3.2.1 Experimental Site

The research study was carried out at the ViclnAqua aquaculture hatcheries in Kisumu, Kenya. All the laboratory analyses were conducted at Jomo Kenyatta University of Agriculture and Technology in the Food Biochemistry Laboratory.

3.2.2 Feed Formulation

Forty kilograms of seaweed (*Sargassum portieranum)* were collected from Shimoni, on the south coast of Kenya, in the month of July, 2020. The seaweeds were hand-picked and then washed with seawater to remove foreign particles. The samples were then transported to the Jomo Kenyatta University of Agriculture and Technology in cold storage (4 ^{o}C). On arrival, they were thoroughly washed in running tap water, dried to a constant weight in a conventional hot-air oven at 40 ^{o}C for 24 hours, and then ground into a fine powder. The other feed ingredients, i.e., fish meal, lake shrimp, wheat bran, cassava flour, and vegetable oil, were purchased from local stores.

For the diet preparation, the dry base ingredients were ground in a mill (Barrisio omniblend, model TM-767) and then sieved through a 1 mm sieve mesh (Endecotts ltd, model BS410/1986). The ingredients were then weighed out in triplicate and homogenised for preparing the experimental diets. The proportions of ingredients used are as shown in Table 3.1. The feed preparation was based on Pearson's square. Fishmeal was used at 10% in each diet, as recommended in organic aquaculture (Shepherd & Jackson, 2013). The seaweed was added to two diets at inclusion levels of 5% and 10%, with a control diet without seaweed at 0%. Sunflower oil and water were added and thoroughly mixed to make a blend of soft dough consistency. The dough was extruded using an automated meat mincer fitted with a 2 mm plate. The 2-mm pellets were sundried to a constant weight. Airtight containers were used for storage of the pellets until the start of the feeding.

3.2.3 Experimental Set-Up

Male Nile tilapia fingerlings were obtained from the experimental site (ViclnAqua) hatcheries. One hundred and eighty fingerlings were distributed randomly in 9 circular cylindrical tanks with a capacity of 200 liters (3 replicates per treatment) at a stocking density of 20 fingerlings per tank in completely randomized block design. The fingerlings began with an average weight of 31.11 ± 0.60 g and 12.5 ± 0.04 cm length (head to tail). Feeding was done at a rate of 4% body weight, three times daily (at 0830, 1300, and 1700 hours) for 12 weeks. The fish were maintained at a natural photoperiod. Dissolved oxygen was maintained above 6 mg/l using an aeration system. The quality of the water was regulated by replacing the water in the tanks three times every week. At the end of the experimental period, the fish were fasted for 24 hours and then sampled.

Plate 3.1: Pictures of the Experimental Set-Up and Feed Formulation Process

Key: A: Set-Up of the tanks used in the experiment; B: Drying of the *Sargassum portieranum* in the oven; C: Extruding the fish feeds and D: Sun drying of the feeds.

Table 3.1: Formulation of the Diets. Ingredients Content in the Feed in g/kg

Ingredients	**Diets (g/kg, dry weight basis)**		
	Diet 1 (0%, control)	**Diet 2 (5%)**	**Diet 3 (10%)**
Fish meal	100	100	100
Shrimp (*Caridina nilotica*)	152	152	152
Wheat bran	222	222	222
Wheat pollard	222	222	222
Sunflower meal cake	169	169	169
Cassava flour (binder)	75	75	75
Vegetable oil	50	50	50
S. portieranum (seaweed)	0	50	100
[a] Mineral and vitamin premixes	10	10	10

Diet 1, 0% inclusion; Diet 2, 5% inclusion; Diet 3, 10% inclusion of the seaweed.

[a] Vitamin and mineral premix composition per Kg of feed: Vitamin A, 600 I.U.; vitamin D3, 100 I.U.; vitamin E, 3 I.U.; vitamin K (menadione), 0.42 mg; vitamin B1, 0.25 mg; vitamin B2, 0.6 mg; vitamin B6, 0.5 mg; vitamin B12, 0.0011 mg; nicotinic acid, 2.5 mg; pantothenic acid, 2.2 mg; folic acid, 0.15 mg; biotin, 0.001 mg; vitamin C, 1 mg; copper, 0.5 mg; manganese, 15 mg; zinc, 4.5 mg; iodide, 0.14 mg; selenium, 0.012 mg; cobalt, 0.02 mg; choline chloride, 15 mg; iron 4 mg.

3.3 Analytical Methods

3.3.1 Growth Performance of the Fish

The growth performance of the fish was determined by measuring its weight, length, survival rate, and food index parameters (specific growth rate and condition factor). The parameters were calculated using equations from Tekinay & Davies, 2001, as follows:

$$Survival\ rate\ (SR,\%) = \frac{Number\ of\ live\ tilapia\ at\ end\ of\ experiment}{Intial\ number\ of\ tilapia} \times 100 \ldots\ldots \text{ Eq. 3.1}$$

$$Weight\ gain\ (WG, g) = \ Average\ final\ weight - Average\ initial\ weight \ldots \text{ Eq. 3.2}$$

$$Length\ gain, (LG, cm) = Average\ final\ length - Average\ initial\ length \ldots\ldots \text{ Eq. 3.3}$$

$$Specific\ growth\ rate\ (SGR,\%) = ((Log\ of\ final\ weight(g) -$$

$Log\ of\ initial\ weight\ (g)))/(Experiment\ period) \times 100$……………Eq. 3.4

$Condition\ factor\ (CF) = \frac{Final\ weight\ (g)}{Final\ lenght\ (cm^3)}$……………..………………...Eq. 3.5

$Feed\ conversion\ ratio\ (FCR) = \frac{Total\ feed\ intake\ (g)}{Total\ wet\ weight\ gain\ (g)}$……………………Eq. 3.6

3.3.2 The Physical Properties of the Feed

The sinking velocities of the pellets were measured by adapting the method of Lekang *et al.* (1991). Sinking velocity test was done in a transparent measuring cylinder, the diameter, and height of the tube was 3 cm and 200 cm respectively. The measuring cylinder was filled with fresh water heated up to 25 ^{o}C to simulate the temperature of the natural growing environment for Nile tilapia. The fixed point was marked on 10 cm and 160 cm around the cylinder. The 10 cm marking from the top of the tube was to allow feed pellets to reach constant velocity before timing. The sinking velocity to travel 150 cm was measured by using stop-watch. Single pellets of approximately the same lengths (1 cm) were randomly selected for sinking velocity measurements and sinking velocities were recorded as cm/s. Forty pellets were randomly chosen for each diet for the test and pellets which came in contact with the wall of measuring cylinder during dropping were excluded.

Bulk density was measured by filling the pellet in a measuring cylinder of known volume and weighing the content on a balance following the method by Aarseth *et al.* (2006). The measurement was done in triplicate and the bulk density was calculated as mass per unit volume of the sample.

3.3.3 Proximate Analysis of the Feed and Fish Muscle

Moisture content, crude protein, crude lipid, crude fat, crude fiber, and ash for experimental diets and fish muscle were determined according to Association of Official Analytical Chemists method specification 950.46 (AOAC, 1995). The moisture content was determined by weighing 2 g of the sample into a moisture dish and transferring it to an oven previously heated to temperatures of 105 °C, where it was dried for 3 hours. The final weight of the sample was taken after the drying period and cooling in a desiccator. The loss in weight was reported as moisture content (AOAC, 1995, method 925. 10). Ash content was determined by incineration of the samples in an Advantec KL-420 electric muffle furnace at 550 ^oC for 12 hours. The crude protein content (N × 6.25) was determined by the semi-micro Kjeldahl method after acid digestion using a Kjeldahl system (Velp scientifica model). The Kjeldahl system was used to digest 5 g of the sample mixed with two catalysts (5 g of K_2SO_4 and 0.5 g $CuSO_4$) and 15 ml of concentrated H_2SO_4. The digest was then distilled and finally titrated to obtain the nitrogen content. The crude protein was obtained by multiplying the nitrogen content by the protein factor. Crude lipid was analysed using the Soxhlet system (Geohardt model). About 5 g of the sample was weighed into thimbles, and lipid extraction was done using petroleum ether in a soxhlet apparatus for 8 hours. The extraction solvent was evaporated, and the remaining lipid was dried in an oven at 70 ^oC to a constant weight to obtain the crude lipid. The crude fibre content was determined using the Hennenberg-Stohmann method (AOAC, 1995), which involves sequential digestion of samples with 1.25% H_2SO_4 and 1.25% NaOH, followed by drying at 105 ^oC for 30 min and ashing at 550 ^oC for 1 hour, and then cooling.

3.3.4 Fatty Acid Profiling

The Bligh & Dyer method (1959) protocol was used to extract lipid from fish muscle. Finely ground samples were homogenised using a methanol-chloroform (2:1, v/v) mixture containing 0.01% butylated hydroxytoluene (BHT), and the extract was filtered with Whatman No. 1 filter paper. A second solvent mixture of methanol, chloroform, and water (2:1:0.8) was added to the extract, and the process was repeated. The mixture was then centrifuged (Hettich zentrifugen, model D-78532) at 3000 rpm for 10 minutes, and the chloroform layer at the bottom was separated from the aqueous layer using a micropipette. The chloroform layer was transferred into a reflux flask and evaporated to dryness using a rotary vacuum evaporator. Five (5) ml of methanolic H_2SO_4 (1% H_2SO_4, v/v) was added to the extract, and esterification was done at 70 ^oC for 3 hours. The fatty acid methyl esters (FAME) were then extracted into 5 mL hexane and 100 mL water. The mixture was transferred into a separating funnel, and the hexane layer (bottom) was withdrawn and passed through anhydrous sodium sulphate. The extract was finally dried to 0.5 ml using the rotary vacuum evaporator. Concentrated FAME extract was then transferred to vials. Gas chromatography-mass spectrophotometry (Agilent Technologies, model 7890B) was used to identify the FAMEs by injecting the FAME extract into a silica capillary column ((SUPELCO, Omegawax^{tm} 530). The injection temperature and detection temperature were 240 ^{o}C and 260 ^{o}C respectively.

3.4 Statistical Analysis

All the experimental data are expressed as the mean ± standard error (SE). Data was subjected to one-way analysis of variance (ANOVA), followed by the Duncan multiple-range test to compare differences among treatments. Statistically significant differences between the means were considered when $P < 0.05$. Statistical analysis was performed using R, version 4.0.2 Software (R, 2020).

3.5 Results

3.5.1 Growth Performance of the Fish

The results on the growth performance and survival of Nile tilapia are presented in Table 3.2. The final weight and weight gain of the fish increased with the increasing levels of seaweed supplementation. The Nile tilapia fed on the diet containing 10% seaweed had the highest final body weight (66.12 ± 2.24 g) and weight gain (28.08 ± 1.03 g). Both the length and the condition factor of the Nile tilapia were not significantly affected by inclusion of seaweed in the diets. The fish fed the control diet recorded lower levels of survival (93.33%). There was significant difference ($P< 0.05$) in the specific growth rate after 12 weeks of feeding on the control diet and the supplemented diet at a 10% inclusion level. The specific growth rate of the fish increased with the increase in the level of seaweed used in supplementation (0.33-0.38 %).

Table 3.2: Growth Performance and Survival Rate of Nile Tilapia Fed on Three Different Diets for 12 Weeks

Diets	**Weight (g)**		**Length (cm)**		**SR (%)**	**SGR (% per day)**	**FCR**	**CF**
	Final	**Gain**	**Final**	**Gain**				
Diet 1	59.19±1.03^{b}	28.08±1.03^{b}	15.63±0.29^{a}	3.13±0.29^{a}	93.33±3.33^{ab}	0.33±0.01^{b}	1.55±0.03^{b}	0.02±0.00^{a}
Diet 2	62.61±1.54^{ab}	31.50±1.54^{ab}	15.62±0.48^{a}	3.12±0.48^{a}	100±0.00^{a}	0.36±0.01^{ab}	1.60±0.06^{ab}	0.02±0.00^{a}
Diet 3	66.12±2.24^{a}	35.00±2.24^{a}	16.37±0.26^{a}	3.87±0.26^{a}	100±0.00^{a}	0.38±0.02^{a}	1.64±0.02^{a}	0.02±0.01^{a}
LSD	5.797	5.797	1.230	1.230	6.660	0.047	0.040	0.002

The values are the ± SE of the means. n = 3. The values in the same column with different superscript letters differ significantly ($P < 0.05$). Abbreviations: SGR (specific growth rate); SR (survival rate); CF (condition factor); LSD (least significant difference). Diet 1, 0% inclusion; Diet 2, 5% inclusion; and Diet 3, 10% inclusion of seaweed

3.5.2 The Physical Properties of the Feed

The bulk densities and velocity of the pellets are shown in Figure 3.1 below. Bulk densities of the control diet and diets supplemented with seaweed did not vary significantly. The diet supplemented at 10% with seaweed had the highest bulk density of 354 g/l. The velocity of the pellets varied significantly ($p < 0.05$) among the three diets. The control diet pellets recorded the lowest velocity (7.42 cm/sec). A positive correlation was observed between the sinking velocities of the feed pellets and their bulk densities from the graph.

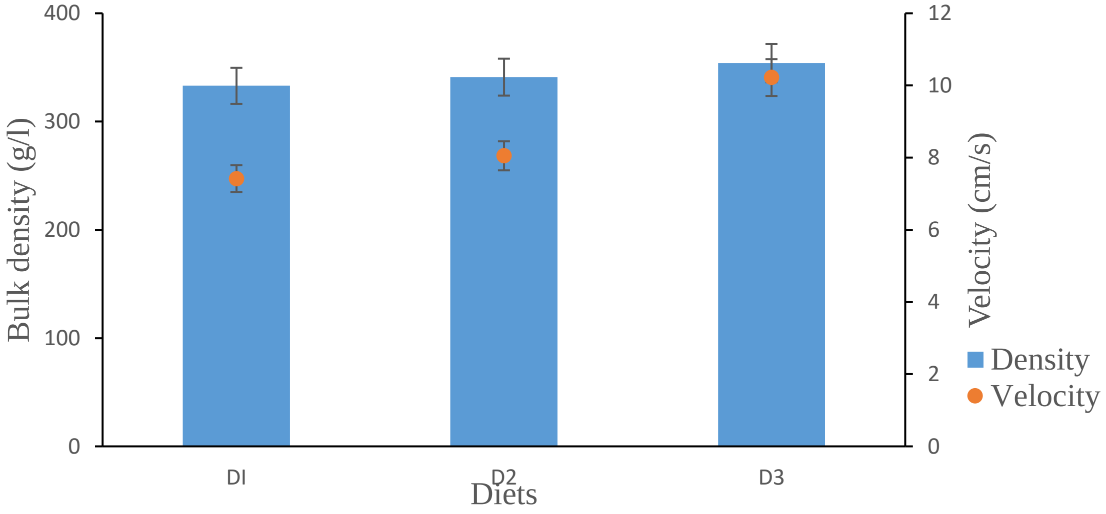

Figure 3.1: The Sinking Velocities and Bulk Densities of the Three Diets. Diet 1, 0% Inclusion (Control); Diet 2, 5% Inclusion; Diet 3, 10% Inclusion

3.5.3 Proximate Analysis of the Feed and Fish Muscle

The crude protein and ash content of the feeds were significantly higher in diet 3 (10% inclusion) than the other diets ($P \leq 0.05$). The highest protein content was recorded in diet 3 at 34.90%. Dietary inclusion of seaweed had no significant effect on the crude fibre content of the feed. Both dry matter and nitrogen free extract were significantly high in the control diet than in the feeds with seaweed.

As shown in Table 3.4, the crude fibre and crude lipid content of the Nile tilapia increased significantly ($P < 0.05$) when fed diets supplemented with seaweeds compared to the control (0%). Fish fed on the 10% supplemented diet showed the highest crude lipid and fibre content at 0.93% and 0.39%, respectively. The three diets did not result in any significant difference ($P < 0.05$) in the dry matter or crude protein levels in the fish muscle. The results of crude ash content showed no significant difference ($P < 0.05$) between the unsupplemented diet and the diet supplemented with 10% seaweed. Crude ash content of the fish was significantly lower when fed the diet 2 compared to diet 3.

Table 3.3: Proximate Composition of the Fish Feed

[a] Proximate composition (% dry matter basis)	Diet 1	Diet 2	Diet 3	P value
Dry matter	95.86 ± 0.18^{a}	94.68 ± 0.03^{b}	94.92 ± 0.13^{b}	0.005
Crude protein	30.96 ± 0.27^{b}	32.76 ± 0.67^{ab}	34.90 ± 0.81^{a}	0.048
Crude lipids	5.57 ± 0.22^{a}	5.76 ± 0.21^{a}	5.55 ± 0.08^{a}	0.683
Crude ash	6.53 ± 0.27^{b}	7.18 ± 0.56^{b}	8.75 ± 0.24^{a}	0.016
Crude fibre	10.83 ± 0.38^{a}	10.67 ± 0.21^{a}	10.48 ± 0.21^{a}	0.682
[b] NFE	41.92 ± 0.18^{a}	37.82 ± 0.03^{ab}	35.62 ± 0.13^{b}	0.053

Diet 1, 0% inclusion; Diet 2, 5% inclusion; Diet 3, 10% inclusion of the seaweed. The values in the same row with different superscript letters differ significantly ($P < 0.05$).

[a] Proximate values are the mean ± S.E.

[b] Nitrogen-free extract (NFE) = 100 - (content of moisture + crude protein + crude lipids + crude ash + fiber)

Table 3.4: Proximate Composition of the Fish Muscle

% Proximate composition (wet weight basis)	Dry matter	Crude protein	Crude lipid	Crude ash	Crude fibre
Diet 1	21.76±0.21^{a}	19.26±0.17^{a}	0.78±0.07^{b}	1.13±0.02^{a}	0.23±0.02^{b}
Diet 2	22.17±0.38^{a}	20.02±0.27^{a}	0.91±0.05^{a}	0.88±0.08^{b}	0.36±0.03^{a}
Diet 3	22.52±0.42^{a}	20.38±0.54^{a}	0.93±0.05^{a}	1.18±0.02^{a}	0.39±0.02^{a}
LSD	1.451	1.261	0.111	0.159	0.081

Data are expressed as the mean ± SE; n = 3. The superscript letters in the same column differ significantly (p<0.05). Diet 1, 0% inclusion; Diet 2, 5% inclusion; and Diet 3, 10% inclusion of the seaweed, LSD, Least significant difference

3.5.4 Fatty Acid Profiling

At the end of the experimental period, fish supplemented with seaweeds showed no significant difference in the saturated fatty acids (palmitic and stearic acid) and monounsaturated fatty acids (palmitoleic), except for the oleic acid, which increased significantly (P<0.05) in the fish fed with a diet of 5% inclusion. The linoleic fatty acid displayed an initial decrease followed by an increase with the increase in seaweed content, while the maximum content was obtained in the control at 37.34%. For the three dietary treatments, linoleic acid was the most abundant fatty acid in the fish muscle. Seaweed supplementation at two levels (5% and 10%) significantly increased omega-3 content while decreasing omega-6 content. The diet with 5% seaweed supplementation showed the highest omega-3/omega-6 ratio. The total omega-6 levels were significantly higher than the other unsaturated fatty acid groups (MUFA and omega-3) for the three dietary treatments, as shown in Figure 3.2.

Table 3.5: Fatty Acid Profile of the Fish-Muscle for Fish Fed on Three Different Diets

% Fatty acids	Diets			
	Diet 1	Diet 2	Diet 3	P-Value
SFA				
C16:0	21.91±0.02^{a}	20.33±0.44^{a}	21.23±0.90^{a}	0.223
C18:0	9.41±0.59^{a}	12.11±0.93^{a}	9.88±0.78^{a}	0.103
MUFA				
C16:1	2.30±0.26^{a}	3.08±0.40^{a}	1.85±0.69^{a}	0.215
C18:1	2.33±0.39^{b}	10.82±0.65^{a}	1.45±0.33^{b}	0.000
Omega 6				
C18:2	37.34±0.43^{a}	24.65±0.83^{c}	32.06±0.16^{b}	0.001
C18:3	0.20±0.02^{b}	0.44±0.02^{a}	0.29±0.03^{b}	0.005
C20:4	6.37±0.67^{b}	7.47±0.63^{ab}	8.59±0.33^{a}	0.085
Omega 3				
C20:5 n-3	1.44±0.09^{b}	2.21±0.35^{ab}	2.39±0.21^{a}	0.065
C22:6 n-3	19.51±0.38^{b}	21.97±0.42^{b}	26.16±0.91^{a}	0.011
Totals				
SFA	30.86±0.68^{a}	32.44±0.55^{a}	31.90±0.93^{a}	0.536
MUFA	4.64±0.71^{b}	13.73±0.02^{a}	3.31±0.34^{b}	0.000
LC-PUFA, ω-6	43.26±0.56^{a}	31.94±0.60^{c}	40.61±0.02^{b}	0.000
LC-PUFA, ω-3	21.04±0.35^{c}	23.93±0.03^{b}	28.54±0.55^{a}	0.002
ω-3/ω-6 (ratio)	0.49±0.01^{b}	0.75±0.01^{a}	0.70±0.01^{b}	0.002

The values are the ± SE of the means. n = 3. The values in the same row with different superscript letters differ significantly ($P < 0.05$). Diet 1, 0% inclusion; Diet 2, 5% inclusion; and Diet 3, 10% inclusion. Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; LC-PUFA, long-chain polyunsaturated fatty acid; C16:0, palmitic acid; C18:0, stearic acid; C16:1, palmitoleic acid; C18:1, oleic acid; C18:2, linoleic acid; C20:4, arachidonic acid; C18:3, linolenic acid; C20:5, eicosapentaenoic acid; C22:6, docosahexaenoic acid; ω-6, Omega 6; ω-3, Omega 3

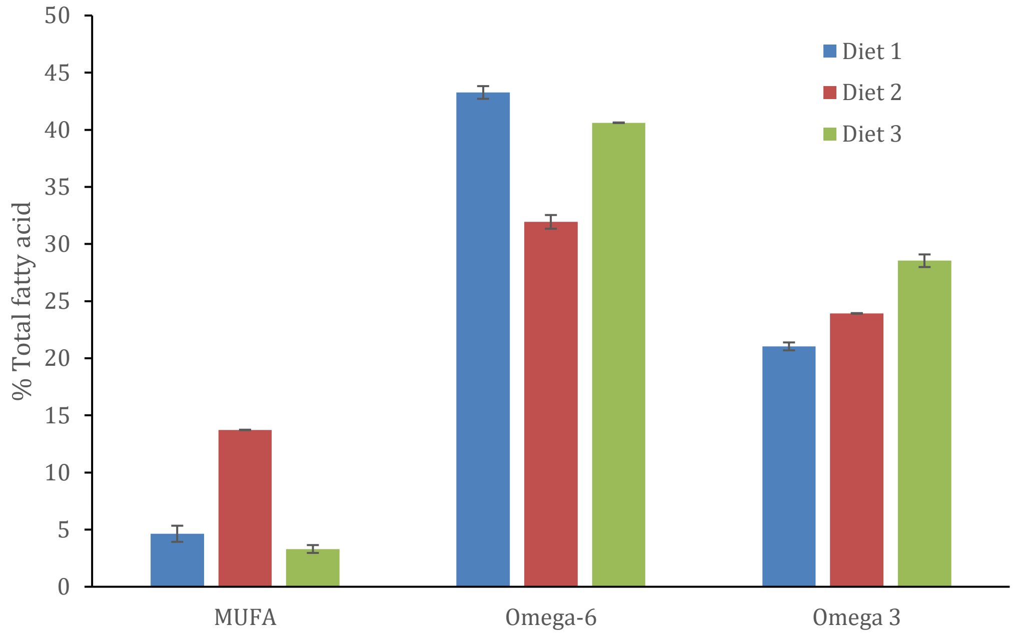

Figure 3.2: Comparison of the Unsaturated Fatty Acid in the Fish Muscle Fed on Three Different Diets for 12 Weeks. Diet 1, 0% Inclusion; Diet 2,5% Inclusion; Diet 3, 10% Inclusion

3.6 Discussions

Previous studies have demonstrated that inclusion of small amounts of seaweeds in fish diets could enhance the growth performance of several cultured fish species (Nakagawa & Montgomery, 2007; Roy *et al*., 2011), including *Sargassum* (Ragaza *et al*., 2013; Serrano *et al*., 2015). In the present study, supplementation of fish feed with *S. portieranum* showed improved fish growth, indicating that inclusion of seaweed in tilapia feeds can improve weight gain without greatly affecting other growth performance parameters. A study by Ergün *et al*. (2008) showed similar results when *Ulva sp*. was included at a rate of 5% in the Nile tilapia diet. The current study is also consistent with those of Wassef *et al*., (2013) and Khalafalla & El-Hais (2015), who found that diets containing seaweeds improved fish growth and feed efficiency. Furthermore, in another study the performance of rainbow trout was enhanced when they were fed diets supplemented with 0.4% of brown seaweed (Ribeiro *et al.,* 2017). However, differences that arise in trials have been attributed to the species of seaweed, fish species, and type of feed, water quality, age, weight, or level of inclusion (Abdelrhman *et al.,* 2022).

The survival rate of the fish fed on the control diet was significantly low compared to the supplemented diets as the mortality rate of the fish was higher. The higher survival rate in the supplemented diet could be attributed to the immunoactivity of seaweeds since the rearing conditions (water quality) were similar in all the treatments (Araújo *et al.,* 2016).

The positive effect could also be attributed to the bioactive phytochemical molecules found in seaweeds, which have been shown to improve immune responses and growth performance (Van Doan *et al*., 2017), as well as the presence of sulphate carbohydrates in seaweeds (Fernández *et al*., 2011), which trigger nonspecific immunity, making them a major amplifier to immunity and growth accelerators (Telles *et al.,* 2018). Furthermore, including seaweeds and their extracts in the diets of aquatic animals improves immunity and growth parameters (Sharawy *et al*., 2020).

The specific growth rate was comparable in the 5% and 10% supplementation groups, with both outperforming the control diet. Earlier studies had demonstrated that high inclusion levels (>10%) have a detrimental effect on fish growth or provide no additional benefit to the fish (Azaza *et al.,* 2008; El-Tawil, 2010; Güroy *et al*., 2007). Moreover, recommendations for the maximum inclusion level in Nile tilapia have been established at 10% for the green seaweed *Ulva sp.* and 5% for the red seaweed *Gracilaria sp.* (Marinho *et al*., 2013; Silva *et al*., 2015). Thiessen *et al*. (2004) demonstrated that growth performance could be affected when using seaweeds in fish diets since plant ingredients constitute a certain amount of fibre that may be detrimental to their nutritional value and palatability.

The physical quality of feed pellets such sinking velocities and bulk densities are affected by the type of ingredients used and the diet composition (Aarseth, 2004). These factors may interfere with the growth performance of fish. Bulk densities help determine the sinking or floating characteristic of a pellet. According to Pandey, (2018), pellets that sink within 15 seconds are considered sinking pellets. The present results showed that the pellets from the control and the supplemented diets were all sinking pellets. Nile tilapia are adapted to picking feed pellets from the water surface or within the water column immediately after feeding (Obirikorang *et al*., 2015). Therefore, the sinking velocities of the feed from the present study were well suited to the feeding habit of Nile tilapia. With increase in seaweed inclusion level, a concomitant rise in the sinking velocity was also observed, indicating that the low inclusion of seaweed in the diets significantly affected the sinking velocity of the feeds. The bulk densities of the three diets were directly related to their respective sinking velocity.

Analysis of the body composition is an effective measure of the health and physiological condition of fish (Saliu *et al.,* 2007); further, it is vital in optimising their utilisation of feeds (Martin *et al*., 2000). The proximate components, lipid and fibre, in the fish muscle were positively affected by the increase in the incorporation of seaweed in the feeds, while for the protein and ash content, were similar to the control. This increase suggests that seaweeds could have influenced the absorption and synthesis of the two nutrients in Nile tilapia. Additionally, Nile tilapias have high amylase activity and hence prioritise carbohydrates as an energy source and spare proteins (Kamunde *et al*., 2019). Indeed, several studies on the utilisation of seaweed as a feed ingredient have demonstrated that the assimilation of nutrients in fish is dependent on the level of inclusion and species of both the seaweed and fish (Peixoto *et al*., 2016; Valente *et al*., 2006; Wan *et al*., 2019).

The nutritional profile of fish, especially the fatty acid profile, is often a reflection of its diet (Bell *et al*., 2003; Rosenlund *et al*., 2002; Keriko *et al*., 2021). Therefore, feeding a diet rich in PUFA leads to high levels of PUFA in the muscle of various fish species (Li *et al.,* 2013; Tonial *et al*., 2009; Visentainer *et al*., 2005). In this study, the results showed a similar pattern: the PUFA content in the fish muscles that were fed the supplemented diets improved with the increase in inclusion levels. These results are similar to what EL-Tawil (2010) found for red tilapia fed on a diet containing the green seaweed *Ulva sp.* at 10% inclusion. The omega-3 fatty acid levels were increased by about 14% and 36% in the fish feed diets supplemented with 5% and 10%, respectively. These results could be attributed to an increase in the quantity of seaweed incorporated into the diets. All three diets resulted in fish tissue with higher docosahexaenoic acid (DHA) levels than eicosapentaenoic acid (EPA), with the fish fed the diet supplemented with 10% seaweed showing the highest levels of DHA. PUFAs play an important role in the growth and survival of marine fish (Tocher, 2010). These processes utilise more EPA, conserving more DHA relative to the EPA, hence resulting in high DHA levels in the fish muscle (Rønnestad *et al.*, 1995; Villalta *et al.*, 2005). Moreover, omega-6 levels were relatively high in all the fish fed the two supplemented experimental diets, with linoleic acid contributing the highest levels. Omega-6 has been shown to have better growth promoting effect than omega-3. The omega-3/omega-6 ratio increased with the increase in the seaweed proportion in the diet, suggesting active metabolism of the essential fatty acid from the dietary source (Lim *et al.*, 2009). In addition, the higher the omega-3/omega-6 ratio, the higher the ability of the body to utilize the omega-3 fatty acid (Ridha *et al.*, 2020). The ratio omega-3/omega-6 from our study is similar to that reported by Sarker *et al*. (2018).

3.7 Conclusions

From the findings in this study, incorporation of 5% and 10% of the brown seaweed *Sargassum portieranum* in aquafeeds improves the fatty acid content of Nile tilapia while at the same time promoting its growth performance and proximate composition. The study indicated feeding Nile tilapia with diets containing 10% of the brown seaweed significantly improved the growth performance indicators, weight and the specific growth rate. Inclusion of seaweed also promoted the level of protein, ash and nitrogen free extract in the feed and the lipid and fibre in the fish muscle. Nile tilapia that were fed diets containing the seaweed showed improved omega 3; moreover, the omega 6 of the fish muscle were enhanced optimally at 5% seaweed inclusion level. Thus, including brown seaweed at low levels in fish feeds has the potential to improve the polyunsaturated fatty acid content of fish while enhancing growth.

CHAPTER FOUR

MEMBRANE BIOREACTOR TREATED WASTEWATER INFLUENCE ON THE GROWTH PERFORMANCE AND BIOCHEMICAL COMPOSITION OF NILE TILAPIA

Abstract

The aquaculture sector in Africa has great potential for growth; however, it faces several challenges, one of them being the scarcity of clean water. This prompts the need for water recycling. The present study was conducted to investigate the effects of rearing Nile tilapia (*Oreochromis niloticus*) using municipal wastewater treated with membrane bioreactor (MBR) technology. A total of 270 Nile tilapia fingerlings (0.15 ± 0.05g) were reared in three treatment groups in triplicate. There were 2 treatments, including; MBR treated wastewater and stabilization pond treated wastewater (maturation pond), while the municipal tap water was used as the control. The growth performance (weight and length) of the fish was monitored over a 24-week period. After the experimental period, the proximate composition of the fish muscle was analysed using standard AOAC methods. The results showed that the highest weight gain, length gain, survival rate, and specific growth were obtained in the fish in the control followed by the MBR treatment. Additionally, the crude protein, as well as the crude fiber and dry matter, were higher in the fish in the maturation ponds at 23.10%, 0.29%, and 25.35%, respectively, while the crude ash was highest in the MBR at 1.22%. Results also showed that the MBR and maturation pond treatments meet the permissible levels for BOD, COD, NH_4, and NO_3 for water to be used in aquaculture. The bioaccumulation of heavy metals in the fish was mainly from the feed, with copper being the highest contaminant at 1.75 mg/100 g. In conclusion, both the MBR and maturation pond treated wastewater are viable for use in the rearing of Nile tilapia without adverse effect on the growth. However, MBR treatment showed better growth performance, suggesting that it could be used to increase productivity in fish farming.

4.1 Introduction

With the global population estimated to rise to 9.7 billion by 2050 from the current 7.7 billion, the demand for food will also proportionally increase (United Nations, 2019). To improve food security, eradicate hunger and malnutrition presently and in years to come, there is need sustainable food production systems. Fish and aquatic plants are major contributor to healthy and nutritious human diet. Over the last five decades, the global fish consumption has increased at a rate almost double that of the global population growth in the same period (FAO, 2020). This has in turn fueled the demand for fish. Fish is an important part of the diet because it is a source of high-quality protein, rich in essential amino acids and long chain polyunsaturated fatty acids and when consumed whole with skin, head and bones it provides essential micronutrients like selenium, calcium, iron, zinc, vitamins D and A (Khalili & Sabine, 2018; Tacon & Metian, 2013).

The world supply of fish is from wild catch and aquaculture, with aquaculture accounting for almost half of the total global fish production at 82.1 million tonnes in 2018 (FAO, 2020). There has been overexploitation of capture fisheries through illegal, unregulated and unreported fishing leading to decline in the wild fish stock hence shifting the fish production system to aquaculture. Aquaculture has great potential to meet the demands for fish and fish products for the growing world population (Cao *et al.*, 2013). In addition, it reduces over reliance on fisheries and enhances preservation of natural aquatic resources. In developing countries, the growth of inland aquaculture is faced with the challenge of water shortage due to competition from other uses (FAO, 2014). Wastewater reuse is therefore a great alternative and valuable resource in sustainable aquaculture. Membrane technology is an effective wastewater treatment technology, in particular the membrane bioreactor (MBR) technology, in improving the quality of wastewater for reuse in aquaculture (Bouhadjar *et al.*, 2016; Stephenson *et al.*, 2000). MBR technology employs combined conventional activated sludge process with microfiltration or ultrafiltration process (Judd, 2011). The bioreactor is involved in the biodegradation of the organic waste while the membrane separates the treated water and the mixed liquor (Hoinkis *et al.*, 2012).

MBR has gained major interest over the years as it gives high quality effluent, small environmental footprint and good disinfection capabilities (Assayie *et al.,* 2017; Mutamim *et al.,* 2013). The MBR technology has been widely applied in recirculating aquaculture systems as biological filters for the wastewater produced in the system. This technology creates a new alternative to produce fish to promote food and nutrition security especially in developing countries where water for aquaculture is scarce but it also poses the question of fish safety for human consumption. Due to increasing interest in food quality and safety as a result of stringent food standards at national and international levels (FAO, 2016), there is the need to establish the quality of fish reared in treated wastewater.

Fish normally interact involuntarily with its culture environment (Ibrahem, 2015), this therefore mean they can draw and accumulate components in the water. For instance, they can bioaccumulate heavy metals. In fish farming the quality of water used is important in determining yields and survival of the fish. In this study, the effect of using MBR treated municipal wastewater to rear Nile tilapia (*Oreochromis niloticus*) was investigated and compared with conventional pond treated wastewater at the maturation pond. It is from the maturation pond that the treated wastewater is released into the environment and can be used in agriculture (Von Sperling & Chernicharo, 2005). Specific focus of this study was on the growth performance of the tilapia fish, muscle biochemical composition, microbial load and heavy metal concentrations.

4.2 Materials and Methods

The experimental set-up was carried out at the ViclnAqua pilot site, in Kisumu, Kenya. All the laboratory analysis was done at Jomo Kenyatta University of Agriculture and Technology, in the Food Biochemistry Laboratory.

4.2.1 Experimental Set-Up

The Nile tilapia fingerlings were obtained from the study site (ViclnAqua project) hatcheries. Following acclimatization for two weeks, male Nile tilapia fingerlings of average weight 0.15±0.05 g (mean ± SE) and 1.32±0.11 cm length were randomly distributed in 9 circular cylindrical tanks of 200 L at a stocking density of 30 fingerlings per tank. Three tanks were assigned to each of the treatment; MBR treated wastewater, maturation pond water, and control (tap water) in a completely randomized block design. All the tanks were provided with aeration to maintain the dissolved oxygen levels above 3 mg/L with a photoperiod of 12:12 h light: dark. Throughout the experiment period the water quality (dissolved oxygen, pH and temperature) was monitored daily with a multiparameter analyzer (OxyGuard, probes). The water quality was maintained by changing the water regularly (thrice a week).

The specifications of the membranes used in the membrane bioreactor are as follows: ultrafiltration membranes with polymer of polyethersulfone (PES), molecular weight cut-off (MWCO) of 150 kDalton and pore size of nominal 35 nm (MARTIN Systems, 2019).

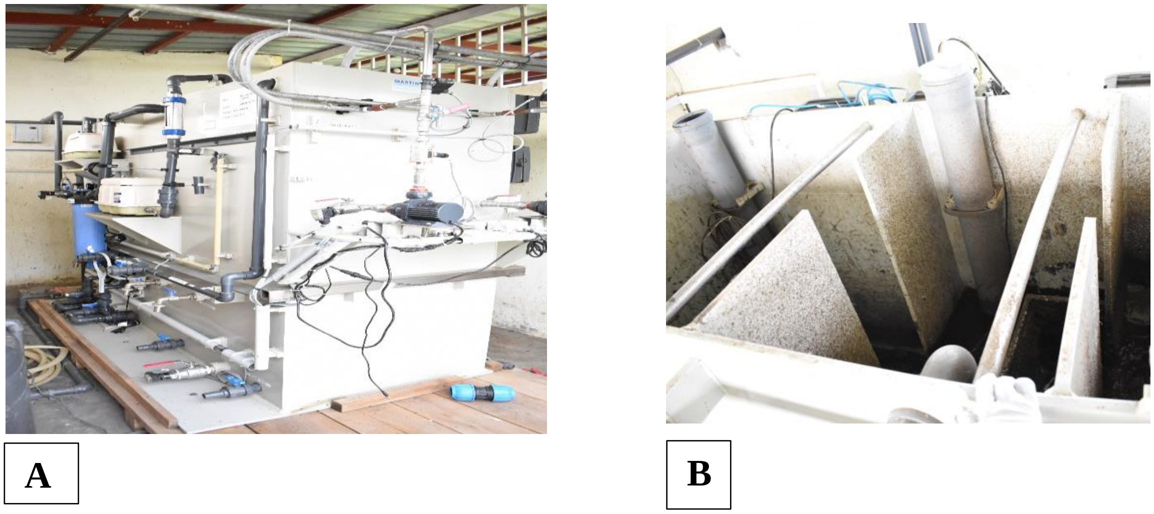

Plate 4.1: Membrane Bioreactor Set-Up (A) and the Partitioning of the Bioreactor (B)

4.2.2 Experimental Diets

The fingerlings were initially fed on an isonitrogenous diet (mash) at a daily rate of 40 % of their body weight with frequencies of four times a day (8:00 h, 11:00 h, 14:00 h and

17:00 h) for the four weeks. After four weeks the diet was changed to an isonitrogenous and isoenergetic (pellets) and the feeding done three times a day (08:00 h, 12:00 h and 17:00 h) to apparent satiation, for twenty weeks. The feed preparation was based on Pearson's square. The Ingredients in the experimental diets (% dry weight basis (dwb)) is presented in Table 4.1 below. During the entire experimental period the fish (10%) were randomly sampled at intervals of 14 days and the weight and length taken, then released back to their respective tanks.

At the end of the experiment, the fish were fasted for 24 h, prior to sampling. They were then weighed individually. Thereafter, the fish were slaughtered, degutted and the muscle collected for further analysis.

Table 4.1: Ingredients in the Experimental Diets (g/kg)

Ingredients (g /kg)	**Experimental diets**	
	Diet 1(mash) (g/kg)	**Diet 2 (pellets) (g/kg)**
Fish meal	_	262
Shrimp (*Caridina nilotica*)	1000	262
Wheat bran	_	121
Wheat pollard	_	95
Sunflower oil	_	100
Cassava flour (binder)	_	150
Mineral and vitamin premixes [a]	_	10

[a] Vitamin and mineral premix composition per Kg of feed: vitamin A, 600 I.U; vitamin D3, 100 I.U.; vitamin E, 3 I.U; vitamin K (menadione), 0.42 mg; vitamin B1, 0.25 mg; vitamin B2, 0.6 mg; vitamin B6, 0.5 mg; vitamin B12, 0.0011 mg; nicotinic acid, 2.5 mg; pantothenic acid, 2.2 mg; folic, 0.15 mg; biotin, 0.001 mg; vitamin C, 1 mg; copper, 0.5 mg; manganese, 15 mg; zinc, 4.5 mg; iodide, 0.14 mg; selenium, 0.012 mg; cobalt, 0.02 mg; choline chloride, 15 mg; iron 4 mg.

4.3 Analytical Methods

4.3.1 Physicochemical Analysis of Water

The water samples pH, dissolved oxygen and conductivity were monitored using portable devices; pH meter (HANNA model, HI 2211), Oxygen Handy Polaris probe (OxyGuard model, Hv 3.12 Eu) and digital conductivity meter (HANNA DisT3 model, HI 98303) respectively. The nitrates and ammonia levels were determined using rapid colorimetric methods. Chemical oxygen demand (COD) was analyzed using the potassium permanganate method while the biological oxygen demand (BOD) was determined with the five days incubation method (Li et al., 2018, Jouanneau et al., 2014). The BOD was determined using dissolved oxygen meter, an incubator and BOD bottles. The BOD bottles were filled with the water sample and the dissolved oxygen (DO) measured and recorded before incubating at 20 ^{o}C for 5 days. The DO was measured after incubation and recorded. The BOD was calculated as the difference in the DO before and after incubation. COD was quantified by injecting potassium permanganate and liquid sample into an analysis kit, thermally reacting, cooling, then analyzing by comparing absorbance in a spectrophotometer.

4.3.2 Analysis of Growth Performance

The growth parameters (weight gain, length gain), survival rate and food index parameters (specific growth rate, condition factor and feed conversion ratio) were calculated using equations from Tekinay & Davies, (2001), as summarized below.

$$Survival\ rate(SR, \%) = \frac{Number\ of\ live\ tilapia\ at\ end\ of\ experiment}{Initial\ number\ of\ tilapia} \times 100 \qquad \text{….Eq. 4.1}$$

$$Weight\ gain\ (WG, g) = \ Average\ final\ weight - Average\ initial\ weight \qquad \text{…. Eq.4.2}$$

$$Length\ gain, (LG, cm) = Average\ final\ length - Average\ initial\ length \qquad \text{Eq. 4.3}$$

$$Specific\ growth\ rate\ (SGR,\%) = \frac{(Log\ of\ final\ weight(g) - Log\ of\ initial\ weight\ (g))}{Experiment\ period} \times 100 \quad \ldots \text{Eq. 4.4}$$

$$Condition\ factor\ (CF) = \frac{Final\ weight\ (g)}{Final\ lenght\ (cm^3)} \times 100 \quad \ldots \text{Eq. 4.5}$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{Total\ feed\ intake(g)}{Total\ wet\ weight\ gain(g)} \quad \text{Eq. 4.6}$$

4.3.3 Proximate Analysis of Fish Muscle

The proximate analysis of the fish muscle was conducted as described in section 3.3.2.

4.3.4 Heavy Metal and Mineral Analysis of Fish Muscle

The mineral concentration in the fish samples were determined using the atomic absorption spectrometry (Shimadzu, AAS AA/AE) as described by Alvin and Gardner, (1986). About 5 g of the fish samples were placed in a previously weighed porcelain crucible, charred and then put in a furnace at 550 ^{o}C. The resulting white ash was weighed, dissolved in 100ml of 0.5N nitric acid. The solution was then used to determine the minerals. Similarly, the water samples, were analyzed for minerals and heavy metal using the AAS after digestion with nitric acid. The detection (flame ionization detector) wavelengths of the minerals were as follows: Mg, 285.42 nm; Na, 589.79 nm; Ca, 422.40 nm; K, 766.12 nm; Fe, 510.11 nm; Zn, 214.15 nm; Mn, 279.48 nm; Cd, 228.70 nm; Cr, 357.87 nm; Cu, 324.80 nm and Pb, 283.52 nm.

4.3.5 Microbiological Analysis of Fish Muscle

Standard methods for microbial analysis of food were used to determine the bacterial counts in the Nile tilapia samples (Dijk *et al.*, 2007). Twenty grams of fish muscle was pulverized before analysis according to the method by Stoops *et al.* (2016). An aliquot of 10 g of the fish sample was aseptically transferred into a sterile stomacher bag and 90 ml peptone was added. The mixture was then homogenized for one minute. A ten-fold serial dilution series of 1 ml was plated on different media using the pour plate method. Total viable counts (TVC) were determined on Plate Count Agar incubated at 30°C for 72 hours, lactic acid bacteria on De Man Rogosa Sharpe medium with an overlay of the same medium and incubated at 30 °C for 72 hours and Enterobacteriaceae on Violet Red Bile Glucose medium with an overlay of the same medium and incubated at 37 °C for 24 hours. For the yeasts and molds, 0.1 ml was plated using the spread plate method on Dichrolan Rose-Bengal Chloramphenicol Agar and incubated at 25 °C for 120 hours.

The presence of *Salmonella* was determined according to ISO 6579-1:2007 method. Twenty-five grams of the fish muscle sample was added in 225 ml of buffered peptone water and incubated at 37°C for 24 hours. Then 0.1 ml of the pre-enrichment culture was added to 10 ml of tetrathionate broth and incubated at 37°C for 24 hours. loopful inoculums were then streaked into Salmonella Shigella agar and incubated at 37°C for 24 hours. Presence of black-centered colonies was examined. An additional confirmation test of the black-centered colonies entailed incubation in triple sugar-iron agar at 37°C for 24 hours. The presence of *Escherichia coli* was determined according to ISO 7251-1: 2005 method. Ten-fold serial dilution series of fish muscle sample was plated on violet red bile lactose agar using the pour plate method and incubated at 37°C for 24 hours, and the presence of colonies checked. All microbial counts were expressed as cfu/g.

4.4 Statistical Analysis

Triplicate samples were used in all the experimental analysis. The experimental data were expressed as the mean ± standard error (SE). The analysis of variance was performed by applying one-way analysis of variance (ANOVA) followed by the Duncan multiple-range test to compare difference between treatments. Statistically significant differences between the means were considered when $P < 0.05$. Statistical analysis was performed using R, version 4.0.2 Software (R, 2020).

4.5 Results

4.5.1 Physicochemical Properties of Water

The results in Table 4.2 show that the water quality of the three treatments were significantly different ($p< 0.05$) except for the dissolved oxygen. The values for the dissolved oxygen ranged from 7.39 to 7.47. Conductivity was highest in the MBR treated water at 692.67 µS/cm while the maturation pond recorded the highest values in BOD and COD at 8.52 and 20.42 mgO_2/l respectively. The concentration of NH_4 was lowest in the control at 0.06 mg/l, while it was highest in the maturation pond (2.07 mg/l), above the permissible level for use in aquaculture (0.2 mg/l).

Table 4.2: Water Physicochemical Properties in the Three Treatments

Water parameters	**MBR water**	**Maturation pond water**	**Tap water**	**Permissible level for aquaculture (FAO)**
Dissolved oxygen (mg/l)	7.39 ± 0.04^a	7.39 ± 0.00^a	7.47 ± 0.04^a	Min 3
pH	7.31 ± 0.03^b	7.98 ± 0.04^a	6.47 ± 0.14^c	6.5-8.5
Conductivity (µS/cm)	692.67 ± 0.88^a	578.67 ± 1.86^b	157.33 ± 0.88^c	Max 2500
BOD_5 days at 20 ^OC (mgO_2/l)	4.14 ± 0.19^b	8.52 ± 0.26^a	1.05 ± 0.02^c	Max 15
COD (mgO_2/l)	16.03 ± 0.03^b	20.41 ± 0.19^a	0.02 ± 0.00^c	Max 30
NO_3 (mg/l)	19.33 ± 0.36^b	25.17 ± 0.60^a	3.60 ± 0.52^c	Max 44
NH_4 (mg/l)	0.09 ± 0.02^b	2.07 ± 0.08^a	0.06 ± 0.01^b	Max 0.2

Data are expressed as the mean ± SE. n=3. Values in the same row with different superscript letters are significantly different ($P < 0.05$). Abbreviations: BOD, biological oxygen demand; COD, chemical oxygen demand; NO_3, nitrate; NH_4, ammonium; MBR, membrane bioreactor

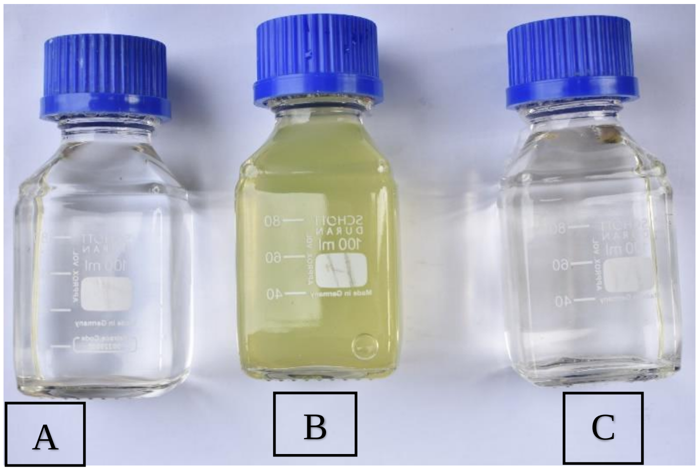

Plate 4.2: Pictures of the Water from the Three Treatments. A: Tap Water; B: Maturation Pond Water and C: MBR Treated Water

4.5.2 Growth Performance and Survival

The data on the growth performance and survival rate of the Nile tilapia in the three treatments are shown in Table 4.3. There was significant difference ($P<0.05$) in all the growth parameters in the three treatments. After the 24 weeks feeding period, the mean weight differed significantly in the three treatments at 62.55 g, 57.84 g and 50.48 g for tap water, MBR treated water and maturation pond water respectively. The fish reared in the maturation pond water showed significantly lower length (14.70 cm) than the control (15.29 cm). The survival rate was significantly different with a high survival rate in the tap water (95.56%) followed by the MBR treated water (86.67%) and the lowest in the maturation pond water (76.67%). Although the Nile tilapia reared in the maturation pond water showed the lower levels of specific growth rate, condition factor and feed conversion ratio, compared with control (tap water), these parameters did not differ significantly between the control and the MBR samples. The maturation pond water showed the lowest performance in terms of the specific growth rate and feed conversion ratio while the tap water had the best performance. The results indicated that the condition factor were between 0.016 to 0.017 in the three water treatments.

The weight growth curve showed a sigmoid pattern in all the treatments while the length gain over time was not significantly different among the treatments.

Table 4.3: Growth performance and Survival Rate of *O. niloticus* Fed for 24 Weeks

	Weight (g)		Length (cm)		SR (%)	SGR (% per day)	FCR	CF
	Final	**gain**	**Final**	**Gain**				
MBR	57.84±0.77^{b}	57.69±0.77 ^{b}	15.10±0.11^{ab}	13.78±0.11^{ab}	86.67 ± 1.92^{b}	1.54±0.00^{a}	1.42±0.03^{a}	0.017±0.00^{a}
MP	50.48±0.22^{c}	50.33±0.22^{c}	14.70±0.08^{b}	13.38±0.08^{b}	76.67 ± 1.92 ^{c}	1.50±0.00^{b}	1.26±0.06^{b}	0.016±0.00^{b}
TW	62.55±1.03^{a}	62.40±1.03^{a}	15.29±0.20^{a}	13.97±0.20^{a}	95.56 ± 1.11^{a}	1.56±0.02^{a}	1.53±0.02^{a}	0.017±0.00^{a}
P-Value	0.00	0.00	0.06	0.06	0.00	0.02	0.00	0.06

Values are the means ± SE. *n = 3*. Values in the same column with different superscript letters are significantly different ($P < 0.05$). Abbreviations: SGR, Specific growth rate; SR, survival rate; FCR, feed conversion ratio; CF, condition factor; MBR, membrane bioreactor treated water; MP, maturation pond water; TW, tap water.

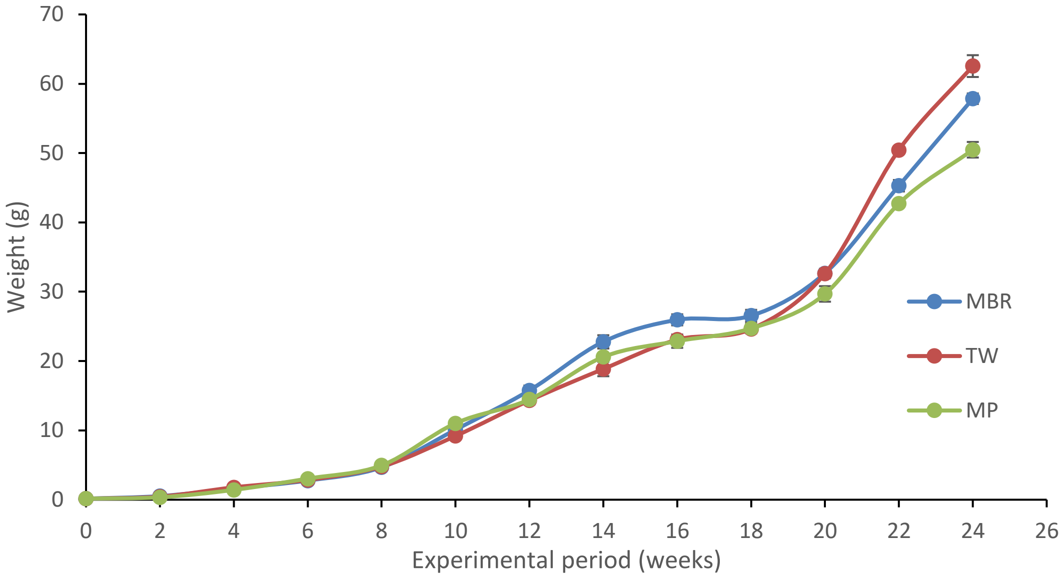

Figure 4.1: Fish Weight Gain over a Period of 24 Weeks in Three Different Water Qualities

Abbreviations: MBR, membrane bioreactor treated water; MP, maturation pond water; TW, tap water.

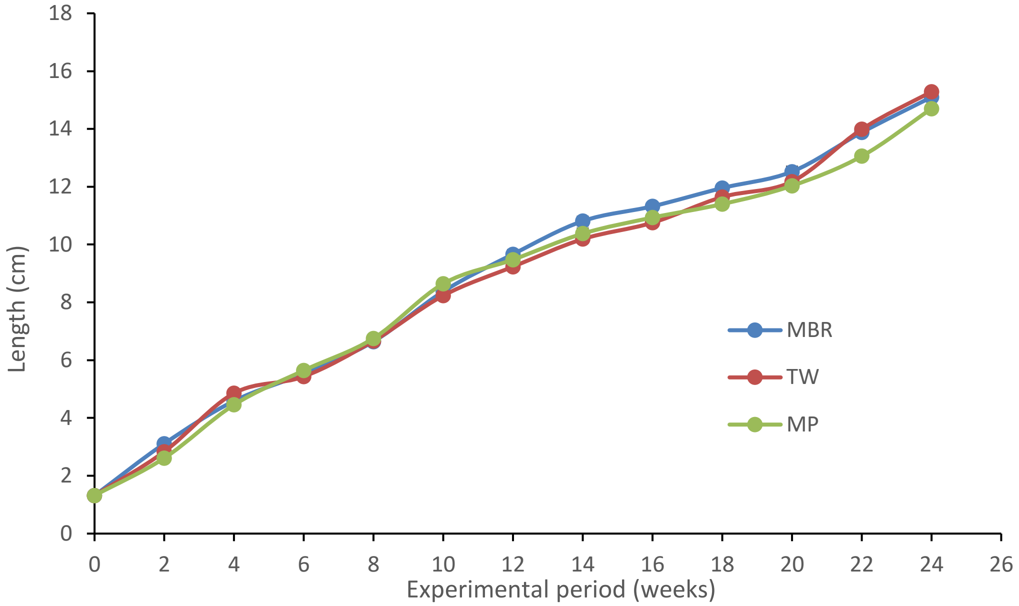

Figure 4.2: Fish Length Gain over a Period of 24 Weeks in Three Different Water Qualities

Key: MBR, membrane bioreactor treated water; MP, maturation pond water; TW, tap water.

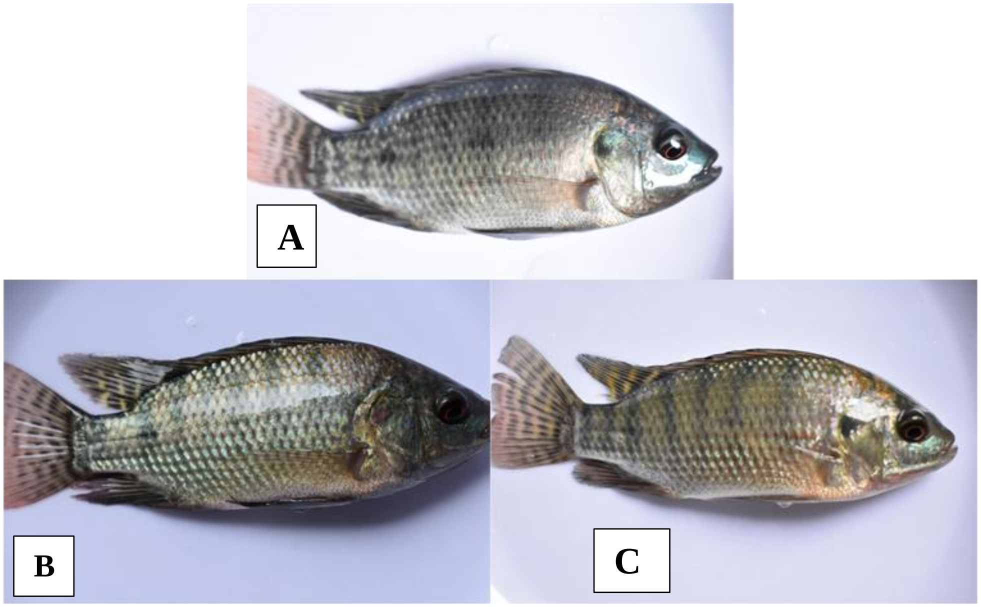

Plate 4.3: Picture of the Nile Tilapia Reared in Tap Water (A), Maturation Pond Water (B) and MBR Treated Water (C)

4.5.3 Feed and Muscle Proximate Composition

Nile tilapia reared in the three treatments exhibited varying proximate composition except for the ash content as shown in Table 4.5. The dry matter, protein, lipid and fibre content in the three water qualities varied significantly (P<0.05). Tilapia fish reared in maturation pond water demonstrated the highest content of fibre at 0.29% and protein at 23.10%. The tap water reared tilapia recorded the highest lipid content (1.04%) while the MBR water reared tilapia had the lowest lipid content (0.74%). The results showed that the fish achieved the highest content of dry matter in the maturation pond water. The feed proximate composition (% dry weight basis (dwb)) is presented in Table 4.4 below. The first diet is mash made of lake shrimp only and therefore, the high protein content (62.16%) compared to the pellets (33.09%) which have varied sources of protein. In addition, the fibre content was highest in the pellets (28.36%) compared to the low level in the mash (2.32%). The mash was used in the first 4 weeks of the experiment period and the pellets for the following 20 weeks.

Table 4.4: % Proximate Composition of Feed (Mash And Pellets)

^{a}Proximate composition (%)		
	Mash	**Pellets**
Dry matter	91.00±0.10^{a}	94.15±0.06^{b}
Crude protein	62.16±1.26^{a}	33.09±0.34^{b}
Crude lipids	6.23±0.05^{a}	5.47±0.19^{b}
Crude ash	20.27±0.21^{a}	8.91±0.05^{b}
Crude fibre	2.32±0.48^{a}	28.36±1.17^{b}
NFE^{b}	0.02±0.10^{a}	18.32±0.06^{b}

^{a} Proximate values are the means ± S.E.

^{b} NFE; Nitrogen free extract=100- (moisture content +crude protein +crude lipids +crude ash +fibre)

Table 4.5: % Proximate Composition of Fish Muscle

Treatment	**Dry matter**	**Crude protein**	**Crude lipid**	**Crude ash**	**Crude fibre**
MBR	24.38±0.28^{ab}	22.25±0.27 ^{ab}	0.74±0.01^{c}	1.22±0.08 ^{a}	0.17±0.01 ^{b}
MP	25.35±0.35^{a}	23.10±0.30 ^{a}	0.86±0.01 ^{b}	1.10±0.05 ^{a}	0.29±0.03 ^{a}
TW	23.60±0.58^{b}	21.38±0.51 ^{b}	1.04±0.03 ^{a}	1.01±0.07 ^{a}	0.16±0.01 ^{b}
P-Value	0.07	0.05	0.00	0.17	0.00

Data are expressed as the mean± SE. n=3. Different superscript letters in the same column are significantly different (p<0.05). MBR, membrane bioreactor treated water; MP, maturation pond water; TW, tap water

4.5.4 Minerals and Heavy Metals in the Fish Muscle

The comparison of mineral and heavy metal concentration in the tilapia fish muscle in the three treatments are shown in Figure 4.3. All the treatments exhibited high levels of Ca and low Mg levels in the macro minerals category. Significant difference (P<0.05) was observed for Na, Mg, Zn and Fe among the treatments. The concentration of Mn, Ca and K did not show clear variation between the MBR and the maturation pond treatments. For the relative abundance of the examined minerals, the sequence of concentration in the fish muscle was Ca > K > Na > Mg > Fe > Zn > Mn. There was a significant difference (P < 0.05) in the heavy metal composition of Nile tilapia muscle, with Pb content being the highest (0.50 mg/100 g) from the MBR samples. Analysed Pb, Cu and Cr were more or less similar in the water samples of the MBR and maturation pond. In the case of the control, the heavy metals were below levels of detection. Compared to the water samples, the feed was the major source of contamination, with the content of Pb, Cu and Cr at 0.36

mg/100 g, 1.75 mg/100 g and 0.50 mg/100 g respectively. The analysis showed that the fish samples from all the three water treatments didn't contain Cadmium metal.

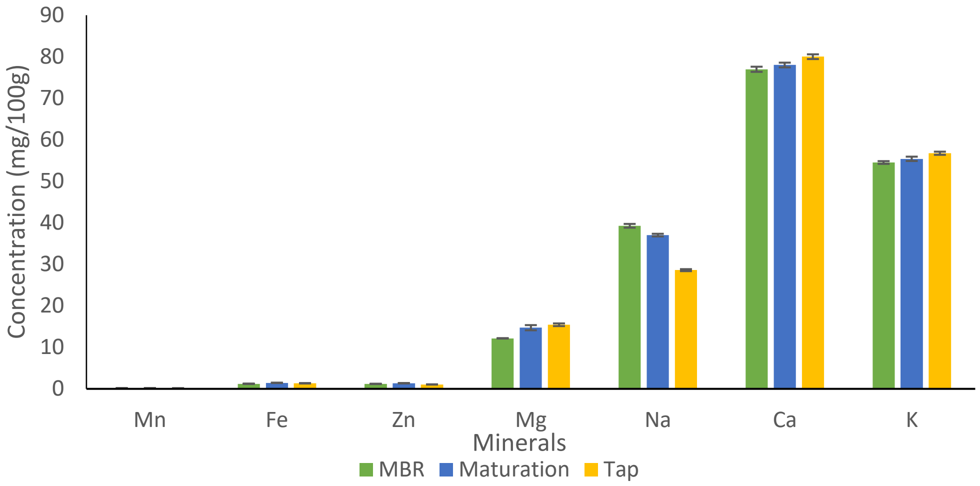

Figure 4.3: Minerals Concentration in the Fish Muscle from the Different Water Quality (MBR, Membrane Bioreactor Treated Water; MP, Maturation Pond Water; TW, Tap Water)

Table 4.6: Comparative Analysis of the Heavy Metal Concentration in the Feed, Water and the Fish Muscle

		Heavy metal Concentration (mg/100 g)			
Treatment		**Pb**	**Cu**	**Cr**	**Cd**
MBR	Fish	0.50±0.01	0.11±0.06	0.25±0.01	ND
	Water	0.07±0.00	0.01±0.00	0.06±0.00	ND
MP	Fish	0.46±0.02	0.18±0.04	0.34±0.04	ND
	Water	0.06±0.00	0.03±0.00	0.06±0.00	ND
TP	Fish	0.37±0.00	0.09±0.03	0.17±0.02	ND
	Water	ND	ND	ND	ND
Feed		0.38±0.01	1.75±0.01	0.50±0.01	ND

Values are the means ± SE. *n* = 3. Abbreviations: MBR, membrane bioreactor treated water; MP, maturation pond water; TW, tap water; ND, not detected

4.5.5 Microbiological Analysis of Fish Muscle

Table 4.7 compares the microbial quality of the fish sampled at harvest when reared in MBR treated water, maturation pond water and tap water (control). Comparatively high levels of total viable counts up to 10^4 CFU/g were found in the fish reared in the maturation pond water than the corresponding MBR. No significant difference ($p>0.05$) was found in the microbial counts, Lactic acid bacteria and Enterobacteriaceae in the fish reared in the MBR and the tap water. Except for fish reared in tap water, *Salmonella* and *Escherichia coli* were detected in the raw fish samples reared in the MBR and maturation pond water, and the *Salmonella* was above the Maximum Permissible Limit for fresh water fish in 25g sample (East African standard, 2000). Fungal growth was observed in raw fish samples reared in the MBR (3.9×10^3) and maturation pond water (4.0×10^3).

Table 4.7: Microbial Counts of the Fish Muscle

	Microbial counts			
Treatment	**Total viable count**	**Lactic acid bacteria**	**Enterobacteriaceae**	**Yeast and molds**
MBR	$5.10 \pm 0.60 \times 10^3$	$4.60 \pm 0.07 \times 10^3$	$5.50 \pm 0.02 \times 10^3$	$3.90 \pm 0.20 \times 10^3$
MP	$1.10 \pm 0.01 \times 10^4$	$7.20 \pm 0.30 \times 10^3$	$6.90 \pm 0.07 \times 10^3$	$4.00 \pm 0.40 \times 10^3$
TW	$1.70 \pm 0.07 \times 10^3$	$4.80 \pm 0.04 \times 10^3$	$5.30 \pm 0.05 \times 10^3$	ND

4.6 Discussion

In fish farming, the quality of water used is important in determining yields and survival of the fish as the fish involuntarily interacts with its culture environment (Ibrahem, 2015). The quality of water for use in rearing fish need to meet specifics standards for the survival of the fish, to obtain high yield and even for the safety of the consumer. In a stressful environment (poor water quality), fish tend to expend more energy to cope with the stressor and therefore the weight gain is less in comparison to fish in good quality water (Lugert *et al.*, 2016). The present study shows that both treatments (MBR and maturation pond) meet the FAO recommended limits for water for use in aquaculture. However, both treatments were significantly different compared to the control. MBR treatment showed lower levels of BOD, COD, NO_3 and NH_4 than the maturation pond. The variation in the quality could be attributed to difference in treatment techniques; the MBR employs both filtration and bioreactor while the maturation ponds are based on oxidation. In addition, the MBR system is fitted with UV treatment point to reduce the microbial load. Therefore, there appears to be room to further improve the maturation pond water by adding two more steps: filtration and UV treatment step. The findings from the study are in agreement with Muralikrishna & Manickam (2017) who reported that the effluent at the outlet of maturation ponds could reduce ammonia and BOD levels of about 10-15 mg/l and 5 mg/l respectively which is comparative to our findings. The ammonia levels in this experiment were even lower at 2.07 mg/l.

The Nile tilapia fish was used in this study because it is well adapted and tolerant to a wide range of environmental conditions and has a high resistance to diseases and infections (Ndiwa *et al*., 2014; Ng & Romano, 2013). Low survival rates (76.67%) of the fish were experienced in the maturation pond due to deaths resulting from infections such as eye abnormalities (corneal cloudiness or opacity). Several studies have identified *Streptococcus* infection as major cause of this abnormalities in fish especially with low water quality for aquaculture (Amal *et al.*, 2015; Siti-Zahrah *et al*., 2008). By introducing a filtration step and sterilizing the water with UV light before using it in fish ponds, the mortality rate in maturation ponds can be decreased as demonstrated in the MBR treatment. Rearing fish in the MBR treated water and maturation pond water had positive effect on the final weight, FCR and CF. Similar results were reported by Osman & El-Khateeb, (2016) for tilapia when reared in treated water and canal water where they found that the weight increased from 8.7 to 236.5 g and 8.2 to 384.5 g when reared in raw canal water and treated water (combined sand filter and activated carbon) respectively.

The nutritional profile of tilapia fish is influenced by its diet, its surrounding environment and its ability to convert its feed into the different nutrients (Shearer, 1994). The results in this study demonstrated that the water quality affected the proximate composition of tilapia except for the ash content. MBR treated water performed better than the maturation pond water in terms of the protein, lipid and fibre composition. In a study to investigate whether the proximate composition of tilapia reared in pond water differ from that from the lake; the authors reported better performance in the lake fish (Desta *et al*., 2019). Their findings on the crude ash results (1.51-1.89%) are comparable to those in the present study (1.01-1.22%).

As fish interact involuntarily with its environment it draws components from the water through bioaccumulation. However, uptake of heavy metals is not exclusively by water exposure but depends also on the diet and feeding mode (Orata & Birgen, 2016). In the present study, the levels of heavy metals in the feed are included to substantiate how diet affects the overall composition of the fish. The high levels of these metals in the fish could be due to the inclusion of aquatic-based ingredients like fish meal and lake shrimp hence passing on the heavy metals through biomagnification along the food chain. Heavy metals in fish represent a threat not only to the fish themselves but also to the health of human beings (Vieira *et al*., 2011; Hill *et al.*, 2005). From our results copper was the major contaminant in the feed with 1.75 mg/100 g while lead bioaccumulation was highest in the fish muscle at 0.50 mg/100 g in the MBR treatment. The lead level in the fish muscle was above the Codex Alimentarius Commission maximum recommended safe levels of 0.03 mg/100 g in the fish, posing a potential risk for human health if consumed (CAC, 2015). Several studies have reported unsafe levels of lead in the muscle of fish caught in inland waters like rivers (Junejo *et al*., 2019; Jooste *et al*., 2015; Nevárez *et al*., 2015; Addo-Bediako *et al*., 2014). These findings suggest that fish caught in freshwaters may pose a risk of contamination with heavy metals just like fish reared in treated wastewater. The heavy metal content in both the MBR treated water and maturation pond did not meet the permissible levels for discharge in the environment as set in the Kenyan legislation. The maximum limits for Cu, Cr and Pb are 1.0, 2.0 and 0.01 mg/l respectively (NEMA, 2006). The high levels of the heavy metals in the treated water could be attributed to industrial waste present in the wastewater discharged into the treatment plant and the persistent nature of heavy metals (Duruibe *et al*., 2007). In addition, this shows that fish can be good bioindicator of pollution in the environment. A previous study by Orata & Birgen, (2016) carried out on the same site as our study did not detect Cd as was the case with our results. Furthermore, studies on some freshwater fish species did not also detect Cd (Korkmaz *et al.*, 2017; Jooste *et al.*, 2015) and indicated a lower bioaccumulation in the fish muscle (Ibrahim *et al*., 2018; El-Moselhy *et al*., 2014).

In low concentrations, certain minerals such as manganese, zinc, copper, iron, and magnesium are essential and have recognized role in the metabolism of aquatic organisms (Sheppard, 2001). These minerals are actively regulated through the process of bile elimination and their concentration in the fish muscle is maintained at certain levels depending on the fish need to maintain homeostasis (Murugan *et al*., 2008; Widianarko *et al*., 2000). However, there exist international legislation on maximum permissible levels for Zn (4 mg/100 g) and Mn (0.25 mg/100 g) in fish (FAO, 1983). The legal limits for these minerals were not exceeded in our present study with Zn ranging from 1.06 to 1.35 mg/100 g and Mn from 0.13 to 1.15 mg/100 g. The concentration of the essential minerals Mg, Na, Zn and Fe in the fish muscle showed variation in the maturation pond and MBR treated water. Our study showed that the muscle of the fish from the maturation pond water contained relatively higher concentration of the micro-minerals Mn, Fe and Zn while the muscle of the fish from the MBR water had higher concentrations of the macro-minerals, Na and Ca. This accumulation pattern is likely related to their feeding; the presence of suspended green algae in the maturation water that could have added to the diet of pellet feeds. A study by Santhakumaran *et al*. (2020), showed that algae are a valuable mineral source and some species contain Fe ranging from 0.08 to 10.21 mg/g while Zn content can range from 0.01 to 1.10 mg/g. The bioavailability of the minerals from ingested food can be influenced by the ingestion rate, the nature of the food and the effectiveness of food assimilation (Marengo *et al*., 2018). The micro-minerals concentration in the maturation pond and the MBR were listed in the order Fe > Zn > Mn. A similar trend was reported by Islam et al. (2021) in tilapia collected from the wild and cultured (pond, *gher* and cage) in Bangladesh. Besides, Jim *et al.* (2017) reported similar range of concentration of Zn in Nile tilapia from three lakes in Zimbabwe. Our results for the macro-minerals in the fish from the three water qualities were in the same order of magnitude (Ca > K > Na > Mg), compared to those reported for *Oreochromis* *mossambicus* (Ullah *et al.,* 2022). However, these findings did not match with those found in literature for some fresh water species (Guerra-García *et al*., 2023; Islam *et al*., 2021; Jim *et al*., 2017), but in all studies K > Na. Our findings therefore suggest that MBR and maturation pond treated water does not affect the capacity of fish to absorb and assimilate these trace minerals from water and diet.

Water is a suitable environment for the growth of microorganisms which make their habitat within the bodies of aquatic animals that live within this environment. Microbial evaluation of fish gives information about the hygienic status and possible contamination of aquatic environments, such as lakes, rivers, ponds, and fish farms (Novoslavskij *et al*., 2016). Bacterial colonization of fish muscle is limited. However, contamination of the fish muscles is also possible when immunological resistance is compromised (Guzmán *et al*., 2004). In the present study, both the fish muscle and skin were used to assess the microbial quality as it is generally expected that bacteria found on fish skin are the same as those found in the contaminated water (Novoslavskij *et al*., 2016). The highest bacterial loads (total viable count, lactic acid bacteria and Enterobacteriaceae) were observed in the fish reared in the maturation pond. These results agree with those obtained by Lan *et al*. (2007), for tilapia reared in wastewater fed ponds. The low microbial counts in the MBR reared fish indicated low bacteria penetration into the fish flesh. Buras *et al*. (1987) developed a classification scheme for the quality (presence of bacteria) of fish reared in wastewater, “for bacteria that grow on nutrient agar the bacteriological quality should be expressed as: 0−10 bacteria ml^{-1}, very good; 10−30 bacteria ml^{-1}, medium quality; more than 50 bacteria ml^{-1}, not acceptable”. Thus, based on this ordering, the tilapia fish from the MBR treated wastewater were “very good”, but not acceptable for human consumption on the basis of the presence of pathogenic bacteria *Salmonella* and *Escherichia coli*. In addition, the findings from the present study indicate that the fish from the MBR and maturation pond water did not exceed the set limits by the International Commission on Microbiological Specifications for Foods, for total viable plate counts (10^7 CFU/g) in fresh and frozen fish (ICMS, 2008).

The Enterobacteriaceae contamination was found in low amounts in the three treatment samples in this investigation, but the concentration was unacceptable when compared to the limit of 10^2 CFU/g for fresh and frozen fish (Popovic *et al*., 2010). In summary, from the results obtained, the existence of pathogenic bacteria in the MBR treated and maturation pond water was evidenced by the fish muscle microbial quality. Bacterial pathogens, *Salmonella* and *Escherichia coli* were detected in the fish samples based on the colonies formed in agar plates. This suggests that by using MBR technology, the contamination by pathogenic bacteria in wastewater is not effectively reduced similar to the maturation pond. In addition, there is possible presence of drug resistant microorganisms in the wastewater and therefore the inability of the UV installed in the MBR to eliminate all the microorganism. UV radiation wavelength of 200–280 nm is required to damage the DNA or RNA of the drug-resistant microorganism (Fan *et al.*, 2017). Furthermore, the presence of *E. coli* in water bodies like lakes and rivers has been reported (Manjengwa *et al*., 2019; Xue *et al*., 2018; Prasanna *et al*., 2012), suggesting that the water quality of natural water bodies could be compromised just as in treated wastewater.

4.7 Conclusions

From this study, it is clear that water quality plays an important role in the growth of fish altering how they utilize feed nutrients with the exception of trace minerals. The use of MBR was found to be performing satisfactorily in achieving the standards set for water for aquaculture, indicating the technology is viable for use in aquaculture. In addition, the study showed variation in the growth of tilapia in MBR-treated water and maturation pond, indicating better growth in MBR, hence suggesting that MBR technology can be used to promote productivity in fish farming. However, an additional polishing step has to be considered to remove the heavy metals in the MBR-treated water. A nanofiltration membrane is recommended to further upgrade the water quality by removing the divalent ions. Although fish can bioaccumulate heavy metals from the water, feeds also play a role in the final quality of the product and regular monitoring of feeds and fish habitat is recommended. Both the maturation pond and MBR waters produced fish with total viable counts and lactic acid bacteria that were at levels acceptable for human consumption. However, the presence of pathogenic bacteria E. *coli* made them unsafe for consumption.

CHAPTER FIVE

WEIGHT PREDICTION MODEL OF NILE TILAPIA USING EMPIRICAL KNOWLEDGE

Abstract

Fish weight is a fundamental parameter for commercial decision making in aquaculture. It is vital for determining the time to market and fish feeding practices (including feed quantities) at the various growth stages of fish. Existing fish weight measurement methods during rearing are generally manual, time consuming, impractical and often causes injuries to fish. Therefore, to improve this process, establishing a weight prediction model based on empirical knowledge extraction of historical production data is proposed. Production data set of Nile tilapia (*Oreochromis niloticus*) from two different rearing scenarios was used. In the first dataset, Nile tilapia was reared in three different water qualities (tap water, membrane bioreactor treated and stabilization pond treatment) while in the second dataset the fish were reared on three different diet containing different seaweed levels (0%, 5% and 10%). In both cases, weight was measured manually fortnightly. A scatter plot to visualize the relationship between fish weight and feed intake over time was first plotted. Secondly, the fish production data was trained to obtain the coefficients of determination. Subsequently, the fish's weight was estimated by a trained model based on regression learning. Linear regression was developed to determine the best models for weight estimation. There was a significant correlation between feed intake, culture period and fish weight, which can be used to estimate the weight of fish. The results showed that the established models achieved root mean square errors (RMSE) of the range of 0.0038 to 0.0043 for the different water environment and 0.0079 to 0.0084 for the different diets. The water quality models had high coefficient of determination, R^2 (0.98-0.99), indicating that the water quality model can more accurately predict fish weight than the diet model. Furthermore, when compared with the actual weights, the model predicted weights were very close, indicating that the proposed method can accurately estimate the fish weight.

5.1 Introduction

Growth of the aquaculture sector is driven by adoption of novel scientifically proven practices and technologies. Due to development of the sector, there has been rapid shift from manual and mechanized systems to automation and intelligent, unmanned equipment with the potential of producing higher output and turnover (Wu *et al*., 2022). Nevertheless, there are limitations to the use of precision aquaculture technology such as the employment of a large number of sensors, like integrated multiparametric probes used for water quality analysis and computer vision systems for fish traits analysis, making the initial operational and maintenance costs quite substantial (Zhang *et al*., 2020c; Tonachella *et al*., 2022). On the other hand, smart aquaculture that employs machine learning by using trained algorithm models to recognize and learn traits from the data such as size, weight, length, grading, disease detection, and species classification, the cost is moderate, and the practicability is validated, providing a feasible approach for assessing fish welfare in aquaculture (Vo *et al.,* 2021).

Machine learning helps in solving problems that exist based on the algorithms and learning data to create mathematical models that ameliorate the performance of a system. Models are crucial tools for tackling production optimization and sustainability issues associated with aquaculture growth and development on a global, regional, and farm scale. Mathematical modeling uses equations and parameters that have been fitted statistically, to describe the dynamic processes of a system (Chary *et al*., 2022), and can predict a variety of outputs such as fish weight, waste and by-products. For fish growth modelling, the main approaches are mechanistic and empirical modelling. Mechanistic also called theoretical models are based on theories about the processes that cause the phenomena of growth and include nutrient based models and dynamic energy budget models (Dumas *et al*., 2010; Stavrakidis-Zachou *et al*., 2019; Chary *et al*., 2022). In contrast, empirical models depict observed patterns using parameters and equations that are statistically fitted through regression, without explicitly describing the core processes (Reid *et al*., 2020). However, mechanistic models for practical aquaculture production have complex forms and comprise a large number of parameters that may be difficult to determine accurately making their practical usage limited. Therefore, for new fish species and environments, empirical models are preferred because they require fewer input parameters, are easier to calibrate and quicker to run (Li *et al*., 2021). Data driven modelling or empirical modelling approach has an extensive application in various scenarios in aquaculture, such as fish weight estimation, water quality monitoring and fish counting (Yang *et al*., 2019; Zhang *et al.,* 2020a; Zhang *et al*., 2020b; Yu *et al*., 2022).

Estimation of the weight of cultured fish is an important practice in aquaculture. The weight of fish plays a critical role in optimizing daily feeding to avoid under- or overfeeding, control stocking densities, antibiotic dose and determining the optimal harvest time (Li *et al*., 2020). It also reveals important details about the maturity level of fish, fish quality at harvest and the commercial value of the fish (Lopes *et al*., 2017). Conventional weighing of fish, involves periodically capturing fish and weighing them individually. This can be time consuming, labor intensive, and costly, hindering the estimation of large number of fish (Romero *et al*., 2015). In addition, the manual handling during weighing, subjects' fish to stress and may lead to growth retardation, nerve damage, and in severe cases death (Abinaya *et al.*, 2022). Even during postharvest, manual handling of fish for weight determination degrades its texture and quality. Therefore, this highlights the need for automatic, accurate, effective and non-invasive method for weight estimation such as mathematical models.

Fish weight prediction model can provide real-time fish weight data with high accuracy rate to optimise feeding decision and cut down on production cost. Until now, the feeding decision of fish has been mainly based on the appetite of the fish and their behaviour (Wu *et al*., 2015; Martins *et al*., 2012). However, in fish production practice, the common method is to determine feeding amount based on fish weight. This is due to the fact that feed demand in fish is varied in their different growth stages, as a result of the difference in individual fish size. Therefore, the measurement of fish weight is of great significance for reasonably feeding and can help predict precise feed quantities for fish growth. Therefore, the focus of this study was on the exploration of fish weight prediction model based on the analysis of historical production data. The historical data will be run through forecasting models to anticipate how parameters affect fish production This approach is applied here to Nile tilapia (*Oreochromis niloticus*), a fast-growing aquaculture species, tolerant to a wide range of growth environments, which produces high quality fillet (Abd El-Hack *et al*., 2022).

5.2 Materials and Methods

5.2.1 Experimental Set-Up

The experimental set-up to obtain the production data of Nile tilapia are as described in section 3.2 and 4.2 for the different diets and water quality respectively.

5.3 Building the Weight Prediction Model

Building the fish weight prediction model was implemented as shown in figure 5.1 below.

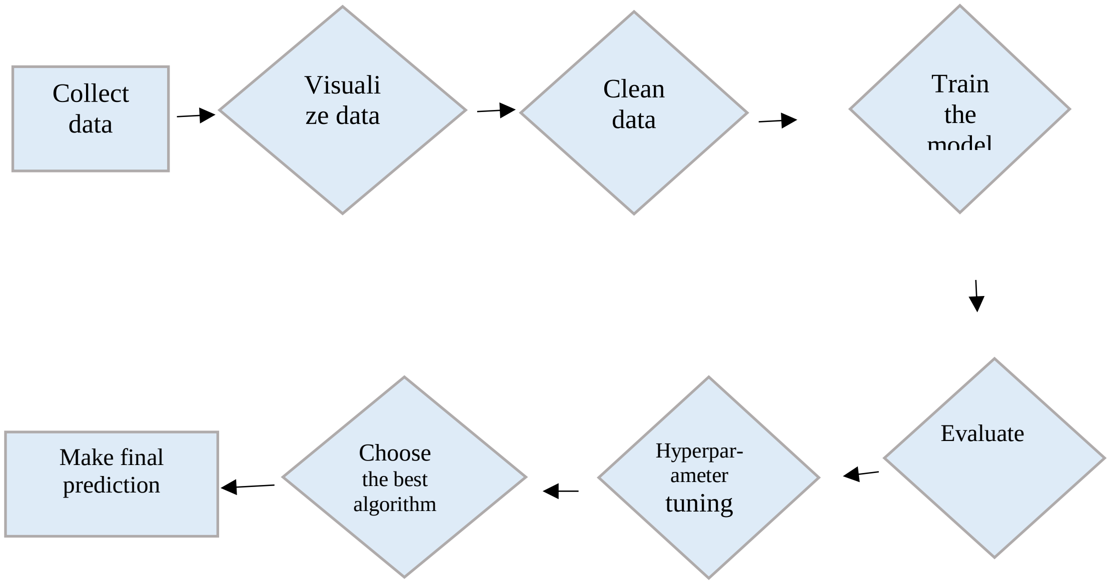

Figure 5.1: Process in Building the Weight Prediction Model

The production data set (feeding amount, fish weight, fish number and culture time) makes the establishment of fish weight prediction model possible. The fish weight prediction model is expressed by several input variables as shown by the equation 5.1 adapted from Li *et al.* (2021);

$BW_t = f_g(BW_o, FA, t, N, P_g)$……………………………………………………...Eq. 5.1

Where; BW_O is the initial fish weight

FA is the total feeding amount
N is the cultured fish number
t is the culture time in weeks
P_g is the parameter of function f_g
BW_t is the fish weight at any given point in time

Since the required weight is for individual fish, the equation can be simplified by reducing the number of parameters to determine the relationship of f_g. Therefore, the number of input variables can be reduced to two as shown in the equation 5.2 below:

$BW_t = a_g \times IFA + b_g \times t + c_g$ ……………………………………………….. Eq. 5.2

Where; IFA is the average feeding amount for individual fish and a_g, b_g and c_g are the parameter functions of f_g. The root mean square error (RMSE) between measurement and model fitting was used to assess model goodness-of-fit.

5.4 Statistical Analysis

The analysis of regression and weight prediction was performed using a multiple linear regression approach. Variable coefficients and root mean square error were obtained from the regression models. The statistical modelling was performed using R, version 4.0.2 Software.

5.5 Results

5.5.1 Production Data

The data on production of Nile tilapia are shown in Tables 5.1 and 5.2. The first production dataset was obtained over a period of 24 weeks while the second dataset was obtained over a period of 12 weeks and this was based on the variation of the initial weights of the two groups of fish. The initial weight of the Nile tilapia was 0.15 g and 31.11 g for the different water qualities and the diet respectively. In addition, the final weights were relatively close in the two-treatment ranging from 50.48 g to 62.61 g.

Table 5.1: Production Dataset of Nile Tilapia in Different Water Quality

	Treatment					
	MBR		**Tap**		**Maturation pond**	
Time (weeks)	**Final weight (g)**	**Feed intake (g)**	**Final weight (g)**	**Feed intake (g)**	**Final weight (g)**	**Feed intake (g)**
0	0.15	0.08	0.15	0.08	0.15	0.085
2	0.52	0.29	0.46	0.25	0.35	0.20
4	1.62	0.91	1.78	0.99	1.42	0.79
6	2.76	1.55	2.85	1.60	3.04	1.70
8	4.67	2.61	4.78	2.67	4.95	2.77
10	10.10	5.65	9.189	5.15	11.02	6.17
12	15.77	8.83	14.31	8.01	14.45	8.09
14	22.7	12.76	18.86	10.56	20.59	11.53
16	25.95	14.53	23.09	12.93	22.87	12.81
18	26.54	14.86	24.61	13.78	24.71	13.84
20	32.67	18.30	32.63	18.27	29.68	16.62
22	45.29	25.36	50.45	28.25	42.72	23.92
24	57.84	32.39	62.55	35.03	50.48	28.27

Data are expressed as the mean. n=3. Number of fish in each tank is 30. MBR, membrane bioreactor treated water; MP, maturation pond water; TW, tap water

Table 5.2: Production Dataset of Nile Tilapia Feed Diets With Different Inclusion Levels of Seaweed

	Treatment					
	Diet 1		**Diet 2**		**D3**	
Time (weeks)	**Final weight (g)**	**Feed intake (g)**	**Final weight (g)**	**Feed intake (g)**	**Final weight (g)**	**Feed intake (g)**
0	31.11	17.42	31.11	17.42	31.11	17.42
2	41.98	23.51	42.47	23.78	43.17	24.18
4	45.88	25.70	45.64	25.56	46.15	25.84
6	48.88	27.37	49.48	27.71	49.50	27.72
8	55.56	31.11	54.62	30.59	59.54	33.34
10	58.82	32.94	61.21	34.28	64.21	35.96
12	59.19	33.15	62.61	35.06	66.12	37.02

Data are expressed as the mean; n = 3. Number of fish in each tank is 20. Diet 1, 0% inclusion; Diet 2, 5% inclusion; and Diet 3, 10% inclusion of the seaweed

5.5.2 Model Calibration Results

The visualization of the data is as shown in Figure 5.2. The weight of fish had a positive correlation with the feed intake over time. The presented model was calibrated using both measurements of feed intake and culture period from the growth trials. The regression coefficient, a_g for feed intake was similar (1.786) in all the models. For the water quality, the maturation pond had the lowest rmse (0.0038) while for the diets, D1 had the lowest rmse (0.0079). The correlation of the weight and feed intake based on linear regression had an R^2 values of 0.98-0.99 and 0.95 for the water quality models and diet variation models respectively. The model prediction and true value are close to each other as shown in figure 5.3 and 5.4 for the water quality and diets respectively.

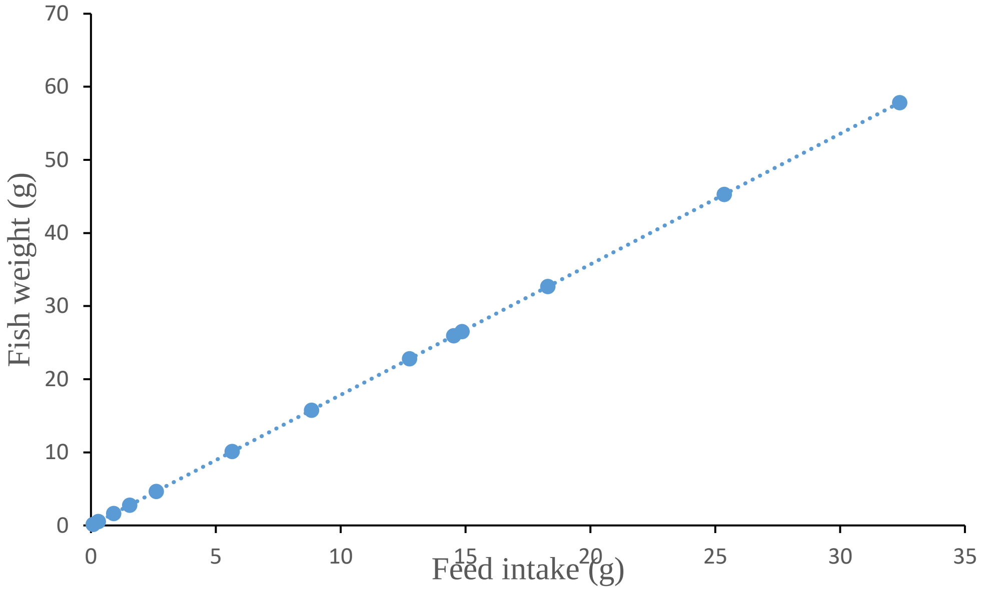

Figure 5.2: Relationship between Weight and Feed Intake of Nile Tilapia

Table 5.3: Calibration Results for the Fish Weight Prediction Model

Treatment		**Parameters**			
Water quality	**a_g,**	**b_g**	**c_g**	**RMSE**	**R^2**
MBR	1.786	-3.927e-10	2.076e-09	0.0041	0.98
Tap	1.786	-7.352e-10	2.291e-09	0.0043	0.98
Maturation pond	1.786	5.082e-10	-1.923e-09	0.0038	0.99
Diet					
D1	1.786	1.692e-09	2.501e-08	0.0079	0.95
D2	1.786	-2.204e-09	-2.234e-08	0.0081	0.95
D3	1.786	4.551e-09	4.382e-08	0.0084	0.95

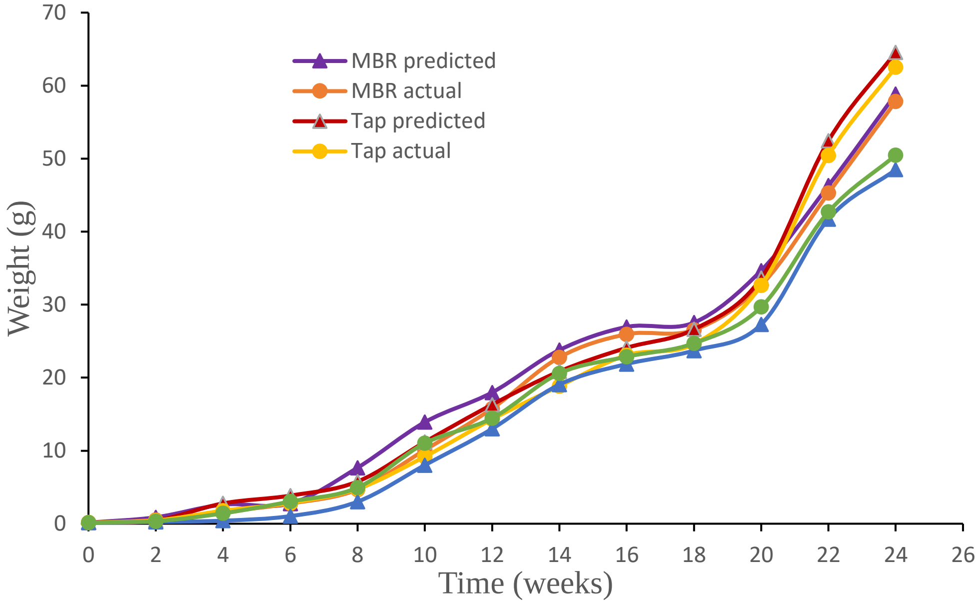

Figure 5.3: Comparison Results of Nile Tilapia Weight Prediction for Different Water Quality

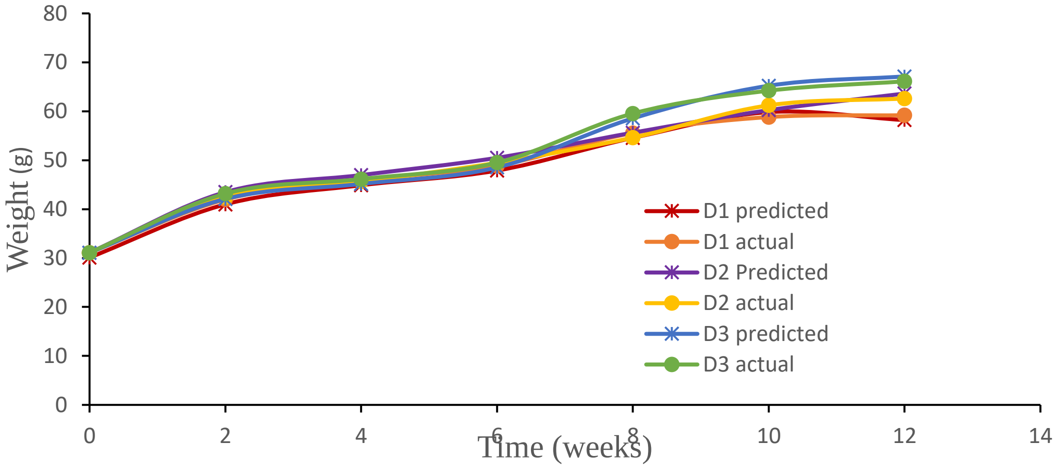

Figure 5.4: Comparison Results of Nile Tilapia Weight Prediction for Different Diets

5.6 Discussion

Regression learning is a machine learning approach that has been broadly used to develop models for the prediction of body weight of fish and animals like cows and sheep (Tengtrairat *et al*., 2022). Weight prediction using regression learning requires animal features that are significantly related to weight to be used for learning. In the present study, the Nile tilapia weight prediction model was trained by learning data consisting of the fish feed intake, fish weight and culture period, in view of the fact that calibration equations for mass estimation have been found to be dependent upon fish weight group and level of ration consumed by the fish (Zion *et al.*, 2007). The feed intake and fish weight were significantly and positively correlated when measured over a period of time. Therefore, the higher the quantity of feed intake, the faster the fish gains weight. Froese *et al*. (2014) deployed a measure of fish weight based on a single feature, length while Ault & Luo, (2013) proposed a mechanical model to estimate Atlantic tarpon mass using multiple fish features, fork length and dorsal girth. However, multifactor regression equations have been shown to predict the weight of individual fish more accurately (98%), than single factor regression equations (Zion, 2012).

The weight function parameters (b_g and c_g) had relatively low influence on model variables. This indicates that little errors in the determination of the normalized maintenance. Fish weight is directly related to the feeding amount, and is therefore used to determine daily fish feed intake successfully in practice (Papandroulakis *et al*., 2000). In the present study, the daily feeding quantity was determined fortnightly based on the fish weight measurement. Therefore, for the model development the data from these historical fish production dataset was analysed to establish a Nile tilapia weight prediction model.

Statistical indicators such coefficient of determination (R^2) and the root mean square error (RMSE) were used to test the model's goodness-of-fit. Large values of R^2 combined with low RMSE values, show the best model (Hernández *et al*., 2003). The best overall weight predictive model for the water quality achieved an R^2 of 0.99 and RMSE of 0.0038, while for the different diets the R^2 was 0.95 and RMSE of 0.0079. As the result shows, the water quality variation model yields the best results compared to the diet variation model. Visualizing the prediction and actual weights helps evaluate coefficient of determination to confirm the model fitness. Therefore, in the visualization of this study, the prediction value showed a close resemblance to the actual weight indicating the correctness of the proposed model. Related to this approach, Li *et al*. (2021) conducted an experiment using spotted knifejaw fish (*Oplegnathus punctatus*) to develop weight prediction model established by regression analysis methods to obtain weight prediction from empirical knowledge. The RMSE obtained from the three scenarios in their experiment ranged from 0.0042 to 0.0498, which were in good agreement with results from our study. A lower accuracy performance of R^2 score of 0.62 was obtained in the weight prediction of sheep using similar regression as the present study, multiple linear regression analysis in addition to a generalized linear model (Hussain *et al*., 2019).

5.7 Conclusion

This work illustrates how models can be powerful tools for predicting the weight of Nile tilapia in aquaculture production as the models fitted the historical production data well. The fish growth model developed here explicitly identified the optimal feed quantity as a function of fish weight and culturing time and, therefore, is useful for minimizing the left-over/portion of uneaten food. Furthermore, this models could be tools for monitoring fish growth, health, product quality and production efficiency as seen in the deviations between the two scenarios (water quality variation and diet variation). Although the study focused on Nile tilapia, the same approach can be applied to other fish species after the model parameters are recalibration.

Additionally, creating a database can allow historical production data to be run through forecasting models to anticipate how parameters affect fish production. As such, to predict fish weight, feeding quantities need to be known since they provide important information on input to the prediction of fish weight.

CHAPTER SIX

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

In order to support and grow aquaculture, there is need to address the quality of feed used and water safety to boost productivity and protect the health of the consumer. Aquaculture is on upward growth trend and sustainability of the resources used is important. The major ingredient in fish feeds is fish oil but its use has gotten to unsustainable level due to competition from other uses such as supplements and food. Fish oil is a source of lipid in feeds and therefore alternative sources are required. The nutritional profiling of seaweeds based on literature showed that brown seaweeds are higher in lipid content compared to their green and red counterpart and can be used as feed ingredients in fish feed.

Seaweeds have a huge potential for use in aqua feed. They are rich in nutrients like carbohydrates, protein, lipids, minerals, vitamins and bioactive compounds. The brown seaweeds (Phaeophyta) have been shown to have high levels of polyunsaturated fatty acids (PUFA) that are important in fish growth. The total lipids in the brown seaweed species, sargassum can rise up to 15% dry matter, with 40% of the total fatty acids being the omega 3 PUFAs. Variation in lipid content is influenced by species, environmental conditions, season of harvest and geographical location. In cold seasons, production of PUFAs is high and therefore, cold environmental conditions can be harnessed to increase lipid production in seaweed. Studies have also demonstrated the beneficial effect of diets supplemented with seaweeds, on the growth, muscle composition and nutrient utilization of a number of finfishes such as tilapia, trout, catfish, and seabass. Low levels of supplementation (≤10%) with seaweeds had improved growth performance compared to high levels, which had detrimental effect on fish growth. Thus, in order to promote growth in fish, seaweeds can be exploited in low quantities for use in aquafeeds.

Rearing Nile tilapia with diets supplemented with the brown seaweed, *Sargassum portieranum* at 10% resulted in fish with higher weight, specific growth rate and survival than the control (unsupplemented). In addition, the muscle fibre, lipid and ash content significantly improved with the supplementation. This demonstrated the positive influence on growth performance and muscle composition of the brown seaweed when included in diets at low levels. Thus, the present findings stress importance of low-level supplementation of fish diets with seaweeds and their potential as fish feed ingredients. Supplementing the fish diets with seaweed did not significantly affect the muscle saturated fatty acids content. In regards to the effect on the fish muscle unsaturated fatty acids, 10% seaweed inclusion improved the omega-3 better than 5% inclusion while the 5% inclusion level improved the MUFAs better than the 10%. This indicates that Nile tilapia utilize and retain different fatty acids at different rates depending on the level of inclusion of the brown seaweed.

Fish interacts with its culture environment and therefore represents an interesting model for risk–benefit assessments of the nature and probability of health effects in humans exposed to contaminants at present and in the future. It is therefore necessary to monitor acceptability of water quality for use in aquaculture and control the dangerous levels of fish contamination both from the water and feed. The present study investigated the effects of rearing Nile tilapia, in wastewater treated using membrane bioreactor (MBR) and compared to oxidation pond treatment. In oxidation pond treatment, the last pond where the water is discharged to the environment is the maturation pond. The water quality parameters tested included dissolved oxygen, pH, biological oxygen demand, chemical oxygen demand, conductivity, nitrates and ammonia. Both methods of wastewater treatment produced water that met the standards set for use in aquaculture according to guidelines by FAO. However, the ammonia content was above the permissible level in the maturation pond water. Nevertheless, the level of ammonia can be reduced by allowing more retention time in the maturation pond to allow for more nitrification of the ammonia to nitrates. These findings proved that MBR can be used to effectively treat wastewater for reuse in aquaculture.

When the Nile tilapia were reared in the MBR treated water, the growth performance (weight, length, survival rate, specific growth rate, feed conversion ratio and condition factor) were significantly higher than in the maturation pond water. Similar feed conversion ratio, condition factor and specific growth rate were observed in the control and the MBR treatment. The use of MBR treated wastewater to rear fish is therefore considered viable and its productivity coincides with that of clean tap water. Apart from the ash content, the biochemical composition of fish reared in the maturation pond and the MBR treated water differed significantly. Fish muscle from the maturation pond had the highest content of dry matter, protein, and fibre, thus suggesting that the water possibly contained dissolved water-borne nutrients that benefitted the fish. In addition, it is possible that the state of the maturation pond water provided better conditions for the specific nutrient accumulation.

The bioaccumulation of heavy metals and minerals in the muscle from the water and feed were investigated to determine the safety of the Nile tilapia for human consumption. The study showed the concentration of the minerals in the fish muscle were in the order Ca > K > Na > Mg > Fe > Zn > Mn. On the other hand, the macrominerals Na and Ca were highest in the fish reared in the MBR treated water while the microminerals Mn, Fe and Zn were higher in the fish reared in the maturation pond water. For the regulated minerals Zn and Mn, they did not exceed the set limits for safety. This showed that fish actively regulate minerals in muscle based on their need to maintain homeostasis and the water quality plays a slightly small part in the process. Wastewater treatments in this study were incapable to eliminate the heavy metals Pb, Cu and Cr to levels that are recommended for discharge in the environment by the National Environmental Management Authority. In addition, no significant difference was observed in the heavy metal content of the MBR and maturation pond water. Thus, to remove heavy metals, a further treatment step such as filtration by reverse osmosis is suggested. The high uptake of the heavy metals by the fish, constituted to high levels that posed health risk for human consumption. Apart from the water, the feed was shown to also contribute to the high levels of the heavy metals in the fish muscle. The findings of this study have shown that although fish bioaccumulates heavy metals from water, feeds also play an important role in the bioaccumulation. Therefore, the safety of fish feeds needs be assessed to promote safety of aquatic food.

This study showed that the bacteria counts in the Nile tilapia fish samples reared in the three treatments (maturation pond, MBR and tap water) were acceptable for human consumption given that the samples complied with reference standards. On the contrary, pathogenic bacteria, *Salmonella* and *Escherichia coli* were present in the fish samples reared in the MBR and maturation pond water. Some pathogenic bacteria can be eliminated through cooking, however their presence in fish poses a health risk to humans especially for people who consume raw or smoked fish. Therefore, using a UV treatment in the MBR at 200-280 nm radiation is suggested to eliminate all the pathogens and particularly the drug resistant bacteria.

For the weight prediction model development, two different environmental scenarios were used. They are the water quality variations (MBR water, maturation pond water and tap water) scenario, and the diet variation scenario, which includes seaweed content variation at 0%, 5 % and 10%. The water quality models achieved high predictive accuracy based on the coefficient of determination, R^2 and root mean square error (RMSE). From this result, it can be deduced that empirical modelling is an efficient tool for estimating fish weight as well as determining feed quantity. Therefore, it is suggested that this method can also be used in fisheries production processes in further work.

6.2 Conclusion

In conclusion;

1. The optimum level of the brown seaweed *Sargassum portieranum* in Nile tilapia diets, to improve growth and muscle composition was 10%, while 5% was optimum for MUFAs and 10% for omega 3. Inclusion of seaweed in tilapia diet improve the growth performance and essential fatty acid composition of the tilapia

and therefore help reduce on the overexploitation of fish oil in feeds and instead shift its use to food.

2. Both wastewater treatments techniques (oxidation ponds and membrane bioreactor) produce water suitable for reuse in aquaculture. However, the MBR was identified as a more promising technique due to better growth performance and productivity of Nile tilapia, which were similar to clean tap water.
3. The model was able to predict the fish body weight accurately without the need of weighing the fish on a scale, speeding up the measurement process since the weight can be measured consistently in the proposed model.

6.3 Recommendations

Recommendations suggested from this study include:

1. The present study recommend the use of seaweeds in aqua feeds at low inclusion levels to promote growth and improve the essential fatty acid composition.
2. Membrane bioreactor effectively treated the wastewater for reuse in aquaculture and it’s recommended that the MBR be used in aquaculture production for treatment of wastewater for rearing fish.
3. Since the membrane bioreactor used in this study had an ultrafiltration membrane, some large molecules like heavy metals passed through. As such, further work is needed using reverse osmosis membrane that is capable of removing divalent and monovalent ions in wastewater.
4. Further research is recommended on seaweeds and membrane bioreactor effects on other finfish species and abalones.
5. Since fish feed play an important role in the quality of harvested fish and fish products, regular assessment of feed is recommended to ensure safety against heavy metals in aquatic products.
6. Further testing of the weight prediction model for Nile tilapia and other fish species is recommended. In addition, modelling can be used in fisheries production processes for efficiency, safety and health of the fish.

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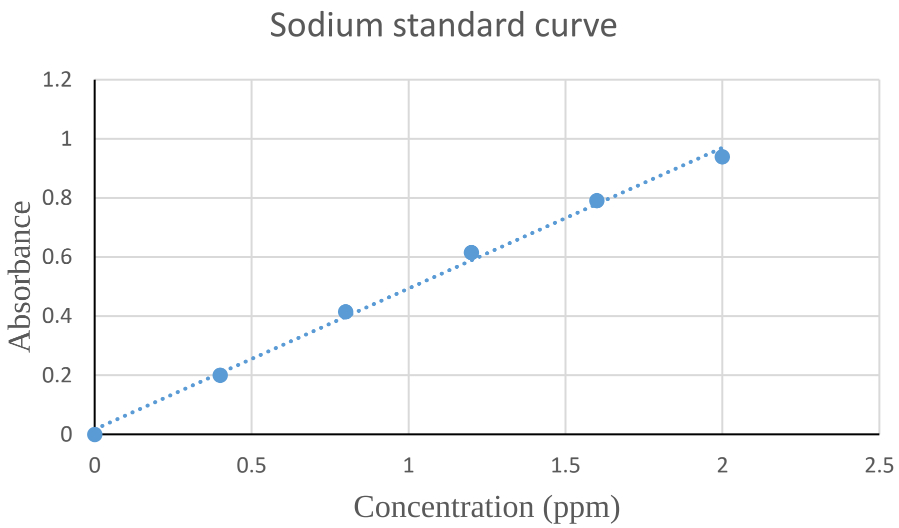
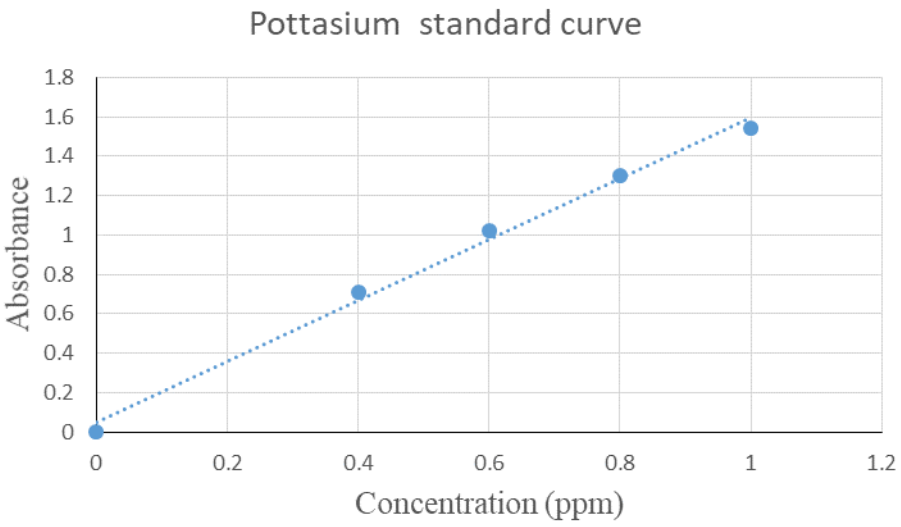
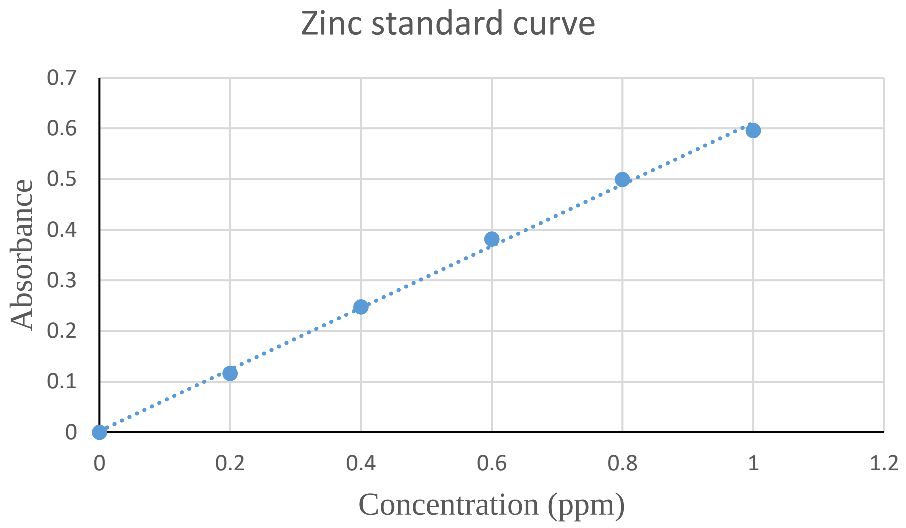
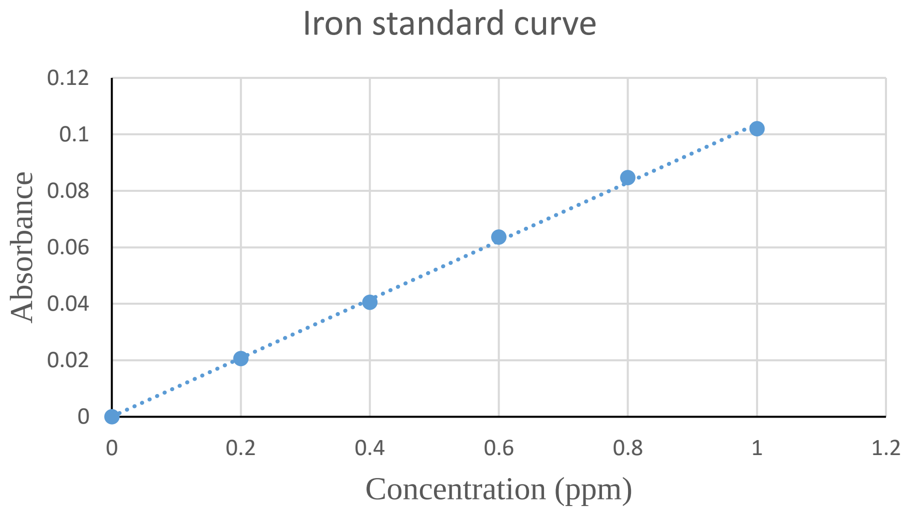
APPENDICES

Appendix I: Minerals Standard Curves






Zinc standard curve
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.2
0.4
0.6
0.8
1
1.2
Concentration (ppm)


Iron standard curve
Absorbance
0.12
0.1
0.08
0.06
0.04
0.02
0
0
0.2
0.4
0.6
0.8
1
1.2
Concentration (ppm)

Appendix II: R codes for Modelling

```
#######READING THE DATA
Hdata<-read.csv("C:/Users/Administrator/Desktop/mwesh.csv")
Hdata
######TREATMENT 1
weight1<-Hdata$final.weight1
weight1
intake1<-Hdata$feed.intake1
intake1
weeks<-Hdata$Week
weeks
ddata<-data.frame(weight1,intake1,weeks)
ddata
# Scatter plot to visualize the relationship between feed intake and fish weight
ggplot(ddata, aes(x = intake1, y = weight1)) +
  geom_point() +
  labs(x = "Feed Intake", y = "Fish Weight") +
  theme_minimal()
# REPRODUCIABILITY
set.seed(123)
# SPLITTING THE DATA
train_size <- 0.8
train_size
train_index <- 1:round(train_size * nrow(ddata))
train_index
train_data <- ddata[train_index, ]
train_data
test_data <- ddata[-train_index, ]
test_data
```

```
#THE LINEAR REGRESSION
lm_model <- lm(weight1 ~ intake1+weeks, data = train_data)
lm_model
# Print the summary of the model
summary(lm_model)
# MAKING PREDICTIONS
predictions <- predict(lm_model, newdata = test_data)
predictions
# ######### (RMSE) FOR PERFORMANCE EVALUATION
rmse <- sqrt(mean((test_data$weight1 - predictions)^2))
rmse
##FORECASTING WEEK 27
week24_weight<- 24
week24_weight
weeks_to_forecast <- 27
weeks_to_forecast
# Prepare the data for prediction for week 27
week27_data <- data.frame(
  intake1 = intake1[weeks == 24],
  weeks = 27 )
week27_data
# Make the prediction for week 27 using the trained linear regression model
predicted_weight_week27 <- predict(lm_model, newdata = week27_data)
predicted_weight_week27
# Forecasted weight at week 27
forecasted_weight_week27 <- week24_weight + predicted_weight_week27
forecasted_weight_week27
```

Appendix III: List of Publications

1. Mwendwa, R., Wawire, M., & Kahenya, P. (2023). Potential for Use of Seaweed as a Fish Feed Ingredient: A Review. Journal of Agricultural Science, 15(2), 96-108.

2. Mwendwa, R., Wawire, M., & Kahenya, P. (2023). Effect of dietary supplementation with seaweed on growth and nutritional quality of Nile tilapia. *Journal of agriculture, science and technology*, *22*(2), 100-116.

3. Mwendwa, R., Wawire, M., Kahenya, P. & Oyoo, E. (2023). Growth Performance and Biochemical Composition of Nile Tilapia Reared in Membrane Bioreactor (MBR) Treated Wastewater. *Journal of agriculture science and technology, 15*(12), 61-73.

4. Mwendwa, R., Wawire, M., & Kahenya, P. (2023).Weight prediction model of Nile tilapia using empirical knowledge. (Under review).