NUTRITIVE VALUE OF *Arthrospira platensis, (*Gomont, 1892*)* AND *Oocystis* sp. AS PROTEIN SOURCES IN NILE TILAPIA FISH FEEDS

MARVIN GAYE MUKOMA

A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Limnology of Egerton University, Kenya

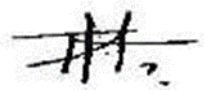
EGERTON UNIVERSITY

APRIL, 2023

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

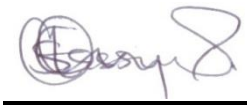
Signature:  Date: 02/04/2023

Marvin Gaye Mukoma

SM18/13710/19

Recommendation

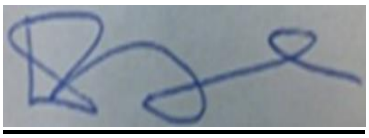
This thesis has been submitted with our approval as university supervisors.

Signature:  Date: 03/04/2023

Dr. Steve Oduor

Department of Biological Sciences

Egerton University

Signature:  Date: 03/04/2023

Dr. Elick Otachi

Department of Biological Sciences

Egerton University

DEDICATION

To my mom, Ms. Joy Olumala Sande, thank you for being the pillar of strength in my life, for pushing me to achieve my dreams and guiding me in every step of the way. My heart can't thank you enough for giving me such a strong foundation and helping me to be the independent adult I am today. I am grateful and I love you too.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to God for the gift of life. To my supervisors, Dr. Steve Oduor and Dr. Elick Otachi I acknowledge their support, encouragement, guidance, and critiques while writing this thesis. I acknowledge the Kenya National Government and the World Bank, for funding my studies and research through the Kenya Climate Smart Agriculture Project (KCSAP). I also thank the KCSAP team for their continuous support and guidance during my studies. I acknowledge Ms. Shakila Muendo for her encouragement while writing this thesis. My gratitude is also extended to the Department of Biological Sciences, Egerton University for giving me space and equipment to carry out my research work. I also acknowledge the help and technical guidance of Mr. Lewis Mungai, Mr. Erick Otieno and Ms. Priscilla Muriithi in addition to availing the relevant field and laboratory materials and apparatus. The assistance of Ms. Janice Kimuli and her crew at the Agro-Science Farm at Egerton, is also greatly appreciated for welcoming me and their assistance during my research work.

ABSTRACT

Fish farming is a rapidly developing industry globally due to the increasing demand on protein for feeding the rapidly increasing human population. On the other hand, capture fisheries have significantly declined. One major impediment to aquaculture is access to affordable fish feeds, due to the expensive crude protein component. To address this challenge, this study investigated the potential of two algal species: *Arthrospira platensis*, and *Oocystis* sp. as sources of crude protein in fish feeds. The objectives of this study were to establish the most appropriate fertilizer regime that would allow for the optimum *Oocystis* sp. growth to generate adequate biomass, formulate fish feeds from the two algal species, analyse their proximate composition, and determine their efficiency in the growth of *Oreochromis niloticus*. Two experimental set-ups were made in the study, the first set-up involved the culturing of *Oocystis* sp., and determining its biomass and crude protein contents. The growth rate, doubling time, and divisions per day were determined from the changes in chlorophyll-*a* concentration. Proximate analysis was done on subsamples of *Oocystis* sp. and *A. platensis*. The second experimental set-up involved the assessment of the performance of *O. niloticus* fed on seven formulated diets namely, diet 1 was the control with *Caridina nilotica* as the protein source and the other six diets had 10%, 20%, and 40% replacement of *C. nilotica* with *Oocystis* sp. (diet 2-diet 4), and *A. platensis* (diet 5-diet 7). To achieve this, a ten-week experiment was conducted at the Agro-science Fish Park at Egerton University, where 21 hapa nets, each containing of 30 *O. niloticus* fry were subjected to the seven diets in triplicates. The results of the first experiment showed that there were no significant differences in the mean *Oocystis* sp. biomass ($p > 0.05$), growth rate ($p > 0.05$), divisions per day ($p > 0.05$), and doubling time ($p > 0.05$) from the different treatments. The overall body weight of the fish increased significantly ($p < 0.05$) from 0.240 g to 8.486 g. There were significant differences ($p < 0.001$) in the final body weight between diet 1 (7.778 ± 0.498 g) against diet 2 (9.985 ± 0.504 g) and diet 5 (9.937 ± 0.366 g). Therefore, this study showed that inorganic fertilizers can be used as cost-effective media in the mass scale culture of *Oocystis* sp. and that 40% substitution of *C. nilotica* as a protein source with either *A. platensis*, or *Oocystis* sp. enhances the growth of *O. niloticus* fry and reduces production cost, while having no adverse effects on the growth performance. In conclusion, the study revealed that there is great potential in the use of *A. platensis*, and *Oocystis* sp. as protein sources in fish feeds. This study recommends the potential inclusion of *A. platensis* or *Oocystis* sp. to lower the cost of production so that aquaculture not only becomes more profitable but contributes to increased food and nutritional security.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE
INTRODUCTION

1.1 Background information

Fish is a very important food item, rich in proteins that supports good health while fisheries offer livelihoods to many households all over the world. There has been a decreasing trend in the quantity of capture fisheries in most water bodies (Belton *et al*., 2014), yet the demand for fish as a protein source continues to rise as the world population increases correspondingly (Béné *et al*., 2015). This calls for increase in fish production through aquaculture to match the increasing demand (Obiero *et al*., 2019). Aquaculture as a means of enhancing food production, has been receiving increased attention as it is envisaged to increase the world's fish production by up to 50% (FAO, 2018).

Aquaculture is the growth of aquatic organisms in a controlled or semi-controlled aquatic environment, to provide human food, recreational fishing, enhancement of commercially valuable stocks, recovery of endangered species, bait, and ornamental species (Pillay & Kutty, 2005). Fish production from aquaculture has been on an increasing trend in the world, reaching an all-time high of 82.1 million tonnes in 2018 (FAO, 2020). On the other hand, aquacultural fish production in Kenya has been recording a decline from 24,096 tonnes in 2014 to 18,656 tonnes in 2015 and further to 14,952 tonnes in 2016 (KMFRI, 2017; Opiyo *et al*., 2018). This decline has been attributed to various challenges with fish feeds standing out as a primary causal factor depicted by the lack of inexpensive and appropriate fish feeds for different fish growth stages (Munguti *et al*., 2014a).

Intensification of aquaculture requires artificial feeding, to enable fish to grow at a faster rate to maximise productivity. In their natural habitat, fish derive their food from natural sources such as algae, zooplankton, macroinvertebrates, and some types of organic matter. Consequently, the source of nutrients for fish in aquaculture cannot be achieved through natural supply alone, but has to be supplemented through artificial feeds (Howe, 1996). While growth of natural food in ponds is normally enhanced through pond fertilisation, artificial feeds are formulated from different ingredients to improve nutritional supply and fish yields (Liti *et al*., 2006). They are made by mixing different ingredients to make a balanced diet, consisting of either simple mixtures or nutritionally complete mixtures (Gatlin, 2010). The feeds need to be nutritionally complete consisting of crude proteins, carbohydrates, minerals, lipids, vitamins, and essential fatty acids (Munguti *et al*., 2014b).

Aquaculture production is highly dependent on the quality of protein in the feeds. The high protein, fat, and amino acid composition, of *Caridina nilotica* (Freshwater shrimp) and *Rastrineobola argentea* (Silver cyprinid) make them the primary source of protein for fish feeds in Kenya (Munguti *et al*., 2014b). However, due to direct consumption as human food of *R. argentea*, its application in fortifying foods, and in making animal feeds; resulting to scarcity and higher prices; has driven scientists to look for other sources of protein for fish feeds to decrease overdependence on this two.

Several plants have been fronted to provide crude proteins in fish feeds for example corn gluten meal, rice protein concentrate, wheat gluten and soy protein concentrate (Kaushik & Hemre, 2008). However, the main shortcoming of plant proteins used in fish feeds is that they lack many essential amino acids such as lysine, methionine, threonine, and tryptophan (Torres-Tiji *et al*., 2020). Some species of algae are the base of the aquatic food chains in natural aquatic habitats on which other aquatic faunal organisms including fish are dependent on either directly or indirectly. However, analyses of the amino acid content of various species of algae have shown that while there is considerable variation, they usually have high content of essential amino acids (Brown *et al*., 1997).

Arthrospira platensis is a filamentous blue-green micro-algae, found growing in alkaline aquatic environments and is known to have high protein content, which ranges between 55 and 70% depending on the source (Habib *et al*., 2008). *Oocystis* sp. is a genus of planktonic microalgae widely distributed in freshwater bodies.

This study aimed to assess the nutritional values of the two algal genera to use them to formulate fish feeds, and determine the growth performance and survival rates of *O. niloticus* fed on the formulated feeds of *A. platensis* and *Oocystis* sp.

1.2 Statement of the problem

Fish offers a high protein food source and supports livelihood of many people globally through capture fisheries and fish farming. The demand for fish has been rising due to increase in human population, while fish production from capture fisheries has been declining. To increase fish production, promotion of fish farming is pertinent. However, fish farming is an expensive venture due to the expensive fish feeds resulting from high cost of crude protein component of fish feeds which is mostly sourced from animal proteins. There is an urgent need to find high quality, cost-effective, and sustainable alternative protein sources to be used in the formulation of fish feeds. Such feeds would enhance optimum growth of fish and allow aquaculture

production to remain economically and environmentally viable. Some algae species have been identified to have high protein sources that may be used in formulating fish feeds. This study investigated the possibility of using *A. platensis* and *Oocystis* sp. as possible sources of crude protein that may promote fish farming.

1.3 Objectives

1.3.1 General objective

To contribute to the development of fish feeds that would enhance fish production through aquaculture.

1.3.2 Specific objectives

i. To determine the right nutritional formulation for faster growth of *Oocystis* sp. using urea, DAP, and NPK media as cheaper nutrient sources.
ii. To determine the nutritional values of *A. platensis* and *Oocystis* sp.
iii. To determine the growth performance of *O. niloticus* fed on *A. platensis* and *Oocystis* sp. formulated fish feeds.

1.4 Hypotheses

H_{01}. There is no significant difference in the growth of *Oocystis* sp. cultured in different nutritional formulations of urea, DAP, and NPK media.

H_{02}. There is no significant difference between the nutritional values of *A. platensis* and *Oocystis* sp.

H_{03}. There is no significant difference in the growth performance of *O. niloticus* fed on *A. platensis* and *Oocystis* sp. formulated fish feeds.

1.5 Justification

Kenya's fish production is mostly based on capture fisheries, while fish farming is only beginning to be adopted strongly. In 2015, projections showed that the fisheries sector could contribute more than 8% to Kenya's GDP, an improvement compared from 5%. Just 0.014 % of land (1.4 million ha) in Kenya is reported to be used, with 95% of the nation being technically ideal for aquaculture. Fish feeds play a key role in aquaculture development and usually constitute 40-60% of the total production costs, mainly due to the crude protein components' cost. This is expensive and therefore necessitates the development of sustainable and cheaper sources of fish feeds. Consequently, there is need for new ingredients to be included in the formulation of fish feeds that have an equally high percentage of crude protein.

This study is relevant in aquaculture by informing on sustainable *A. platensis* and *Oocystis* sp. production systems and their use as fish feed components to promote profitable fish farming. As a result, leading towards efficient utilisation of resources, and decreasing dependence on capture fisheries as the main source of fish proteins and crude proteins for fish feeds. Aquaculture is part of the agricultural sectors contributing to increase in food security in the country, which is key in Kenya's vision 2030. This study will contribute towards the achievement of this important item. Additionally, this study is in line with the United Nations Sustainable Development Goal number 3 of ensuring healthy lives to promote well-being for all at all ages, as growth of aquaculture will provide animal protein for consumption, and goal number 14 of conserving and sustainably using the oceans, seas, and marine resources for sustainable development, by allowing conservation, and sustainable use of inland and marine resources.

1.6 Scope of the study

With the increase in aquaculture production globally, there has been corresponding increase in the demand of sources of fish feeds of key importance the protein sources especially in a country like Kenya. In view of this, the present study assessed the possibility of using *A. platensis* and *Oocystis* sp. as possible sources of crude protein that may promote fish farming via formulation of diets. To, this end the study covered the potential production of *Oocystis* sp., using inorganic fertilizers as a cheaper source of nutrients; mainly focusing on the optimization level of urea to be utilized in the culture of *Oocystis* sp. However, the study did not cover the production of *A. platensis*. Further, the study focused on proximate analysis of *A. platensis*, *Oocystis* sp., and formulated diets. The effects of the formulated diets on *O. niloticus* growth were ultimately tackled, via looking at the growth parameters such as body weight gain, specific growth rate, and the relative condition factor. The researcher limited the study to the effect on the growth of *O. niloticus* fries to the juvenile stage.

CHAPTER TWO
LITERATURE REVIEW

2.1 Status and trends of global aquaculture

Aquaculture was defined by Marshall (2017) as the sustainable farming and harvesting of fish to keep aquatic biodiversity and ecosystems intact. It also broadly refers to the culturing of aquatic organisms that includes fish, crustaceans, molluscs, and aquatic plants. Aquaculture is the world's fastest-growing and most diverse food based sector, with approximately 95.6% of its production being done in developing nations (Tacon, 2020). There has been a trend of increase in the world fish production through aquaculture, reaching 82.1 million tonnes (FAO, 2020), from 80.0 million tonnes (FAO, 2018). Further algae production through aquaculture reported 32.4 million tonnes of aquatic algae harvested, and 26,000 tonnes of ornamental seashells and pearls, bringing the total to an all-time high of 114.5 million tonnes (FAO, 2020).

Aquaculture has recorded the fastest growth, from 25.7% in 2001 to 46.8% of the total fisheries production by 2016 (FAO, 2018), consequently increasing the gross production from capture fisheries and aquaculture to a total of 362 billion United Sates Dollars (USD). Smith *et al*. (2010) noted that aquaculture's steady growth, while the world capture fisheries stagnated, maintained the global per capita fish consumption, despite an increase in the global population. Tacon (2020) stated that aquaculture offers the most diverse food source, with over 277 different species of organisms cultured in 2016, including 20 species of aquatic plants, 59 species of molluscs, 27 species of crustaceans, and over 171 fish species.

The global growth of aquaculture differs among regions with, Asia (89%) leading in production where China had the highest amount of aquaculture production than the rest of the world combined since 1991, recording approximately 63.7 million tonnes annually. Africa has registered the fastest growth rate of the sector, from a low base, currently recording 7.3% and 9.28% increase in annual percentage in aquaculture quantity and value respectively, compared to the Asia region (Nadarajah & Flaaten, 2017). Aquaculture statistically had a total of 19.3 million people involved in 2016 (FAO, 2018) and this has grown to 20.5 million in 2018 (FAO, 2020), registering an increase of those employed in the sector.

2.1.1 Aquaculture in Kenya

Aquaculture practice in Kenya is reported to have taken root in the early 1900s through introduction of trout (*Oncorhynchus* sp.) in rivers for sport fishing (Ngugi *et al*., 2007). Later, in the 1920s the sport fishing advanced to static pond culture which involved the culturing of

tilapia, common carp, and catfish species. Sagana and Kiganjo trout fish farms were the pioneers of small-scale rural fish farming. The farms focused on rearing the warm water and cold-water species respectively (Ngugi & Manyala, 2004; Ngugi *et al*., 2007). By the year 1960, aquaculture had become popular in most regions around the country with a dramatic growth of the sector being observed over the last decade. Currently, aquaculture is majorly practiced in Kakamega, Bungoma, Siaya, Busia, Kisii, Meru, Nyeri, Kisumu, Muranga, and Embu counties with less practice in Kitui, Lamu, and Elgeyo Marakwet (Opiyo *et al*., 2018).

The growth of the sector was slow until the introduction of the economic stimulus programme in 2009, where the Kenyan government injected a total of 10.5 KES billion into the sector, which aimed at increasing fish production from aquaculture (Munguti *et al*., 2014a). The economic stimulus program resulted in a 55% increase in aquaculture production (Garlock *et al*., 2020). As a result of the economic stimulus project, there was an increase in the aquaculture production for the subsequent three years with a minor decrease to 21,488 tonnes in 2012 from 22,135 tonnes in 2011. However, there was a steady decrease from 24,096 tonnes in 2014 to 18,656 tonnes in 2015 and further to 14,952 tonnes in 2016 (KMFRI, 2017) as illustrated in Figure 2.1.

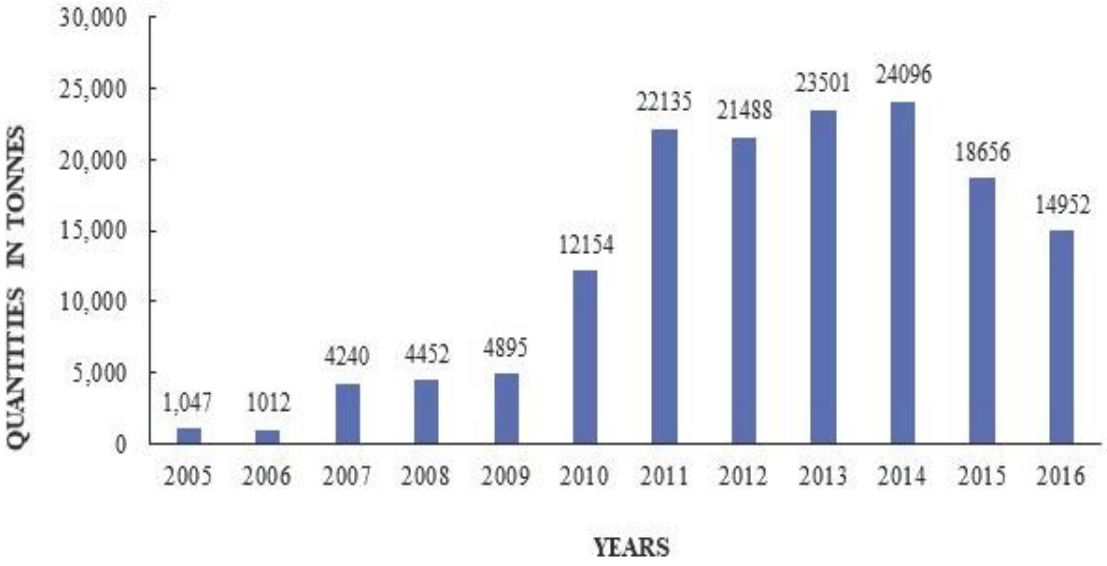


Figure 2.1: Kenya Aquaculture Production Trends (KMFRI, 2017)

Aquaculture in Kenya consists of freshwater aquaculture and mariculture, where most of the aquaculture production is from freshwater aquaculture (98%) while mariculture (2%) is mostly underexploited (Munguti *et al*., 2014a). *O. niloticus* (80%) and *Clarias gariepinus* (14%) account for 94% of the total aquaculture production (Ngugi & Manyala, 2004). Other

freshwater species cultured include *Cyprinus carpio*, *Oncorhynchus mykiss*, *Micropterus salmoides*, *Carassius auratus*, *Xiphophorus hellerii*, *Labeo victorianus*, *Oreochromis jipe*, and *Protopterus aethiopicus* (KMFRI, 2017). *Chanos chanos* at 90% and *Mugil cephalus* at 9% (Ngugi & Manyala, 2004) are the most produced fish species under mariculture. Other species include *Scylla serrata*, *Trachnotus blochii*, *Penaeus monodon*, *Euchema* sp., and *Artemia* sp. (KMFRI, 2017).

Aquaculture is further categorised by the cultivation system into extensive, semi-intensive, and intensive, with each system having its unique features. In Kenya, semi-intensive aquaculture is the most practised system, which consists of mainly ponds (Opiyo *et al*., 2018). Intensive aquaculture is also practised in Kenya; however, due to the massive amount of capital associated with these systems, it is rare. Its practise consist of using cages, aquaponics, and recirculating systems (Munguti *et al.*, 2014a). Cage culture is slowly growing, mostly within the Lake Victoria region depicted by an increase in the number of cages to 3,398 in 2017 from 1,663 in 2016 (KMFRI, 2017).

2.1.2 Challenges facing aquaculture in Kenya

While fish farming is an industry with a promising future due to the ever-increasing demands, in Kenya it is however faced with a number of challenges. One major challenge is the lack of cheap fish feeds for different stages of fish (Munguti *et al*., 2014b). This has resulted into stagnation in aquaculture growth. Fish feeds are very expensive in the market and their quality in some cases is wanting. Though standards have been developed to be followed in making fish feeds (Shitote *et al*., 2012) lack of enforcement and poor feeding strategies stand out as setbacks in the development of aquaculture in Kenya (Obwanga *et al.*, 2017). Feeding strategies positively correlate to fish growth where under- or over-feeding results in bad feed efficiencies and growth of the fish. Lack of monitoring of fish biomass, and water temperature by many farmers has resulted in poor feeding strategies (Munguti *et al*., 2014b).

Locally formulated diets by farmers act as the primary source for fish feeds by many fish farmers in Kenya (Munguti *et al*., 2014b). However, lack of knowledge on feed formulation for specific target species at different stages of growth is a challenge in the sector as the farmers end up formulating feeds without taking the growth stage aspects into account (Rana & Hasan, 2013). Farmers further lack the techniques and knowledge in transportation, handling, and storage of feeds, leading to the spoilage or contamination of feeds (Munguti *et al*., 2014b).

Availability of fish fries to be used in aquaculture is also a significant challenge despite of an increase in the number of hatcheries in the country from 21 in 2009 to 127 in 2015 (KMFRI, 2017). Fish farmers often end up buying fries of genetically inferior quality since the hatcheries do not produce quality seeds. The availability of good quality seeds is of significant concern as it ensures the fish's survival from stocking through the entire growth stages until it is ready for harvesting. This ensures maximum returns from the venture (Ngugi *et al*., 2007).

Inadequate extension services, poor husbandry practices, and poor quality of fish farms' inputs are other challenges facing the sector (Opiyo *et al*., 2018). The formation of county governments and subsequent devolving of aquaculture duties from the national government to the county governments that lacked support services for fish farming, is a major challenge in the growth of the sector due to poor transition from national to county government. The absence of a comprehensive policy on aquaculture including legislation have derailed the development of the sector (Mbugua, 2008). Shitote *et al*. (2012) summarized the challenges of fish farmers in western Kenya into high cost, unavailability and low quality of feeds, drying up of ponds during drought, lack of fingerlings, flooding, siltation of ponds, poor pond maintenance, and poor security. These identified shortcomings broadly reflect the challenges facing the aquaculture farming in the country.

2.2 Fish nutrition

The fast growth of feed aquaculture practices has led to an increase in production in fish feeds. The production from aquaculture practices lacking supplementary feeding decreased from 40.5% in 2000 to 30.5% in 2016; however, the volume produced from aquaculture practices with supplementary feeding increased to 24.4 million tonnes in 2016 (FAO, 2018). Consequently, the growth in aquaculture production is directly related to the increase in nutritionally quality feeds.

Fish feeds constitute 40 to 60% of fish production costs in most intensive and semi-intensive aquaculture practices. This requires availability of affordable and quality feeds for the farming to be profitable. Studies carried out reveal that commercially formulated diets are more economical than locally compounded diets as they provide the fish with all nutrients within the correct concentrations required for growth, and adhere to the set standards as illustrated in Table 2.1 (Munguti *et al*., 2014b).

Table 2.1: The Kenya commercial fish feed standards for fry, fingerlings, growers, and brooders (Munguti *et al*., 2014b)

Feed parameters	Fry	Fingerlings	Growers	Brooders
Feeding rate %	5% body weight	6-8% body weight	3% body weight	3% body weight
Crude protein %	40-45%	35-40%	30-34%	40%
Energy (millijoules/kilogram)	≥10	≥10.5-11	≥11.5-12.5	≥11.5-12.5
Crude fiber %	≥4%	≥4%	≥6%	≥6%
Lipids %	≥8%	≥8%	≥10%	≥10%
Lysine %	≥12%	≥12%	≥12%	≥12%
Methionine %	≥5%	≥5%	≥5%	≥5%
Moisture content %	≤12%	≤12%	≤12%	≤12%
Pellet size (millimeters)	Mash	2	2-5	2-5
Floating pellets (minutes)	N/A	≥2	≥2	≥2

2.2.1 Importance of protein in fish feeds

Protein is stated as the most expensive macro-nutrient in fish feeds (Munguti *et al*., 2014b). For a fish diet to be complete, it must contain a certain percentage of crude protein depending on the fish species. Proteins in fish feeds are vital, as they act as transporters of essential amino acids (Kaushik & Seiliez, 2010). Amino acids are either essential or non-essential, where the organism produces the non-essential amino acid while essential amino acids are provided for an organism; hence they need to be included in the diet.

There are twenty-two combinations of amino acids that act as building blocks for proteins in animals, of which ten are essential amino acids in fish hence need to be included in the fish feed (Lopez & Mohiuddin, 2020). These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The quality of crude protein depends on the composition of amino acids; hence a balanced protein; should have the correct composition of amino acids and thus a higher nutritional value (Miles & Chapman, 2015).

2.2.2 Sources of crude protein

There are various sources of crude proteins; as earlier mentioned *C. nilotica* and *R. argentea* are the most common sources of crude proteins used in fish feed formulation in Kenya (Charo-Karisa *et al*., 2012). Apart from the two other familiar sources of crude proteins include fish meal, soybean meal, animal by-products, and other oilseed meals. The percentage of crude protein varies with the different sources and further on feed production from raw ingredients.

Fish meal for instance, has crude protein raging between 60 and 70% of the total body weight (Miles & Chapman, 2015). Fish meal is highly used as a source of crude protein in fish feeds as it has an excellent amino acid profile. Soybean meal and other oilseed meals though used their amino acid profile is not as good therefore limiting the provision of amino acids required (Chou *et al*., 2004; Ogello *et al*., 2014). *C. nilotica* and *R. argentea* also have a good amino acid profile, hence their high demand in the production of fish feeds. They further have high percentages of digestibility and palatability as fish meal. Soybean meal and other oilseed meals, which are plant protein, have lower digestibility percentages as they contain cellulose and oligosaccharides, which are absent in animal proteins (Salmeán *et al*., 2015).

2.3 *Arthrospira platensis* and *Oocystis* sp. nutrient contents

A. platensis was declared the best food for the future during the United Nations World Food Conference in 1974 (Jung *et al*., 2019) this is because it is one of the most nutritious foods by weight when compared to other food, both plant and animals based. *A. platensis* has a high concentration of quality protein ranging between 55 and 70% (Habib *et al*., 2008; Jung *et al*., 2019). In comparison to other standard plant proteins, *A. platensis* stands out as superior as it contains all essential amino acids. When compared to animal proteins, it has reduced amounts of cysteine, lysine, and methionine. Habib *et al*. (2008) noted that 6 to 6.5% constitutes lipids, 1.3 to 15% consist of unsaturated fatty acids, of which 25 to 60% consists of polyunsaturated acids. All essential minerals are found in *A. platensis*, which is of importance as it bioaccumulates minerals.

Oocystis sp. is reported to have lipid content ranging between 10 and 20%, with an ash range of 6.3 to 7.7%. The carbohydrate percentage of *Oocystis* sp. is relatively high, ranging between 25 and 37%, with a notable increase in the carbohydrate with a decrease in the protein percentage of the *Oocystis* sp. cells (Lee & Picard, 1982).

2.3.1 *Arthrospira platensis* and *Oocystis* sp. as possible sources of crude protein for fish feeds

A. platensis has gained much attention as a source of protein not only for fish feeds but also as a human food supplement. Though available in specialised natural habitats, *A. platensis* can be cultured cost-effectively and then be incorporated in fish feeds as a crude protein source in these feeds. De Chavez and Bolivar (2018) state that selecting fish feeds should be based on the suitability as reflected on its nutritional profile, viability, and its growth potential for sustained supply. *A. platensis* exhibit a high protein efficiency ratio (Habib *et al*., 2008). The absence of cellulose in the structure of *A. platensis* is of vital importance as it makes it readily digestible (Habib *et al*., 2008) unlike other plant sourced proteins in which break down of cellulose is necessary before digestion. Therefore amino acid assimilation (Salmeán *et al*., 2015) in *A. platensis* feed occurs very fast.

Polyunsaturated fatty acids found in *A. platensis* (Habib *et al*., 2008; Jung *et al*., 2019) are important to aquatic organisms as they maintain normal cell-to-cell permeability even in extreme environments. *A. platensis* is stated to have higher energy efficiency; therefore, it requires less amount of energy per kilo of protein produced (Andrade & Costa, 2008; Habib *et al*., 2008). High yields coupled with efficient water use (Habib *et al*., 2008), are more merits as *A. platensis* is produced in high yields for feeds with a minimal amount of water use. *A. platensis* is stated to increase the growth rate, improve and intensify fish colouration if used as an ingredient in fish feeds (De Chavez & Bolivar, 2018).

Oocystis sp. has been reported to vary on the percentage of proteins found in the cells, ranging between 15 and 40% (Lee & Picard, 1982). The difference in the protein percentage is attributed to the culture medium of the *Oocystis* sp. cells. The high percentage of protein found in the cells, forming a reasonable basis for its use as an ingredient if fish feeds.

2.4 Culturing *Arthrospira platensis* and *Oocystis* sp.

There are various ways of culturing *A. platensis* which include ponds, raceways, and tanks (Habib *et al*., 2008). The culture system is often influenced by the scale of production. *Oocystis* sp. has been reported to withstand a wide range of changing environmental conditions, cultured mainly in open concrete ponds with various media. The *Oocystis* sp. cells are characterised by a fast growth rate, with the maximum number of cells reported after 12 days (Zhaowei, 2020).

2.4.1 Growth requirements for *Arthrospira platensis* and *Oocystis* sp.

The culturing requirements of *A. platensis* can be summarised by eight main factors to macro and micronutrients presence (C, Ca, N, Cl, P, K, S, Na, Mg, and Se, Fe, Co, Zn, Ni, Cu), water quality, luminosity (photo-period 12/12, 4 lux), temperature (30^0C), pH (8.5-10.5), dissolved solids (10-60 g/litre), inoculation size, and stirring speed (Habib *et al*., 2008). The culture media is essential as the dominance of *A. platensis* in the natural environment is positively correlated with alkalinity. The higher the alkalinity, the higher the dominance of *A. platensis* in natural water bodies. High pH ranging from 8.5 to 11 is the best for growth.

The temperature range required for optimum growth by *A. platensis* is between 35 ^{0}C and 37 ^{0}C; however, it can still grow at temperatures between 16 ^{0}C and 40 ^{0}C. Light photo-period is also an essential requirement, as Moraes *et al*. (2013) states that *A. platensis* has higher production with higher light photo-period. The light intensity is also a crucial factor, as Soni *et al*. (2019) states the suitable range is between 1500 and 4500 lux, where higher intensities may cause the cells to die. Innoculation size of 15% is stated as the best for cultivation by Moraes *et al*. (2013) while, Andrade and Costa (2008) state 20% as the best inoculation size, with both being harvested after 18 days with other factors at optimum. The culture medium should be in constant motion to ensure all *A. platensis* growing to get illumination. However, fast stirring speeds are stated by Moraes *et al*. (2013) as harmful as it ends up damaging the cells.

Oocystis sp. farming requires the provision of carbon, nitrogen, phosphorus sources, and trace nutrients. Therefore the culture medium of *Oocystis* sp. should have the following concentration components: 1.5-3g/L carbon source, 0.03-0.08g/L phosphorus source, 0.2-0. 5g/L nitrogen source, 0.03-0.08g/L iron salt, and trace water-soluble vitamins, of which the carbon source is NaAc, pH:7-8 in the culture system (Zhaowei, 2020).

2.4.2 Challenges in culturing *Arthrospira platensis* and *Oocystis* sp.

Arthrospira platensis is an obligate autotroph hence requires light and nutrients for its growth in the artificial media which must be well formulated for growth. The sedimentation of *A. platensis* to the bottom of culture media is also another challenge, mostly in small-scale production, as the media needs to be in constant motion, and the means of achieving the constant motion is through use of a shaker resulting in an increase in the cost of production. Contamination during *A. platensis* culture is also a challenge as other species may have toxins, hence not edible as a result, there is a need to ensure the culture remains pure during the entire

cultivation period (Yu *et al*., 2019). The culture media used is also a challenge as it determines the availability of required macro- and micro-nutrients required for growth.

Oocystis sp. culture is limited as with open pond culture systems which are easy to pollute, they further require a large area for culturing, the system is also affected by the weather (via influence on the light photoperiod and temperature) as such controlling amount of biomass produced, and as such not easy to control during production (Zhaowei, 2020).

2.4.3 Summary of literature review and identification of research gaps

Arthrospira platensis has been used in the formulation of fish feeds. The research on its application on *O. niloticus* in Kenya, has not yet been conducted in ponds, but in aquariums. Information on the nutrient profile of *Oocystis* sp. is not comprehensively mentioned in literature. *Oocystis* sp. incorporation in fish feeds has not yet been done, despite reports of it having high proteins and a fast growth rate (as they are smaller), as such having good potential for faster culture for incorporation into fish feeds.

CHAPTER THREE

SUSTAINABLE FISH FEEDS: OPTIMIZATION OF LEVELS OF INORGANIC FERTILIZERS FOR MASS PRODUCTION OF *Oocystis* sp. FOR CLIMATE SMART AQUACULTURE

Abstract

Use of microalgae as a source of food in aquaculture production is gaining recognition due to their rapid growth rates that promise high biomass generation within a short time. The challenge faced is getting good and inexpensive nutrients to be used in mass production of the required microalgae. This study investigated the effect of different nutrient combination in influencing the growth rate of the green algae *Oocystis* sp. which has been identified as a possible protein source for the raising of *Oreochromis niloticus* fingerlings for fish farming. Modified Bolds 3N Medium and commercial agricultural fertilisers (urea, NPK and DAP) media were compared to establish the appropriate combinations that would result in high biomass generation but at the lowest cost possible. The Modified Bold 3N Medium acted as the control, at a cost of 11.28 KES per litre, the other media were derived from urea, NPK and DAP (varying the ratio of each) at a cost of treatment 1 (0.14 KES per litre), treatment 2 (0.18 KES per litre) and treatment 3 (0.22 KES per litre). The algae was cultured for five weeks with samples taken daily for biomass analyses using chlorophyll-*a* concentration as the surrogate for *Oocystis* sp. biomass for 30 days, from each treatment was determined. The growth rate, doubling time, and divisions per day were then estimated based on this chlorophyll-a concentration. The results showed that the mean *Oocystis* sp. biomass in treatment 1 was highest (7.715±0.667 µg/ml) while treatment 3 (6.441±0.555 µg/ml) had the lowest. There were no significant differences in the mean *Oocystis* sp. biomass in the four treatments (Kruskal-Wallis H test: $p > 0.05$). The *Oocystis* sp. biomass varied significantly in each treatment with time (Kruskal-Wallis H test: $p < 0.001$). There were no significant differences in the growth rate (Kruskal-Wallis H test: $p > 0.05$), divisions per day (Kruskal-Wallis H test: $p > 0.05$), and doubling time (Kruskal-Wallis H test: $p > 0.05$) from the different treatments. The results of this study showed that inorganic fertilizers can be used as cost-effective media in the mass scale culture of *Oocystis* sp.

3.1 Introduction

Microalgae are associated with diverse applications such as animal feed sources biodiesel production due to their faster growth rates that yield high biomass within a short time (Khan *et al*., 2018; Mata *et al*., 2010). Some species of microalgae have high lipid accumulation in

their dry cells, ranging between 50 and 60% that makes them good candidates for use as energy sources (Hu *et al*., 2008; Mata *et al*., 2010).

Saadaoui *et al*. (2021) observed that some microalgae have been used as food in livestock, poultry, and aquaculture production due to their diverse nutritional properties. For example *Chlorella vulgaris* was used as a substitute for fish meal in feed for *Clarias gariepinus* (Enyidi, 2017). In another study, El-Sheekh *et al*. (2014) used *Arthrospira platensis* as feed for hybrid red tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*). These studies revealed that the inclusion of algae in the feeds was beneficial to the cultured fish through an increase in the feed conversion ratio.

Some other species of such as *Oocystis* sp. have been reported to have varying amounts of proteins in the cells, ranging between 15 and 40% (Lee & Picard, 1982). The differences in the protein contents have been attributed to the culture medium. However, *Oocystis* sp. incorporation in fish feeds has not yet been done investigated comprehensively.

The expansion of microalgae production is critical in any perceived applications, hence the need for development of cost-effective media for microalgae cultivation. The factors that affect microalgae growth are namely light, temperature, and nutrients (Chowdury *et al*., 2020; da Silva Ferreira & Sant'Anna, 2017; Gani *et al*., 2019; Kazbar *et al*., 2019). Their nutrient requirements varies, with each algal species having a specific requirement to optimize growth (Ghafari *et al*., 2018; Khan *et al*., 2018). Nitrogen and phosphorus are essential as they are limiting factors to microalgae growth (Yaakob *et al*., 2021). Consequently, nitrogen and phosphorus sources are usually emphasized while upscaling the culture of microalgae.

Raising interest in the use of inorganic fertilizers in microalgae culture, as a source of nutrients; which stands out as a major limitation in large-scale production of algae (Hu *et al*., 2008; Mata *et al*., 2010; Ravindran *et al*., 2016) is critical. Various studies have been done to assess the use of inorganic fertilizers (either singly or combined) in microalgae culture (Ashraf *et al*., 2011; Michael *et al*., 2019; Nayak *et al*., 2016).

The inorganic fertilizers commonly used in mass-scale production are urea, di-ammonium phosphate (DAP), and nitrogen-phosphorus-potassium (NPK) (Ansari *et al*., 2017; Arenas *et al*., 2017; Renuka *et al*., 2018; Win *et al*., 2018). Urea is a nitrogenous fertilizer that consists of 46% nitrogen (having the highest nitrogen content). DAP is important as it provides both nitrogen and phosphorus for growth, found having different ratios depending on the fertilizers end-use. NPK is also an inorganic fertilizer that is wholesome as it provides nitrogen,

phosphorus, and potassium nutrients for the growth of organisms, with individual concentrations differing based on the end-use. Different concentrations of the inorganic fertilizers affect the growth of microalgae; further, a combination of different fertilizers is used to provide microalgae with all the nutrients required for growth.

The aim of this study was to establish the appropriate concentration of inorganic fertilizers combination that could be used to generate good production of *Oocystis* sp. for use in fish culture.

3.2 Materials and methods

3.2.1 Study area

The study was conducted at Egerton University in the Biological Sciences Department laboratories. The University is located in Nakuru County, Njoro Sub- County, and is approximately 25 km south-west of Nakuru city, at an altitude of 1890-2190 m above sea level. The area's temperature ranges between 17 ^{0}C and 27 ^{0}C (Waithaka *et al*., 2017).

3.2.2 Culturing of *Oocystis* sp.

The algae was cultured using commercial agricultural fertilizers (urea, NPK and DAP) media for a period of five weeks. The inoculum for culturing *Oocystis* sp. was obtained from the University of Texas Culture Collection of Algae (UTEX). Modified Bolds 3N Medium (UTEX, n.d.) was used as a control. The other treatments were as shown in Table 3.1, which was derived based on past studies by Nayak *et al*. (2016) on *Scenedesmus* sp. and Rahardini *et al*. (2018) on *Chlorella* sp. Pure cultures were raised through isolation and growing the culture in the incubators in the laboratory to raise suitable biomass before transferring them into 3-liter plastic bottles as growth chambers or bioreactors containing the culture media.

Table 3.1: Composition of different treatment culture media

Treatments	NPK (g/L)	UREA (g/L)	DAP (g/L)
Treatment 1	0.75	0.25	0.25
Treatment 2	0.75	0.5	0.25
Treatment 3	0.75	0.75	0.25

3.2.3 Sampling and biomass estimation

Samples were taken daily after 24 hours for 30 days, where 20ml was filtered through glass fibre carbon filters from each treatment bottle. The *Oocystis* sp. biomass was analysed through

the determination of chlorophyll-*a* concentration which was done according to the standard method as given in the American Public Health Association (APHA) (2005).

The filters and seston were folded, wrapped in aluminium foil, and kept in the freezer all night to help the chlorophyll-*a* cells rupture during the extraction process using acetone. Then, 5 ml of 90% aqueous acetone was added after the seston and filters had been homogenized in a tissue grinder (Heidolph, 637 69, Germany) at about 5,000 rpm. The grinder was then rinsed with 90% acetone (the volume used was documented), and the rinsed slurry was added to the extraction slurry after the samples had been put into a centrifuge tube. For the whole chlorophyll-*a* extraction, the volume was then increased to 10 ml with 90% acetone and left for at least 8 hours at 4 °C in the dark. After incubation, the samples were centrifuged at 3,000 rpm for 10 minutes. Decanting the cleared extract into a clean test tube. A spectrophotometer (Pharmacia Biotech Novaspec II, Sweden) was used to determine the light absorbance of the chlorophyll-a extract at 750 and 663 nm.

Chlorophyll-*a* concentration was calculated using, the equation according to Talling and Driver (1963):

Biomass; $N = \frac{11.4*(B-A)*Ve}{Vf*Lp}$ 1

Where, B= absorbance at 663 nm

A= absorbance at 750 nm

Ve= volume of extract used (ml)

Vf= volume of sample filtered (ml)

Lp= light path length of the cuvette (cm)

After which the chlorophyll-*a* concentration was used to derive the growth rate, divisions per day, and the generation time as critical parameters of importance. These were all derived from the equations developed by Levasseur *et al*. (1993):

Growth rate; $K' = \frac{Ln\left(\frac{N2}{N1}\right)}{t2-t1}$ 2

Where N1 and N2 = biomass at time1 (t1) and time2 (t2) respectively.

Divisions per day and the doubling time were calculated based on the specific growth rate.

Divisions per day; $\frac{Div}{day} = \frac{K'}{Ln2}$ 3

Where Ln2= Natural logarithm to the base 2

Doubling time; $Doub'\ t\ = \frac{1}{\frac{Div}{day}}$ 4

3.2.4 Statistical analyses

The biomass, growth rate, divisions per day, and doubling time of *Oocystis* sp. cultured in the different treatments were compared using Sigma Plot software (version 14) through Kruskal-Wallis H test, as the data failed Normality Test (Shapiro-Wilk: $p < 0.05$).

3.3 Results

3.3.1 Water quality

Mean temperature of the culture during the study ranged from 24.2^0C to 27.3^0C. Temperature variations during the study period were not significant among the treatments (Kruskal-Wallis H test: $p > 0.05$). The pH for Treatment 1 ranged from 6.8 to 7.7, where there was a steady increase from the beginning of the study. On the other hand, pH for Treatment 2 ranged from 6.6 to 7.4, and Treatment 3 ranged from 6.4 to 7.2, all showing a trend of temporal increase as observed for Treatment 1. Finally, the control treatment had a pH ranging from 6.1 to 7.1, also showing a trend of temporal increase as observed in the other three treatments.

3.3.2 Comparison of *Oocystis* sp. biomass

The *Oocystis* sp. biomass in Treatment 1 was highest (7.715±0.667 µg/ml) followed by the Control (6.963±0.788 µg/ml) and Treatment 2(6.862 ±0.617 µg/ml), with the lowest in Treatment 3 (6.441±0.555 µg/ml). There were no statistically significant differences in mean *Oocystis* sp. biomass among different treatments (Kruskal-Wallis H test: $p > 0.05$).

3.3.3 Temporal variations in *Oocystis* sp. biomass

Generally, the biomass increased gradually in all treatments until it reached its peak on different days for all the treatments. The control treatment (Modified Bolds 3N Medium) achieved the highest *Oocystis* sp. biomass on the 25th day (12.977±0.788 µg/ml). Treatment 1 followed, reaching its highest *Oocystis* sp. biomass on the 17th day (11.4437±0.667 µg/ml). Treatment 2 achieved its highest biomass on the 21st day (11.3696±0.617 µg/ml), and finally Treatment 3 had the lowest biomass, which was achieved on the 16th day (10.2714±0.555 µg/ml). Overall, the *Oocystis* sp. biomass in all the treatments fluctuated once the optimum biomass was achieved. At the end of the experiment, the *Oocystis* sp. biomass in the treatments followed a similar pattern, with the control having the highest (11.2442±0.788 µg/ml) while treatment 3 had the lowest (5.8197±0.555 µg/ml) (Figures 3.1).

The *Oocystis* sp. biomass varied significantly in each treatment with time (Kruskal-Wallis H test: $p < 0.001$) for all the treatments. At the beginning of the study, all the treatments had equal biomass (where 100 ml of stock *Oocystis* sp. cultured having a biomass of 10.0719 µg/ml was introduced into 2.49 litres of respective treatment media).

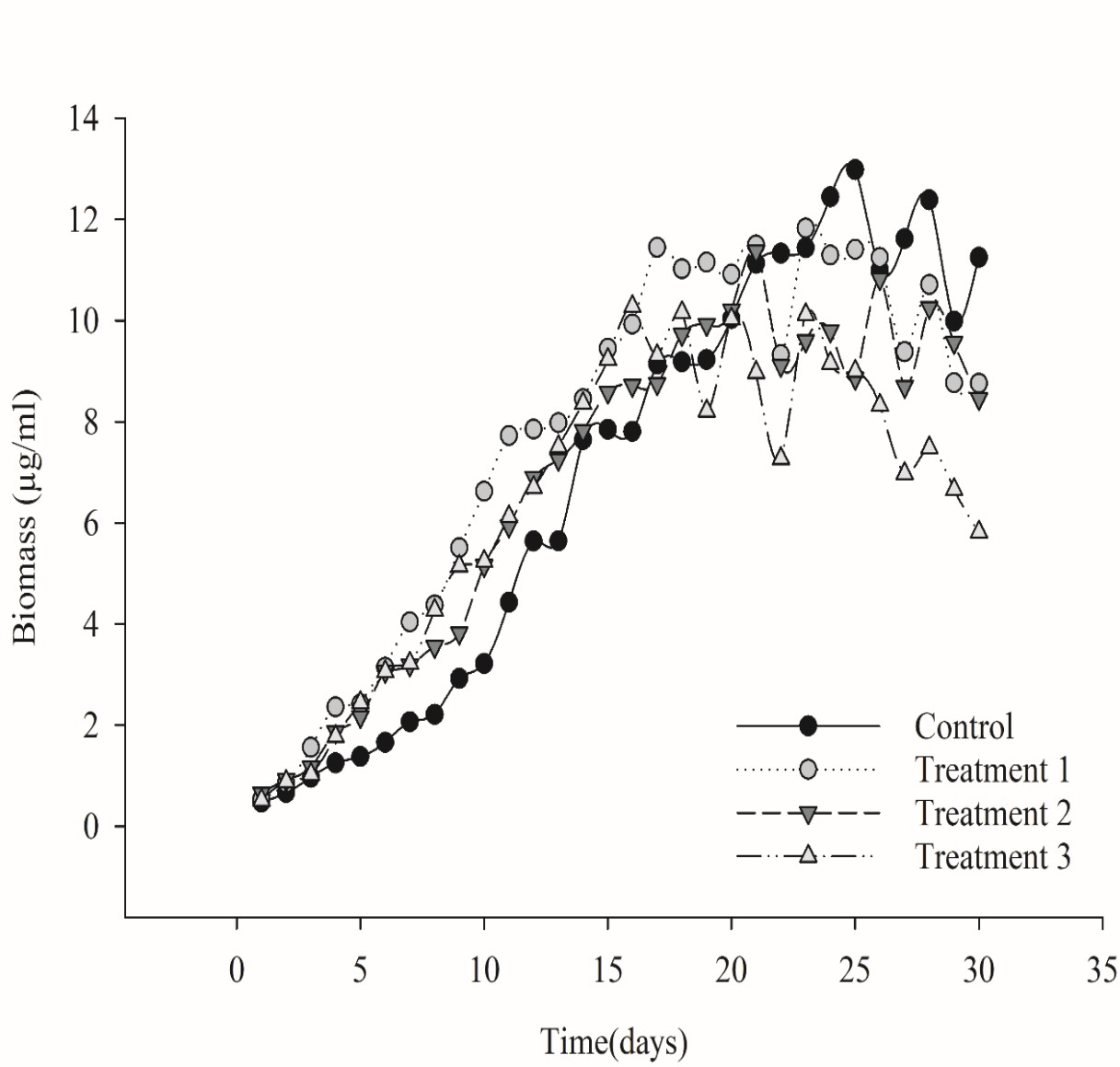


Figure 3.1: Temporal variations in biomass of *Oocystis* sp. in different treatments

3.3.4 Growth rate, divisions per day, and doubling time

The maximum, mean, and least values of the growth rate, divisions per day, and doubling time in different treatments are shown in Table 3.2. There was no significant difference in the mean growth rate (Kruskal-Wallis H test: $p > 0.05$), divisions per day (Kruskal-Wallis H test: $p > 0.05$), and doubling time (Kruskal-Wallis H test: $p > 0.05$) for the treatments.

Table 3.2: The maximum, mean, and least values of the growth rate, divisions per day, and doubling time of different treatment culture media

Parameters	Treatment			
	Control	**Treatment 1**	**Treatment2**	**Treatment 3**
Growth rate (K') $_{max}$	0.376	0.601	0.469	0.545
Growth rates (K') $_{mean}$	0.109 ± 0.0262	0.0963 ± 0.0345	0.0885 ± 0.0293	0.0837 ± 0.0370
Growth rate (K') $_{least}$	-0.215	-0.209	-0.219	-0.213
Divisions per day (Div.day^{-1}) $_{max}$	0.543	0.867	0.676	0.786
Divisions per day (Div.day^{-1}) $_{mean}$	0.157 ± 0.0378	0.139 ± 0.0498	0.128 ± 0.0423	0.121 ± 0.0534
Divisions per day (Div.day^{-1}) $_{least}$	-0.310	-0.302	-0.316	-0.307
Doubling time (cells) $_{max}$	1028.630	70.142	187.374	37.900
Doubling time (cells) $_{mean}$	49.722 ± 35.978	-11.267 ± 19.150	13.352 ± 6.659	1.412 ± 2.192
Doubling time (cells) $_{least}$	-124.118	-532.684	-9.929	-39.418

3.3.5 Media cost and biomass

The control (Modified Bold 3N Medium) was produced at a cost of 11.28 KES per litre, the other media were derived from urea, NPK, and DAP at a cost of 0.14 KES per litre for treatment 1, 0.18 KES per litre for treatment 2 and 0.22 KES per litre for treatment 3. Distilled water was generated by a distiller in the laboratory for use in generating the control media (Modified Bold 3N Medium) while harvested rainwater was used for the experiment for treatment 1, 2 and 3; hence, their cost were not captured in the above calculation.

3.4 Discussion

The nature of the culture media is the main determining factor in the growth of microalgae, their productivity, and ultimately their biomass as long as the pH, light intensity, and temperature needs have been fulfilled (Chowdury *et al*., 2020; da Silva Ferreira & Sant'Anna, 2017; Gani *et al*., 2019). The light provided to the culture was by white light-emitting diode tubes (3200K, 9W). Csavina *et al*. (2011) illustrated that *Oocystis* sp. requires the optimum light intensity of 150 μmol m^{-2} s^{-1}. Latala *et al*. (1991) found that the growth of *Oocystis* sp. was completely inhibited at high light intensities ranging from 270-380 μE/m^{2}s.

pH influences the quantity of free carbon in the culture media and the balance between carbonate and bicarbonate. In this study, the pH was adjusted to range between 6.0 and 8.0, as reported by Rao (1963), to suit the growth of *Oocystis* sp. optimally. Mayo (1997) found similar

results while culturing *Chlorella* sp. with the maximum growth rate at pH ranging from 6.31 to 6.84. Changes in the media pH were attributed to the photosynthetic and decomposition processes of the cultured *Oocystis* sp. (Rahardini *et al*., 2018; Tucker & D'Abramo, 2008).

During the study, the water temperature was in the optimal range as reported by Nalley *et al*. (2018), where they found the best temperature for optimum growth of *Oocystis* sp. ranged from 26.60^0C to 27.78^0C. Chowdury *et al*. (2020) state that temperature is critical in microalgae culture as it affects the algal growth rate, cell size, biochemical composition, and nutrient requirements.

The temporal *Oocystis* sp. biomass varied amongst the treatments, mainly due to the different constituents in the culture treatment media, with the differences being significant with time in all the treatments. Sutkowy *et al*. (2019) state that the composition of culture media influences the growth of micro-algae *in vitro*. Overall, all treatments followed a similar trend where there was a decrease in the chlorophyll-*a* concentration after attaining its highest biomass. The pattern achieved is similar to that obtained by Rahardini *et al*. (2018) while assessing the growth of *Chlorella* sp., a green algae in the Chlorophyceae class as *Oocystis* sp.

At the beginning of the experiment, there was a steady increase (exponential phase) in the *Oocystis* sp. biomass due to sufficient nutrient concentration in all the treatments. There was no lag phase observed during the study, which can be attributed to the conditions of the inoculum (Spencer, 1954). Talling (1966) notes that an inoculum obtained from a healthy exponentially growing population is unlikely to have a lag phase when transferred to a freshly prepared media under similar growth conditions (light, temperature, and pH); consequently, there is no need for physiological adaptations for growth. The lack of a lag phase is crucial as it reduces the time required for upscaling the culture, allowing the harvesting of cultured cells sooner. The exponential phase is characterised by the production of materials that are also capable of growth (Fogg & Thake, 1987), with its length depending on the size of the inoculum, the growth rate, and the capacity of the medium and culturing conditions to support algal growth.

The exponential phase was followed by the stationary phase, characterised by a decline in population growth, resulting from reduced nutrient concentration in the culture media, causing the number of cells to remain constant. The stationary phase is characterised by zero net growth, as the rate of the growth of the cells is equal to the rate of cell death (Maier & Pepper, 2015; Nyström, 2004). Finally, the death phase follows, as nutrients in the culture media run

out, resulting in the death of cultured algal cells; therefore, a decline in the *Oocystis* sp. biomass was witnessed. The death rate of cells in the media became higher than the rate of cell growth (Maier & Pepper, 2015).

The study demonstrated that the inorganic fertilizers' specific compositions and comparison between the culture media did not have any significant impact on the growth rate, divisions per day, and the doubling time of cultured *Oocystis* sp. Similar results were also obtained by Rahardini *et al*. (2018) who also found no significant differences in the growth rate and doubling time of *Chlorella* sp. cultured in media with different compositions. The divisions per day obtained was higher than those from Csavina *et al*. (2011), which can be attributed to the continuous agitation of the algal cells during culturing, consequently not allowing the clamping of cells together, resulting in deaths, and ultimately decline in the growth rate and divisions per day. Research carried out by Sobczuk *et al*. (2006) showed an increase in the growth rate of microalgae with an increase with agitation; it should also be noted that excessive agitation can result in cell death.

3.5 Conclusion

This study found that inorganic fertilizers can be used as an option of cost-effective media in the culture of *Oocystis* sp., with the ratio of 3:1:1 while using NPK, urea, and DAP fertilizers respectively to achieve the highest biomass within the shortest period. The finding that there were no significant differences in the *Oocystis* sp. biomass using either medium is an encouraging result as it offers opportunities to explore the use of low-cost media which is equally competitive with the standard media in mass production of *Oocystis* sp. for potential use in fish feeds in Aquaculture.

CHAPTER FOUR

SUSTAINABLE FISH FEEDS: EVALUATION OF THE EFFECT OF PARTIAL REPLACEMENT OF *Caridina nilotica* WITH *Arthrospira platensis* AND *Oocystis sp.* ON THE GROWTH PERFORMANCE OF *Oreochromis niloticus* FOR CLIMATE SMART AQUACULTURE

Abstract

Aquaculture was introduced in Africa with the intention of enhancing rural nutrition, generating additional income, diversifying the agricultural sector to lower the risk of crop failure, and creating rural jobs. However, despite the steady growth globally, and progressive growth in Africa, there are challenges in the sector, with the sourcing of high-quality cheap fish feeds being a major impediment. Fish feeds are composed of different ingredients, with crude proteins being the most expensive, and less diversified. The objective of this study was to assess the performance of a newly formulated feeds from algal species as alternative sources of crude protein for *O. niloticus* feed. Proximate analysis was done on subsamples of *A. platensis*, and *Oocystis*, sp., and the formulated feeds. This was followed by a ten-week experiment that was conducted at the Agro-science Fish Park at Egerton University, where seven diets (diet 1-diet 7) were formulated. This consisted of diet 1 (a control with a commercial feed) and three (diet 2- diet 4) having 10%, 20%, and 40% replacement of *C. nilotica* with *Oocystis* sp. and another three (diet 5-diet 7) with 10%, 20%, and 40% replacement of *C. nilotica* with *A. platensis* respectively. A total of 630 *O. niloticus* fries were stocked, 30 in each hapa net, with each of the seven treatments randomly applied in triplicates. Fish were fed twice daily at 10% body weight. Sampling fish for growth performance (total and weekly body weight gain, specific growth rate, food conversion factor, relative condition factor, and survival rate) was done weekly. Selected water quality measurements were done. There was a significant difference ($p \leq 0.05$) in the crude protein content between *A. platensis* (53.667 ± 1.074 %), and *Oocystis*, sp. (41.927 ± 0.51 %). The overall body weight of the fish increased significantly ($p \leq 0.05$) from 0.240 g to 8.486 g. Diet 2 (9.985 ± 0.504 g) had highest final body weight followed with diet 5 (9.937 ± 0.366 g), diet 6 (8.380 ± 0.292 g), diet 3 (8.040 ± 0.307 g), diet 7 (7.988 ± 0.361 g), diet 4 (7.840 ± 0.329 g), and finally diet 1 (7.778 ± 0.498 g) had the lowest. There were significant differences ($p < 0.001$) in the final body weight between diet 1 (7.778 ± 0.498 g) against diet 2 (9.985 ± 0.504 g) and diet 5 (9.937 ± 0.366 g). This study revealed that 40% substitution of *C. nilotica* as a protein source with *A. platensis*, or *Oocystis*, sp. enhances the growth of *O. niloticus* fry, while reducing the costs of production.

4.1 Introduction

Aquaculture broadly refers to the rearing of aquatic animals and plants in fresh, brackish, and marine environments (Pillay & Kutty, 2005). There has been a rapid growth of the aquaculture sector to meet the growing population demand of protein source (FAO, 2020). Troell *et al.* (2017) states that aquaculture will be the main way of obtaining food from the aquatic environments in the future. This has been supported with the constant increase in the quantities of fishes harvested from aquaculture sector as compared to the capture fisheries sector, coupled with the stagnating phase being experienced from the capture fisheries stocks. FAO (2020) recorded 82.1 million tonnes of live weight fish from aquaculture in 2018, while capture fisheries recorded 96.4 million tonnes. The trend of the capture fisheries from the 1980's has been fluctuating between 86 and 93 million tonnes. Aquaculture is important as an alternative source of proteins to human (Hinrichsen *et al*., 2022).

Globally fish production through aquaculture has been steadily increasing (FAO, 2020), with Africa also registering a steady growth though at a slower pace despite the large volume of unexploited area suitable for aquaculture (Hinrichsen *et al*., 2022; Msangi & Batka, 2015). This slow growth has kept the contribution from the African continent at a small percentage (2.7%) (Halwart, 2020), which is still insignificant (Adeleke, 2020). Adeleke (2020) states that aquaculture was introduced in Africa with the goals of improving rural nutrition, supplementary income generation, diversification to reduce crop failure risks, and rural employment creation. The slow growth of the aquaculture sector in Africa is attributed to various challenges, which are described by Hinrichsen *et al*. (2022) as a lack of infrastructure and development capital, inadequate information, limited technological know-how, and poor governance. Satia (2016) notes that the burgeoning demand for capital, inadequate quantities and quality of seed and feeds, resource (land/water/feed) competition, requirement to reinforce aquaculture management, and overall governance of the sector, as the challenges being faced by the aquaculture sector in Africa.

Fish farming was first introduced in Kenya in the 1900's by colonialist, via stocking trout in rivers for sport fishing (Munguti *et al*., 2014a), with further campaigns aimed towards diversifying the agricultural sector and enhancing rural nutrition (Adeleke, 2020). Opiyo *et al*. (2018) and Adeleke (2020) concur that the first successful aquaculture practise in Kenya was done in the 1920's, characterised with production of *O. niloticus* in ponds. The most widely used form of aquaculture being practised in Kenya is the earthen pond based semi-intensive culture system (Opiyo *et al*., 2018). The other form of aquaculture is mariculture, the

production of aquatic organisms using marine aquatic ecosystems. However, most of the aquaculture production in Kenya is from freshwater with the mariculture sector being underexploited (Munguti *et al*., 2014a; Opiyo *et al*., 2018). The uptake of aquaculture in the country got a major boost through government facilitated projects such as the Economic Stimulus Program between 2009 and 2014 (Munguti *et al*., 2014a). Opiyo *et al*. (2018) notes that the Economic Stimulus Program supported various aquaculture activities such as subsidizing fingerlings, feed, and pond construction costs. However, when the program came to an end there was a reduction in the production which was concurrent with the reduction in the number of operational ponds in the country.

Aloo *et al*. (2017) notes that 300,000 metric tons of fish are required in Kenya per year, however the national production is below the required level, even though aquaculture is the fastest growing food sector in the world (Anderson *et al*., 2017; Subasinghe *et al*., 2009; Obiero *et al*., 2019). The low production when compared to the demand, is attributed to various challenges that the aquaculture sector faces in Kenya; lack of quality fish feeds and seeds, lack of enforcement of regulations for proper management of aquatic resources and poor feeding strategies, inadequate extension services, and poor husbandry practices (KMFRI, 2017; Munguti *et al*., 2014b; Obwanga *et al*., 2017; Opiyo *et al*., 2018; Shitote *et al*., 2012).

Fish feeds constitute 40 to 60% of fish production costs in most intensive and semi-intensive aquaculture practices (Munguti *et al*., 2021). Fish feeds being the most expensive input in aquaculture, results in the need of creation of high-quality fish feeds that can be utilized in aquaculture. In the quest to reduce the cost of fish feeds, small-scale aquaculture farmers often opt for self-formulation of fish feeds, rather than buying commercial fish feeds.

Fish feeds are often formulated with different components in-order to provide the cultured fish with the desired nutrition. The most prominent primary source of crude protein in fish feeds in Kenya are *Caridina nilotica* (freshwater shrimp) and *Rastrineobola argentea* (silver cyprinid) (Munguti *et al*., 2014b). However, *R. argentea* is also used as human food, putting fish feed and human food in conflict. Because of the high protein, fat, and amino acid composition, they are the industry's key drivers of success. However, because of overexploitation, excessive pesticide usage, deforestation, and the climate change phenomena, their catches are reducing (Rana *et al*., 2009). The scarcity of *C. nilotica* and *R. argentea* has a direct influence on commodity prices, resulting in higher fish farming production costs (Ayoola, 2010). This has led researchers to look for other protein sources for fish feed composition, such as black soldier

fly larvae, cotton and sunflower seed cakes, blood meal, meat and bone meal, and algae of high quality which could be less costly crude protein (Schiavone *et al*., 2017).

This study assessed the nutritional values of two algal species *A. platensis*, and *Oocystis*, sp., and used them to formulate fish feeds for raising *O. niloticus* fingerlings. The growth performance and survival rates of *O. niloticus* fed on the formulated feeds of *A. platensis,* and *Oocystis* sp. was determined and compared with the commercially formulated feeds.

4.2 Materials and methods

4.2.1 Study area

The study was conducted at the Agro-science Fish Park at Egerton University located in Nakuru County, Njoro Sub- County, (described in chapter 3, see figure 4.1).

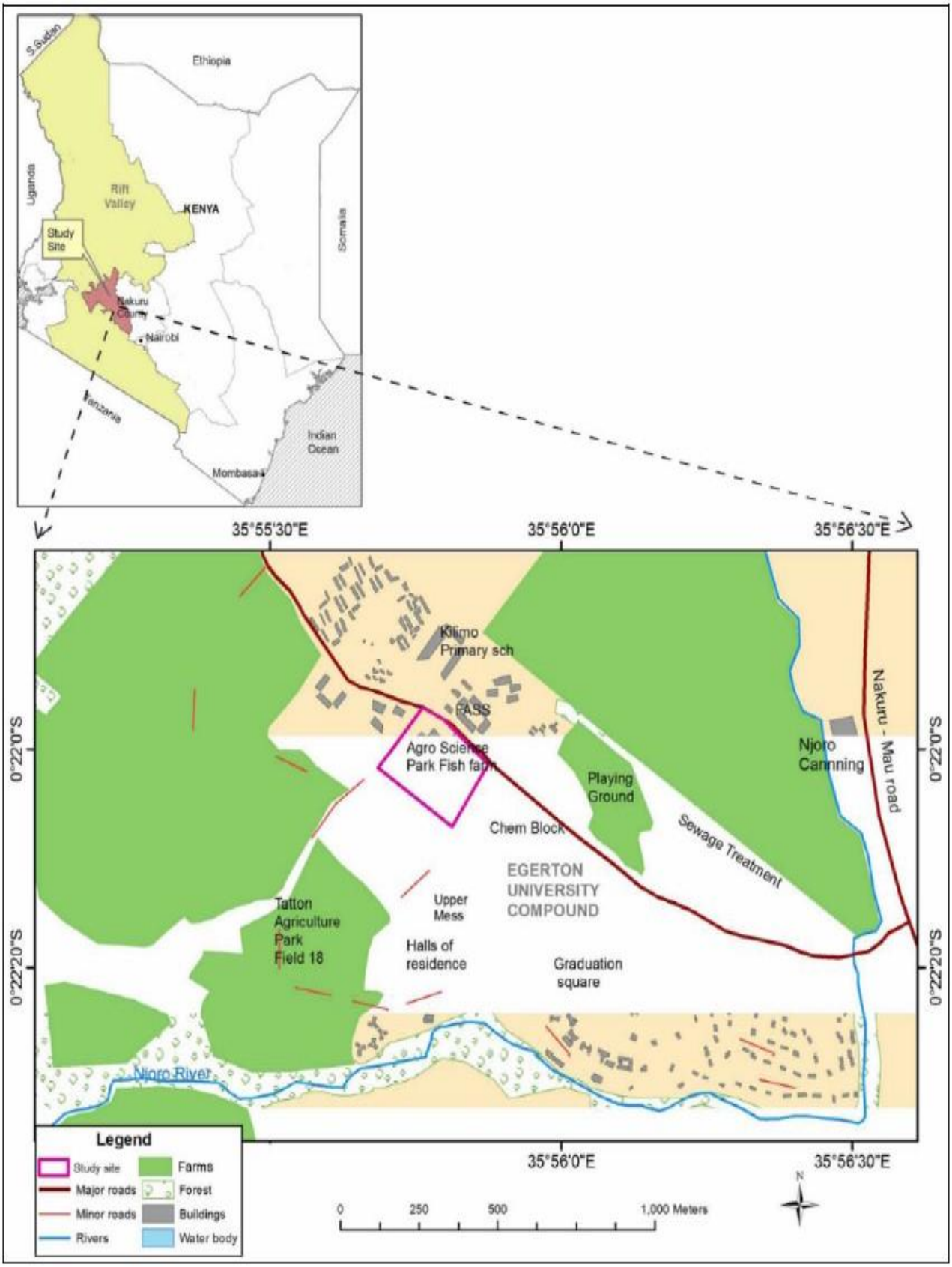


Figure 4.1: A map of the study area showing the Agro-science Fish Park (Modified from: Akidiva *et al*., 2020)

4.2.2 Nutritional analyses and formulation of fish feeds with the algae

4.2.2.1 Nutritional analyses of *Arthrospira platensis* and *Oocystis* sp.

The *A. platensis* and *Oocystis* sp. were dried and weighed. The proximate analysis of individual algae was done. These consisted of determining the moisture content, crude protein, crude fibre, crude lipids, ash, and nitrogen-free extract content of the samples. Moisture was determined through drying in the oven (AOAC, 1995). 5 grams of sample was placed in crucibles in an oven and dried for six hours to constant weight at 105^{o}C. The samples were cooled in a desiccator at room temperature and weighed. The moisture content was worked out using the Formula 5.

$$\text{Moisture (\%)} = \left[\frac{\text{weight of initial sample (g)} - \text{weight of dried sample (g)}}{\text{initial sample weight}}\right] \times 100 \quad (5)$$

Crude protein was determined based on the Micro Kjeldahl method (Chow *et al*., 1980), using a sample size of 0.4 grams, a Behroset InKje M digestion apparatus and a Behr S 1 steam distillation. The ammonia distillate was trapped in 4 % boric acid solution prior to titration with 0.1N Hcl.

Crude protein was estimated by:

$$\text{\% Nitrogen} = \frac{[(\text{ml standard acid} - \text{ml blank}) \text{x N of acid x } 1.4007]}{\text{weight of sample in grams}} \quad (6)$$

$$\text{Crude Protien} = (\text{\% total nitrogen x } 6.25) \quad (7)$$

Crude lipids were determined based on Soxhelet's method (AOAC, 1995), using a sample weight of 2 grams in a Soxhlet extractor with petroleum ether at a boiling point of 40 – 60^{o}C. To calculate for crude lipid, Formula 8 was used.

$$\text{Crude lipid (\%)} = \left[\frac{\text{weight of dry flask} - \text{weight of empty flask}}{\text{weight of the sample}}\right] \times 100 \quad (8)$$

Crude fibre was determined by boiling 1 gram of the sample in a standard solution of 3.13 % H_2SO_4 for 10 minutes. The remaining sample was rinsed with hot water followed by boiling in 3.13 % NaOH for another 10 minutes. Thereafter, the remaining sample was rinsed repeatedly with water followed by acetone. The residue was then oven dried at 60^{o}C for 4 hours, cooled in a desiccator and weighed. After which the residue was ashed at 550^{o}C in a muffle furnace overnight. Crude fibre was quantified as shown in Formula 9:

$$\text{CF (\%)} = \left[\frac{\text{dried sample (g)} - \text{ashed sample (g)}}{\text{initial sample weight}}\right] \times 100 \quad (9)$$

Ash content of the sample was determined based on the incineration method (Joslyn, 2012), by heating the samples in a muffle furnace set at 600°C for three hours. The nitrogen-free extract was determined as illustrated in Formula 10:

$$\text{NFE}\ (\%) = 100 - (\text{A} + \text{B} + \text{C} + \text{D} + \text{E}) \quad 10$$

Where:

A = moisture content (%)

B = crude protein content (%)

C = crude lipid content (%)

D = crude fibre content (%)

E = ash content (%)

4.2.2.2 Formulation of fish feeds with the algae

Two different sets of treatments, predetermined with the percentage of *A. platensis* and *Oocystis* sp. were in-cooperated to the diet where the first to the fourth had 0%, 10%, 20%, and 40% of *A. platensis* and *Oocystis* sp. in the diets respectively. This consisted of diet 1(a control with a commercial feed) and three (diet 2- diet 4) having 10%, 20%, and 40% replacement of *C. nilotica* with *Oocystis* sp. and another three (diet 5-diet 7) with 10%, 20%, and 40% replacement of *C. nilotica* with *A. platensis* respectively. A total of 630 *O. niloticus* fries (sourced from the Agro-science Fish Park at Egerton University) were stocked, 30 in each hapa net, with each of the seven treatments randomly applied in triplicates.

Table 4.1: The composition of the formulated diets used in this study

Ingredients (grams)	**0%**	**10%**	**20%**	**40%**
C. nilotica	88	79.2	70.4	52.8
A. platensis* or *Oocystis sp.	-	8.8	17.6	35.2
Wheat Bran	11	11	11	11
Premix	1	1	1	1

The fish were placed in acclimatisation hapa nets (4 x 3 m) mounted in one 800 m^2 earthen ponds for one week before commencing the experiment. During the acclimatisation period, the fish were fed at 09:00 and 16:00hrs with commercial feeds. Which was followed by the actual study where feeding was carried out twice at 09:00hrs and 16:00hrs. The fish feeds were fed 10% of their body weight for a period of 9 weeks (28th March to 30th May), and the quantity of

feed was recorded throughout the experimental period. The feeding rate was adjusted after each sampling based on mortality and the weight of sampled fish. Feeding rings were used for each hapa to avoid drifting of feeds outside of the hapa.

4.2.3 Fish sampling

Fish sampling was done weekly, where the total length and weight of fish was measured to determine their growth rate. The growth of *O. niloticus* was measured alongside the physico-chemical parameters of the water in the fishponds for two months. Ten fish were randomly sampled from each hapa in the pond using a seine net of 10mm mesh size. The fish were transported using a bucket of 25mm/100ltr of water before taking measurements to avoid stressing them. Total length in centimetres and weight in grams was taken immediately using a measuring board and an electric weighing scale. After that, fish were transferred into a bucket containing clean water for stabilisation before releasing them back to their respective hapas in the pond.

4.2.4 Determination of routine pond limnological parameters

4.2.4.1 *In situ* determination of selected physico-chemical variables

Water temperature, electrical conductivity, pH, and dissolved oxygen (DO) concentration were measured *in situ* using a portable multi-sensor probe (HACH HQ-40d meter [USA]). Triplicate water samples (500 ml) were collected from the pond using acid-washed bottles for nutrient analyses. The collected water samples were transported in ice-cool boxes to the laboratory. In the laboratory, the samples were filtered using 0.45µm Whatman glass fibre carbon filters for the analyses of dissolved nutrients such as ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and soluble reactive phosphorus (SRP). Unfiltered samples were used to measure total phosphorus (TP) concentrations.

4.2.4.2 Nutrient analyses

The concentrations of nutrients were determined using conventional calorimetric techniques (APHA, 2005). The ammonium-nitrogen concentration was evaluated using the sodium salicylate technique using a hypochlorite solution as a catalyst, as specified in APHA (2005). The sodium-salicylate technique was used to determine nitrate-nitrogen using the APHA (2005) procedures. Nitrite-nitrogen was detected by forming a reddish-purple azo dye at pH 2.0-2.5 by coupling diazotized sulfanilamide with N-(1-naphthyl) ethylenediamine dihydrochloride (NED dihydrochloride), as outlined in APHA (2005).

Soluble reactive phosphorous (SRP) was evaluated spectrophotometrically using the molybdenum blue technique, as described in APHA (2005), whereas Total Phosphorus was measured by first digesting the sample with potassium persulphate, followed by SRP analysis using the molybdenum blue method.

4.2.5 Data collection on the performance of the feeds

4.2.5.1 Determination of the total and weekly body weight gain of *Oreochromis niloticus*

The total and weekly body weight gain of the *O. niloticus* was determined by taking a census of the cultured fish in different culturing units during the 10 weeks. Their current weights were taken, and the mean derived.

The total and weekly body weight gain were calculated using equations 11 and 12 (Ricker, 1979):

$$WBW = \frac{W_f - W_i}{t} \quad 11$$

Where:

WBW= Weekly body weight gain

W_f = Mean Final weight after sampling

W_i = Mean Initial weight

t = time

$$TBW = W_f - W_i \quad 12$$

Where:

TBW= Total body weight gain

W_f = Mean Final weight after sampling

W_i = Mean Initial weight

4.2.5.2 Determination of the specific growth rate of *Oreochromis niloticus*

The specific growth rates of the *O. niloticus* was determined by taking weights of the cultured fish in different culturing units during the 10 weeks. Their weights were taken, and the mean derived. The initial mean was subtracted from the weight obtained after sampling.

The growth rates were calculated using equation 13 (Ricker, 1979):

$$GR = 100 * (\frac{LNW_f - LNW_i}{t}) \quad 13$$

Where:

GR= Growth rate

LN= Natural logarithm

W_f = Mean Final weight after sampling

W_i = Mean Initial weight

t = time

4.2.5.3 Determination of the feed conversion ratio of *Oreochromis niloticus*

The feed conversion ratios were determined by the feeds provided to the fish weekly and divided by the live weight gain of the fish.

The feed conversion ratios were calculated using equation 14:

$$FCR = \frac{F_p}{W_g} \quad 14$$

Where:

FCR= Feed conversion ratio

F_p = Feed provided

W_g = Mean weight gain

4.2.5.4 Determination of the survival rate of *Oreochromis niloticus*

Survival rates of the fish was determined by comparing the number of *O. niloticus* that were still alive to the initial number of fish stocked. Formula 15 was used to calculate the survival rate.

$$SR = \left(\frac{S_i - S_d}{S_i}\right) 100 \quad 15$$

Where:

SR= Survival rate

S_i = Initial stocked a number

S_d = Dead fish in the culturing units

4.2.5.5 Determination of the relative condition factor of *Oreochromis niloticus*

The relative condition factor for the fish was determined based on the regression coefficient, with the equations for obtaining the relative condition factor are illustrated in the formulae 16, 17 and 18 (Gayanilo, 1997):

The length-weight relationship was estimated as:

$$W = aL^b \quad 16$$

Where:

W= The weight (g) of fish in grams

L = The Total length of fish in centimetres

a = Exponent describing the rate of change of weight with length (= the intercept of the regression line on the Y-axis)

b = The slope of the regression line (also referred to as the Allometric coefficient)

The log-transformed data gave regression equation 17:

$$\log w = \log a + b \log L \quad 17$$

Where:

a = Constant

b = The regression co-efficient

Relative condition Factor: The relative condition factor (k) of the experimental fish was estimated from the relationship in equation 18.

$$K = \frac{100w}{L^b} \quad 18$$

Where:

W = Weight of the fish in grams

L = The total length of the fish in centimetres

b = The value obtained from the length-weight equation formula.

4.2.6 Statistical analyses

Descriptive statistics was used to present variations in physico-chemical water quality parameters, length, and weight of fish from different treatments. Before inferential statistical analysis, data was tested for normality using Shapiro–Wilk test and Levene's test of homogeneity of variance. One -way Multivariate ANOVA was used to determine if there were significant differences between the water quality, proximate analysis and growth parameters results of the different treatments, with group means being compared using the Tukey's HSD test. All statistical analyses were performed with Sigma Plot, Minitab and Microsoft Excel software at a significance level of $p < 0.05$.

4.3 Results

4.3.1 Variations in *in-situ* measurements

The mean dissolved oxygen concentration varied significantly temporally (Kruskal-Wallis H test: $p< 0.001$) in the pond. The dissolved oxygen during the period ranged from 3.93 mg/l to 6.39 mg/l, with a mean 5.047 ± 0.149 mg/l. The temperature of the pond during the study period had a range between 20.0 ^{0}c and 26.2 ^{0}c with a mean of 23.153 ± 0.407 ^{0}c, where it varied significantly temporally (Kruskal-Wallis H test: $p< 0.001$). Conductivity varied

significantly temporally (Kruskal-Wallis H test: $p < 0.001$); where it had a range between 360 µS/cm and 500 µS/cm, with a mean of 411.50 ± 9.030 µS/cm. pH on the other hand ranged from 6.97 to 8.39. The values of selected physico-chemical parameters during the study period are presented in table 4.2.

Table 4.2:Values of selected physico-chemical parameters in the pond (minimum, maximum, and mean ± standard error)

Parameter	Minimum	Maximum	Mean
Dissolved Oxygen (mg/l)	3.93	6.39	5.047 ± 0.149
Temperature (^{0}c)	20.00	26.20	23.153 ± 0.407
Conductivity (µS/cm)	360.00	500.00	411.50 ± 9.030
pH	6.97	8.39	pH≤ 7 pH≥7
			6.98 8.03

4.3.2 Variation in nutrient concentrations

The nitrite concentrations during the study were minimal having a range from 0.000 mg/l to 0.002 mg/l. Nitrite had a mean concentration of 0.00067 ± 0.00002 mg/l. Nitrate on the other hand, had a mean of 0.107 ± 0.007 mg/l, with a range between 0.052 mg/l and 0.156 mg/l. Ammonia concentration recorded was also low having a range between 0.003 mg/l and 0.042 mg/l with a mean of 0.0148 ± 0.00294 mg/l. Soluble reactive phosphorus had a mean of 0.0622 ± 0.00572 mg/l during the research with a range from 0.0233 mg/l to 0.0900 mg/l. Total phosphorus had the highest concentration ranging from 1.9280 mg/l to 8.2480 mg/l with a mean of 4.2968 ± 0.637 mg/l. The minimum, maximum, and mean ± standard error values of selected nutrients during the study period are presented in table 4.3.

Table 4.3:Nutrients concentrations in the pond (minimum, maximum, and mean ± standard error)

Parameter	Minimum	Maximum	Mean
Nitrite (mg/l)	0.000	0.002	0.00067 ± 0.00002
Nitrate (mg/l)	0.052	0.156	0.107 ± 0.007
Ammonia (mg/l)	0.003	0.042	0.0148 ± 0.00294
Soluble Reactive Phosphorus (mg/l)	0.0233	0.0900	0.0622 ± 0.00572
Total Phosphorus (mg/l)	1.9280	8.2480	4.2968 ± 0.637

4.3.3 Temporal variation in nutrient concentrations

The nitrite values recorded were less variable, at a constant level with no significant differences (One Way ANOVA, $F = 1.200$, df (5, 12), $p > 0.05$) observed temporally. The lowest nitrite concentration was found in week 7 (0.000 ± 0.000 mg/l) whereas the highest was recorded in week 9 (0.013 ± 0.012 mg/l). The highest nitrate concentration of 0.130 ± 0.009 mg/l was recorded in week 7 while the lowest was recorded at week 5 (0.072 ± 0.011 mg/l), however there were no significant differences in the nitrate concentration temporally (One Way ANOVA, $F = 2.079$, df (5, 12), $p > 0.05$). Ammonia on the other hand was recorded highest at week 9 (0.038 ± 0.002 mg/l), while it was lowest at week 1 (0.004 ± 0.001 mg/l).

There was a significant difference in the ammonia concentration recorded temporally (One Way ANOVA, $F = 21.049$, df (5, 12), $p < 0.001$). Tukey's HSD Test for multiple comparisons found that the mean value of ammonia concentration at week 9 was significantly different from week 1 (t-value = 8.94, $p < 0.001$), initial (t-value = 7.82, $p < 0.001$), week 5 (t-value = 7.65, $p < 0.001$), week 3 (t-value = 6.963, $p < 0.001$), and at week 7 (t-value = 4.99, $p < 0.001$). Tukey's HSD Test for multiple comparisons also found significant differences in the mean value of ammonia concentration between week 7 and week 1 (t-value = 3.95, $p < 0.001$).

The soluble reactive phosphorus had no significant differences in the concentrations temporally (One Way ANOVA, $F = 2.091$, df (5, 12), $p > 0.05$) recorded, notably the highest concentration was found at week 9 (0.09000 ± 0.000 mg/l), while the lowest at week 3 (0.0400 ± 0.0098 mg/l). Total phosphorus was the only nutrient that was found in high concentrations during the study, with the highest value recorded at week 3 (7.4947 ± 0.374 mg/l), while the least was recorded at week 1 (2.8147 ± 0.489 mg/l). There was no significant difference found in the total phosphorus value recorded temporally (One Way ANOVA, $F = 1.058$, df (5, 12), $p > 0.05$). The temporal mean ± standard error values of selected nutrients are presented in table 4.4.

Table 4.4: The temporal variation of selected nutrients in the pond

Time	Nitrite (mg/l)	Nitrate (mg/l)	Ammonia (mg/l)	Soluble Reactive Phosphorus (mg/l)	Total Phosphorus (mg/l)
Initial	0.0007 ± 0.0003	0.119 ± 0.025	0.008 ± 0.004	0.0567 ± 0.0167	4.248 ± 1.930
Week 1	0.0007 ± 0.0007	0.107 ± 0.017	0.004 ± 0.001	0.0510 ± 0.0147	2.8147 ± 0.489
Week 3	0.0003 ± 0.0003	0.088 ± 0.017	0.011 ± 0.001	0.0400 ± 0.0098	7.4947 ± 0.374
Week 5	0.0010 ± 0.0000	0.072 ± 0.011	0.009 ± 0.001	0.0623 ± 0.0147	4.1347 ± 2.058
Week 7	0.0000 ± 0.0000	0.130 ± 0.009	0.019 ± 0.006	0.0733 ± 0.0095	5.5280 ± 1.641
Week 9	0.0013 ± 0.0012	0.124 ± 0.007	0.038 ± 0.002	0.09000 ± 0.000	5.3413 ± 1.828

4.3.4 Proximate analysis of *Arthrospira platensis, Oocystis* sp., and formulated feeds

The proximate analysis for *A. platensis*, *Oocystis* sp., and the formulated diets was done with the mean ± standard error values illustrated in table 4.5. The moisture content was less variable, having no significant difference between *A. platensis*, *Oocystis* sp., and the formulated diets (One Way ANOVA, $F = 1.341$, df (8, 18), $p > 0.05$), *Oocystis* sp. had the highest moisture content (5.878 ± 0.584 %) as a protein source. The moisture content for the formulated diets recorded were diet 1 with the lowest (3.997 ± 0.382 %), while diet 3 had the highest (5.183 ± 0.314 %). The ash content was also less variable, as there was no significance found between *A. platensis*, *Oocystis* sp., and the formulated diets (One Way ANOVA, $F = 2.088$, df (8, 18), $p > 0.05$), *A. platensis* had the highest ash content (11.505 ± 0.576 %) as a protein source. Diet 4 recorded the highest ash content (9.935 ± 1.181 %) for the formulated diets, while diet 1 recorded the lowest ash content (7.466 ± 0.0876 %).

A. platensis had the highest crude protein content (53.667 ± 1.074 %) for the protein sources while *Oocystis* sp. had the lowest (41.927 ± 0.51 %). The formulated diets crude protein was less variable with diet 1 having the highest (49.875 ± 0.334 %) while diet 7 had the lowest (46.813 ± 1.079 %). There was a significant difference in the crude protein content between *A. platensis*, *Oocystis* sp., and the formulated diets (One Way ANOVA, $F = 15.463$, df (8, 18), $p < 0.001$). Tukey's HSD Test for multiple comparisons found that the mean value of crude protein content of *A. platensis* was significantly different from *Oocystis* sp. (t-value = 10.46,

p< 0.001), diet 7 (t-value = 6.10, p< 0.001), diet 6 (t-value = 5.78, p< 0.001), diet 5 (t-value = 5.78, p< 0.001), diet 2 (t-value = 4.55, p< 0.001), diet 4 (t-value = 4.09, p< 0.001), and diet 3 (t-value = 3.96, p< 0.001). Tukey's HSD Test for multiple comparisons also found significant differences between *Oocystis* sp. and diet 1 (t-value = -7.08, p< 0.001), diet 3 (t-value = -6.49, p< 0.001), diet 4 (t-value = -6.36, p< 0.001), diet 2 (t-value = -5.91, p< 0.001), diet 5 (t-value = -4.68, p< 0.001), diet 6 (t-value = -4.68, p< 0.001), and diet 7 (t-value = -4.35, p< 0.001).

Oocystis sp. had the highest crude lipid content (13.627 ± 0.722 %) for the protein sources. Diet 2 had the highest crude lipid content (7.455 ± 0.32 %), while diet 5 had the lowest (6.938 ± 0.186 %). There was a significant difference in the crude lipid content between *A. platensis*, *Oocystis* sp., and the formulated diets (One Way ANOVA, F = 20.921, df (8, 18), p<0.001). Tukey's HSD Test for multiple comparisons found that the mean value of crude protein content of *Oocystis* sp. was significantly different from diet 5 (t-value = 2.40, p< 0.001), diet 6 (t-value = 2.27, p< 0.001), diet 1 (t-value = 2.18, p< 0.001), diet 7 (t-value = 1.99, p< 0.001), diet 4 (t-value = 1.78, p< 0.001), diet 3 (t-value = 1.70, p< 0.001), diet 2 (t-value = 1.62, p< 0.001), and *A. platensis* (t-value = -7.73, p< 0.001).

Oocystis sp. had the highest crude fibre content (0.813 ± 0.0836 %) for the protein sources while *A. platensis* had the lowest (0.7 ± 0.0896 %). The formulated diets crude fibre content was less variable with diet 3 having the highest (8.948 ± 0.65 %) while diet 7 had the lowest (8.197 ± 1.275 %). There was a significant difference in the crude fibre content between *A. platensis*, *Oocystis* sp., and the formulated diets (One Way ANOVA, F = 18.893, df (8, 18), p<0.001). Tukey's HSD Test for multiple comparisons found that the mean value of crude fibre content of *A. platensis* was significantly different from diet 3 (t-value = -7.33, p< 0.001), diet 1 (t-value = -7.15, p< 0.001), diet 2 (t-value = -7.14, p< 0.001), diet 4 (t-value = -7.00, p< 0.001), diet 6 (t-value = -6.99, p< 0.001), diet 5 (t-value = -6.76, p< 0.001), and diet 7 (t-value = -6.66, p< 0.001). Tukey's HSD Test for multiple comparisons also found significant differences between *Oocystis* sp. versus diet 3 (t-value = -7.23, p< 0.001), diet 1 (t-value = -7.05, p< 0.001), diet 2 (t-value = -7.04, p< 0.001), diet 4 (t-value = -6.90, p< 0.001), diet 6 (t-value = -6.89, p< 0.001), diet 5 (t-value = -6.66, p< 0.001), and diet 7 (t-value = -6.56, p< 0.001).

Oocystis sp. had the highest nitrogen free extract content (28.919 ± 0.723 %) for the protein sources. Diet 5 had the highest nitrogen free extract content (24.776 ± 1.337 %), while diet 4 had the lowest (20.133 ± 1.603 %). There was a significant difference in the nitrogen free

extract content between *A. platensis*, *Oocystis* sp., and the formulated diets (One Way ANOVA, $F = 3.562$, df (8, 18), $p<0.001$). Tukey's HSD Test for multiple comparisons found that the mean value of nitrogen free extract content of *Oocystis* sp. was significantly different from *A. platensis* (t-value = 4.05, $p< 0.001$), diet 4 (t-value = 4.05, $p< 0.001$), and diet 3 (t-value = 3.71, $p< 0.001$).

Oocystis sp. had the highest metabolizable energy content (3936.501 ± 23.889 kcal/Kg) for the protein sources while *A. platensis* had the lowest (3588.337 ± 14.779 kcal/Kg). The formulated diets metabolizable energy content was less variable with diet 7 having the highest (3454.474 ± 48.067 kcal/Kg) while diet 4 had the lowest (3333.013 ± 11.445 kcal/Kg). There was a significant difference in the metabolizable energy content between *A. platensis*, *Oocystis* sp., and the formulated diets (One Way ANOVA, $F = 10.918$, df (8, 18), $p<0.001$). Tukey's HSD Test for multiple comparisons found significant differences between *Oocystis* sp. and diet 4 (t-value = 7.76, $p< 0.001$), diet 3 (t-value = 7.26, $p< 0.001$), diet 2 (t-value = 6.66, $p< 0.001$), diet 5 (t-value = 6.62, $p< 0.001$), diet 6 (t-value = 6.54, $p< 0.001$), diet 1 (t-value = 6.26, $p< 0.001$), diet 7 (t-value = 6.20, $p< 0.001$), and *A. platensis* (t-value = 4.48, $p< 0.001$).

Table 4.5: Nutritive contents for *A. platensis*, *Oocystis* sp. and the formulated diets (mean ± standard error)

Diet	Moisture (%)	Ash (%)	Crude Proteins (%)	Crude Lipids (%)	Crude Fibre (%)	Nitrogen Free Extract (%)	Metabolizable Energy (kcal/Kg)
Diet 1 (0% Control)	3.997 ± 0.382	7.466 ± 0.0876	49.875 ± 0.334	7.083 ± 0.196	8.749 ± 0.631	22.83 ± 1.135	3449.62 ± 23.102
Diet 2 (10% *Oocystis* sp.)	4.563 ± 0.714	8.213 ± 2.04	48.563 ± 0.455	7.455 ± 0.32	8.737 ± 0.849	22.469 ± 3.02	3418.194 ± 137.878
Diet 3 (20% *Oocystis* sp.)	5.183 ± 0.314	8.386 ± 0.583	49.219 ± 0.455	7.398 ± 0.676	8.948 ± 0.65	20.866 ± 1.356	3371.69 ± 54.685
Diet 4 (30% *Oocystis* sp.)	4.924 ± 0.533	9.935 ± 1.181	49.073 ± 0.193	7.35 ± 0.618	8.584 ± 1.001	20.133 ± 1.603	3333.013 ± 11.445
Diet 5 (10% *A. platensis*)	4.86 ± 0.425	7.941 ± 0.495	47.177 ± 1.271	6.938 ± 0.186	8.308 ± 1.007	24.776 ± 1.337	3421.823 ± 25.624
Diet 6 (20% *A. platensis*)	4.623 ± 0.345	7.846 ± 0.522	47.177 ± 0.956	7.02 ± 0.241	8.564 ± 0.709	24.769 ± 1.007	3428.097 ± 28.316
Diet 7 (40% *A. platensis*)	4.788 ± 0.447	7.628 ± 0.204	46.813 ± 1.079	7.21 ± 0.549	8.197 ± 1.275	25.364 ± 1.141	3454.474 ± 48.067
A. platensis	5.474 ± 0.349	11.505 ± 0.576	53.667 ± 1.074	8.523 ± 0.263	0.7 ± 0.0896	20.131 ± 1.314	3588.337 ± 14.779
Oocystis sp.	5.878 ± 0.584	8.836 ± 0.763	41.927 ± 0.51	13.627 ± 0.722	0.813 ± 0.0836	28.919 ± 0.723	3936.501 ± 23.889

4.3.5 Growth performance parameters

The growth parameters analysis for the cultured *O. niloticus* was done with the mean ± standard error values illustrated in table 4.6. There was no significant difference in the initial body weight (One Way ANOVA, F = 4.474, df (6, 203), p>0.05). The final body weight of the cultured *O. niloticus* varied, with the highest recorded in diet 2 (9.985 ± 0.504 g), while the lowest was recorded in diet 1 (7.778 ± 0.498 g). A significant difference (One Way ANOVA, F = 5.878, df (6, 163), p<0.001) was observed in the final body weights of the cultured *O. niloticus*. Tukey's HSD Test for multiple comparisons found significant differences between diet 2 versus diet 1 (t-value = 3.12, p< 0.001) and diet 5 versus diet 1 (t-value = 4.09, p< 0.001).

The total body weight gain of the cultured *O. niloticus* varied, diet 2 (9.753 ± 0.179 g) having the highest while diet 1 (7.540 ± 0.111 g) had the least. However, there was no significant difference in the weekly body weight gain (One Way ANOVA, F = 0.522, df (6, 56), p> 0.05). Diet 2 had the highest weekly body weight gain (1.084 ± 0.179 g) while diet 1 had the lowest (0.838 ± 0.111 g). The specific growth rate of the *O. niloticus* showed no significant difference (One Way ANOVA, F = 0.0104, df (6, 56), p> 0.05), with diet 2 registering the highest (5.969 ± 2.127), while diet 7 registered the lowest (5.379 ± 2.069).

The feed conversion ratio was not significantly different among the different diet treatments (One Way ANOVA, F = 0.186, df (6, 56), p> 0.05). The highest feed conversion ratio was found in diet 1 (1.734 ± 0.308), while the lowest was found in diet 2 (1.251 ± 0.274). The relative condition factor for the cultured *O. niloticus* showed no significant difference (One Way ANOVA, F = 0.579, df (6, 56), p> 0.05), diet 7 recording the highest (1.032 ± 0.0214), while diet 2 recorded the lowest (1.005 ± 0.00723). There were no mortalities recorded during the study, thus survival rate (100.00 ± 0.000) for all the diets was the same.

Table 4.6: The growth parameters of *Oreochromis niloticus* fed on the formulated diets (mean ± standard error)

Parameter	Diet 1 (0% Control)	Diet 2 (10% *Oocystis* sp.)	Diet 3 (20% *Oocystis* sp.)	Diet 4 (40% *Oocystis* sp.)	Diet 5 (10% *A. platensis*)	Diet 6 (20% *A. platensis*)	Diet 7 (40% *A. platensis*)
Initial Body Weight (g)	0.237 ± 0.00516	0.232 ± 0.0119	0.256 ± 0.0113	0.226 ± 0.0126	0.237 ± 0.0116	0.207 ± 0.00969	0.282 ± 0.0140
Final Body Weight (g)	7.778 ± 0.498	9.985 ± 0.504	8.040 ± 0.307	7.840 ± 0.329	9.937 ± 0.366	8.380 ± 0.292	7.988 ± 0.361
Total Body Weight Gain (g)	7.540 ± 0.111	9.753 ± 0.179	7.784± 0.171	7.614 ± 0.131	9.700 ± 0.154	8.682 ± 0.111	8.073 ± 0.151
Weekly Body Weight Gain (g)	0.838 ± 0.111	1.084 ± 0.179	0.865 ± 0.171	0.846 ± 0.131	1.078 ± 0.154	0.965 ± 0.111	0.897 ± 0.151
Specific Growth Rate	5.539 ± 2.844	5.969 ± 2.127	5.471 ± 1.686	5.627 ± 2.705	5.930 ± 2.945	5.968 ± 2.697	5.379 ± 2.069
Feed Conversion Ratio	1.734 ± 0.308	1.251 ± 0.274	1.479 ± 0.443	1.476 ± 0.385	1.459 ± 0.248	1.426 ± 0.310	1.460 ± 0.317
Relative Condition Factor (k)	1.008 ± 0.00867	1.005 ± 0.00723	1.010 ± 0.00832	1.009 ± 0.00818	1.011 ± 0.00832	1.015 ± 0.00974	1.032 ± 0.0214
Survival Rate (%)	100.00 ± 0.000	100.00 ± 0.000	100.00 ± 0.000	100.00 ± 0.000	100.00 ± 0.000	100.00 ± 0.000	100.00 ± 0.000

All cultured *O. niloticus* exhibited a steady increase in weight following their exposure to feed, as illustrated in figure 4.2. Immediately in the first week of sampling the fish in different treatments varied, with diet 3 recording the least growth, while diet 6 had the most. The subsequent weeks were characterised by overlaps in the growth curves. At the end of the experiment diet 5 had the most weight recorded while diet 3 still had the least weight registered.

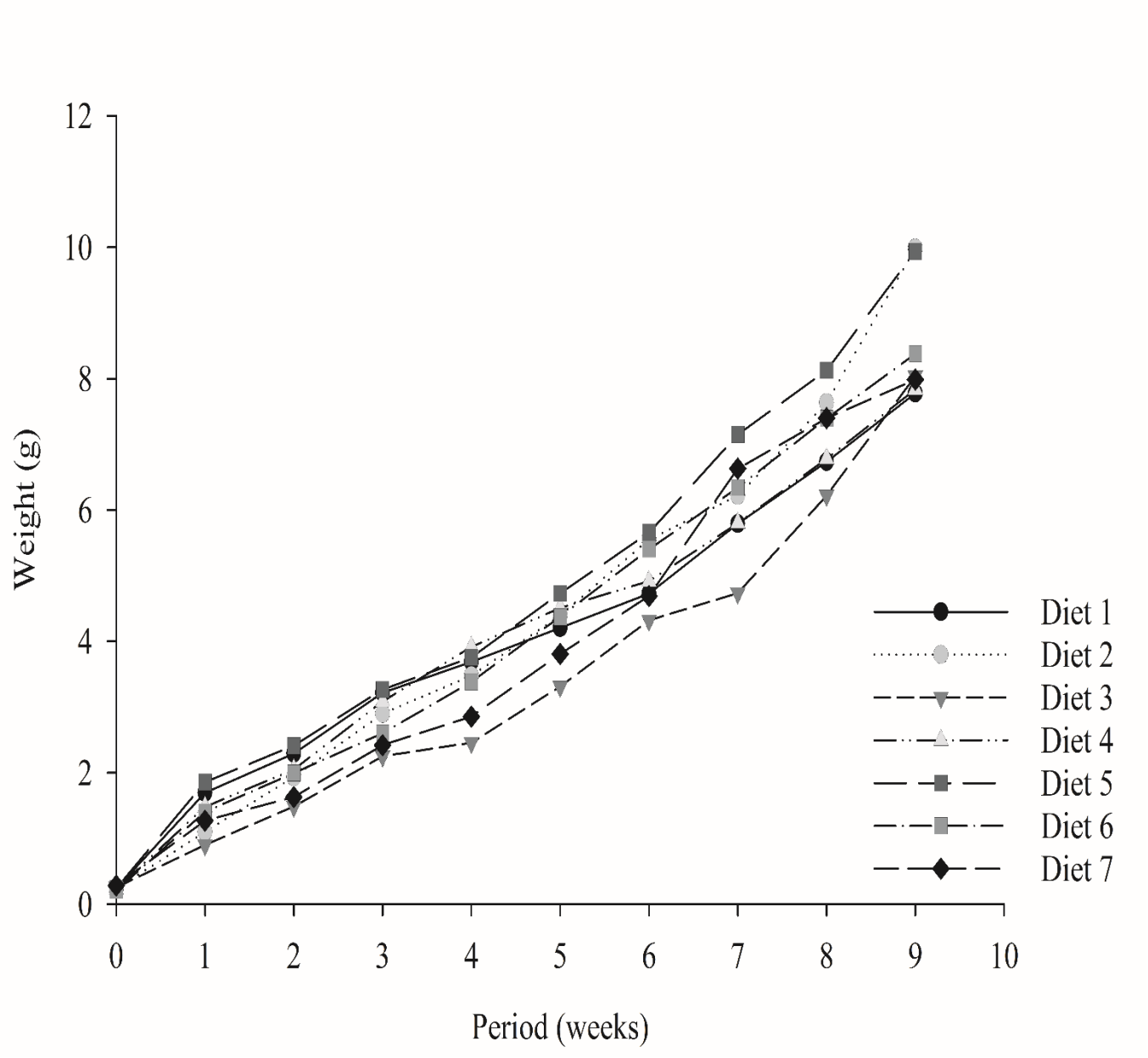


Figure 4.2: Growth curves of *Oreochromis niloticus* fed on formulated diets

4.4 Discussion

4.4.1 Water quality parameters

Water is a critical component of aquaculture since fish are fully vulnerable to shifts in water quality as they live, breathe, eat, develop, reproduce, and excrete waste in water (Akhter *et al*., 2021; Boyd & Tucker, 1998; Little & Bunting, 2016). Consequently, the water quality needs to be monitored and managed, so that the fish prevent stress and diseases to maintain an optimal level of health (Segner *et al*., 2012; Van Wyk & Scarpa, 1999). There are various parameters used to assess water quality in aquatic ecosystems. This study narrowed down to monitoring dissolved oxygen concentration, temperature, conductivity, and pH for physico-chemical parameters. Nitrite, nitrate, ammonia, soluble reactive phosphorus, and total phosphorus sum up the nutrients that were monitored in this study.

The concentration of dissolved oxygen in aquacultural ponds is often dependent on the oxygen diffused from the atmosphere and the one produced inside the system as a by-product of photosynthesis by algae (Boyd *et al*., 2018; Francis-Floyd, 1997). Mallya (2007) states that the recommended dissolved oxygen concentration for tropical freshwater fish is a minimum of 5 mg/l at 80% saturation. The recommended dissolved oxygen level for *O. niloticus* is at a range of between 5 and 7.5 mg/l (DeLong *et al*., 2009). The dissolved oxygen during this study was above 3 mg/l with a mean that was within the recommended range. The recorded dissolved oxygen concentration in this study were similar to those found by Makori *et al*. (2017) who recorded dissolved oxygen range from 4.86 to 10.53 mg/l in earthen ponds in Busia County in Kenya, and Keremah *et al.* (2014) who found a range of 2.8 to 6.6 mg/l in earthen ponds in Nigeria. However, they differed with those found by Akidiva *et al*. (2020) in Egerton University ponds, which can be attributed to the seasonal changes during the period which the data was collected and/or the time when sampling was done. Ojwala *et al*. (2018) recorded a higher mean of dissolved oxygen concentration (15.80 mg/l) at the fish ponds in Egerton University, contrasting those found in this study, but it is important to note the difference in sampling time. During their study sampling was done mostly at mid-day, while in this study sampling was done at dawn.

Temperature is an important factor, as it influences the growth and survival of cultured fish, as they are poikilothermic (Adhikari *et al*., 2018; Maulu *et al*., 2021; Ngoan, 2018). Ngugi *et al*. (2007) states that the recommended temperature range for *O. niloticus* is 20-35^{0}C. Qiang *et al*. (2018) noted that *O. niloticus* stopped eating when the temperature dropped to 18^{0}C, while at higher temperatures, Ngugi *et al*. (2007) notes that death may also occur due to increased

metabolic activities. The temperature range recorded during this study was within the acceptable range for optimum growth, because the pond was in a greenhouse. The temperature recordings in this study were similar to those obtained by Akidiva *et al*. (2020) who recorded a temperature range of 22-28 ^{0}C. Jesse *et al*. (2018) recorded temperature of similar range to this study, in a study also carried out in a greenhouse.

Conductivity is an important parameter as it gives an idea of the concentration of dissolved ions in a particular aquatic system. The conductivity in this study was within the desirable range of 100-2000 μS/cm as recommended by Syed *et al*. (2022) and Stone *et al*. (2013). Ogello *et al*. (2017), recorded similar conductivity values, in a study carried out in earthen ponds at Kegati Aquaculture Research Station in Kisii, Kenya.

pH is an important parameter, that requires continuous monitoring in aquaculture. Stone *et al*. (2013), notes that pH below 6.0 can results in fish death, and high pH above 9.0, results in increased unionized ammonia from ionized ammonia as such increasing toxicity of ammonia. Both Keremah *et al*. (2014) and Stone *et al*. (2013), note that the desirable range of pH is between 6.5 and 9, which consequently includes the values recorded in this study.

Kuhn *et al*. (2010) states that ammonia and nitrite are toxic to aquatic fauna and that they should not be allowed to accumulate in the culturing system as they can impair the growth, health, and survival rates of the organisms. Ammonia is important depending on the form (ionized or unionized) found in the system. Gogoi *et al*. (2021) states that aquaculture systems are very sensitive to ammonia concentrations and the recommended level of ammonia is at $\leq$ 0.5 mg/l. The ammonia concentration in this study was within the recommended range. The significance difference in the ammonia concentration temporally, can be attributed to the accumulation of wastes in the system but at very minimal levels.

Stone and Thomforde (2004) noted that the desired concentration of nitrite should be from 0-1mg/l, with the nitrite concentration recorded in this study falling in the desirable range. The ammonia and nitrite levels were like those found by Niyotwambaza *et al*. (2010), in a study at Rwasave Fish Farm and Research Station, Rwanda. Syed *et al*. (2022) notes that a concentration of up to 0.3 mg/l of nitrates is suitable for the culture of *O. niloticus*, the concentrations of this study consequently were within the desirable range.

Boyd and Tucker (1998) recommended up to a maximum of 0.2 mg/l of soluble reactive phosphorus in aquaculture, with the values recorded in this study being in this range. Elnady *et al*. (2010) found soluble reactive phosphorus ranging from 0.061 mg/l to 0.093 mg/l, similar

to the values recorded in this study only that the minimum values were lower. The total phosphorus levels recorded in this study were higher than those observed by Akidiva *et al*. (2020), which can be attributed to the accumulation of sediments in the pond where the study was carried out. Total phosphorus and soluble reactive phosphorus are important water quality parameters in aquaculture, as they stimulate algal growth (Stone *et al*., 2013); however, when in excess concentrations they can result to algal blooms in the pond, which in turn can cause fish death due to oxygen depletion in the system.

4.4.2 Proximate composition

4.4.2.1 Moisture content

The moisture content of *A. platensis* and *Oocystis* sp. were similar with very little difference. Patwary *et al*. (2013) notes that moisture is an important parameter in fish feeds as it dictates the shelf-life of the feed. Craig *et al*. (2017) stated that mould development and feed degradation are accelerated by high moisture content in the feeds. Russo and Yanong (2010) noted that high moisture levels of above 14% are among the factors that contribute to aflatoxin production in fish feeds. The moisture content of the formulated feeds in this study was at $\leq$ 5%, which was within the recommended range of $\leq$ 12% by Munguti *et al*. (2014b), implying the feeds will have a high shelf-life. Similar results were obtained by Appler (1985), in a study involving the use of *Hydrodictyon reticulatum* as a protein source for *O. niloticus* where the moisture content for the formulated feeds was < 5%.

4.4.2.2 Ash content

Divakaran and Duerr (1987) stated that the ash content of algae, is dependent on the culture medium used during culturing. De Souza *et al*. (2020) points out that the mineral content of algae can depend on several factors such as the cultivation temperature, salinity, physiological state of the strain, and geographic distribution. *A. platensis* had higher ash content than *Oocystis* sp., which can be as result of the culture medium. *A. platensis* are usually cultured in high alkaline media to promote growth as compared to *Oocystis* sp. which requires less alkaline environment. Mobin *et al*. (2019) stated that more than 6.7% dry eight of microalgae is ash, which is similar to the results found in this study. Shearer *et al*. (1992) noted that high ash content in fish feeds resulted to increased mortality, reduced growth and increased in susceptibility to diseases and parasites in cultured fish; consequently, the desired ash content in fish feeds should be low. Terpstra (2015) stated that fish feeds usually have an ash content ranging from 6 to 10%, with the ash content of the formulated diets used in this study falling within this range.

4.4.2.3 Crude protein content

Protein sources economically are important in fish feeds owing to the fact that they are the most expensive component. Jeong *et al*. (2019) stated that *A. platensis* has a protein content range of 60 to 70%, this value is slightly higher than the one obtained in this study. Falquet & Hurni (1997) gives a possible explanation of this difference, by stating that the crude protein of *A. platensis* often ranges between 50 and 70% depending on the time of harvesting in relation to daylight similar to the crude protein measured in this study. In a study carried out by Na *et al*. (2021) involving the culture of *Oocystis* sp., it was found that the harvested biomass had a protein content of 43.9% of the wet weight. This is similar to the findings in this study. The crude protein during the study was formulated to meet the nutritional requirements of *O. niloticus* fry, as they require a range of 40-50% (El-Sayed & Teshima, 1992; Munguti *et al*., 2014b). Craig *et al*. (2017) points that it is critical to determine the protein requirements for each species and life stage of cultured fish, as it is the most expensive component of fish feeds. Terpstra (2015) states that proteins are important in fish feeds as they are used for primarily tissue accretion and growth; they can also be utilized as a source of energy.

4.4.2.4 Crude lipid content

Sajjadi *et al*. (2018) notes that algae have varying levels (2-23% dry weight) of lipids and composition influenced by various individual or a combination factor such as geographical region, salinity, and sunlight intensity. *Oocystis* sp. had significantly higher crude lipid content in this study; similar to that found by Na *et al*. (2021). Yang *et al*. (2020) states that the lipid content of *A. platensis* ranges between 5 and 10% of its dry weight; consequently, placing the value observed in this study within this range. Kim *et al*. (2012) states that the lack of dietary lipids may lead to an increased reliance on protein for energy, which would increase ammonia excretion and cause water pollution in aquaculture. Consequently, for fish to develop normally and expand to their full potential, lipids are necessary to perform crucial physiological roles in supplying energy, essential fatty acids, and fat-soluble nutrients (Oliva-Teles, 2012). Munguti *et al*. (2014b) recommends a provision of $\geq$ 8% crude lipids for *O. niloticus* fry's, with all the formulated diets providing the required level of crude lipids during the study.

4.4.2.5 Crude fibre content

Crude fibre contents of *A. platensis* and *Oocystis* sp. were significantly lower compared to the ones in the formulated diets. The high crude fibre in the formulated diets was mainly from the contribution of other ingredients, which assisted to meet the required crude fibre levels for *O. niloticus* fries of $\geq$ 4% (Munguti *et al*., 2014b). The right amount of fibre in fish feeds is

important as it assists in moderating passage of food in the alimentary canal. However, too much or > 13% fibre is not recommended as it decreases the digestibility of feeds and growth of fish (Adewolu *et al*., 2010; Agbabiaka *et al*., 2013). Metabolizable energy was within the recommended range by Orlando *et al*. (2017). Metabolizable energy is important in sustaining the growth, reproduction, and health of the cultured fish.

4.4.3 Growth performance of *Oreochromis niloticus* from the different treatments

There were significant differences in the final body weight of fries in diets 2 and 5 from diet 1, which suggests that partial replacement of *C. nilotica* with either *A. platensis* or *Oocystis* sp. improves the growth performance of *O. niloticus* fries. The highest body weight gain of the fries was observed in diets 2 and 5; however, there was no significant difference. These results agree with those found by Badwy *et al*. (2008), who noted that partial substitution of fishmeal with *Chlorella* sp. and *Scenedesmus* sp. in fish feeds resulted in improved growth performance of *O. niloticus*. Mustafa *et al*. (1994) observed that supplementation of fish meal with *A. platensis* resulted in increase in growth and better protein digestibility in the red sea bream (*Pagrus major*). Similarly, Roy and Pal (2015) point out that algae-based feeds result in improved growth performance and better carcass quality of *O. niloticus*. In contrast, Walker & Berlinsky (2011) noted a proportional decrease in the growth of the Atlantic cod when *Nannochloropsis* sp. and *Isochrysis* sp. replaced fishmeal as sources of crude protein. However, according to this study, partial replacement of *C. nilotica* with either *A. platensis* or *Oocystis* sp. at 10%, 20% and 40%, all resulted in an increase in the body weight gain of the *O. niloticus*, but the highest body weight gain was at 10% replacement of *C. nilotica* with either *A. platensis* or *Oocystis* sp.

Specific growth rate in this study was a bit higher in the diets 2 to diet 6 pointing out that partial replacement of *C. nilotica* with either *A. platensis* or *Oocystis* sp. resulted in increase in the growth of *O. niloticus*. Kim *et al*. (2013), also found that there was increase in the specific growth rate of parrot fish (*Oplegnathus fasciatus*) when fish meal was partially replaced with *A. platensis* in their diets. Similar results were also found by Sarker *et al*. (2020), in a study utilizing *Nannochloropsis oculata* substitution for fish meal on the growth performance of *O. niloticus*. Vizcaíno *et al*. (2014) found similar results with the use of *Scenedesmus almeriensis* as fishmeal alternative in diets for gilthead sea bream (*Sparus aurata*) juveniles, with partial replacement at 25% having highest specific growth rate.

The feed conversion ratio is an important parameter during the assessment of the impact of feed on the growth of cultured organisms as it indicates the efficiency of the feed in the production of cultured organisms (Luo *et al*., 2021). Fry *et al*. (2018) points out that the feed conversion ratio in aquaculture range from 1.0 to 2.4, with a lower value indicating a higher efficiency of the feed. The values obtained in this study were within this range, with diet 2 being the most efficient feed. Mengistu *et al*. (2020) states that a feed conversion factor below 2.0, is considered as acceptable in the culture of *O. niloticus*; consequently, all the diets in this study had an acceptable ratio. Kim *et al*. (2013) obtained similar results as they noted a reduction in the feed conversion ratio of cultured parrot fish (*Oplegnathus fasciatus*) at 5% and 10% replacement of fishmeal with *A. platensis*.

Gubiani *et al*. (2020) states that body condition of an organism is an important indicator of the health of that individual or population. Relative condition factor is an example of an index amongst various indices that are utilized to assess the body condition and indicate the nutritional and physiological status of fishes (Labocha *et al*., 2014; Le Cren, 1951; Gubiani *et al*., 2020). The values of the relative condition factor in this study were all above 1.0, indicating that the fish were in good condition (Ayoade, 2011), as such they were in good health during the study and there was isometric growth, which is desired for aquaculture. The relative condition factor results were similar to those obtained by Ighwela *et al*. (2011) while assessing the growth of *O. niloticus* fingerlings. The survival rate across all diets was 100%, implying that the replacement of *C. nilotica* with either *A. platensis* or *Oocystis* sp. had no effect on the survival rate of the fish, and optimum conditions were provided for the growth and survival of the fish. These results are in agreement with those obtained by Sarker *et al*. (2018) where the substitution of fishmeal with *Nannochloropsis oculata* had no significant effect on the survival of *O. niloticus* juveniles.

4.5 Conclusion

This study revealed that 40% substitution of *C. nilotica* as a protein source in fish feeds with either *A. platensis* or *Oocystis* sp. enhances the growth of *O. niloticus* fry at the same time lowering the cost. The finding that there was no significant difference in the body weight gain, specific growth rate, feed conversion factor, and relative condition factor, implies that partial replacement of *C. nilotica* with either *A. platensis* or *Oocystis* sp. is encouraged at 40% to optimize the growth of *O. niloticus* fry at a lower cost.

CHAPTER FIVE
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

This study was conducted in the Biological Sciences Department and Agro-science Fish Park at Egerton University from October 2021 to May 2022. *Oocystis* sp. was first cultured and, subsequently diets were formulated incorporating partial substitution of *C. nilotica* with *Oocystis* sp. and *A. platensis*, which was fed to *O. niloticus* fries. The objective was to assess the nutritive value of *A. platensis,* and *Oocystis* sp. ingredients as potential crude protein sources, and the growth performance of *O. niloticus* fed on these alternative sources of protein. The growth rate, divisions per day, and doubling time for *Oocystis* sp. was determined. The nutritive values of *A. platensis*, *Oocystis* sp., and the formulated diets, and the growth performances of the formulated diets on *O. niloticus* were determined. The major findings from the study are as follows: the research showed that the inorganic fertilizers' specific compositions and comparison between the culture media had no impact on the growth rate, divisions per day, and the doubling time of cultured *Oocystis* sp. However, the time required to attain optimum growth differed based on fertilizer combination. *A. platensis* had higher percentage of crude protein than *Oocystis* sp., with the vice versa occurring for the crude lipids' percentage. The highest growth rate was achieved in diet 2 and 6, the feed conversion ratio was within the recommended range for all the diets, there was isometric growth of fish fries in all the diets with the relative condition factor being above 1 and the feed conversion ratio were within the acceptable range for *O. niloticus* culture.

5.2 Conclusions

i. For specific objective 1, it was determined that inorganic fertilizers can be used as an option of cost-effective media in the culture of *Oocystis* sp., with the ratio of 3:1:1 while using NPK, urea, and DAP fertilizers respectively to achieve the highest biomass within the shortest period.

ii. For specific objective 2, *A. platensis* had higher crude protein content, while *Oocystis* sp. had higher crude lipids and nitrogen free extract content. The moisture, ash, and crude fibre content were all similar for the two algae species.

iii. For specific objective 3, 40% substitution of *C. nilotica* as a protein source in fish feeds with either *A. platensis* or *Oocystis* sp. enhances the growth of *O. niloticus* fry, at the same time lowering the cost of production, while the water quality

parameters were within the optimum range for the growth and survival of the fish. The relative condition factor indicated all fish were healthy and in good conditions.

5.3 Recommendations

i. This study recommends the culture of *Oocystis* sp. using inorganic fertilizers as a cost-effective media, for the formulation of Nile tilapia feeds.

ii. This study recommends the partial replacement of *C. nilotica* with either 40% of: *A. platensis* or *Oocystis* sp. in Nile tilapia feeds to lower the cost of production and to make aquaculture more profitable.

iii. This study recommends further studies on the applicability of the *A. platensis* or *Oocystis* sp. for the formulation of feeds for other species of fish.

REFERENCES

Adeleke, B., Robertson-Andersson, D., Moodley, G., & Taylor, S. (2020). Aquaculture in Africa: A comparative review of Egypt, Nigeria, and Uganda *vis-a-vis* South Africa. *Reviews in Fisheries Science & Aquaculture*, *29*(2), 167-197.

Adewolu, M. A., Ikenweiwe, N. B., & Mulero, S. M. (2010). Evaluation of an animal protein mixture as a replacement for fishmeal in practical diets for fingerlings of *Clarias gariepinus* (Burchell, 1822). *Israel Journal of Aquaculture-Bamidgeh, 62*(4), 237-244.

Adhikari, S., Keshav, C. A., Barlaya, G., Rathod, R., Ikmail, S., Saha, G. S., & Sundaray, J. K. (2018). Adaptation and mitigation strategies of climate change impact in freshwater aquaculture in some states of India. *Journal of Fisheries Sciences*, *12*(1), 16-21.

Agbabiaka, L. A., Okorie, K. C., & Ezeafulukwe, C. F. (2013). Plantain peels as dietary supplement in practical diets for African catfish (*Clarias gariepinus* Burchell 1822) fingerlings. *Agriculture and Biology Journal of North America*, *4*(2), 155-159.

Akhter, F., Siddiquei, H. R., Alahi, M. E. E., & Mukhopadhyay, S. C. (2021). Recent advancement of the sensors for monitoring the water quality parameters in smart fisheries farming. *Computers*, *10*(3), 26.

Akidiva, A., Yasindi, A., & Nzula, K. (2020). Influence of greenhouse technology on selected pond water quality parameters and growth performance of Nile tilapia in high altitude areas. *International Journal of Fisheries and Aquatic Studies 8*(4), 142-147.

Aloo, P. A., Charo-Karisa, H., Munguti, J., & Nyonje, B. (2017). A review on the potential of aquaculture development in Kenya for poverty alleviation and food security. *African Journal of Food, Agriculture, Nutrition and Development*, *17*(1), 11832-11847.

American Public Health Association (APHA), (2005). *Standard method for the examination of water and wastewater* (21st edition). Washington DC: America Water Works Association and Water Control Federation. USA.

Anderson, J. L., Asche, F., Garlock, T., & Chu, J. (2017). Aquaculture: Its role in the future of food. In *World Agricultural Resources and Food Security, 17,* 159-173.

Andrade, M. D. R., & Costa, J. A. V. (2008). Culture of microalga *Spirulina platensis* in alternative sources of nutrients. *Ciência e Agrotecnologia*, *32*(5), 1551-1556.

Ansari, F. A., Singh, P., Guldhe, A., & Bux, F. (2017). Microalgal cultivation using aquaculture wastewater: integrated biomass generation and nutrient remediation. *Algal Research, 21,* 169-177.

Appler, H. N. (1985). Evaluation of *Hydrodictyon reticulatum* as protein source in feeds for *Oreochromis niloticus* (Tilapia) and *Tilapia zillii*. *Journal of Fish Biology, 27*(3), 327-

334.

Arenas, E. G., Rodriguez Palacio, M. C., Juantorena, A. U., Fernando, S. E. L., & Sebastian, P. J. (2017). Microalgae as a potential source for biodiesel production: techniques, methods, and other challenges. *International Journal of Energy Research*, *41*(6), 761-789.

Ashraf, M., Javaid, M., Rashid, T., Ayub, M., Zafar, A., Ali, S., & Naeem, M. (2011). Replacement of Expensive Pure Nutritive Media with Low-Cost Commercial Fertilizers for Mass Culture of Freshwater Algae, *Chlorella vulgaris*. *International Journal of Agriculture & Biology*, *13*(4), 484-490.

Association of Official Analytical Chemists (AOAC), (1995). *Official Methods of Analysis* (16th edition). Arlington, VA, USA: Association of Official Analytical Chemists. http://www.sciepub.com/reference/141205

Ayoade, A. A. (2011). Length-weight relationship and diet of African carp *Labeo ogunensis* (Boulenger, 1910) in Asejire Lake Southwestern Nigeria. *Journal of Fisheries and Aquatic Science*, *6*(4), 472-478.

Ayoola, A. (2010). *Replacement of Fishmeal with Alternative Protein Sources in Aquaculture Diets.* (Master's thesis, North Carolina State University) North Carolina State University Institutional Repository. https://repository.lib.ncsu.edu/bitstream/handle/1840.16/6546/etd.pdf?sequence=1

Badwy, T. M., Ibrahim, E. M., & Zeinhom, M. M. (2008). Partial replacement of fishmeal with dried microalga (*Chlorella* spp. and *Scenedesmus* spp.) in Nile tilapia (*Oreochromis niloticus*) diets. In *8th International Symposium on Tilapia in Aquaculture, 2008*, 801-810.

Belton, B., van Asseldonk, I. J. M., & Thilsted, S. H. (2014). Faltering fisheries and ascendant aquaculture: Implications for food and nutrition security in Bangladesh. *Food Policy*, *44*, 77-87.

Béné, C., Barange, M., Subasinghe, R., Pinstrup-Andersen, P., Merino, G., Hemre, G. I., & Williams, M. (2015). Feeding 9 billion by 2050–Putting fish back on the menu. *Food Security*, *7*(2), 261-274.

Boyd, C. E., & Tucker, C. S. (1998). Water quality requirements. In *Pond Aquaculture Water Quality Management* (pp. 87-153). Springer, Boston, MA.

Boyd, C. E., Torrans, E. L., & Tucker, C. S. (2018). Dissolved oxygen and aeration in ictalurid catfish aquaculture. *Journal of the World Aquaculture Society*, *49*(1), 7-70.

Brown, M. R., Jeffrey, S. W., Volkman, J. K., & Dunstan, G. A. (1997). Nutritional properties

of microalgae for mariculture. *Aquaculture*, *151*(1-4), 315-331.

Charo-Karisa, H., Nzayisenga, L., Opiyo, A., & Munguti, J. (2012). Nutritive Value and Availability of Commonly Used Feed Ingredients for Farmed Nile Tilapia (*Oreochromis niloticus* L.) and African Catfish (*Clarias gariepinus*, Burchell) in Kenya, Rwanda, and Tanzania. *African Journal of Food, Agriculture, Nutrition and Development*, *12*(3), 6135-6155.

Chou, R. L., Her, B. Y., Su, M. S., Hwang, G., Wu, Y. H., & Chen, H. Y. (2004). Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. *Aquaculture*, *229*(1-4), 325-333.

Chow, K. W., Rumsey, G. L., & Waldroup, P. W. (1980). Linear programming in fish diet formulation. In *FAO/UNDP Training Course in Fish Feed Technology, Seattle, Wash. (USA), 9 Oct 1978*. https://agris.fao.org/agris-search/search.do?recordID=XF8004181

Chowdury, K. H., Nahar, N., & Deb, U. K. (2020). The Growth Factors Involved in Microalgae Cultivation for Biofuel Production: A Review. *Computational Water, Energy, and Environmental Engineering*, *9*(4), 185-215.

Craig, S. R., Helfrich, L. A., Kuhn, D., & Schwarz, M. H. (2017). *Understanding fish nutrition, feeds, and feeding*. Virginia Cooperative Extension. https://vtechworks.lib.vt.edu/bitstream/handle/10919/80712/FST-269.pdf

Csavina, J. L., Stuart, B. J., Guy Riefler, R., & Vis, M. L. (2011). Growth optimization of algae for biodiesel production. *Journal of Applied Microbiology*, *111*(2), 312-318.

da Silva Ferreira, V., & Sant'Anna, C. (2017). Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications. *World Journal of Microbiology and Biotechnology*, *33*(1), 1-8.

De Chavez, P. D., & Bolivar, R. B. (2018). *Spirulina platensis* as an alternative protein source for the African catfish *Clarias gariepinus*. *Aquaculture, Aquarium, Conservation & Legislation*, *11*(2), 481-494.

De Souza, R. A. S., Saldanha-Corrêa, F. M. P., Gallego, A. G., & Neto, A. M. P. (2020). Semi-quantitative determination of ash element content for freeze-dried, defatted, sulfated and pyrolysed biomass of *Scenedesmus* sp. *Biotechnology for Biofuels*, *13*(1), 1-12.

DeLong, D. P., Losordo, T., & Rakocy, J. (2009). *Tank culture of tilapia* (Vol. 282). Stoneville, Mississippi: Southern Regional Aquaculture Center. https://appliedecology.cals.ncsu.edu/wp-content/uploads/282.pdf

Divakaran, S., & Duerr, E. O. (1987). Characteristics of a blue-green alga (*Spirulina platensis*) preserved by acidulation with sulfuric acid. *Journal of Agricultural and Food*

Chemistry, *35*(4), 568-570.

Elnady, M. A., Alkobaby, A. I., Salem, M. A., Abdel-Salam, M., & Asran, B. M. (2010). Effect of fertilization and low quality feed on water quality dynamics and growth performance of Nile tilapia (*Oreochromis niloticus*). *Journal of American Science*, *6*(10), 1044-1054.

El-Sayed, A. F. M., & Teshima, S. I. (1992). Protein and energy requirements of Nile tilapia, *Oreochromis niloticus*, fry. *Aquaculture*, *103*(1), 55-63.

El-Sheekh, M., El-Shourbagy, I., Shalaby, S., & Hosny, S. (2014). Effect of feeding *Arthrospira platensis* (Spirulina) on growth and carcass composition of hybrid red tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*). *Turkish Journal of Fisheries and Aquatic Sciences*, *14*(2), 471-478.

Enyidi, U. D. (2017). *Chlorella vulgaris* as protein source in the diets of African catfish *Clarias gariepinus*. *Fishes*, *2*(4), 1-12.

Falquet, J., & Hurni, J. P. (1997). *The nutritional aspects of Spirulina*. Antenna Foundation. https://www.antenna.ch/wp-content/uploads/2017/03/AspectNut_UK.pdf

Fogg, G. E., & Thake, B. (1987). *Algal cultures and phytoplankton ecology*. University of Wisconsin Press.

Food and Agriculture Organization (FAO), (2018). *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals.* Food and Agriculture Organization of the United Nations. http://www.fao.org/documents/card/en/c/I9540EN/

Food and Agriculture Organization (FAO), (2020). *The State of World Fisheries and Aquaculture 2020 - Sustainability in action.* Food and Agriculture Organization of the United Nations. www.fao.org/documents/card/en/c/ca9229en/

Francis-Floyd, R. (1997). *Dissolved oxygen for fish production*. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Fry, J. P., Mailloux, N. A., Love, D. C., Milli, M. C., & Cao, L. (2018). Feed conversion efficiency in aquaculture: do we measure it correctly? *Environmental Research Letters*, *13*(2), 1-8.

Gani, P., Sunar, N. M., Matias-Peralta, H. M., & Apandi, N. (2019). An overview of environmental factor's effect on the growth of microalgae. *Journal of Applied Chemistry and Natural Resources*, *1*(2), 1–5.

Garlock, T., Asche, F., Anderson, J., Bjørndal, T., Kumar, G., Lorenzen, K., Andrew, R., Martin, D., & Tveterås, R. (2020). A global blue revolution: Aquaculture growth across regions, species, and countries. *Reviews in Fisheries Science & Aquaculture*, *28*(1), 107-116.

Gatlin, I. D. M. (2010). *Principles of fish nutrition* (No. 5003). Southern Regional Aquaculture Center (SRAC). https://fisheries.tamu.edu/files/2013/09/SRAC-Publication-No.-5003-Principles-of-Fish-Nutrition.pdf

Gayanilo, F. C. (1997). *Fisat: FAO-ICLARM stock assessment tools, reference manuals* (No. 8). Food and Agriculture Organization of the United Nations. https://agris.fao.org/agris-search/search.do?recordID=XF1998081063

Ghafari, M., Rashidi, B., & Haznedaroglu, B. Z. (2018). Effects of macro and micronutrients on neutral lipid accumulation in oleaginous microalgae. *Biofuels*, *9*(2), 147-156.

Gogoi, M., Bhattacharya, P., Sen, S. K., Mukherjee, I., Bhushan, S., & Chaudhuri, S. R. (2021). Aquaculture effluent treatment with ammonia remover *Bacillus albus* (ASSF01). *Journal of Environmental Chemical Engineering*, *9*(4), 105697.

Gubiani, É. A., Ruaro, R., Ribeiro, V. R., & Fé, Ú. M. G. D. S. (2020). Relative condition factor: Le Cren's legacy for fisheries science. *Acta Limnologica Brasiliensia*, *32*, 1-9.

Habib, B., Ahsan, M., Parvin, M., Huntington, T. C., & Hasan, M. R. (2008). Review on culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish. In: *Fisheries and Aquaculture Circular* (No. 1034). Food and Agriculture Organization of the United Nations. https://www.fao.org/3/i0424e/i0424e00.pdf

Halwart, M. (2020). Fish farming high on the global food system agenda in 2020. In: *Food and Agriculture Organization (FAO) Aquaculture Newsletter*, (No. 61). Food and Agriculture Organization of the United Nations. https://www.fao.org/3/ca8302en/CA8302EN.pdf

Hinrichsen, E., Walakira, J. K., Langi, S., Ibrahim, N. A., Tarus, V., Badmus, O., & Baumüller, H. (2022). *Prospects for Aquaculture Development in Africa: A Revie of Past Performance to Access Future Potential* (Report No. ZEF Working Paper Series No. 211). University of Bonn, Center for Development Research (ZEF), Bonn.

Howe, J. C. (1996). Fish nutrition in aquaculture. *Fisheries Research*, *26*(3–4), 387–390.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., & Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal*, *54*(4), 621-639.

Ighwela, K. A., Ahmed, A. B., & Abol-Munafi, A. B. (2011). Condition factor as an indicator of growth and feeding intensity of Nile tilapia fingerlings (*Oreochromis niloticus*) feed on different levels of maltose. *American-Eurasian Journal of Agricultural and Environmental Science*, *11*(4), 559-563.

Jeong, S. J., Choi, J. W., Lee, M. K., Choi, Y. H., & Nam, T. J. (2019). Spirulina crude protein

promotes the migration and proliferation in IEC-6 cells by activating EGFR/MAPK signaling pathway. *Marine Drugs*, *17*(4), 205.

Jesse, D. R., David, D. K., & Siyun, W. (2018). Culturing tilapia in greenhouse-enclosed intensive recirculating aquaculture system in British Columbia, Canada. In *Aquaculture America 2018: Posters, Freshwater Fish Posters*.

Joslyn, M. (2012). *Methods in Food Analysis: Applied to Plant Products*. Elsevier. https://books.google.co.ke/books?id=8b8_f68cyv4C

Jung, F., Krüger-Genge, A., Waldeck, P., & Küpper, J. H. (2019). *Spirulina platensis*, a super food? *Journal of Cellular Biotechnology*, *5*(1), 43-54.

Kaushik, S. J., & Hemre, G. I. (2008). Plant proteins as alternative sources for fish feed and farmed fish quality. In *Improving farmed fish quality and safety* (300-327). Woodhead Publishing.

Kaushik, S. J., & Seiliez, I. (2010). Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. *Aquaculture Research*, *41*(3), 322-332.

Kazbar, A., Cogne, G., Urbain, B., Marec, H., Le-Gouic, B., Tallec, J., Takache, H., Ismail, A., & Pruvost, J. (2019). Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. *Algal Research*, *39*, 101432.

Kenya Marine and Fisheries Research Institute (KMFRI), (2017). *Kenya's Aquaculture Brief 2017: Status, Trends, Challenges and Future Outlook.* Kenya Marine and Fisheries Research Institute (KMFRI). https://kmfri.go.ke/images/pdf/reports/Kenya_Aquaculture_Brief_2017.pdf

Keremah, R. I., Davies, O. A., & Abezi, I. D. (2014). Physico-chemical analysis of fish pond water in freshwater areas of Bayelsa State, Nigeria. *Greener Journal of Biological Sciences*, *4*(2), 033-038.

Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*, *17*(1), 1-21.

Kim, D. K., Kim, K. D., Seo, J. Y., & Lee, S. M. (2012). Effects of dietary lipid source and level on growth performance, blood parameters and flesh quality of sub-adult olive flounder (*Paralichthys olivaceus*). *Asian-Australasian Journal of Animal Sciences*, *25*(6), 869-879.

Kim, S. S., Rahimnejad, S., Kim, K. W., & Lee, K. J. (2013). Partial replacement of fish meal with *Spirulina pacifica* in diets for parrot fish (*Oplegnathus fasciatus*). *Turkish Journal of Fisheries and Aquatic Sciences*, *13*(2), 197-204.

Kuhn, D. D., Drahos, D. D., Marsh, L., & Flick Jr, G. J. (2010). Evaluation of nitrifying bacteria product to improve nitrification efficacy in recirculating aquaculture systems. *Aquacultural Engineering*, *43*(2), 78-82.

Labocha, M. K., Schutz, H., & Hayes, J. P. (2014). Which body condition index is best? *Oikos*, *123*(1), 111-119.

Latala, A., Hamoud, N., & Plinski, M. (1991). Growth dynamics and morphology of plankton green algae from brackish waters under the influence of salinity, temperature and light. *Acta Ichthyologica et Piscatoria. Supplementum*, *21*, 101-116.

Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *The Journal of Animal Ecology*, 201-219.

Lee, B. H., & Picard, G. A. (1982). Fractionation and properties of *Oocystis* algal proteins. *Canadian Institute of Food Science and Technology Journal*, *15*(2), 120-130.

Levasseur, M., Thompson, P. A., & Harrison, P. J. (1993). Physiological acclimation of marine phytoplankton to different nitrogen sources 1. *Journal of Phycology*, *29*(5), 587-595.

Liti, D. M., Mugo, R. M., Munguti, J. M., & Waidbacher, H. (2006). Growth and economic performance of Nile tilapia (*Oreochromis niloticus* L.) fed on three brans (maize, wheat and rice) in fertilized ponds. *Aquaculture Nutrition*, *12*(3), 239-245.

Little, D. C., & Bunting, S. W. (2016). Aquaculture technologies for food security. In *Emerging Technologies for Promoting Food Security* (pp. 93-113). Woodhead Publishing.

Lopez, M. J., & Mohiuddin, S. S. (2020). Biochemistry, Essential Amino Acids. In: *StatPearls [Internet]*. StatPearls Publishing https://www.ncbi.nlm.nih.gov/books/NBK557845/?report=classic

Luo, S., Li, X., Onchari, M. M., Li, W., Bu, Y., Lek, S., Zhang, T., Wang, Z., & Jin, S. (2021). High feeding level alters physiological status but does not improve feed conversion efficiency and growth performance of juvenile red swamp crayfish *Procambarus clarkii* (Girard, 1852). *Aquaculture*, *537*, 736507.

Maier, R. M., & Pepper, I. L. (2015). Bacterial Growth. In *Environmental Microbiology: Third Edition,* 37–56. Academic Press.

Makori, A. J., Abuom, P. O., Kapiyo, R., Anyona, D. N., & Dida, G. O. (2017). Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticus*) growth in earthen ponds in Teso North Sub-County, Busia County. *Fisheries and Aquatic Sciences*, *20*(1), 1-10.

Mallya, Y. J. (2007). *The effects of dissolved oxygen on fish growth in aquaculture* (Final Project, The United Nations University Fisheries Training Programme). The United

Nations University. https://www.grocentre.is/static/gro/publication/58/document/yovita07prf.pdf

Marshall, B. (2017). *Aquaculture and Fish Farming*. Larsen and Keller Education

Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Reviews*, *14*(1), 217-232.

Maulu, S., Hasimuna, O. J., Haambiya, L. H., Monde, C., Musuka, C. G., Makorwa, T. H., Munganga, B. P., Phiri, K. J., & Nsekanabo, J. D. (2021). Climate change effects on aquaculture production: sustainability implications, mitigation, and adaptations. *Frontiers in Sustainable Food Systems*, *5*, 609097.

Mayo, A. W. (1997). Effects of temperature and pH on the kinetic growth of unialga *Chlorella vulgaris* cultures containing bacteria. *Water Environment Research*, *69*(1), 64-72.

Mbugua, H. M. (2008). *Aquaculture in Kenya; Status, Challenges and Opportunities.* Directorate of Aquaculture Development, Kenya. https://aquadocs.org/handle/1834/7367

Mengistu, S. B., Mulder, H. A., Benzie, J. A., & Komen, H. (2020). A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (*Oreochromis niloticus*). *Reviews in Aquaculture*, *12*(2), 524-541.

Michael, A., Kyewalyanga, M. S., & Lugomela, C. V. (2019). Biomass and nutritive value of Spirulina (*Arthrospira fusiformis*) cultivated in a cost-effective medium. *Annals of Microbiology*, *69*(13), 1387-1395.

Miles, R. D., & Chapman, F. A. (2015). The Benefits of Fish Meal in Aquaculture Diets. *University of Florida IFAS Extension*, 1–6.

Mobin, S. M., Chowdhury, H., & Alam, F. (2019). Commercially important bioproducts from microalgae and their current applications–A review. *Energy Procedia*, *160*, 752-760.

Moraes, I. D. O., Arruda, R. D. O. M., Maresca, N. R., Antunes, A. D. O., & Moraes, R. D. O. (2013). *Spirulina platensis*: process optimization to obtain biomass. *Food Science and Technology*, *33*, 179-183.

Msangi, S. & Batka, M. (2015) The rise of aquaculture: The Role of Fish in Global Food Security. In *IFPRI (ed.) 2014-2015 Global Food Policy Report Washington D.C.* (60-72) International Food Policy Research Institute.

Munguti, J. M., Kim, J. D., & Ogello, E. O. (2014a). An overview of Kenyan aquaculture: Current status, challenges, and opportunities for future development. *Fisheries and Aquatic Sciences*, *17*(1), 1-11.

Munguti, J. M., Musa, S., Orina, P. S., Kyule, D. N., Opiyo, M. A., Charo-Karisa, H., & Ogello, E. O. (2014b). An overview of current status of Kenyan fish feed industry and feed management practices, challenges and opportunities. *International Journal of Fisheries and Aquatic Studies 2014; 1*(6), 128-137.

Munguti, J., Odame, H., Kirimi, J., Obiero, K., Ogello, E., & Liti, D. (2021). Fish feeds and feed management practices in the Kenyan aquaculture sector: Challenges and opportunities. *Aquatic Ecosystem Health & Management*, *24*(1), 82-89.

Mustafa, M. G., Umino, T., & Nakagawa, H. (1994). The effect of Spirulina feeding on muscle protein deposition in red sea bream, *Pagrus major*. *Journal of Applied Ichthyology*, *10*(2-3), 141-145.

Na, H., Jo, S. W., Do, J. M., Kim, I. S., & Yoon, H. S. (2021). Production of Algal Biomass and High-Value Compounds Mediated by Interaction of Microalgal *Oocystis* sp. KNUA044 and Bacterium *Sphingomonas* KNU100. *Journal of Microbiology and Biotechnology*, *31*(3), 387-397.

Nadarajah, S., & Flaaten, O. (2017). Global aquaculture growth and institutional quality. *Marine Policy*, *84*, 142-151.

Nalley, J. O., O'Donnell, D. R., & Litchman, E. (2018). Temperature effects on growth rates and fatty acid content in freshwater algae and cyanobacteria. *Algal Research*, *35*, 500-507.

Nayak, M., Thirunavoukkarasu, M., & Mohanty, R. C. (2016). Cultivation of freshwater microalga *Scenedesmus* sp. using a low-cost inorganic fertilizer for enhanced biomass and lipid yield. *The Journal of General and Applied Microbiology*, *62*(1), 7-13.

Ngoan, L. D. (2018). Effects of climate change in aquaculture: Case study in Thua Thien Hue Province, Vietnam. *Biomedical Journal of Science and Technical Research*, *10*(1), 7551-7552.

Ngugi, C. C., & Manyala, J. O. (2004). Aquaculture extension services in Kenya. In: *Aquaculture Extension Services in Sub-Saharan Africa. Fisheries Department Circular* (No. 1002), 35-45. Food and Agriculture Organization of the United Nations.

Ngugi, C. C., Bowman, J. R., & Omolo, B. (2007). *A new guide to fish farming in Kenya*. Aquaculture Collaborative Research Support Program.

Niyotwambaza, C. H., Nhapi, I., Wali, U. G., & Tabaro, S. R. (2010). Nutrient Distribution in a Fish Pond at Rwasave Fish Farming and Research Station, Rwanda. *Rwanda Journal*, *21*, 29-49.

Nyström, T. (2004). Stationary-phase physiology. *Annual Review of Microbiology*, *58*, 161-181.

Obiero, K., Meulenbroek, P., Drexler, S., Dagne, A., Akoll, P., Odong, R., Kaunda-Arara, B., & Waidbacher, H. (2019). The contribution of fish to food and nutrition security in Eastern Africa: Emerging trends and future outlooks. *Sustainability*, *11*(6), 16-36.

Obwanga, B., Lewo, M. R., Bolman, B. C., & van der Heijden, P. G. M. (2017). *From aid to responsible trade: driving competitive aquaculture sector development in Kenya: quick scan of robustness, reliability and resilience of the aquaculture sector* (Report No. 2017-092 3R Kenya). Wageningen University & Research.

Ogello, E. O., Kembenya, E. M., Githukia, C. M., Aera, C. N., Munguti, J. M., & Nyamweya, C. S. (2017). Substitution of fish meal with sunflower seed meal in diets for Nile tilapia (*Oreochromis niloticus* L.) reared in earthen ponds. *Journal of Applied Aquaculture*, *29*(1), 81-99.

Ogello, E. O., Munguti, J. M., Sakakura, Y., & Hagiwara, A. (2014). Complete replacement of fish meal in the diet of Nile tilapia (*Oreochromis niloticus* L.) grow-out with alternative protein sources. A review. *International Journal of Advanced Research 2*(8), 962-978.

Ojwala, R. A., Otachi, E. O., & Kitaka, N. K. (2018). Effect of water quality on the parasite assemblages infecting Nile tilapia in selected fish farms in Nakuru County, Kenya. *Parasitology Research*, *117*(11), 3459-3471.

Oliva-Teles, A. (2012). Nutrition and health of aquaculture fish. *Journal of Fish Diseases*, *35*(2), 83-108.

Opiyo, M. A., Marijani, E., Muendo, P., Odede, R., Leschen, W., & Charo-Karisa, H. (2018). A review of aquaculture production and health management practices of farmed fish in Kenya. *International Journal of Veterinary Science and Medicine*, *6*(2), 141-148.

Orlando, T. M., Oliveira, M. M. D., Paulino, R. R., Costa, A. C., Allaman, I. B., & Rosa, P. V. (2017). Reproductive performance of female Nile tilapia (*Oreochromis niloticus*) fed diets with different digestible energy levels. *Revista Brasileira de Zootecnia*, *46*, 1-7.

Patwary, Y. A., Sarker, B. S., Minar, M. H., & Shamsuddin, M. (2013). Study of mechanical effects on the quality of fish feed during different stages of manufacturing. *Pakistan Journal of Biological Sciences: PJBS*, *16*(17), 865-870.

Pillay, T. V. R., & Kutty, M. N. (2005). *Aquaculture: principles and practices* (No. Ed. 2). Blackwell publishing.

Qiang, J., Cui, Y. T., Tao, F. Y., Bao, W. J., He, J., Li, X. H., Xu, P., & Sun, L. Y. (2018). Physiological response and microRNA expression profiles in head kidney of genetically

improved farmed tilapia (GIFT, *Oreochromis niloticus*) exposed to acute cold stress. *Scientific Reports*, *8*(1), 1-15.

Rahardini, R. A., Helmiati, S., & Triyatmo, B. (2018). Effect of inorganic fertilizer on the growth of freshwater *Chlorella* sp. *IOP Conference Series: Earth and Environmental Science*, *139*(1), 1-9.

Rana, K. J., Siriwardena, S., & Hasan, M. R. (2009). *Impact of rising feed ingredient prices on aquafeeds and aquaculture production* (Report No. 541). Food and Agriculture Organization of the United Nations.

Rana, K. J., & Hasan, M. R. (2013). On-farm feeding and feed management practices for sustainable aquaculture production: an analysis of case studies from selected Asian and African countries. *FAO Fisheries and Aquaculture Technical Paper*, (583), 21-67.

Rao, K. V. N. (1963). Determination of optimum pH range for the growth of *Oocystis marssonii* Lemm. in three media differing in nitrogen source. *Indian Journal of Plant Physiology*, *6*(2), 135-141.

Ravindran, B., Gupta, S. K., Cho, W. M., Kim, J. K., Lee, S. R., Jeong, K. H., Lee, D. J., & Choi, H. C. (2016). Microalgae potential and multiple roles—current progress and future prospects—an overview. *Sustainability*, *8*(12), 1215.

Renuka, N., Guldhe, A., Prasanna, R., Singh, P., & Bux, F. (2018). Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. *Biotechnology Advances*, *36*(4), 1255-1273.

Ricker, W. E. (1979). Growth rates and models. *Fish Physiology*, 673-743.

Roy, S. S., & Pal, R. (2015). Microalgae in aquaculture: a review with special references to nutritional value and fish dietetics. In *Proceedings of the Zoological Society*, *68*(1), 1-8. Springer India.

Russo, J. R., & Yanong, R. P. (2010). Molds in fish feeds and aflatoxicosis. *Institute of Food and Agriculture Sciences, University of Florida*.

Saadaoui, I., Rasheed, R., Aguilar, A., Cherif, M., Al Jabri, H., Sayadi, S., & Manning, S. R. (2021). Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. *Journal of Animal Science and Biotechnology*, *12*(1), 1-15.

Sajjadi, B., Chen, W. Y., Raman, A. A. A., & Ibrahim, S. (2018). Microalgae lipid and biomass for biofuel production: A comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. *Renewable and Sustainable Energy Reviews*, *97*, 200-232.

Salmeán, G. G., Castillo, L. H. F., & Chamorro-Cevallos, G. (2015). Nutritional and

toxicological aspects of Spirulina (*Arthrospira*). *Hospital Nutrition: Official Organ of the Spanish Society of Parenteral and Enteral Nutrition, 32*(1), 34-40.

Sarker, P. K., Kapuscinski, A. R., Bae, A. Y., Donaldson, E., Sitek, A. J., Fitzgerald, D. S., & Edelson, O. F. (2018). Towards sustainable aquafeeds: Evaluating substitution of fishmeal with lipid-extracted microalgal co-product (*Nannochloropsis oculata*) in diets of juvenile Nile tilapia (*Oreochromis niloticus*). *PLoS One, 13*(7), e0201315.

Sarker, P. K., Kapuscinski, A. R., McKuin, B., Fitzgerald, D. S., Nash, H. M., & Greenwood, C. (2020). Microalgae-blend tilapia feed eliminates fishmeal and fish oil, improves growth, and is cost viable. *Scientific Reports*, *10*(1), 1-14.

Satia, B. P. (2016). An overview of the large marine ecosystem programs at work in Africa today. *Environmental Development, 17*,11–19.

Schiavone, A., De Marco, M., Martínez, S., Dabbou, S., Renna, M., Madrid, J., Hernandez, F., Rotolo, L., Costa, P., Gai, F., & Gasco, L. (2017). Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology*, *8*(1), 1-9.

Segner, H., Sundh, H., Buchmann, K., Douxfils, J., Sundell, K. S., Mathieu, C., & Vaughan, L. (2012). Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. *Fish Physiology and Biochemistry*, *38*(1), 85-105.

Shearer, K. D., Maage, A., Opstvedt, J., & Mundheim, H. (1992). Effects of high-ash diets on growth, feed efficiency, and zinc status of juvenile Atlantic salmon (*Salmo salar*). *Aquaculture*, *106*(3-4), 345-355.

Shitote, Z., Wakhungu, J., & China, S. (2012). Challenges facing fish farming development in Western Kenya. *Greener Journal of Agricultural Sciences*, *3*(5), 305-311.

Smith, M. D., Roheim, C. A., Crowder, L. B., Halpern, B. S., Turnipseed, M., Anderson, J. L., Asche, F., Bourillón, L., Guttormsen, A.G., Khan, A., & Selkoe, K. A. (2010). Sustainability and global seafood. *Science*, *327*(5967), 784-786.

Sobczuk, T. M., Camacho, F. G., Grima, E. M., & Chisti, Y. (2006). Effects of agitation on the microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum*. *Bioprocess and Biosystems Engineering*, *28*(4), 243-250.

Soni, R. A., Sudhakar, K., & Rana, R. S. (2019). Comparative study on the growth performance of *Spirulina platensis* on modifying culture media. *Energy Reports*, *5*, 327-336.

Spencer, C. P. (1954). Studies on the culture of a marine diatom. *Journal of the Marine Biological Association of the United Kingdom*, *33*(1), 265-290.

Stone, N. M., & Thomforde, H. K. (2004). *Understanding your fish pond water analysis report* (1-4). Cooperative Extension Program, University of Arkansas at Pine Bluff, US Department of Agriculture and county governments cooperating.

Stone, N. M., Shelton, J. L., Haggard, B. E., & Thomforde, H. K. (2013). *Interpretation of water analysis reports for fish culture*. Stoneville, Mississippi: Southern Regional Aquaculture Center.

Subasinghe, R., Soto, D., & Jia, J. (2009). Global aquaculture and its role in sustainable development. *Reviews in Aquaculture*, *1*(1), 2-9.

Sutkowy, M., Lenarczyk, J., & Kłosowski, G. (2019). Effect of culture medium on the growth of microscopic algae (Chlorophyceae) biomass showing biosorption potential: A case study *Pseudopediastrum boryanum*. *Phycological Research*, *67*(2), 112-119.

Syed, R., Masood, Z., Hassan, H. U., Khan, W., Mushtaq, S., Ali, A., & Ullah, A. (2022). Growth performance, haematological assessment, and chemical composition of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fed different levels of Aloe vera extract as feed additives in a closed aquaculture system. *Saudi Journal of Biological Sciences*, *29*(1), 296-303.

Tacon, A. G. (2020). Trends in global aquaculture and aquafeed production: 2000–2017. *Reviews in Fisheries Science & Aquaculture*, *28*(1), 43-56.

Talling, J. F. (1966). [Review of *Algal Cultures and Phytoplankton Ecology.*, by G. E. Fogg]. *Journal of Applied Ecology*, *3*(1), 215–216.

Talling, J. F., & Driver, D. (1963). *Some problems in the estimation of chlorophyll-a in phytoplankton.* In proceedings of the conference on primary productivity measurement, marine and freshwater, held at University of Hawaii, August 21–Sept. 6, 1961.

Terpstra, A. H. (2015). *The Composition and Production of Fish Feeds.* Universitate Vadensi: Orando, The Netherlands. https://vdocuments.mx/the-composition-and-production-of-fish-ahm-terpstra-2015-de-alimenti-piscibus.html?page=1

Torres-Tiji, Y., Fields, F. J., & Mayfield, S. P. (2020). Microalgae as a future food source. *Biotechnology Advances*, *41*, 1-13.

Troell, M., Kautsky, N., Beveridge, M., Henriksson, P., Primavera, J., Rönnbäck, P., Folke, C., & Jonell, M. (2017). Aquaculture, In Reference Module in Life Sciences, *Elsevier*, ISBN: 978-0-12-809633-8, http://dx.doi.org/10.1016/B978-0-12-809633-8.02007-0

Tucker, C. S., & D'Abramo, L. R. (2008). *Managing high pH in freshwater ponds*. Stoneville: Southern Regional Aquaculture Center.

University of Texas Culture Collection of Algae (UTEX), (n.d.). *Modified Bold 3N Medium.* https://utex.org/products/modified-bold-3n-medium?variant=30991514763354

Van Wyk, P., & Scarpa, J. (1999). Water quality requirements and management. In: *Farming Marine Shrimp in Recirculating Freshwater Systems* (141-162). United States of America: Florida Department of Agriculture & Consumer Services.

Vizcaíno, A. J., López, G., Sáez, M. I., Jiménez, J. A., Barros, A., Hidalgo, L., Camacho-Rodríguez, J., Martínez, T.F., Cerón-García, M.C., & Alarcón, F. J. (2014). Effects of the microalga *Scenedesmus almeriensis* as fishmeal alternative in diets for gilthead sea bream, *Sparus aurata*, juveniles. *Aquaculture*, *431*, 34-43.

Waithaka, P. N., Gathuru, E. M., Githaiga, B. M., & Kimani, S. N. (2017). Control of passion fruit fungal diseases using essential oils extracted from rosemary *(Rosmarinus officinalis*) and eucalyptus (*Eucalyptus agglomerata*) in Egerton University Main Campus Njoro, Kenya. *International Journal of Microbiology*, *2017*,1-6.

Walker, A. B., & Berlinsky, D. L. (2011). Effects of partial replacement of fish meal protein by microalgae on growth, feed intake, and body composition of Atlantic cod. *North American Journal of Aquaculture*, *73*(1), 76-83.

Win, T. T., Barone, G. D., Secundo, F., & Fu, P. (2018). Algal biofertilizers and plant growth stimulants for sustainable agriculture. *Industrial Biotechnology*, *14*(4), 203-211.

Yaakob, M. A., Mohamed, R. M. S. R., Al-Gheethi, A., Ravishankar, G. A., & Ambati, R. R. (2021). Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: an overview. *Cells*, *10*(2), 1-19.

Yang, Y., Du, L., Hosokawa, M., & Miyashita, K. (2020). Effect of Spirulina lipids on high-fat and high-sucrose diet induced obesity and hepatic lipid accumulation in C57BL/6J mice. *Journal of Functional Foods*, *65*, 1-9.

Yu, J., Hu, H., Wu, X., Wang, C., Zhou, T., Liu, Y., Ruan, R., & Zheng, H. (2019). Continuous cultivation of *Arthrospira platensis* for phycocyanin production in large-scale outdoor raceway ponds using microfiltered culture medium. *Bioresource Technology*, *287*, 1-12.

Zhaowei, W. (2020). *CN110760446-Oocystis algae culture process.* (No. 110760446) China National Intellectual Property Administration https://patentscope.wipo.int/search/en/detail.jsf;jsessionid=4FD097BD34A0EA385A112E6FD906095D.wapp1nA?docId=CN288648188&tab=PCTDESCRIPTION

APPENDICES

Apppendix A: Research permit granted by the National Commission for Science, Technology and Innovation (NACOSTI)

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **746840**

Date of Issue: **01/February/2022**

RESEARCH LICENSE

This is to Certify that Mr.. Marvin Gaye Mukoma of Egerton University, has been licensed to conduct research in Nakuru on the topic: ASSESSMENT OF THE NUTRITIVE VALUE OF Arthrospira platensis, Cladophora sp. AND Oocystis sp. AS PROTEIN INGREDIENTS IN NILE TILAPIA FISH FEEDS for the period ending : 01/February/2023.

License No: **NACOSTI/P/22/15536**

746840

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix B: Key data analysis outputs

B.1 One Way Analysis of Variance for the crude protein content in the formulated diets and algae

One Way Analysis of Variance Wednesday, June 22, 2022, 16:11:51

Data source: Crude protiens in Proximate analysis.JNB

Normality Test (Shapiro-Wilk): Passed (P = 0.832)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.360)

Group Name	N	Missing	Mean	Std Dev	SEM
Spirulina	3	0	53.667	1.860	1.074
Oocystis	3	0	41.927	0.884	0.510
Diet 1	3	0	49.875	0.579	0.334
Diet 2	3	0	48.563	0.789	0.455
Diet 3	3	0	49.219	0.789	0.455
Diet 4	3	0	49.073	0.334	0.193
Diet 5	3	0	47.177	2.202	1.271
Diet 6	3	0	47.177	1.656	0.956
Diet 7	3	0	46.813	1.869	1.079

Source of Variation	DF	SS	MS	F	P
Between Groups	8	233.934	29.242	15.463	<0.001
Residual	18	34.038	1.891		
Total	26	267.972			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

B.2 One Way Analysis of Variance for the final bodyweight of the *Oreochromis niloticus* subjected to the different treatments

One Way Analysis of Variance Thursday, June 30, 2022, 21:07:53

Data source: Final bodyweight in Fish Growth.JNB

Normality Test (Shapiro-Wilk): Passed (P = 0.198)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.678)

Group Name	N	Missing	Mean	Std Dev	SEM
Diet 1	20	0	7.778	2.228	0.498
Diet 2	10	0	9.985	1.595	0.504
Diet 3	30	0	8.040	1.683	0.307
Diet 4	20	0	7.840	1.470	0.329
Diet 5	30	0	9.937	2.002	0.366
Diet 6	30	0	8.380	1.600	0.292
Diet 7	30	0	7.988	1.977	0.361

Source of Variation	DF	SS	MS	F	P
Between Groups	6	117.732	19.622	5.878	<0.001
Residual	163	544.172	3.338		
Total	169	661.905			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.990

Appendix C: Publication; Sustainable Fish Feeds: Optimization of Levels of Inorganic Fertilizers for Mass Production of *Oocystis* Sp. for Climate Smart Aquaculture

Aquaculture International
https://doi.org/10.1007/s10499-022-00986-5

Sustainable fish feeds: optimization of levels of inorganic fertilizers for mass production of *Oocystis* sp. for climate smart aquaculture

Marvin Gaye Mukoma[1] · Steve Omondi Odour[1] · Elick Onyango Otachi[1]

Received: 9 May 2022 / Accepted: 20 September 2022

Abstract

Use of microalgae as a source of food in aquaculture production is gaining recognition due to their rapid growth rate that promises high biomass generation within a short time. The challenge faced is getting good and inexpensive nutrients source to be used in mass production of the required microalgae. This study investigated the effect of different nutrient combination in influencing the growth rate of the of the green algae *Oocystis* sp. which has been identified as a possible protein source for the raising of *Orechromis niloticus* fingerlings for fish farming. Modified Bolds 3 N Medium and commercial agricultural fertilizers (urea, NPK and DAP) media were compared to establish the appropriate combinations that would result into high biomass generation but at the lowest cost possible. The Modified Bold 3 N Medium acted as the control, at a cost of 11.28 KSh per litre; the other media were derived from urea, NPK, and DAP (varying the ratio of each) at a cost of treatment 1 (0.14 KSh per litre), treatment 2 (0.18 KSh per litre), and treatment 3 (0.22 KSh per litre). The algae was cultured for 5 weeks with samples taken daily for biomass analyses using chloropyhll-a concentration as the surrogate for *Oocystis* sp. biomass for 30 days, from each treatment was determined. The growth rate, doubling time, and divisions per day were then estimated based on this chlorophyll-a concentration. The results showed that the mean concentrations of chlorophyll-a in treatment 1 was highest (7.715 ± 0.667 µg/ml), while treatment 3 (6.441 ± 0.555 µg/ml) had the least. There were no significant differences in the mean concentrations of chlorophyll-a in the four treatments (Kruskal–Wallis H test: $P > 0.05$). The chlorophyll-a concentration varied significantly in each treatment with time (Kruskal–Wallis H test: $P < 0.001$). There was no significant difference in the growth rate (Kruskal–Wallis H test: $P > 0.05$), divisions per day (Kruskal–Wallis H test: $P > 0.05$), and doubling time (Kruskal–Wallis H test: $P > 0.05$) from the different treatments. The results of this study showed that inorganic fertilizers can be used as cost-effective media in the mass scale culture of *Oocystis* sp.

Keywords *Oocystis* sp. · Mass production · Inorganic fertilizers · Climate smart

Handling Editor: Ronan Sulpice

✉ Marvin Gaye Mukoma
marvinmukoma@gmail.com

[1] Biological Sciences Department, Egerton University, Egerton Njoro, P.O. Box 523-20115, Nakuru, Kenya

Published online: 01 October 2022