DIETERY UTILIZATION OF BLACK SOLDIER FLY LARVAE (*Hermetia illucens*) IN AFRICAN CATFISH (*Clarias gariepinus*) CULTURED UNDER SPINACH (*Spinacia oleracea*) PROPAGATED AQUAPONIC SYSTEM USING DIFFERENT SUBSTRATES

MARANGA BENARD OMBOGA

DOCTOR OF PHILOSOPHY

(Zoology)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2024

Dietery Utilization of Black Soldier Fly Larvae (*Hermetia illucens*) in African Catfish (*Clarias gariepinus*) Cultured Under Spinach (*Spinacia oleracea*) Propagated Aquaponic System Using Different Substrates

Maranga Benard Omboga

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Zoology (Aquatic Ecology and Fisheries) of the Jomo Kenyatta University of Agriculture and Technology

2024

DECLARATION

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

Signature: …….. Date: ……

Maranga Benard Omboga

This thesis has been submitted for examination with our approval as supervisors.

Signature: …….. Date: ……

Dr. Robert Nesta Kagali, PhD

JKUAT, Kenya

Signature: …….. Date: ……

Dr. Kevin Mbogo Omolo,PhD

JKUAT, Kenya

Signature: …….. Date: ……

Dr. Paul Sagwe Orina, PhD,

KEMFRI, Kenya

DEDICATION

This thesis is dedicated to Almighty God for his everlasting support and providence throughout the research period, to my family members, wife Mercy Chelangat, daughter Ellah Kemunto, Dad Francis Ototo, mum Esther Moraa, brothers Donald Nyanchoka, Joshua Machuka and David Nyamwange and sisters Joyce Kwamboka and Florence Kerubo, thank you all for the support and prayers.

ACKNOWLEGEMENTS

I am grateful to the almighty God for the gift of life, knowledge and strength to overcome challenges. I appreciate the assistance given to me by my supervisors, Dr. Robert Nesta Kagali (Zoology Department, JKUAT), Dr. Kevin Mbogo Omolo (Biochemistry Department, JKUAT) and Dr. Paul Sagwe Orina, Kenya Marine and Fisheries Research Institute (KMFRI), Kegati station for their guidance, supervision and support throughout the research period and writing of this thesis.

I would also like to thank the Government of Kenya through the Ministry of Agriculture Livestock, Fisheries and Co-operative under the Kenya Climate Smart Agricultural Program (KCSAP) for financing the studies through a scholarship that was funded by World Bank, Mr. David Chebon for having accepted the trials to be conducted on his farm in Mogotio, Baringo County.

I am also grateful to Mr. David Fotha of Food Science Department, Dr. Jonathan Munguti for your support towards this research. I would also like to thank Mr. Conrad Barongo and Mr. Joshua Kipkulei for the assistance in nutrients analysis, Kenya Marine and Research Institute, Sagana and Baringo stations for providing Laboratory space and instrumentation that enabled the success of the research.

Thank you, Mercy Chelangat and daughter Ellah. I would spend most of the time away from home in an attempt to make this dream a reality. You supported me spiritually and psychologically always reminding me that I can make it. To uncle David Mose the late, thank you for believing and encouraging me to pursue education when you had not departed from us. This would have remained just a dream if you did not have encouraged me.

TABLE OF CONTENTS

DECLARATION ..... **ii**

DEDICATION ..... **iii**

ACKNOWLEGEMENTS ..... **iv**

TABLE OF CONTENTS ..... **v**

LIST OF TABLES ..... **ix**

LIST OF FIGURES ..... **xi**

LIST OF APPENDICES ..... **xii**

LIST OF ABBRIVIATIONS ..... **xiii**

ABSTRACT ..... **xv**

CHAPTER ONE **1**

INTRODUCTION **1**

1.1 Background Information 1

1.2 Statement of the Problem 4

1.3 Justification 5

1.4 Hypothesis 6

1.4.1 Alternate hypothesis 6

1.4.2 Null hypothesis 6

1.5 Objectives 6

1.5.1 General objective 6

1.5.2 Specific objectives 6

CHAPTER TWO 8

LITERATURE REVIEW 8

2.1 Aquaculture status 8

2.2 Feeds ingredients and formulation 9

2.3 Black Soldier Fly Larvae 10

2.4 Fatty acid composition and its significance 12

2.5 Catfish farming 13

2.6 Water quality and management 14

2.7 Re-circulatory Aquaculture and Hydroponic systems (Aquaponics) 15

CHAPTER THREE 17

MATERIALS AND METHODS 17

3.1 Study Site 17

3.2 Experimental Design and set up 17

3.3 Diet formulation 18

3.4 Stocking and feeding 20

3.5 Fish Sampling and growth determination 20

3.6 Extraction of total lipids and fatty acid analysis 21

3.7 Gas chromatography analysis 21

3.8 Proximate analysis and feeding 22

3.9 Nutrients analysis and filtration efficiency 22

3.9.1 Dissolved reactive phosphorous (DRP) 23

3.9.2 Nitrate 23

3.9.3 Nitrite 24

3.9.4 Ammonium 24

3.10 Preparation of standard solutions and determination of nutrients concentration 25

3.10.1 Determination of Dissolved reactive phosphorous concentration 25

3.10.2 Determination of nitrates and nitrites concentration 25

3.10.3 Determination of ammonium concentration 25

3.11 Data collection and analysis 26

3.11.1 Determination of growth Performance and Survival 26

3.11.2 Planting and determination of Spinacia oleracea growth parameters 27

3.11.3 Statistical analysis 27

CHAPTER FOUR 28

RESULTS 28

4.1 Proximate analysis of ingredients and experimental diets 28

4.2 Fish survival, feed utilization and growth parameters 29

4.3 Growth performance of *C. gariepinus* under different substrates 31

4.4 Productivity of *S. oleracea* under different substrate 34

4.5 Fatty acid profiles of experimental diets 35

4.6 Effect of experimental diets on whole body fatty acid profile of *C. gariepinus* 37

4.7 Effect of experimental diets on liver fatty acid profile of *C. gariepinus* 39

4.8 Effect of experimental diets on muscles fatty acid profile of *C. gariepinus* 41

4.9 Water quality parameters in fish tanks under different dietary treatment 43

4.10 Water quality parameters flowing through the hydroponic unit under different substrates 44

4.11 Percentage reduction efficiency of nutrients under different substrates 46

CHAPTER FIVE 48

DISCUSSION 48

5.1 Proximate analysis of ingredients and experimental diets 48

5.2 Growth performance and survival of *C. gariepinus* fed on BSFL diets 48

5.3 Effect of substrates on growth performance and survival of *C. gariepinus* 51

5.4 Substrate influence on *S. oleracea* productivity 52

5.5 Fatty acid profile in *C. gariepinus* tissues 52

5.6 Water quality parameters under aquaponic system 55

5.7 Effects of nitrifiers on reduction of ammonia toxicity 56

5.8 Effects of substrates on nutrients reduction 56

5.9 Conclusions 58

5.10 Recommendation 58

REFERENCES 60

APPENDICES 80

LIST OF TABLES

Table 3.1: Protein content, percentage composition of ingredients in formulated test diets 18

Table 3.2: Proximate composition of commercial diet used to feed *Clarias gariepinus* under different substrate treatment.....22

Table 4.1: Percentage (%) proximate composition of analyzed ingredients used in diet formulation.....28

Table 4.2: Proximate composition of formulated test diets 29

Table 4.3: Performance of *C. gariepinus* fed on experimental diets 30

Table 4.4: Performance of *C. gariepinus* cultured under aquaponic system using different substrates 32

Table 4.5: Growth parameters of *Spinacia oleracea* in the hydroponic units between different substrates, (Means ± SD).....35

Table 4.6: Fatty acid composition of experimental diets 36

Table 4.7: Fatty acid composition of the whole body of C. gariepinus 38

Table 4.8: Fatty acid composition of the liver of *C. gariepinus* 40

Table 4.9: Fatty acid composition of the muscles of *C. gariepinus*.....42

Table 4.10: Water quality parameters in fish tanks under different dietary treatments..44

Table 4.11: Water quality parameters for water flowing into the hydroponic units under different substrates treatments.....45

Table 4.12: The chemical parameters in water flowing out of hydroponic units under different substrates treatments.....46

LIST OF FIGURES

Figure 2.1: Black Soldier Fly (Hermetia illucens) (a) pre-pupae of Hermetia illucens 18 days after hatching; and (b) mating adults of Black Soldier Fly (Hermetia illucens).....11

Figure 2.2: Structure of omega-3 fatty acids; 3 double bonds (ALA), 5 double bonds (EPA) and 6 double bonds (DHA).....13

Figure 2.3: Pictorial presentation of African catfish (Clarias gariepinus).....14

Figure 3.1: The experimental setup for the aquaponic system (Not drawn to scale). The arrows indicate the direction of flow of water within the system.....18

Figure 4.3: A graph of length of C. gariepinus (g) against time (weeks).....33

Figure 4.4: A bar graphs of percentage reduction efficiency of nutrients in the aquaponic system under different substrates. 47

LIST OF APPENDICES

Appendix I: Abstracts of publications 80

Appendix II: Statistical analysis for BSF formulated and control diets 82

LIST OF ABBRIVIATIONS

ALA	Alpha Linolenic Acid
ANOVA	Analysis of Variance
ASAL	Arid and Semi-Arid Areas
BSFL	Black Soldier Fry Larvae
CSC	Cotton Seed Cake
CP	Crude Protein
DC	Direct Current
DHA	Docosahexaenoic Acid
DM	Dry Matter
DO	Dissolved Oxygen
EFA	Essential Fatty Acids
EPA	Eicopentaenoic Acids
ESP	Economic Stimulus Program
FAO	Food and Agricultural Organization
FCR	Food Conversion Ratio
FFEPP	Fish Farming Enterprise Productivity Program
FM	Fishmeal
FWS	Fresh Water Shrimps
GC	Gas Chromatography
GHGs	Green Houses Gases
ICIPE	International Center of Insect Physiology and Ecology
KCSAP	Kenya Climate Smart Agricultural Project
KMFRI	Kenya Marine and Fisheries Research Institute
MUFA	Monounsaturated Fatty Acid
OCHA	Office of the Coordination of Humanitarian Affairs
PUFA	Polyunsaturated Fatty Acid
RAS	Re-circulative Aquaculture System
SD	Standard Deviation

SFA	Saturated Fatty Acid
SR	Survival Rate
SSC	Sunflower Seed Cake
TDS	Total Dissolved Substance
UN	United Nation
WB	Wheat Bran

ABSTRACT

The rising demand of fish due to its nutritive value has led to over exploitation of capture fisheries thus, paving way for aquaculture development. Being the world's fastest growing agro-food sector, aquaculture faces a number of challenges that hamper the sector from meeting the ever-rising demand of fish and fish products. High cost of fish feeds, use of outdated culture systems that require large tracts of land and plenty of water are some of the factors limiting aquaculture from attaining its full potential. Reduction of fish feeds cost by use of cheaper ingredients and use of modern fish farming technologies such as aquaponics can address the challenges to enhance aquacultural productivity. The study was conducted at a private fish farm in Baringo County, Kenya. The study investigated the effect of partial replacement of fishmeal (FM) with black soldier fly larvae (BSFL) on survival, growth performance and fatty acid composition of *Clarias gariepinus*. Also, the study assessed the suitability of pumice, a mixture of (pumice and charcoal) mixed homogenously at 50 % ratio and charcoal substrates on nutrients removal from water for *C. gariepinus* culture and *Spinacia oleracea* propagation under aquaponic system. In the first experiment; three test diets 35% crude protein content (CP) in which FM was substituted by BSFL at 25%, 50% and 75% were formulated and experimented with commercial diet of 35% CP manufactured by Unga Farm Care Limited. Four weeks old *C. gariepinus* of average weight 5.6±0.5 g and average length 9.63±0.5cm were stocked in 12 tanks at a density of 50 fish/tank and subjected to the diets. Whole body, liver and muscles tissues were obtained from experimental fish and taken to the laboratory where they were analysed for fatty acids profile using gas chromatography at the end of the four months experimental duration. In the second experiment, *C. gariepinus* post fingerlings weighing 14.97±0.5g and length 14.05±0.5cm were stocked at 50 fish/tank in 12, 1000L tanks under aquaponic system. Experimental fish were subjected to a commercial diet manufactured by Unga Farm Care Limited for four months duration. The commercial diet was analysed for its proximate composition and found to contain 35% crude proteins content (CP). In both experiments, fish sampling was conducted every three weeks; water quality parameters were analyzed weekly while mortality was recorded on occurrence. Results on growth performance of fish fed on diets containing BSFL showed 50% BSFL diet obtained food conversion ratio (FCR) of 1.2 compared to FCR of 1.3 obtained by diets containing 25% and 75% BSFL. Weight gain obtained for control diet varied significantly ($p<0.001$) in comparison to weight gain obtained for formulated diets. The results on fatty acids profile showed inclusion of BSFL at different rates influenced fatty acid composition. Saturated fatty acids composition increased while unsaturated fatty acids decreased with increasing BSFL ratio in diets. Total saturated and unsaturated fatty acids varied significantly ($p<0.05$) among the diets and fish tissues. Palmitic acid was the dominant saturated fatty acid while oleic and linolenic were the dominant monounsaturated and polyunsaturated fatty acids in fish tissues respectively. Docosahexaenoic acid (DHA) was the dominant omega-3 fatty acid in fish tissues with liver having significantly higher ($P<0.05$) accumulation than whole body and muscle

tissues. Under different substrate types, the mean weight gained by fish for pumice (93.81g), pumice charcoal (77.57g), charcoal (69.79g) and control (55.37g) treatments showed significant difference ($p<0.03$). The final breadth, length and number of leaves in *S. oleracea* showed significant difference obtaining p values of (0.023), (0.045) and (0.003) respectively between different substrate treatments. Survival rates for *C. gariepinus* ranged between 92-98%. The least survival rate was observed for control treatment (92%), followed by treatment with charcoal substrate (94%), then treatment with pumice substrate (96%) while the highest survival rate was obtained for the mixture of pumice and charcoal substrate (98%). Growth performance and survival demonstrated that BSFL has potential to replace FM up to 75%. Catfish productivity can be improved and feed cost reduced by incorporating fully defatted BSFL with higher CP content compared to 25.3% CP content used for the diets in the present study. The study on fatty acid profile of diets and fish tissues showed that BSFL can replace fishmeal in diets without affecting the composition fatty acid profile of cultured catfish muscles. Thus, BSFL is a suitable protein sourced ingredient for partial FM replacement in catfish diets. The observed variation in growth performance of; *C. gariepinus* and *S. oleracea* and nutrients reduction efficiency suggest pumice is a better substrate for catfish and spinach production under aquaponic system.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Rapid human population growth is exerting pressure to world food security status affecting third world countries by contributing to severe hunger, chronic malnutrition and health related problems (United Nations [UN], 2019) among the majority poor. The situation is worsened in developing countries due to the use of traditional agricultural practices and reliance of unpredictable weather patterns that have adversely affected agricultural productivity especially in Africa (FAO 2020). To overcome the situation, efforts aimed at increasing food supply have to be implemented as step gap measure in addressing food insecurity in the affected countries.

Fish has gained prominence as important source of proteins in developing countries by supplying at least 20 % of the total animal protein requirement to the population of the region hence providing much needed nutrition and health benefits (Obiero *et al.,* 2019; Munguti *et al.,* 2014). In Sub-Saharan Africa, fish has always been sourced from the wild through capture fisheries initiatives for a long time. However, the dwindling natural stock is unable to bridge the growing demand and therefore the shortfall can be met through aquaculture (FAO 2016). Although aquaculture production is seen as a solution to declining capture fisheries, it also faces a number of challenges that prevent it reaching its full potential (Barbu *et al.,* 2016). High cost and low quality of fish feeds, competition for raw materials coupled by use of outdated culture systems have been identified as major setbacks in the growth of aquaculture sector in third world countries (Ngugi *et al.,* 2007). In Kenya, the share of fish in total animal proteins consumption is at 8 % which is much lower in comparison to 20 % in Africa continent (Cai and Leung 2020). Catfish is among the most consumed fish species in Kenya as it is readily available from aquaculture and capture fisheries (Kyule et al., 2016). Catfish ability to tolerate constrained environmental conditions has enabled the species to be among the preferred candidate for aquaculture (Somerville *et. al*., 2014). Under high temperatures and

low oxygen levels catfish can be cultured intensively using limited land and water resources without the risk of mortalities (Huntington *et al.,* 2017).

Fishmeal is the commonly used ingredient for animal feeds formulation with aquaculture sector consuming much of the ingredient globally (Al Mahmud *et al.,* 2012; Liti *et al.,* 2005). Fishmeal is highly sought feed ingredient by commercial animal feeds manufacturers due to its high nutritive value essential in growth and development, (FAO, 2014). Fishmeal is overexploited hence exacerbating the ingredient cost resulting from the competition not only from animal feeds manufacturers but also from human consumption (Liti *et al.,* 2005). As such, incorporation of other protein sourced ingredients for fish feeds formulation will significantly reduce the high cost of production incurred by fish farmers (Ngugi *et al.,* 2007). Insect based ingredients are prospective fishmeal replacement in diet formulation for a number of fish species (Al Mahmud *et al.,* 2012). Studies have proved insects' larvae can be incorporated in fish diets as alternative source of proteins without affecting growth and development on recipient fish (Tilami *et al.,* 2020). Examples of insect species used in fish feeds formulation include cockroach, house cricket, silk worm, common house fly, termites, grasshoppers, black soldier fly among other insects (Nina *et al.,* 2020; Tilami *et al.,* 2020 and Stamer *et al.,* 2014). The black soldier fly larvae have been proved to be a potential alternative protein source to fish meal in fish feeds formulation (Matteo *et al*., 2020). Black soldier fly larvae can be cultured using organic waste generated in the farm making it a cheap source of protein for on-farm fish feeds formulation intended in reducing high production cost incurred from purchasing of expensive manufactured feeds (Munguti *et al.,* 2021; Tran, Gnaedinger & Merlin 2015). Proximate composition of the larvae has shown a higher crude proteins content value of up to 45%, presence of microelements, amino and fatty acids in required quantities essential for fish growth (Xiao *et al.,* 2018).

The changing climate resulting from global warming has resulted to unpredictable precipitation pattern affecting aquaculture productivity negatively (United Nations [UN], 2019). Severe water shortage coupled with limited land resource has affected mostly semi-intensive aquaculture threatening the advances made in fish farming

(Seneviratne *et al.,* 2012). To tackle the issues of limited land and water scarcity, adoption of modern aquaculture technologies such as aquaponic system should be embraced to capitalize on fish and crop production (Barbu *et al.,* 2016). The integration of recirculating aquaculture and hydroponics referred to aquaponics is a promising solution for enhancing intensive fish and crops production in areas constrained with land and water resources (Yesiltas *et al.*, 2020). Under aquaponic system, waste produced in the aquaculture system is directed to the hydroponic units for nutrients removal by the activities of micro-organisms attached to substrates (Boxman *et al.,* 2017) and uptake by plants. To maintain conducive water environment for fish growth under aquaponic system, effective plant growth substrates should be used for efficient restoration of water for fish culture (Maucieri *et al.,* 2018).

Substrates in aquaponic systems influence water status by providing attachment media for nitrogen converting bacteria and acting as particle filter medium to improve on water quality (Geisenhoff et al., 2016). Substrates also provide attachment for plants that are used for nutrients uptake within the aquaponic system. However, clogging, formation of death zones and difficulty in cleaning are challenges experienced after using substrates for a long duration without cleaning or replacement (Yesiltas *et al.,* 2020). Best management practices should be observed in the fish rearing units to ensure the amount of organic load draining to the hydroponic units is minimized to prevent clogging (Boxman *et al.,* 2017). As such, substrates will enhance development of bacteria responsible for breakdown of ammonia to absorbable form by plants. Effectiveness of substrates is essential in holding water and air for maintenance of optimal condition for roots and consequently growth of organisms (Maucieri *et al.,* 2018) within the aquaponic unit. Variation in growth has been observed in aquaponic systems when different substrates are subjected to the organisms (Geisenhoff *et al.,* 2016) cultured under aquaponics. Therefore, it is important to identify right substrates to maximize growth of the cultured organisms in aquaponic systems.

Water is required in appropriate quality and quantity to enable sustainable production for intensive aquaculture to succeed (FAO, 2018). Close monitoring of water quality

under intensive aquaculture system is essential to avoid stunted growth and mortality due to high stocking densities of fish placed in limited water recirculating within the system (FAO, 2016). Water quality parameters such as temperature, pH, dissolved oxygen and nutrients must be maintained at optimal levels to enhance growth of all microorganisms under intensive aquaculture. Excess feeds and poor feed utilization efficiency by fish affects water quality as a result of nutrients enrichment of water within the culture systems (Filbrum *et al.,* 2013). As such, fish productivity and yield are reduced by hypoxia and blooming of aquatic plants such as phytoplankton and algae that compete for oxygen with fish during the process of respiration (FAO, 2014). For optimal water environment for fish production to be achieved, excess feeding should be avoided so as to prevent water quality deterioration and unnecessary wastage of feeds (Kosemani *et al.,* 2017). Plants grown in the hydroponic unit should be able to uptake nutrients from culture significantly to maintain the integrity of water within the system. The effective nutrients reduction may also be achieved by the use of appropriate substrates that will enable biological processes of nutrients conversion by microorganism to take place in the hydroponic units (Espinosa-moya *et al.,* 2018).

The present study was aimed at formulating diets in which BSFL partially replaced FM and tested against a commercial diet to evaluate growth performance and fatty acid of *C. gariepinus* reared under aquaponic system. Also, pumice, charcoal and a 50 % ratio, homogenous mixture of pumice and charcoal substrates were tested on a control (aeroponic) for their suitability in growth of *C. gariepinus* and *S. oleracea* under aquaponic system.

1.2 Statement of the Problem

The major challenge facing aquaculture enterprise is the high cost of production incurred in purchasing of fish feeds that take up between 40% and 60% of the total production cost (Chepkirui *et al.,* 2011). Over time, fish diets have been formulated using fish meal whose supply has been unsustainable due to its use in formulation of feeds for other domesticated animals. Fish meal is most preferred ingredient for diet formulation due to its high crude protein content making it the most sourced

ingredient for animal feeds production. The commercial production of various animal feeds has led to over exploitation of fishmeal, reducing its supply and making it unavailable hence increasing the cost of formulated feed (Munguti *et al.,* 2021). Insect sourced ingredients such as black soldier fly, termites and grasshoppers have been extensively experimented in fish feeds production. However, results from various studies have shown optimal inclusion rate of insect ingredients is yet to be achieved for some culture systems used in the production of certain fish species such as catfish (Tilami *et al.,* 2020).

Despite being viewed as an alternative of reducing the widening gap between fish supply and demand, aquaculture face constraints of limited land and availability of sufficient quality water to enhance productivity. With declining land, water scarcity and changing climate, the use of olden culture systems such as ponds cannot sustain fish farming to meet ever rising market demand for fish, (FAO 2014).

1.3 Justification

Diversification of protein sourced ingredients for formulating fish feeds will result to significant reduction of the cost of fish production thus encouraging more farmers to take up fish farming as alternative source of food and livelihood. Black soldier fly larval is an alternative proteins sourced ingredient that can effectively be used in formulating fish diets without affecting fish nutrition requirement (Matteo *et al*., 2020). The fly larvae can easily be cultured using a variety of organic waste generated within the farm making it an eco-friendly insect and very cheap source of protein, therefore a prospective ingredient to reduce production cost. Proximate composition of the larvae has shown a higher crude proteins content value of 45%, presence of microelements, amino and fatty acids in required quantities essential for fish growth (Xiao *et al.,* 2018).

Modern aquaculture technologies such as aquaponic system offer an opportunity to address issues of limited land and scarce water to culture fish and propagate crops within the same system to alleviate food insecurity and malnutrition (Barbu *et al* 2016). However, this may require innovative approach of using solar energy to run the aquaponic system at a low cost and spur food and nutrition security as well as

create aquaculture job opportunities for economic empowerment for both women and youths.

1.4 Hypothesis

1.4.1 Alternate hypothesis

1. Dietary utilization of black soldier fly larvae has effect on growth parameters and fatty acid composition of *C. gariepinus* cultured under aquaponic system.

2. Different substrates have effect on growth parameters of *C. gariepinus* and spinach propagated under aquaponic system.

1.4.2 Null hypothesis

1. Dietary utilization of black soldier fly larvae has no effect on growth parameters and fatty acid composition of *C. gariepinus* cultured under aquaponic system.

2. Different substrates have no effect on growth parameters of *C. gariepinus* and spinach propagated under aquaponic system.

1.5 Objectives

1.5.1 General objective

To evaluate the effect of; dietary utilization of black soldier fly larvae on growth parameters of *C. gariepinus* and use of different substrates on growth parameter of spinach propagated under aquaponic system.

1.5.2 Specific objectives

1. To determine the effect of proximate composition of feed ingredients on diet formulation for *C. gariepinus* cultured under aquaponic system

2. To evaluate the effect of dietary utilization of black soldier fly on growth parameters of *C. gariepinus* cultured under aquaponic system.

3. To evaluate the effect of dietary utilization of black soldier fly on fatty acid composition of *C. gariepinus* cultured under aquaponic system.

4. To evaluate the effect of different substrates on growth parameters of spinach propagated under aquaponic system.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aquaculture status

The Food and Agricultural Organization (FAO) ranks aquaculture as the fastest growing food producing enterprise in the world despite the slowed growth rate experienced in the third millennium by the sector (FAO, 2020). The average annual aquaculture growth rate was estimated at 5.8% between 2001 and 2016 having depreciated from 10.8% and 9.5% in 1980s and early 1990s respectively (FAO, 2018). Globally aquaculture contributed 53% of the total amount of fish produced for human consumption with capture fisheries providing the remaining 47% (Bostock *et al.,* 2010). The global aquaculture production is estimated to be 114.5 million tons with Asia dominating the sector with 89.4% of the total production while the rest of the world contribute 10.6% of the total aquaculture production (FAO, 2020). China as a country is the largest fish producer accounting for about 61.5% of global aquaculture production (FAO 2016). African continent contribution in aquaculture production is the least accounting for less than 3% of total fish produced globally (Bartley, 2022). In Africa, Egypt, Nigeria, Uganda, Tunisia, Ghana, Zambia, Kenya, Malawi, Madagascar and South Africa are the leading aquaculture producers. Egypt, Nigeria and Uganda account for 90% of total aquaculture production in the continent (Adeleke *et al.,* 2021).

In early 2000, the intervention of Kenya government with supportive policies led to rapid growth of aquaculture production through small scale fish farming (Munguti *et al.,* 2014). The “eat more fish campaign” initiative by the government accelerated aquaculture production from 1,021 tons to 4,895 tons with subsequent exponential increase in the number of fish farmers and pond area between the year 2003 and 2009 in Kenya (Nyonje *et al.,* 2018). The highest aquaculture contribution in Kenya was achieved in 2014 with a total fish production of 24,098 tons valued at Ksh 4,634 billion through the initiative of Fish Farming Enterprise Productivity Program (FFEPP) under the Economic Stimulus Program (ESP) (KMFRI, 2017). The (FFEPP) initiative facilitated fish farming through pond construction, provision of

fish farming input subsidy and free extension services. The short-term goal of (FFEPP) initiative of rising production from 4,000 tons to 20,000 tons annually was achieved by 2014 (KNBS, 2017). However, the long-term goal of increasing total production to 100,000 tons annually has never been achieved, instead production has been declining since 2014 after the termination of (ESP) by the Kenya government (Munguti *et al.*, 2021). The termination of (ESP) contributed to drastic drop of fish production from 24,098 tons in 2014 to 14,952 tons in 2016 negatively affecting the growth of aquaculture in the country (KNBS, 2017).

Besides Kenya experiencing tremendous development in aquaculture sector, over 50% of the fish farms initiated through (ESP) have closed down (KMFRI, 2017). The major reasons for fish farm closure in Kenya are high cost of fish feeds, low technology adoption and climate change (Obiero *et al.,* 2019). Climate change has caused unpredictable weather patterns that threaten aquatic environment (Ikehi and Zimoghen, 2015). The effects of climate change resulting to prolonged drought in some areas contribute to water scarcity affecting fish farming (OCHA, 2010). As such, food insecurity, poor health and sanitation are exacerbated leading to competition for limited natural resources (Carpenter *et al.,* 2016). As resources get depleted, there is need for the development and adoption of new technologies to enhance sustainability for future utilization (Huntington *et al.,* 2017). In Kenya, new farming technologies such as Re-circulative Aquaculture System (RAS) are being embraced for resources conservation and enhanced productivity (FAO, 2016). Also, the challenge of the high cost of fish feeds is addressed through diversification of protein sourced ingredients for formulating fish feeds which will result to significant reduction of the cost of fish production thus encouraging more farmers to take up fish farming.

2.2 Feeds ingredients and formulation

Feeds are important component in the value chain of fish production, with substantial percentage of production cost incurred in procurement of feeds. Commercial feeds are generally expensive and their prices continue to rise due to competition for ingredients by animal feed manufacturers, (Ngugi *et al.,* 2007). Complete diets

containing plant and animal sourced ingredients that provide all nutritional requirements of fish should be formulated so as to achieve good health and maximum growth of cultured fish (Liti *et al*., 2005). For economic viability, adequate supply of highly nutritious and low-cost ingredients should be supplied for formulation (Al Mahmud *et al.,* 2012). Therefore, it is important to replace the commonly used ingredients that are expensive with non-conventional locally available inexpensive ingredients to reduce the cost of fish feeds (Liti *et al*., 2002).

Fishmeal is a conventional ingredient used for animal feeds formulation with aquaculture industry accounting for 68% of fishmeal consumption in the world (Naylor *et al.,* 2010; Miles & Jacob, 2011). As such, market supply of fish meal is declining and becoming unsustainable due to over exploitation from nature making it very expensive for small scale fish farming (Munguti *et al.,* 2014). Therefore, diversification of protein sourced ingredients for formulating fish feeds will result to significant reduction of the cost of fish production thus encouraging more farmers to take up fish farming (Ngugi *et al.,* 2007). The potential of insect-based ingredients in replacing fishmeal has shown positive results in growth performance on several fish species (Stamer *et al.,* 2014). Thus, insect meals have been incorporated in fish feeds formulation as protein source ingredient (Walker, 2009). Among the promising insect species for commercial feed formulation include the black soldier fly (BSF), common house fly, silk worms and house cricket (Tilami *et al.,* 2020). Termites and grasshoppers are also viable in animal feeds formulation though to a lesser extent.

2.3 Black Soldier Fly Larvae

Recent studies have demonstrated black soldier fly larvae could serve as a replacement of conventional dietary ingredients such as fishmeal (*Rastrineobola argentea*) in fish feeds formulation (Matteo *et al*., 2020). The BSF larvae can easily be cultured using a variety of organic waste generated within the farm making it an eco-friendly insect and very cheap source of protein, hence a prospective ingredient to reduce production cost (Tran *et al.,* 2015). However, more trials including economic analyses are still necessary as reduced performance has been observed in some cases. Type of culture substrate and method of processing affect the utilization

of the fly larvae by fish. The black soldier fly larvae is a high value feed ingredient containing between 23-44% proteins while it has fats content of between 15-25%, (Tran *et al.,* 2015). The black soldier fly larval is rich in calcium 5-8% and phosphorous 0.6-1.5% microelements and also rich in amino acid profile more so in Lysine 6-8%, (Shumo *et al.,* 2019). Dry matter of BSF range between 38-42% which makes it easier and less costly to dehydrate compared to other animal sourced ingredients, (Sprangers *et al.,* 2017). Composition of fatty acid of the fly is dependent on the fatty acid composition of the diet it feeds on, therefore to enrich the final biomass of BSF, it is important to feed the fly with a diet rich in omega-3 fatty acids, (Xiao *et al.,* 2018).

Dietary substitution of BSFL in diets remains controversial due to variation in percentage proximate composition of nutrients of the insect (Matteo *et al.,* 2020). The variation in the nutritional composition of BSFL affect quality of formulated diets influencing fish growth and survival hence creating the need to determine inclusion rate of BSFL in fish diets (Barrosso *et al.,* 2017).

Figure 2.1: Black Soldier Fly (Hermetia illucens) (a) pre-pupae of Hermetia illucens 18 days after hatching; and (b) mating adults of Black Soldier Fly (Hermetia illucens) (Source: Shumo et al., 2019**)**

2.4 Fatty acid composition and its significance

Fatty acids are essential in diets and occur as saturated or unsaturated fatty acids. Saturated fatty acids lack double bonds in between the carbon chain while unsaturated fatty acids have one or more double bonds in between the carbon chain. Examples of saturated fatty acids include; Lauric acid, Myristic acid, Palmitic acid Stearic acid etc. Unsaturated fatty acids are either Monounsaturated or Polyunsaturated and are categorized into omega-6 and omega-3 fatty acids (Mustapha *et al.,* 2014). Oleic and Palmitoleic acids are examples of a monounsaturated fatty acid. Examples of polyunsaturated fatty acids include; linoleic and arachidonic acids that belong to omega-6 fatty acids while Linolenic, Eicosapentaenoic acid and Docosahexaenoic acid represent omega-3 fatty acids (Helena *et al.,* 2014). Omega-3, Alpha Linolenic Acid (ALA) is converted into Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) in a healthy human body (Oguz & Mahmut, 2017). DHA and EPA are essential in mediating inflammation, improve immune response and promote overall good health in human beings. Deficiency of EFA (Essential Fatty Acid) especially omega-3, have been linked to decreased mental abilities, learning disabilities, loss of memory, cause cardiovascular diseases among other disorders (Shumo *et al.,* 2019).

EPA and DHA are important in human health though they cannot be synthesized by human body and therefore must be consumed in diets (Mbogo *et al.,* 2017). Aquatic organisms e.g., bivalves and fish are a major source of Polyunsaturated Fatty Acids (PUFA) from which humans acquire DHA and EPA by consumption of the organisms (Prato *et al.,* 2019). A variety of consumed organisms contribute to the requirement of omega-3 fatty acids in diets with variation of fatty acid profile depending on species type (Kris-Etherton *et al.,* 2003). The levels of fatty acid composition in fish species entirely depends on the type of diet consumed as well as environmental factors such as salinity, temperature and whether the fish species is under aquaculture or in capture fisheries (Milena *et al.,* 2020). As such, analysis to quantify levels and composition of fatty acid in fish is necessary to ensure fish provide nutritional needs for human beings (Helena *et al.,* 2014).

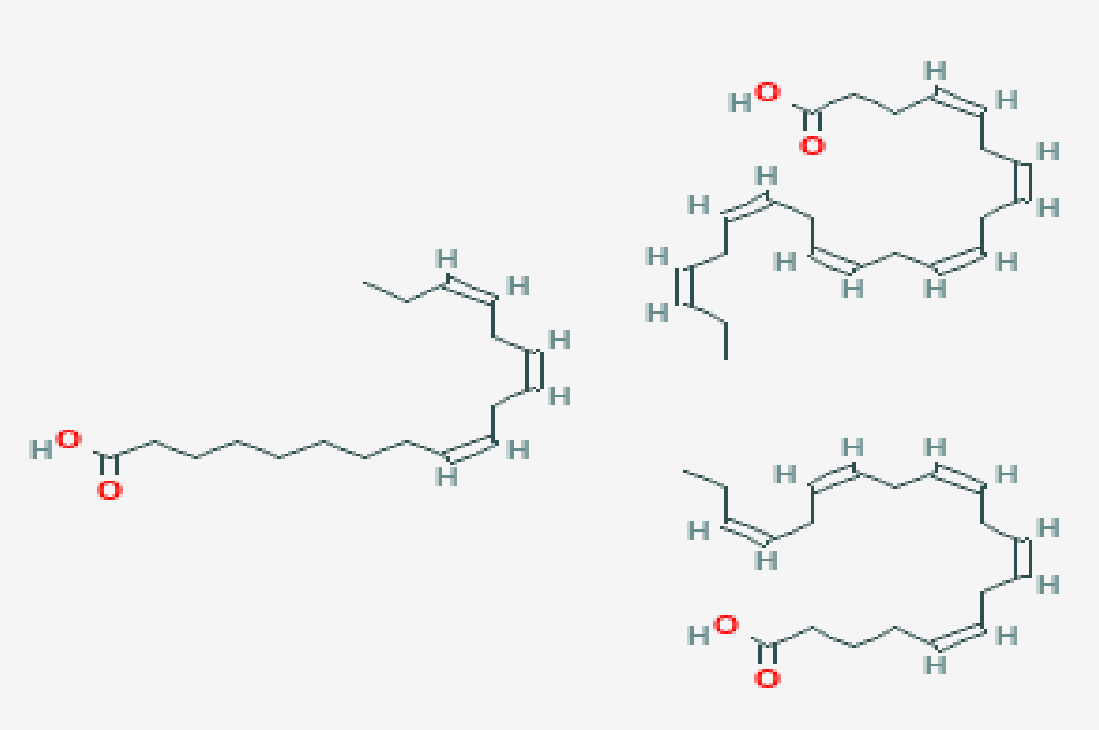

Figure 2.2: Structure of omega-3 fatty acids; 3 double bonds (ALA), 5 double bonds (EPA) and 6 double bonds (DHA) (Source: Mustapha et al., 2014)

2.5 Catfish farming

Fish is the most cultured of aquatic species for food and recreation with over 100 species farmed worldwide (FAO, 2004). *C. gariepinus* is among the most cultured fish worldwide. *C. gariepinus* preference in aquaculture is as a result of its ability to tolerate high environmental temperatures and low dissolved oxygen thus making it a good candidate for intensive farming, (Safran, 2009). Under best management practices, *C. gariepinus* can be cultured intensively (Somerville *et. al*., 2014) under limited land and water resources (Huntington *et al.,* 2017). Factors essential for growth of fish under intensive systems are; adequate oxygen, enough food and effective waste elimination mechanisms from culture systems (Dalsgaard *et al.,* 2013).

Figure 2.3: Pictorial presentation of African catfish (Clarias gariepinus) (Source: Okechi & Jensson 2005)

Demand for *C. gariepinus*, both for food and as bait in capture fisheries has been increasing substantially in Kenya in the last few years (Omondi *et al*., 2001).

Kenya's State Department of Fisheries and the Blue Economy estimates a demand of about 10 million *C. gariepinus* fingerlings per year for aquaculture and 18 million fingerlings per year for Lake Victoria capture fisheries. This adds up to a total demand of about 28 million *C. gariepinus* fingerlings annually, thus creating the need for increased catfish fingerling production to meet the market demand through modern culture systems such as aquaponics (Munguti *et al.*, 2014).

2.6 Water quality and management

Success in aquaculture productivity depends on proper management of water quality to enhance fish health and growth. Poor water management affects health leading to diseases that reduce growth and ultimate mortality of fish. Major water quality issues that occur in fish culture systems are low Dissolved Oxygen (DO) and increased ammonia which exists as toxic unionized (NH_3) and nontoxic ionized (NH^{4+}) forms referred as Total Ammonium Nitrogen (TAN) (Loan *et al.,* 2013). Chronic low DO level is the common cause of stress on cultured fish that reduce feeding, feed

conversion and ultimately affect growth rate in fish. Low DO in fish rearing unit is associated with elevated levels of free ammonia (NH_3) and carbon dioxide (CO_2) gases both of which are toxic to fish (Espinal & Mutalic, 2019). Efficient culture system should maintain adequate DO concentration of at least 6mg/L and CO_2 concentration below 30mg/L for best fish growth rates (Helfrinch & Libey, 1991). Aeration of fish rearing water using aerators is important in achieving required DO and CO_2 concentration. Dire situation should be detected by using water parameter analyzing probes before occurrence of fatalities. Un-ionized ammonia is excreted by fish through gills and becomes toxic if it accumulates beyond 0.25mg/L, (Loan *et al.*, 2013). Upon being acted by bacteria, ammonia is reduced to Nitrites (NO_2) and Nitrates (NO_3) by Nitrosomonas and Nitrobacter respectively through oxidation process to be rendered nontoxic (Badiola *et al.*, 2018). Other parameters that affect the concentration of ammonia in fish rearing water include pH and Temperature.

2.7 Re-circulatory Aquaculture and Hydroponic systems (Aquaponics)

Re-circulatory Aquaculture system has been in existence for over forty years and commonly used in regions experiencing water shortage (Rurangwa & Verdegem, 2014). The system is characterized by intensive fish production unit using series of water treatment steps to remove nutrients and impurities from water to facilitate its reuse (Espinal & Mutalic, 2019). It focuses on environmental regulation of wastewater discharge while utilizing limited water and land resources (Barbu *et al*., 2016) thus increasing productivity per unit area in fish farming (Somerville *et. al*., 2014).

Hydroponics entails the soil-less cultivation of agricultural crops in aqueous medium or by use of various growing substrate that provide plant support (Sorensons, 2009). The substrates are either organic or inorganic and their use depends on a number of properties. The properties include; higher porosity compared to soil, easily root-able hence saving plant from excess use of energy, allow roots to access easily nutrients solution and good drainage property for oxygen penetration from air (Martins *et al.*, 2010). A variety of plants that include high value herbs, vegetables and leafy greens

are grown in the hydroponic unit for nutrients uptake to improve water quality for fish rearing (Dunning *et al.,* 1998).

Advantages of RAS and hydroponics over the conventional fish and crop production methods includes limited land requirement, less water use, increased fish and crops growth rates, year-round production and operational efficiency by equipment sharing and simultaneous multiple production (Pattillo, 2017). Aquaponics is a bio-integrated system that links recirculating aquaculture system with hydroponic unit that utilize nutrients from water hence for reuse by fish (Rinehart, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was carried out over four months duration for each of the two experiments at a private fish farm in Baringo County, Kenya. The farm lies at latitudes N0°39N' S36°05'E and an altitude of 970 meters above sea level, with average annual temperature range of 30^{o}C to 35^{o}C and average annual rainfall of 684mm. Crops grown mostly are under irrigated agriculture which include water melons, tomatoes, mangoes, onions finger millets, cotton, pigeon peas, paw paws, oranges, kales among others. The study site was selected due to its climatic condition, availability of space to support the research and the KCSAP requirement for conducting research in ASAL regions.

3.2 Experimental Design and set up

Performance experiments were conducted under aquaponic systems using a complete randomized block design. The system comprised of; fish rearing units, hydroponic units, water reservoir tank and 1000L sump tanks. Twelve plastic tanks measuring (H1.2 X D1) m were arranged in four sets (treatment) with each set having three tanks (replicates) that were used for rearing fish. Another four plastic tanks measuring (L1X W2X H1) m were used as hydroponic units in which plants were grown on while other four, 1500L plastic tank were used as sump. Four submersible water pump Model DC 12V (Power 120W) with a maximum head of 25m were placed each per sump for pumping water to fish rearing tanks. Two-inch PVC pipes were used to connect the systems to ensure effective circulation of water within aquaponic system. Four solar panels of (DM#698) 250Wp were used to tap energy from the sun and transfer the energy through electric installation cables to 4 Solar Max batteries (6FM-100AH) 12V 100Amp each. The batteries stored energy that enabled the pumps (ACO-328) 250W facilitate water movement within the aquaponic system at night and during the days when there was no sun energy. Four

solar charger controllers were used to regulate the amount of energy getting to the 4 batteries from the 4 solar panels.

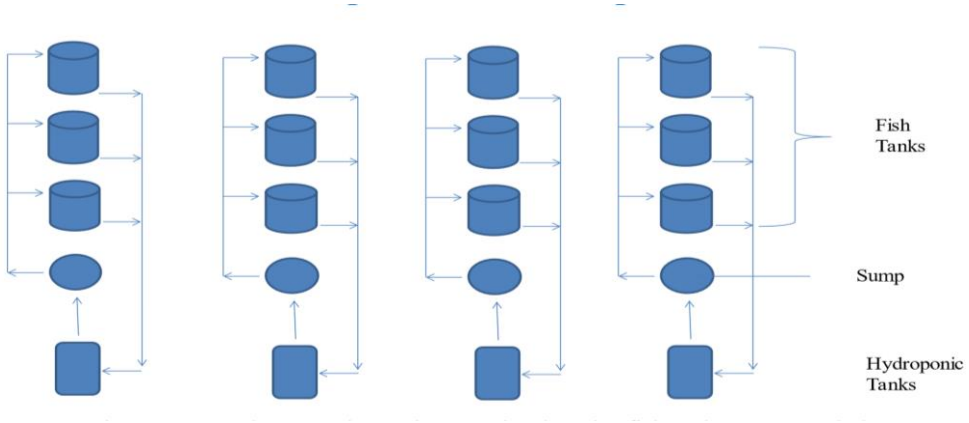

Figure 3.1: The experimental setup for the aquaponic system (Not drawn to scale). The arrows indicate the direction of flow of water within the system.

3.3 Diet formulation

Three different experimental diets of 35% crude proteins (CP) content were formulated using Pearson Square method comprising of the following ingredients; wheat bran (17.1%), cotton seed cake (32.9%), black soldier fly (BSF) (25.3%), sunflower seed cake 26.83% and fish meal (48.8%) (table 3.1).

Table 3.1: Protein content, percentage composition of ingredients in formulated test diets

		Experimental diets		
Parameter	**Unit**	**D_1**	**D_2**	**D_3**
Protein analysis				
Crude proteins	(g $100g^{-1}$)	35.0	35.0	35.0
Ingredients				
BSFL	(g 100g-1)	13.75	6.875	20.625
Fishmeal	(g 100g-1)	13.75	20.625	6.875
Cotton seed cake	(g 100g-1)	16.27	16.27	16.27
Sunflower seed cake	(g 100g-1)	25.495	25.495	25.495
Wheat bran	(g 100g-1)	30.735	30.735	30.735

D_1 =Diet 1, D_2 =Diet 2, D_3 =Diet 3, BSFL= Black soldier fly

Wheat bran and cotton seed cake were sourced from animal feeds raw material outlet within Nakuru town, Black Soldier Fly was sourced from International Centre of Insect Physiology and Ecology (ICIPE) Nairobi City, sunflower seed cake was obtained from an agrovet within Kisumu city in Kisumu County while fish meal was obtained from Lake Victoria (Kendu Bay) located within Homa Bay County. Thereafter the ingredients were directly sun dried separately, ground using a local posho meal grinding machine in Mogotio within Baringo County. The grounded ingredients were then mixed at required ratios for the three different on farm formulated diets that were used in feeding of the experimental fish for the present study. BSF substituted fish meal at (50-50, 25-75 and 75-25) % ratios. The feed ingredients were mixed with appropriate amount of water to form a dough and thereafter pelletized using a meat mincer on dice of 2mm diameter, then sundried appropriately before being packaged and stored at room temperature in 10kg plastic containers.

Proximate analysis was performed in triplicate for all the ingredients that were used for fish feed formulation. Crude protein was quantified by the Kjeldhal method as follows; 100 mg of sample was weighed using weighing machine WTC 2000 and then 3 ml of concentrated sulfuric acid was added. The mixture was then boiled at about 180 ˚C for 2 hours with the acid condensing in the middle of the neck of the Kjeldahl flask. The mixture was cooled and thereafter the Kjeldahl flask was put into the distillation apparatus. Then 12 ml of distilled water was added slowly to the mixture. The mixture was heated at 100 ˚C to liberate ammonia and thereafter distilled by steam for about 10 minutes through a condenser, while the tip of the distillation apparatus was submerged in a flask. After distillation, 25 ml of the mixture was titrated and the protein contented calculated by means of the Kjeldahl factors **F**; **mg protein = mg N · F** and the protein content "c" of the sample; c [%] = (mg protein) 100 weight of sample [mg] 1.0 ml 0.010 N HCl = 10 µMol N = 0.14 mg N

Crude lipid was quantified as the gain in weight of round-bottomed flask after extraction of the sample in petroleum ether (40-600C). Ash was determined by burning dry samples in a muffle furnace at 5500C for 4 hours while crude fibre was

quantified by alkaline/acid digestion followed by burning the dry residue at 5500C in a muffle furnace for 4 hours. Nitrogen free extracts (NFE) was determined by the difference method – (DM – CP – EE –CF –Ash). All the analyses were conducted as described in AOAC (1995). The formulated diets were tested against a commercial diet (Unga Farm Care Limited) of 35% CP which was used as a control diet for the experiment.

3.4 Stocking and feeding

Catfish fingerlings were obtained from Kenya Marine and Fisheries Research Institute (KMFRI), Sagana Aquaculture Station. Since aquaponics entails an intensive culture system in which a small area is used to culture a higher number of fish, 50 catfish fingerlings weighing 5±0.5g each were stocked in all 12 fish rearing tanks each containing 1000 litres of water. Under the stocking, 1 catfish was to occupy 20 litres of water. The diets were randomly allocated to the first four set of tanks and replicated thrice and fish were fed to satiation twice daily except for the sampling day in which fish were not be fed. Feeding was done using respective formulated diets for over four months duration.

3.5 Fish Sampling and growth determination

Buckets of 20 litres capacity were filled halfway with 10 litres of water in which fish were placed after being removed from fish rearing tanks. A scope net of 0.5 mm mesh size and 2m long, was used to scoop fish from the fish rearing tanks and placed in buckets filled halfway with water. A board mounted with a metre rule was used to determine the lengths while weighing machine, WTC 2000, was used to determine the weights of experimental fish respectively. After taking the measurements and recording of the biometrics, fish were put back in a holding container with water supported with aerators until all fish were sampled and thereafter taken back to their respective rearing units. The rearing units were covered with covering nets to prevent fish from jumping out of the units filled with water up to 1m height. Sampling was done after every three weeks to give fish enough time to recover from previous handling/sampling stress. Length and weight relationship was used to determine fish growth rate.

3.6 Extraction of total lipids and fatty acid analysis

Lipid's extraction was done according to the procedure by Bligh and Dyer (1959). Wet liver and muscle tissues were removed from three fish obtained from all the experimental tanks subjected to four different test diets. The wet tissues were preserved in 70% methanol and taken to the laboratory where they were homogenized with a mixture of 10ml chloroform and methanol and then centrifuged to allow dilution of water from tissues with chloroform. The dilution allowed the homogenate to separate into two layers, with chloroform containing all the lipids while the methanol layer contained all the non-lipids. The purified lipids layer was obtained by isolating chloroform layer from the methanol layer.

Fatty acid methyl esters (FAME) were then prepared from extracted total lipid by acid-catalyzed trans-esterification by adding 5ml of 1% H_2SO_4 (v/v) in methanol at 70^0C, for 3hrs. FAME were then extracted into 750ml of distilled water and 10 ml of hexane, dehydrated using anhydrous sodium sulphate, Na_2SO_4 and concentrated to 0.5 ml under vacuum evaporator. The concentrated FAME were then transferred to GC vials for later GC analysis

3.7 Gas chromatography analysis

FAME were separated and quantified by gas-liquid with on-column injection, equipped with a fused silica capillary column (SUPELCO Column Omegawax^{tm}530, 30m x 0.5mm x 0.5µm) with nitrogen as carrier gas and temperature programming from 170^0C to 220^0C for 18 min^{-1} and final time of 47minutes totaling to a run time of 75minutes. Injection and detection temperatures were 240^0C and 260^0C respectively. The programmer rate for both GC and decoder were set at $5min^{-1}$ with an attenuation of 3. All the GC analyses were done under same conditions. Individual methyl esters in the sample were identified by comparison with known FAME standards obtained from Kobian chemicals.

3.8 Proximate analysis and feeding

The commercial diet 35% CP was purchased from Unga Farm Care Limited Nakuru and proximate composition was determined according to method specifications 950.46 (AOAC, 1995). Experimental fish were fed twice a day according to the percentage body weight of fish over four months duration except for the sampling days. Body weight of fish was determined every three weeks after sampling and feed ration adjusted accordingly.

Table 3.2: Proximate composition of commercial diet used to feed *Clarias gariepinus* under different substrate treatment

Parameter	Percentage
Dry matter	91.55
Crude Proteins	35.41
Crude fats	7.26
Ash	10.31
Fibre	6.75
Moisture	8.46
Nitrogen free extract	38.58

3.9 Nutrients analysis and filtration efficiency

Nutrients (dissolved reactive phosphates, nitrites, nitrates and ammonia) were monitored in all 12 fish rearing tanks and the 4 sumps to determine the filtration efficiency of the aquaponic system. Spectrophotometry procedure was used to determine the levels of nitrites, nitrates and ammonia in the Laboratory using Shimadzu spectrometer (UV-1800ENG240V, SOFT).

3.9.1 Dissolved reactive phosphorous (DRP)

Dissolved reactive phosphorous (DRP) was determined through digestion method (Buxton, 2011). Ten (10) milliliters of sampled water was poured into 250ml volumetric flasks and prepackaged powder reagent weighing 100mg consisting of sulfuric acid, potassium antimonyl tartrate, ammonia molybdate and ascorbic acid was added to the volumetric flasks and swirled vigorously to mix thoroughly. The mixture was left to stand for 10 minutes and then poured into a clean sample cell test tube. The Shimadzu spectrophotometer (UV-1800ENG240V, SOFT) was zeroed using a blank standard solution (water sample with no reagent in it) and then the sample cell test tube was placed into the sample cell and covered. Absorbance was read at a wavelength of 885 nm and recorded. The sample cell was rinsed thoroughly with distilled water and wiped with clean cotton wool while avoiding touching its lower side before using it for the next sample analysis.

3.9.2 Nitrate

Nitrate was determined through cadmium method (Buxton, 2011). Nitriver 3 nitrate reagent powder pillow weighing 100mg containing copper sulphate, sulphanilamide, potassium nitrate hydrochloric acid and N-(1 naphthyl)-ethylene diamine-dihydrochloride was added to a sample cell with 10ml of sampled water and the content swirled until pink color appeared. The mixture was left for 20 minutes for the reaction to be completed and thereafter passed through reduction column made of cadmium copper filling treated in copper sulphate solution and rinsed by ammonium chloride solution. The Shimadzu spectrophotometer (UV-1800ENG240V, SOFT) was zeroed using a blank standard solution (water sample with no reagent in it). The sample cell test tube was placed in the sample cell and covered and the absorbance read at a wavelength of 543nm and recorded. The sample cell was rinsed thoroughly with distilled water and wiped with clean cotton wool while avoiding touching its lower side before it was used for the next sample analysis.

3.9.3 Nitrite

Nitrite was determined through diazotization reduction method (Buxton, 2011). Powdered pillow reagent weighing 100mg containing copper sulphate, sulphanilamide, potassium nitrate hydrochloric acid and N-(1 naphthyl)-ethylene diamine-dihydrochloride was added to a sample cell with 10ml sampled water and the mixture was swirled vigorously for three minutes and left for 10 minutes for the reaction to complete. The Shimadzu spectrophotometer (UV-1800ENG240V, SOFT) was zeroed using a standard solution (water sample with no reagent in it) and the sample cell test tube was placed in the spectrophotometer and absorbance read at a wavelength of 543nm and recorded. The sample cell was rinsed thoroughly with distilled water and wiped with clean cotton wool while avoiding touching its lower side before using it for the next sample analysis.

3.9.4 Ammonium

Ammonia was determined through colorimetric indophenol blue method (Loan *et al.,* 2013). Powdered pillow reagent weighing 100mg containing sodium nitroprusside, tri-sodium citrate dihydrate, sodium hydroxide and phenol indicator was added to a sample cell with 10ml sampled water and the mixture was swirled vigorously for three minutes and left for 10 minutes for the reaction to complete. The Shimadzu spectrophotometer (UV-1800ENG240V, SOFT) was zeroed using a standard solution (water sample with no reagent in it) and the sample cell test tube was placed in the spectrophotometer and absorbance read at a wavelength of 630nm and recorded. The sample cell was rinsed thoroughly with distilled water and wiped with clean cotton wool while avoiding touching its lower side before using it for the next sample analysis.

3.10 Preparation of standard solutions and determination of nutrients concentration

3.10.1 Determination of Dissolved reactive phosphorous concentration

Anhydrous potassium hydrogen phosphate 0.816g was dissolved in 1000ml distilled water. Five standards were prepared of between (1, 2, 4, 6 and 8) ml from diluted anhydrous phosphate hydrogen solution and diluted up to 100ml with distilled water. The absorbance of the standards of different concentration was determined at 885nm wavelength and a calibration curve was plotted of absorbance against concentration (Y=MX-C) from which the concentration of phosphates was determined.

3.10.2 Determination of nitrates and nitrites concentration

Sodium nitrate and sodium nitrite were dried at 100^{o}C, 10g of sodium nitrate and 13.5g of sodium hydroxide were diluted separately in 1000ml distilled water to form standard solutions. Each of the standard solution 1ml was pipetted and transferred to 100ml volumetric flask and diluted using distilled water to obtain a concentration of 10 μg/ml. The standard solution of concentration of 10 μg/ml was pipetted into 100ml volumetric flasks at different volumes of between 1ml and 10ml and diluted to the mark and sulfanilic acid 2.5ml was added to each and left for 5 minutes. N-(1 naphthyl)-ethylene diamine-dihydrochloride solution 2.5ml was added and absorbance was determined at 563nm wavelength. A calibration curve was plotted of absorbance against concentration (Y=MX-C) from which the concentration of nitrates and nitrites was determined.

3.10.3 Determination of ammonium concentration

Ammonium chloride was dissolved in distilled water and several standards were obtained by measuring different volumes of between 5ml and 500ml (5, 10, 25, 50, 100, 250, and 500) ml from dilute ammonium chloride solution. Each of the standard was diluted up to 1000ml with distilled water. Powdered pillow reagent weighing 100mg containing sodium nitroprusside, tri-sodium citrate dihydrate, sodium hydroxide and phenol indicator was added to the standards of different

concentrations. The standards mixed with the reagents were stored in a dark place for a minimum of 12 hours and not more than 48 hours before processing. Then the absorbance of the standards of different concentration was determined at 630nm wavelength and a calibration curve was plotted of absorbance against concentration (Y=MX-C) from which the concentration of ammonium was determined.

The nutrient reduction efficiency of different substrates in the hydroponic units was calculated using the formula, (Oladimeji 2018).

$$\% \text{ Reduction Efficiency} = [(a-b)/a] \times 100$$

where, a= concentration in the inlet water, and b= concentration of the outlet water

3.11 Data collection and analysis

3.11.1 Determination of growth Performance and Survival

At the conclusion of the experiment, fish were harvested using a fishing net, counted, weight and length documented. Survival, Growth and feed efficiency were evaluated by the following standard formula.

Daily growth (DG) (g) = Final weight/Time (Exp days)

Body weight gain (BWG) (g) = Final weight – Initial weight

Specific Growth Rate (SGR) (%) = 100% X [(In Final weight (g) – In Initial weight (g)) / Time (Exp days)]

Fish Food Conversion Ratio (FCR) = Feed provided (g) / Weight gain (g)

Survival rate (SR) (%) = 100 X (Final number of fish) / (Initial number of fish)

3.11.2 Planting and determination of Spinacia oleracea growth parameters

Three different types of substrates (pumice, charcoal and pumice mixed with charcoal) were placed in the three hydroponic tanks while the fourth tank did not have substrate (Aeroponic) and was used as a control. Fifteen *Spinacia oleracea* seedlings sourced from a Nakuru city market were planted in the hydroponic unit at a spacing of 30cm by 45cm for water purification by nutrients intake from fish rearing water. The plants were allowed to grow for one and a half months and were harvested continuously monthly for three months. A pair of scissors was used to cut the leaves from the plants. The length of the leaves was measured from the tip of the leafy part to the tip of the stalk while the breadth was measured at the broader region of the leaf from the end of one side of the leaf margin to the end of the other side of the leaf margin and values recorded in a data book. The number of leaves per plant were counted during harvesting and summed up for every treatment. The number of plants that the actual harvesting was done were also counted during every harvesting session for all the treatment on the same day.

3.11.3 Statistical analysis

Statistical analysis was performed using SPSS version 23 for windows. Analysis of weight, length and fatty acid of fish was done using one way analysis of variance (ANOVA). Analysis of spinach length and breadth was carried out by one way (ANOVA) while analysis of water quality parameters (DO, temp, pH, conductivity, TDS, ammonia, nitrates, nitrites and phosphates) was done using Analysis of Variance (ANOVA). Statistical variation for all the inference tests under the present study were performed using the Tukey-HSD post hoc, at 95 significance level. The results were presented using tables and graphs plotted using Microsoft excel spreadsheet for windows 2010.

CHAPTER FOUR

RESULTS

4.1 Proximate analysis of ingredients and experimental diets

The formulated experimental diets contained black soldier fly (BSFL), fishmeal (FM), cotton seed cake (CSC), sunflower seed cake (SSC) and wheat bran (WB). The (table 4.1) shows the parameters analyzed for each of the ingredients used in the diets.

Table 4.1: Percentage (%) proximate composition of analyzed ingredients used in diet formulation

Parameter	Unit	DM	CP	EE	CF	Ash	NFE
BSFL	(g $100g^{-1}$)	95.8	25.3	27.3	8.0	14.7	20.5
FM	(g $100g^{-1}$)	89.94	48.80	12.4	1.28	9.28	18.18
CSC	(g $100g^{-1}$)	92.14	32.29	8.60	6.46	18.54	26.25
SSC	(g $100g^{-1}$)	91.85	26.83	9.90	16.92	9.44	28.76
WB	(g $100g^{-1}$)	90.08	17.10	5.01	10.96	8.43	48.58

DM: Dry matter, CP: Crude proteins, EE: Ether extract, Crude fibre: NFE: Nitrogen free extract. BSF: Black soldier fly larvae, FM: Fishmeal, CSC: Cotton seed cake, SSC: Sunflower seed cake and WB: Wheat bran

Three test diets were formulated with each diet containing same ingredients but different ratios of BSFL and FM. Dietary BSFL: FM inclusion was as follows; diet 1 (1:1), diet 2 (1:3) and diet 3 (3:1). The test diets were evaluated on *C. gariepinus* using diet C (control) purchased from a feed manufacturing company. The (table 4.2) shows the parameters analyzed for the diets used in the study.

Table 4.2: Proximate composition of formulated test diets

		Means ± SD			
Parameter	**Unit**	**D_1**	**D_2**	**D_3**	**D_c**
Dry matter	(g/100g)	91.43±0.81^{a}	91.57±0.35^{a}	91.57±0.09^{a}	91.55±0.63^{a}
Crude proteins	(g/100g)	35.39±0.07^{a}	35.28±0.10^{a}	35.30±0.02^{a}	35.41±0.19^{a}
Ether extract	(g/100g)	9.09±0.14^{b}	10.28±0.10^{c}	11.47±0.93^{d}	7.25±0.32^{a}
Ash	(g/100g)	10.65±0.05^{b}	10.05±0.06^{b}	6.65±0.08^{a}	10.31±0.06^{b}
Crude fiber	(g/100g)	5.36±0.39^{a}	6.20±0.52^{b}	6.54±0.18^{b}	6.75±0.25^{b}
Moisture	(g/100g)	8.57±0.86^{ab}	8.42±0.35^{a}	8.57±0.02^{ab}	8.45±0.07^{a}
NFE	(g/100g)	36.28±0.26^{b}	35.95±0.52^{a}	38.00±0.04^{c}	39.56±0.50^{d}

D_1 =Diet 1, D_2 =Diet 2, D_3 =Diet 3, D_c =Control diet, and NFE: Nitrogen free extract

a<b<c<d at ($P < 0.05$)

4.2 Fish survival, feed utilization and growth parameters

Survival of fish under the set growth conditions and experimental diets was above 95% with relatively low mortality rate experienced in the first week of stocking for fish cultured under diet 3 (table 4.3). There was no significant difference ($P \geq 0.44$) observed in survival rate (SR) among all the dietary treatment and control diet under the present study. Fish that were fed on diets that BSFL substituted FM did not show significant difference ($P \geq 0.091$) in mean growth weight among the dietary treatment. However, fish that were fed on experimental diets exhibited a relative higher feed conversion ratio (FCR) compared to fish that were fed on control (commercial diet) (table 4.3). FCR values obtained for diets that BSFL substituted FM but were relatively higher than the value obtained for fish fed on the control diet. The specific growth rate (SGR) obtained by experimental fish ranged between 2.17 and 2.50 with diet (50%) BSFL obtained the least SGR while the control diet obtained the highest SGR. The diet that had (25%) BSFL obtained SGR of 2.24 while diet with (75%) BSFL obtained 2.22. Body weight gain (BWG) obtained by experimental fish ranged between (64.09 and 97.07) grams with diet containing (50%) BSFL obtained the least BWG while the control diet obtained the highest

BWG. The diet that contained (25%) BSFL obtained BWG of 69.78 grams while diet that had (75%) BSFL obtained BWG of 67.77 grams.

Table 4.3: Performance of *C. gariepinus* fed on experimental diets

	% BSFL in diets			
Variable	**D_1(50%)**	**D_2(25%)**	**D_3(75%)**	**D_c (0%)**
Fingerling initial quantity	50	50	50	50
Fingerlings final quantity	50	50	49	50
Stocking weight (g)	5.1	5.1	5.1	5.1
Harvest weight (g)	69.16	74.78	72.87	102.17
Body weight gain (g)	64.09	69.78	67.77	97.07
Growth rate (g day1)	0.534	0.582	0.565	0.809
Specific growth rate	2.17	2.24	2.22	2.50
Food conversion ratio	1.3	1.2	1.3	1.1
Survival rate (%)	100	100	98	100

D_1 =Diet 1, D_2 =Diet 2, D_3 =Diet 3 and D_c =Control diet

Experimental fish growth curves were exponential for the first five weeks of culture where fish growth assumed more or less linear trends for all the dietary treatments (figure 4.1). Differential growth trends exhibited by experimental fish among dietary treatment occurred between weeks five and seven of culture. During the period of week five and seven, the growth curve of fish fed on control diet separated from the growth curves of fish fed on diets that BSFL substituted FM (figure 4.1). Growth appeared to slow down towards the end of the study period for fish subjected to control diet. The growth curves of the test diets having not separated much from each other is the reason there was no observed significance difference ($P \geq 0.091$) for BSFL formulated diets. However, growth of fish fed on test diets appeared to rise exponentially towards the end of the culture period.

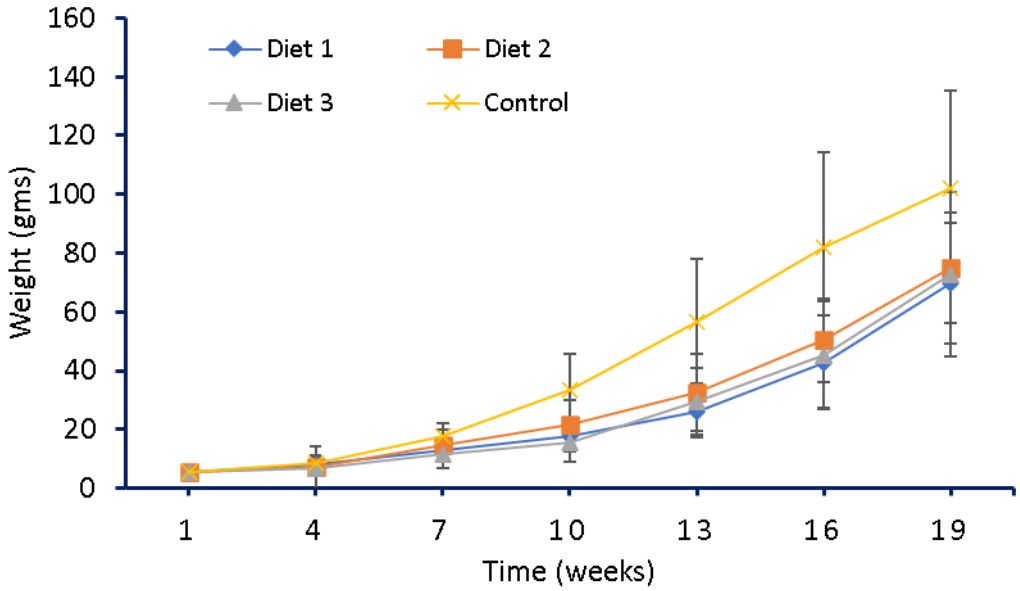

Figure 4.1: Growth curves for C. gariepinus under different BFSL dietary treatments (Diet 1; 50%) (Diet 2; 25%; (Diet 3; 75%) and (Diet 4; control) without BSFL

4.3 Growth performance of *C. gariepinus* under different substrates

Proximate composition of different parameters suggested suitability of the commercial diet in *C. gariepinus* culture at post fingerling stage of growth (table 4.4) The results on growth performance of *C. gariepinus* as presented in (table 4.4) indicated a variation of final average weights of experimental fish after the four-month trial. The treatment with pumice substrate attained the highest final weight of 108.78g, followed by pumice charcoal substrate with 92.54g, then charcoal substrate with 84.76g while the control treatment was least with 70.34g.

Table 4.4: Performance of *C. gariepinus* cultured under aquaponic system using different substrates

	Treatment			
Substrate	**Control**	**Charcoal**	**Pumice & Charcoal**	**Pumice**
Initial fingerling quantity	50	50	50	50
Final fingerlings quantity	46	49	47	48
Initial stocking weight (g)	14.97	14.97	14.97	14.97
Final harvest weight (g)	70.34	92.54	84.76	108.78
Body weight gain (g)	55.37	77.57	69.79	93.81
Initial stocking length (g)	14.05	14.05	14.05	14.05
Final harvest length (g)	20.58	22.12	21.73	23.13
Growth rate (g day1)	0.461	0.645	0.582	0.782
Specific growth rate	3.345	3.625	3.538	3.784
Food conversion ratio	1.4	1.2	1.3	1.1
Survival rate (%)	92	98	94	96

The growth curves for weight and length for *C. gariepinus* subjected on different substrate treatments (figures 4.2 and 4.3), showed there was no significant difference observed ($p \geq 0.234$) between pumice, charcoal and the interaction between pumice and charcoal treatment. However, there was significant difference ($p \geq 0.021$) observed between the control treatment (without any substrate) and the treatments that had substrates. Fish subjected to pumice substrate obtained final length and weight of 23.13 cm and 108.78 g respectively at the end of the experiment. The pumice charcoal substrate obtained a final length and weight of 22.12 cm and 92.54 g while charcoal substrate obtained a final length and weight of 21.24 g and 84.76 cm respectively. The control treatment was the least obtaining length of 20.58 cm and weight of 70.34 g for *C. gariepinus* at the end of the experiment.

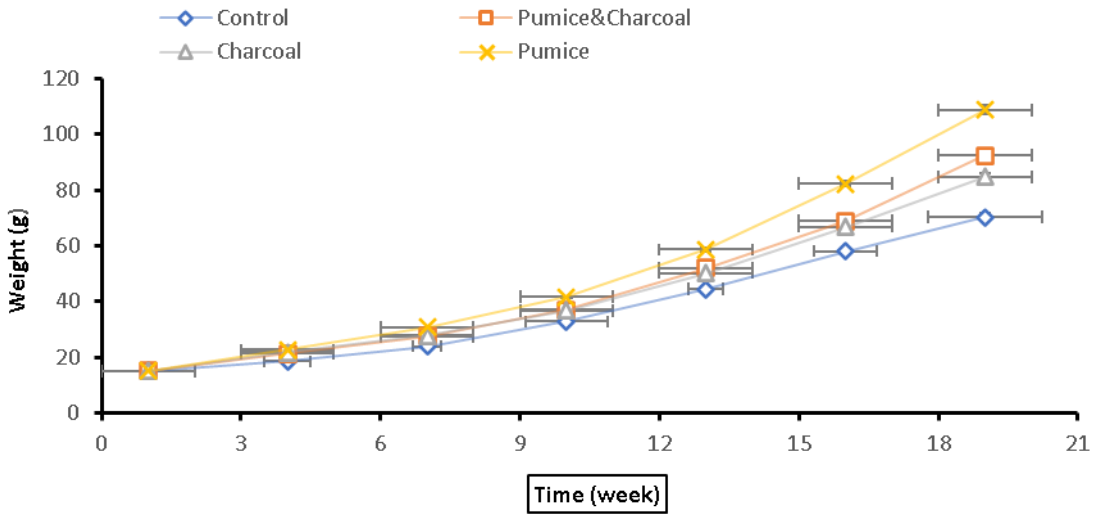

Figure 4.2: A graph of weight of C. gariepinus (g) against time (weeks).

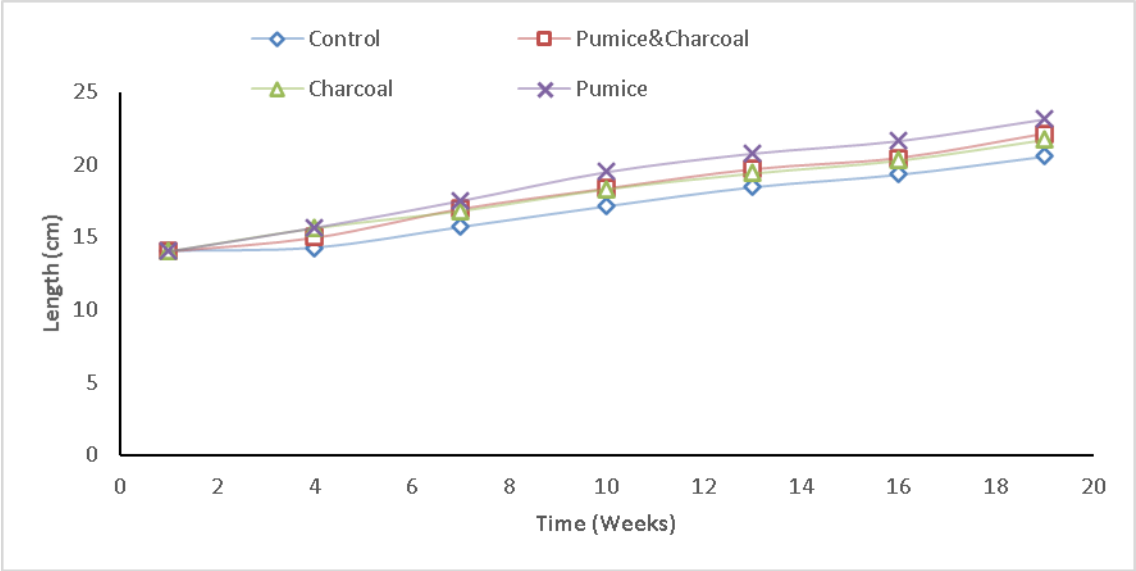

Figure 4.3: A graph of length of C. gariepinus (g) against time (weeks)

The survival rates of fish under the set growth conditions subject to different substrates was between 92-98% with most of fish mortality experienced in the second day after stocking. Highest mortality was recorded under the control

treatment with 8% deaths, followed by the charcoal treatment with 6% deaths, pumice treatment with 4% deaths while the pumice charcoal had the least mortalities with 2% as shown on (table 4.11). There was no significant difference ($P > 0.05$) in survival rate (SR) among all the treatment under the present study.

4.4 Productivity of *S. oleracea* under different substrate

The growth parameters of *S. oleracea* grown in different substrate treatments under aquaponic system indicated significant difference ($p<0.05$) for breadth, length and number of leaves harvested per plant with p values of 0.023, 0.045 and 0.003 respectively. In addition, there was no significance difference ($p<0.05$) in the number of plants harvested during the study period with p value of 0.087. The range of the number of plants harvested during the study period was between 40% and 53% for charcoal treatment while the pumice and the mixture of pumice and charcoal treatments had similar number of plants harvested of between 53% and 73% plants. The number of leaves harvested from the plants during the study duration ranged between (24-32) leaves for charcoal treatment, (40-48) leaves for pumice treatment and (41-48) leaves for a mixture of pumice and charcoal treatment. The range in breadth of harvested leaves were between (6-12.2) cm for charcoal substrate, (6.2-13.2) cm for pumice substrate and (7.2-15) cm for the mixture of pumice and charcoal substrate. The range for length of leaves were between (12-24) cm, (12-24) cm and (14-30) cm for charcoal, pumice and a mixture of pumice and charcoal substrates respectively (Table 4.5). The control treatment under the current study did not have *Spinacia oleracea* and also lacked substrates.

Table 4.5: Growth parameters of *Spinacia oleracea* in the hydroponic units between different substrates, (Means ± SD)

Parameter	Unit	Treatment				
		Control	Charcoal	Pumice	Pumice+ Charcoal	P Value
Breath	(cm)	-	8.43 ± 1.48[a]	8.15 ± 1.81[a]	10.20 ± 1.90[b]	0.023
Length	(cm)	-	16.20 ± 2.34[a]	17.10 ± 2.88[b]	20.96 ± 3.86[c]	0.045
No of Leaves		-	27.67 ± 4.04[a]	44.33 ± 4.04[b]	44.67 ± 0.09[b]	0.003
No of Plants		-	7.00 ± 1.01[a]	9.67 ± 1.53[b]	9.97 ± 1.79[c]	0.087

SD: Standard deviation, P-value: Level of significance, mgL^{-1}: Centimeters, Superscript letters within rows indicate statistical variation in mean values at ($P<0.05$); a<b<c.

4.5 Fatty acid profiles of experimental diets

The total saturated fatty acids analyzed varied significantly ($p< 0.05$) among the diets used for the present study as shown in (table 4.6). The significantly higher value of fatty acid was obtained by diet 3 which contained a higher percentage of BSFL (75%) used in the diet formulation. Diet 1 containing 50% BSFL was second in total saturated fatty acids present while diet 2 with 25% BSFL was third. Diet 4 which was control and a commercial diet contained the least amount of total saturated fatty acids in the diets. Individual saturated acids varied significantly ($p< 0.05$) among dietary treatments. All the saturated fatty acids extracted were present in all the diets except Myristic acid, C 14:0, which was lacking in diet 2 and control diet 4 (commercial diet). Palmitic acid, C16:0, was the dominant saturated acid among the formulated test diets while behenic acid, C21:0 dominated in the commercial diet.

Oleic acid, C18:1, the dominant monounsaturated fatty acid showed no statistical variation ($p> 0.05$) while palmitoleic and nervonic showed statistical valuation ($p< 0.05$) among dietary treatments (table 4.6)

Table 4.6: Fatty acid composition of experimental diets

		Dietary Treatment			
		Diet 1	**Diet 2**	**Diet 3**	**Diet 4**
C 14:0	Myristic	0.38±0.01^{a}	0	0.49±0.21^{a}	0
C 16:0	Palmitic	24.59±0.05^{c}	15.12±0.19^{b}	29.51±0.36^{d}	1.78±0.09^{a}
C 18:0	Stearic	1.71±0.00^{c}	1.24±0.03^{b}	2.24±0.01^{d}	0.09±0.01^{a}
C 20:0	Arachidic	2.63±0.02^{b}	1.53±0.04^{a}	2.48±0.03^{b}	1.96±0.11^{a}
C 21:0	Behenic	1.83±0.03^{a}	2.90±0.04^{b}	1.99±0.02^{a}	3.32±0.13^{c}
C 24:0	Lignoceric	0.82±0.04^{a}	2.49±0.06^{b}	2.40±0.12^{b}	0.75±0.01^{a}
∑SFAs		**31.96±0.03^{c}**	**23.28±0.06^{b}**	**39.11±0.13^{d}**	**7.9±0.06^{a}**
C13:1	Palmitoleic	5.90±0.07^{c}	4.31±0.05^{b}	6.57±0.05^{c}	0.89±0.07^{a}
C 18:1	Oleic	12.35±0.06^{a}	13.53±0.29^{b}	13.37±0.03^{b}	13.83±0.38^{b}
C 24:1	Nervonic	0.64±0.04^{b}	2.38±0.07^{c}	0.53±0.02^{a}	2.66±0.10^{c}
∑MUFAs		**18.31±0.07^{b}**	**20.22±0.14^{d}**	**19.28±0.03^{c}**	**17.38±0.18^{a}**
C 18:2	Linoleic	14.28±0.37^{c}	7.05±0.11^{a}	10.25±0.02^{b}	48.65±3.48^{d}
C 18:3	Linolenic	33.06±0.58^{b}	42.47±0.62^{c}	30.43±0.43^{b}	14.93±0.81^{a}
C 20:5	EPA	1.18±0.03^{b}	3.29±0.31^{c}	0.94±0.01^{a}	1.70±0.05^{b}
C 22:6	DHA	0.65±0.04^{a}	3.66±0.04^{b}	0.43±0.01^{a}	7.77±0.59^{c}
∑n3		34.89±0.22^{c}	49.42±0.32^{d}	31.8±0.15^{b}	24.4±0.24^{a}
∑PUFAs		**49.17±0.26^{b}**	**56.47±0.27^{c}**	**42.05±0.12^{a}**	**73.05±1.23^{d}**
n3/n6		1.91±0.30^{b}	7.01±1.45^{d}	3.10±0.75^{c}	0.50±0.07^{a}

Values indicate are means± standard error. Means within same row with different superscript varied significantly ($p<0.05$), where, a<b<c<d. **∑SFAs;** Summation of saturated fatty acids, **∑MUFAs;** Summation of monounsaturated fatty acids, **∑PUFAs;** Summation of polyunsaturated fatty acids, **∑n3;** Summation of omega-3 fatty acids.

There was statistical variation ($p< 0.05$) in the summation of monounsaturated fatty acids among diets. Diet 2 (25% BSFL) dominated in the summation of monounsaturated fatty acids, followed by diet 3 (75% BSFL), then diet 1 (50% BSFL) while control diet 4 (commercial) had the least value of (17.38±0.18) in the summation of monounsaturated fatty acids for the present study. There was a significantly high statistical variation ($p< 0.05$) in the total polyunsaturated fatty acid for the experimental diets (table 4.6). Control diet 4 (commercial) dominated in the

summation of polyunsaturated fatty acid with (73.05±1.23) followed by diet 2 (25% BSFL) (56.47±0.27), then diet 1 (50% BSFL) (49.17±0.26) and lastly diet 3 (75% BSFL) (42.05±0.12). There was statistical variation ($p< 0.05$) among the polyunsaturated fatty acids for the dietary treatment under the present study. Linoleic acid, C18:2, was the predominant omega-6 polyunsaturated fatty acid while linolenic was the dominant omega-3 fatty acids.

4.6 Effect of experimental diets on whole body fatty acid profile of *C. gariepinus*

The total saturated fatty acids analyzed for the whole body varied significantly ($p< 0.05$) among dietary treatments as shown in (table 4.7). The highest summation value of saturated fatty acid for whole body was obtained by fish fed on control diet 4 (commercial) while the least summation was obtained by fish fed on diet 1. Whole body of fish fed on diet 2 was second in summation of saturated fatty acids present while whole body of fish fed on diet 3 was third. Most individual saturated acids for whole body varied significantly ($p< 0.05$) among dietary treatments except capric acid C10:0, pentadecanoic acid C15:0 and behenic C21:0 which did not show statistical variation ($p> 0.05$). Palmitic acid, C16:0, was the dominant saturated fatty acid obtained for the whole body among the dietary treatments while heptadecanoic acid, C17:0, dominated in the commercial diet.

There was statistical variation ($p< 0.05$) in all the monounsaturated fatty acids for whole body among different dietary treatments with oleic acid, C18:1, being the dominant monounsaturated fatty acid (table 4.7). There was statistical variation ($p< 0.05$) in the summation of monounsaturated fatty acids for whole body among dietary treatments. Whole body of fish fed on control diet 4 (commercial) obtained relatively higher value of monounsaturated fatty acids, followed by whole body of fish fed on diet 1, then whole body of fish fed on diet 2 while whole body of fish fed on diet 3 was the lowest.

Table 4.7: Fatty acid composition of the whole body of C. gariepinus

		Whole body			
		Diet 1	**Diet 2**	**Diet 3**	**Diet 4**
C 4:0	Butyric	2.04±0.09^{c}	1.63±0.66^{b}	0.87±0.34^{a}	0.26±0.12^{a}
C 8:0	Caprylic	0.27±0.04^{d}	0.04±0.019^{a}	0.11±0.01^{c}	0.08±0.03^{b}
C 10:0	Capric	0.24±0.03^{b}	0.01±0.00^{a}	0.25±0.03^{b}	0.40±0.21^{b}
C 12:0	Lauric	0.94±0.34^{a}	0.21±0.02^{a}	0.15±0.01^{a}	0.14±0.02^{a}
C 14:0	Myristic	1.09±0.08^{b}	2.14±0.32^{d}	1.40±0.15^{c}	0.23±0.09^{a}
C 15:0	Pentadecanoic	1.49±0.19^{a}	1.80±0.08^{a}	4.03±0.27^{b}	1.23±0.22^{a}
C 16:0	Palmitic	13.87±1.05b	19.05±1.63^{d}	16.55±0.92^{c}	2.21±0.56^{a}
C 17:0	Heptadecanoic	2.09±0.27^{b}	2.75±0.39^{c}	0.94±0.12^{a}	41.21±0.28^{d}
C 18:0	Stearic	6.87±0.77^{d}	5.67±0.34^{c}	4.62±0.71^{b}	0.68±0.09^{a}
C 20:0	Arachidic	3.34±0.52^{c}	1.79±0.31^{a}	9.97±0.60^{d}	2.76±0.08^{b}
C21:0	Behenic	0.95±0.47^{b}	0.85±0.33^{b}	0.61±0.01^{a}	0.19±0.03^{a}
C 24:0	Lignoceric	2.86±0.15^{c}	1.43±0.14^{b}	1.44±0.19^{b}	0.74±0.33^{a}
∑SFAs		**36.05±0.33^{a}**	**37.37±0.37^{a}**	**40.94±0.28^{b}**	**50.13±0.17^{c}**
C13:1	Palmitoleic	3.64±0.22^{c}	2.52±0.26^{b}	3.48±0.27c	0.25±0.01a
C 17:1	Cis 10 heptadecanoic	0.59±0.00^{b}	1.00±0.01^{d}	0.89±0.06c	0.22±0.11a
C 18:1	Oleic	27.81±0.50^{a}	28.26±0.90^{b}	22.29±0.45a	40.50±1.49c
C 24:1	Nervonic	2.51±0.38^{c}	2.02±0.85^{c}	0.96±0.04b	0.08±0.01a
∑MUFAs		**34.55±0.72^{d}**	**33.80±0.75^{c}**	**27.33±0.32^{a}**	**41.05±0.48^{b}**
C 18:2	Linoleic	20.76±2.53^{d}	18.65±1.71^{c}	17.30±0.77b	7.08±0.78^{a}
C 20:4	Arachidonic	5.10±0.55b	7.24±0.62c	2.95±0.45a	5.56±0.17^{b}
∑n6		**26.86±2.81^{c}**	**25.89±1.17^{c}**	**20.25±0.61^{b}**	**12.64±0.48^{a}**
C 18:3	Linolenic	3.94±0.50^{b}	3.22±0.06^{b}	2.77±0.08^{a}	3.80±0.91^{b}
C 20:5	EPA	3.78±0.29^{b}	4.35±0.08^{c}	4.35±0.60^{c}	2.31±0.16^{a}
C 22:6	DHA	6.67±0.39^{d}	4.08±0.37^{c}	3.05±0.39^{b}	2.81±0.13^{a}
∑n3		**14.39±0.40^{c}**	**11.65±0.17^{b}**	**10.17±0.36^{b}**	**8.92±0.40^{a}**
∑PUFAs		41.25±1.61^{d}	37.54±0.94^{c}	30.42±0.49^{b}	21.56±0.44^{a}
n3/n6		**0.54±0.07^{c}**	**0.45±0.05^{a}**	**0.50±0.03^{b}**	**0.71±0.04^{d}**

Values indicate are means± standard error. Means within same row with different superscript varied significantly ($p<0.05$). **∑SFAs;** Summation of saturated fatty acids, **∑MUFAs;** Summation of monounsaturated fatty acids, **∑n3;** Summation of omega-3 fatty acids, **∑n6;** Summation of omega-6 fatty acids and **∑PUFAs;** Summation of polyunsaturated fatty acids. Superscript letters within rows indicate significant difference in mean values at ($P<0.05$); a<b<c<d

There was statistical variation ($p< 0.05$) in the summation of polyunsaturated fatty acid for whole body for the present study. Whole body of fish fed on diet 1 dominated in the summation of polyunsaturated fatty acid with (41.25±1.61) followed by diet 2 (37.54±0.94), then diet 3 (30.42±0.49) followed by diet 4 (commercial) (21.56±0.44). Statistical variation ($p< 0.05$) was observed for whole body in all the polyunsaturated fatty acids under the present study. Linoleic acid C18:2, was the predominant polyunsaturated fatty acid. Statistical variation ($p< 0.05$) in the summation of omega-3 fatty acids for whole body was observed with fish fed on diet 1 dominating by (14.39±0.40).

4.7 Effect of experimental diets on liver fatty acid profile of *C. gariepinus*

Saturated fatty acids analyzed for the liver varied significantly ($p< 0.05$) among dietary treatments (table 4.8). Fish fed on diet 1 and diet 3 recorded relatively high amount of total saturated fatty acids compared to fish fed diet 2 and commercial diet. Palmitic acid was the dominant saturated fatty acid with no significant difference in the values of palmitic acid obtained in diet 1 and diet 3 (table 4.8). Arachidic acid, C20:0 was the second most dominant saturated fatty acid with lignoceric acid C24:0, being the least dominant among the saturated acid (table 4.8). There was statistical variation ($p< 0.05$) in all the monounsaturated fatty acids for the liver between dietary treatments with oleic acid, C 18:1, being the dominant monounsaturated fatty acid (table 4.8). The liver of fish fed on diet 3 recorded significantly higher value of monounsaturated fatty acids, followed by the liver of fish fed on control diet 4 (commercial), then the liver of fish fed on diet 1, while the liver of fish fed on diet 2 recorded significantly lower monounsaturated summation value.

Table 4.8: Fatty acid composition of the liver of *C. gariepinus*

		Liver			
		Diet 1	**Diet 2**	**Diet 3**	**Diet 4**
C 12:0	Lauric	3.60±0.16^{c}	2.40±0.22^{b}	2.37±0.05^{b}	0.72±0.08^{a}
C 14:0	Myristic	1.09±0.17^{c}	0.87±0.10^{b}	1.01±0.08^{c}	0.54±0.04^{a}
C 15:0	Pentadecanoic	1.69±0.19^{a}	2.69±0.07^{c}	2.8±0.06^{c}	1.87±0.05^{b}
C 16:0	Palmitic	25.36±0.58^{a}	24.79±0.61^{a}	25.48±1.90^{a}	26.26±0.73^{a}
C 17:0	Heptadecanoic	2.41±0.15^{c}	2.15±0.08^{b}	2.23±0.07^{b}	1.47±0.04^{a}
C 18:0	Stearic	0.63±0.12^{b}	0.41±0.12^{a}	0.41±0.18^{a}	0.44±0.16^{a}
C 20:0	Arachidic	5.82±0.12^{a}	6.85±0.04^{b}	5.53±0.29^{a}	8.66±0.49^{c}
C21:0	Behenic	1.36±0.06^{c}	0.41±0.08^{a}	1.11±0.06^{b}	0.70±0.17^{a}
C 24:0	Lignoceric	0.30±0.04^{b}	0.26±0.02^{a}	0.31±0.02^{b}	0.89±0.01^{c}
∑SFAs		**34.78±0.15^{c}**	**33.31±0.09^{b}**	**34.30±0.12^{c}**	**31.30±0.87^{a}**
C13:1	Palmitoleic	0.34±0.10^{b}	0.20±0.03^{a}	0.57±0.24^{b}	0.16±0.01^{a}
C 17:1	Cis 10 heptadecanoic	1.12±0.03^{b}	1.05±0.08^{b}	0.61±0.15^{a}	0.58±0.15^{a}
C 18:1	Oleic	30.95±1.80^{a}	31.52±2.18^{a}	34.54±4.74^{b}	33.60±2.43^{a}
C 24:1	Nervonic	0.33±0.00^{a}	0.56±0.05^{b}	0.57±0.01^{b}	0.28±0.04^{a}
∑MUFAs		**32.74±0.08^{a}**	**33.33±0.59^{b}**	**36.29±1.29^{d}**	**34.62±0.66^{c}**
C 18:2	Linoleic	15.04±0.23^{c}	13.42±0.26^{b}	11.47±0.68^{a}	15.99±1.41^{c}
C 20:6	Arachidonic	4.00±0.11^{b}	4.33±0.15^{c}	3.35±0.07^{a}	4.31±0.15^{c}
∑n6		**19.04±0.17^{c}**	**17.75±0.21^{b}**	**14.82±0.36^{a}**	**20.20±0.78^{d}**
C 18:3	Linolenic	1.72±0.01^{d}	0.63±0.06^{a}	1.20±0.03^{c}	0.78±0.09^{b}
C 20:5	EPA	0.29±0.12^{b}	0.22±0.04^{a}	0.19±0.04^{a}	0.17±0.02^{a}
C 22:6	DHA	6.33±0.50^{b}	7.25±0.94^{c}	6.04±0.68^{b}	3.65±0.47^{a}
∑n3		8.34±0.21^{c}	8.10±0.35^{c}	7.43±0.25^{b}	4.60±0.19^{a}
∑PUFAs		**27.38±0.19^{d}**	**25.85±0.28^{c}**	**22.25±0.31^{a}**	**24.8±0.49^{b}**
n3/n6		0.44±0.06^{b}	0.46±0.04^{c}	0.50±0.04^{d}	0.23±0.03^{a}

Values indicate are means± standard error. Means within same row with different superscript varied significantly ($p<0.05$). **∑SFAs;** Summation of saturated fatty acids, **∑MUFAs;** Summation of monounsaturated fatty acids, **∑n6;** Summation of omega-6 fatty acids, **∑n3;** Summation of omega-3 fatty acids and **∑PUFAs;** Summation of polyunsaturated fatty acids. Superscript letters within rows indicate significant difference in mean values at ($P<0.05$); a<b<c<d

There was statistical variation ($p< 0.05$) in the summation of polyunsaturated fatty acid for liver under the present study. The liver of fish fed on diet 1 dominated in the summation of polyunsaturated fatty acid with (27.38±0.19) followed by diet 2 (25.85±0.28), then control diet 4 (24.8±0.49) with diet 3 (22.25±0.31) recording significantly lower levels of polyunsaturated. There was statistical variation ($p< 0.05$) observed for the liver in the polyunsaturated fatty acids obtained for the present study. Linoleic acid C18:2, which is a representative of omega-6 fatty acid was predominant among polyunsaturated fatty acids in liver samples. Docosahexaenoic acid, C22:6, was the dominant omega-3 fatty acids for the liver samples analyzed for the current study. Diet 2 recorded significantly high docosahexaenoic acid compared to proportions of this fatty acid in other experimental diets (table 4.8). Statistical variation ($p< 0.05$) was observed in liver samples for the summation of omega-3 fatty acids.

4.8 Effect of experimental diets on muscles fatty acid profile of *C. gariepinus*

Fatty acids composition in the muscles of experimental fish is presented in (table 4.9). The sum total of saturated fatty acids analyzed for fish muscles varied statistically ($p< 0.05$) among dietary treatments. The highest summation value of saturated fatty acid for muscles was obtained by fish fed on diet 2 while the least summation was obtained by fish fed on control diet 4 (commercial). There was no significant difference in the values obtained for stearic, C18:0, behenic acid C21:0 and lignoceric, C24.0, acid Palmitic acid, C 16:0, was the dominant saturated fatty acid obtained from the muscles tissue just like in all the other samples that were analyzed for fatty acid composition for the present study. For the six monounsaturated fatty acids obtained from the muscle tissues, only three varied statistically ($p< 0.05$) with oleic acid, C18:1, being the dominant monounsaturated fatty acid analyzed.

Table 4.9: Fatty acid composition of the muscles of *C. gariepinus*

		Muscle			
		Diet 1	**Diet 2**	**Diet 3**	**Diet 4**
C 12:0	Lauric	1.46±0.17^{b}	1.74±0.17^{c}	2.33±0.17^{d}	0.95±0.07^{a}
C 14:0	Myristic	2.30±0.15^{b}	1.45±0.17^{a}	2.38±0.11^{b}	1.47±0.19^{a}
C 15:0	Pentadecanoic	1.81±0.10^{b}	2.72±0.50^{c}	1.70±0.9^{a}	2.46±0.11^{c}
C 16:0	Palmitic	25.99±0.54^{a}	31.29±0.41^{c}	26.57±0.38^{b}	25.38±0.32^{a}
C 17:0	Heptadecanoic	4.44±0.16^{c}	3.35±0.20^{b}	5.14±0.43^{d}	1.30±0.07^{a}
C 18:0	Stearic	0.32±0.11^{a}	0.25±0.02^{a}	0.41±0.15^{b}	0.58±0.04^{b}
C 20:0	Arachidic	2.54±0.11^{c}	1.47±0.11^{a}	1.58±0.21^{a}	0.58±0.04^{b}
C21:0	Behenic	0.81±0.14^{b}	0.45±0.10^{a}	0.59±0.11^{a}	0.54±0.08^{a}
C 24:0	Lignoceric	0.56±0.01^{a}	0.61±0.02^{a}	0.56±0.08^{a}	0.68±0.03^{b}
∑SFAs		40.23±0.13^{b}	43.33±0.21^{c}	41.26±0.87^{b}	33.94±0.09^{a}
C16:1	Palmitoleic	0.55±0.04^{b}	0.46±0.15^{b}	0.38±0.06^{a}	0.83±0.02^{c}
C 17:1	Cis 10 heptadecanoic	0.69±0.04^{b}	0.74±0.10^{b}	1.07±0.01^{c}	0.55±0.08^{a}
C 18:1	Oleic	35.59±2.52^{a}	36.83±1.49^{a}	38.10±1.79^{a}	43.33±3.78^{b}
C 24:1	Nervonic	0.26±0.01^{a}	0.31±0.02^{a}	0.42±0.05^{b}	0.41±0.07^{b}
∑MUFAs		37.09±0.71^{a}	38.34±0.39^{b}	39.97±0.48^{c}	45.12±0.98^{d}
C 18:2	Linoleic	12.78±0.72^{a}	13.20±1.31^{b}	12.13±1.21^{a}	11.84±0.90^{a}
C 20:4	Arachidonic	2.39±0.10^{d}	1.20±0.10^{b}	1.14±0.04^{a}	1.88±0.04^{c}
∑n6		15.17±0.41	14.40±0.71	13.27±0.64^{b}	13.72±0.47^{a}
C 18:3	Linolenic	1.62±0.26^{b}	1.25±0.02^{b}	0.83±0.02^{a}	0.91±0.05^{a}
C 20:5	EPA	1.00±0.04^{c}	0.62±0.12^{b}	0.51±0.05^{b}	0.38±0.08^{a}
C 22:6	DHA	3.98±0.13^{d}	3.13±0.09^{b}	3.70±0.10^{c}	2.39±0.12^{a}
∑n3		6.60±0.14^{c}	5.0±0.12^{b}	5.04±0.06^{b}	3.68±0.08^{a}
∑PUFAs		21.77±0.26^{d}	19.49±0.42^{c}	18.31±0.09^{b}	17.4±0.26^{a}
n3/n6		0.44±0.03^{d}	0.35±0.02^{b}	0.38±0.05^{c}	0.27±0.09^{a}

Values indicate are means± standard error. Means within same row with different superscript varied significantly ($p<0.05$). **∑SFAs;** Summation of saturated fatty acids, **∑MUFAs;** Summation of monounsaturated fatty acids, **∑n3;** Summation of omega-3 fatty acids, **∑n6;** Summation of omega-6 fatty acids and **∑PUFAs;** Summation of polyunsaturated fatty acids. Superscript letters within rows indicate significant difference in mean values at ($P<0.05$); a<b<c<d

The sum total of monounsaturated fatty acids indicated statistical variation ($p< 0.05$) for muscle tissues between dietary treatments. Muscle tissue of fish feed on control diet 4 (commercial) obtained highest sum total of monounsaturated fatty acids,

followed by muscle tissue of fish fed on diet 3, then muscle tissue of fish fed on diet 2 the least sum total value was obtained by fish fed on diet 1.

The sum total of polyunsaturated fatty acid for muscle tissue under the present study showed statistical variation ($p< 0.05$). Muscle tissue of fish fed on diet 1 dominated in the summation of polyunsaturated fatty acid (21.77±0.26) while diet 2 (19.49±0.42), then diet 3 (18.31±0.09) and diet 4 (17.4±0.26). Linoleic acid C18:2, was the dominant among the omega-6 polyunsaturated fatty acids with significant higher composition in diet 2. There was no statistical variation ($p< 0.05$) for the muscle tissue samples analyzed for diet 1, diet 3 and control diet. Docosahexaenoic acid, C22:6, dominated in omega-3 fatty acids for muscles samples. High statistical variation ($p< 0.05$) was observed in muscle samples for omega-3 fatty acids that included linolenic acid, C18:3, eicosapentaenoic acid, C20: 5 and docosahexaenoic acid, C22:6.

4.9 Water quality parameters in fish tanks under different dietary treatment

The experimental diets affected the water quality parameters contributing to marked significant difference observed for dissolved oxygen (DO) ($P<0.001$) and temperature ($P<0.003$) between treatments (Table 4.4). However, pH, conductivity and total dissolved substance did not show significant difference ($P>0.05$) between the treatments as shown on (table 4.10). There was significant difference ($P>0.05$) for phosphates that attained significant difference of ($P<0.001$) between dietary treatment while the rest of the analyzed nutrients did not show significant difference as shown on (table 4.10).

Table 4.10: Water quality parameters in fish tanks under different dietary treatments

		Means ± SD				
Parameter	**Unit**	**D_1**	**D_2**	**D_3**	**D_c**	**P value**
DO	(mgL^{-1})	3.03 ± 0.9^a	3.89 ± 0.74^c	3.66 ± 0.82^b	3.96 ± 0.46^d	0.001
Temperature	(^oC)	24.53 ± 1.1^a	25.22 ± 1.7^b	25.98 ± 1.4^b	26.52 ± 1.65^c	0.003
pH		7.72 ± 0.52^b	7.72 ± 0.36^b	7.69 ± 0.54^a	7.72 ± 0.52^b	0.155
Conductivity	(μScm^{-1})	47.43 ± 3.39^b	45.9 ± 3.93^a	46.76 ± 3.45^b	46.81 ± 3.86^b	0.891
TDS	(mgL^{-1})	23.71 ± 1.89^b	22.86 ± 1.9^a	$23.29 \pm 1.68b$	32.3 ± 2.02^c	0.500
Ammonia	(mgL^{-1})	0.54 ± 0.51^a	0.61 ± 0.59^b	0.48 ± 0.46^a	0.71 ± 0.73^b	0.550
Nitrites	(mgL^{-1})	0.24 ± 0.15^a	0.22 ± 0.13^a	0.21 ± 0.12^a	0.30 ± 0.16^b	0.265
Nitrates	(mgL^{-1})	1.31 ± 0.78^b	1.0 ± 0.89^a	1.0 ± 0.92^a	1.16 ± 0.99^a	0.734
Phosphates	(mgL^{-1})	0.61 ± 0.07^c	0.52 ± 0.04^b	0.51 ± 0.1^b	0.47 ± 0.07^a	0.001

D_1, Diet 1; D_2, Diet 2; D_3, Diet 3; D_c Control Diet; DO, Dissolved oxygen; TDS; Total dissolved substance; a<b<c<d

4.10 Water quality parameters flowing through the hydroponic unit under different substrates

Physical water parameters and nutrients are presented in (tables 4.11 and 4.12). Water quality analysis indicated significant difference ($P \leq 0.05$) observed for dissolved oxygen (DO), temperature, ammonia, nitrates and nitrites between treatments. Dissolved oxygen ranged from 2.2- 4.2 and the water temperature which was affected by the prevailing weather condition of the study area was maintained between 19.4^{o}C and 23.5 ^{o}C in the fish rearing tanks (Table 4.11). The other parameters including pH, conductivity, total dissolved substance and phosphates didn't vary significantly ($P>0.05$) between fish rearing tanks. The water pH ranged between 6.81 and 8.18, while conductivity and total dissolved substance ranged between 50-68 μScm^{-1} and 25-34 μScm^{-1} respectively. The maximum value for ammonia, nitrates, nitrites and phosphates analyzed were 3.32m/L, 5.22m/L, 0.99m/L and 0.94m/L respectively. Low ammonia levels were observed in fish tanks that had a mixture of pumice and charcoal and high level of ammonia was observed in the control fish tanks that had no substrates. The nutrients varied significantly

(P<0.05) except for nitrates which were statistically indifferent (P>0.05) between the treatments.

Table 4.11: Water quality parameters for water flowing into the hydroponic units under different substrates treatments

Parameter	Unit	Treatment				P Value
		Control	Pumice+ Charcoal	Charcoal	Pumice	
DO	(mgL^{-1})	2.70 ± 0.40^{a}	3.84 ± 0.21^{d}	3.38 ± 0.35^{b}	3.79 ± 0.30^{c}	0.05
Temperature	(^{o}C)	20.30 ± 0.79^{a}	20.66 ± 0.77^{b}	21.03 ± 0.76^{c}	21.39 ± 0.78^{d}	0.041
pH		7.36 ± 0.49^{b}	7.40 ± 0.41^{b}	7.40 ± 0.51^{b}	7.19 ± 0.17^{a}	0.178
Conductivity	(μScm^{-1})	60.00 ± 6.00^{b}	59.67 ± 5.54^{a}	59.33 ± 6.30^{a}	59.67 ± 5.54^{a}	0.975
TDS	(mgL^{-1})	30.00 ± 3.00^{b}	29.67 ± 2.54^{a}	29.61 ± 3.15^{a}	29.65 ± 3.50^{a}	0.971
Ammonia	(mgL^{-1})	2.51 ± 0.40^{d}	1.95 ± 0.65^{a}	2.14 ± 0.72^{b}	2.40 ± 0.90^{c}	0.015
Nitrates	(mgL^{-1})	1.72 ± 1.11^{d}	1.95 ± 1.42^{b}	2.64 ± 1.75^{a}	2.68 ± 1.97^{a}	0.043
Nitrites	(mgL^{-1})	0.56 ± 0.36^{a}	0.41 ± 0.34^{b}	0.29 ± 0.16^{b}	0.29 ± 0.13^{b}	0.001
Phosphates	(mgL^{-1})	0.37 ± 0.07^{b}	0.33 ± 0.12^{a}	0.47 ± 0.13^{c}	0.49 ± 0.27^{c}	0.008

SD: Standard deviation, P-value: Level of significance, DO: Dissolved oxygen, mgL^{-1}: Milligrams per litre, ^{o}C: Degrees Celsius, μScm^{-1}: Micro-siemens per centimeter, TDS: Total dissolved substance, Superscript letters within rows indicate statistical variation in mean values at (P<0.05); a<b<c<d

Descriptive statistics of nutrients in the hydroponic unit is presented in (table 4.12). The mean statistical levels for ammonia, nitrates, nitrites and phosphates were similar (P>0.05). The nutrients, ammonia, nitrate, nitrates and phosphates ranged between 0.66 mg/L-2.91 mg/L, 0.09 mg/L-4.11 mg/L, 0.03 mg/L-0.92 mg/L and 0.02 mg/L-0.61 mg/L respectively.

Table 4.12: The chemical parameters in water flowing out of hydroponic units under different substrates treatments

Parameter	Unit	Control		Treatment Pumice+ Charcoal	Charcoal	Pumice	P Value
Ammonia	(mgL^{-1})	2.30 0.46[b]	±	1.67 ± 0.72[a]	1.87 ± 0.73[a]	1.72 ± 0.59[a]	0.174
Nitrates	(mgL^{-1})	1.59 0.36[b]	±	1.59 ± 0.20[a]	2.18 ± 1.62[b]	2.04 ± 1.71[b]	0.75
Nitrites	(mgL^{-1})	0.50 0.36[b]	±	0.26 ± 0.91[a]	0.25 ± 1.16[a]	0.17 ± 0.09[a]	0.20
Phosphates	(mgL^{-1})	0.32 0.15[b]	±	0.22 ± 0.15[a]	0.20 ± 0.22[a]	0.19 ± 0.16[a]	0.19

SD: Standard deviation, P-value: Level of significance, $mgL^{-}1$: Milligrams per litre, Superscript letters within rows indicate statistical variation in mean values at ($P<0.05$); a<b.

4.11 Percentage reduction efficiency of nutrients under different substrates

The (figure 4.4) show bar graphs of percentage nutrients reduction efficiency of the substrates that were used for the present study. Pumice substrate had the highest reduction efficiency while the control had the least reduction efficiency for the nutrients analyzed. A mixture of pumice and charcoal substrate was second best in nutrients reduction efficiency.

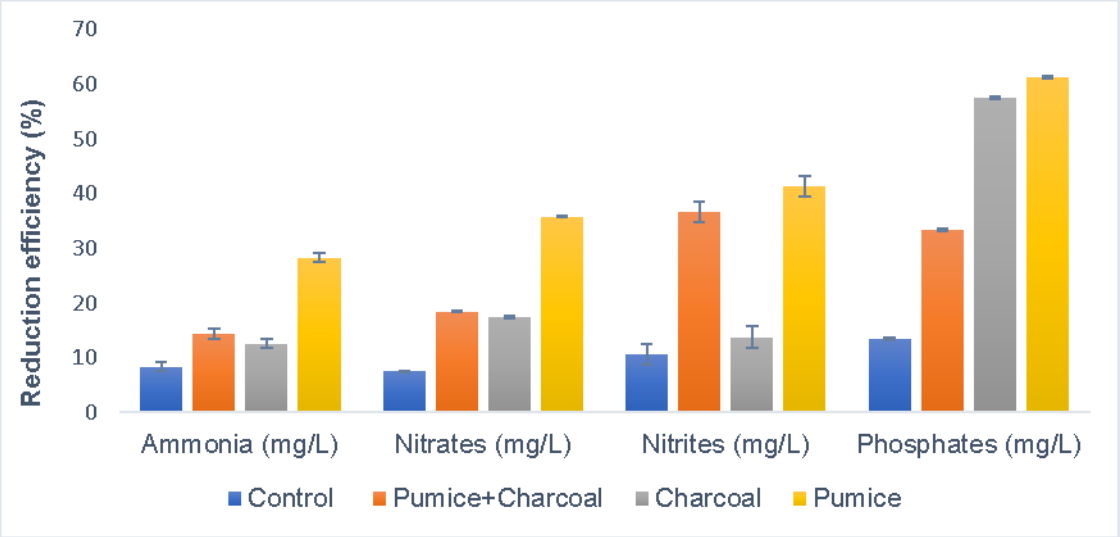

Figure 4.4: A bar graphs of percentage reduction efficiency of nutrients in the aquaponic system under different substrates.

CHAPTER FIVE

DISCUSSION

5.1 Proximate analysis of ingredients and experimental diets

Parameter analyzed for proximate composition of ingredients under the present study were within acceptable range as shown in (table 3.2 and table 4.1) for catfish diets. Percentage crude protein (CP) content of BSFL used for the present study was 25.3 % which is low compared to the percentage CP values used in other studies. Studies by (Renna *et al.,* 2017; Muin *et al.,* 2017 and Sheppard *et al.,* 1994), on Rainbow trout (*Oncorhynchus mykiss*), on tilapia (*Oreochromis niloticus*) and in the management of swine manure reported CP values of 44.86-45.20%, 41.74% and 40% respectively for BSFL used in their experiments. Low-cost insect sourced ingredients e.g., BSFL are used in substituting highly demanded conventional ingredients such as FM in diets formulation when the ingredient(s) meet fish nutritional requirements (Matteo *et al.,* 2020). Dietary substitution of ingredients remains controversial due to variation in percentage proximate composition of nutrients in diets when ingredients are included at varying ratios (Matteo *et al.,* 2020). The variation in the nutritional composition of ingredients influence proximate composition of formulated diets ultimately affecting growth performance and survival of fish when incorporated in diets (Barrosso *et al.,* 2017) as proven under the present study. The variation in proximate composition of ingredients observed for the present study is attributed to factors which include; geographical area, soil type, and weather for plant sourced ingredients, e.g., sunflower seed cake (Liti *et al.,* 2007) and type of culture media, stage of development and type food for animal sourced ingredients, e.g., BSFL (Barragan-Fonseca *et al.,* 2017). Proximate analysis for the ingredients used in the present study in catfish feeds enabled the formulation of experimental diets with uniform CP value of 35% for all the dietary treatments.

5.2 Growth performance and survival of *C. gariepinus* fed on BSFL diets

Food conversion ratio (FCR) is an important tool to determine efficiency, suitability and acceptability of formulated diets for fish (Gabriel *et al*., 2007; Nadaf *et al.,* 2010

and Devic *et al.,* 2017). Under optimal environmental condition and best pond management practices, high fish growth rate is achieved (USAID 2011) thus, better FCR. Other factors that influence FCR are type of fish; species and health status that contribute to variation in FCR (Amisah *et al.,* 2009; Newton *et al.,* 2005). Under the current study, FCR of between 1.1 – 1.3 obtained among test diets were lower than FCR values obtained by (Tiamiyu *et al.*, 2017) who tested *C. gariepinus* performance with *Allium sativum,* and *Leucaena leucocephala* dietary replacement and reported FCR values ranging between 5.71 – 6.32 and 4.95 – 6.39 respectively. The difference in FCR observed in various studies aforementioned may be attributed to feed ingredients variation, culture systems and varying stocking densities. The FCR under this study for *C. gariepinus* fed on 50% FM replacement indicated significantly higher growth performance compared to 25% and 75% FM replacement diets. Similar results were reported by (Muin *et al.,* 2017) for Nile tilapia fingerlings fed on diet containing 50% FM replacement with FCR of 1.91. In another study, (St-Hilaire *et al.,* 2007), better growth performance with favorable FCR was reported on rainbow trout fed on 25% FM replacement diet. This is explained by Koprucu and Ozdemir (2005), who reported that BSFL contain chitin made of insoluble fiber that make digestion difficult for diets with high BSFL percentage hence affecting growth performance.

In the current study, fish appeared healthy and there was no sign of diseases outbreak throughout the trial period. All diets used for the trials were accepted by *C. gariepinus* irrespective of the percentage BSFL used in the substitution of FM paralleling reports by (Fawole *et al.,* 2019 and Adewolu *et al.,* 2010). The single mortality observed under the study may have not been diet related but due to mishandling during sampling time thus, a higher survival rate (SR) was reported among the trial diets. The SR observed for this study was higher than that reported by (Nairuti *et al* 2021) on *O. niloticus* but was similar to SR obtained by (Maina *et al.,* 2020) on *C. gariepinus* under semi-intensive culture systems. Feed acceptance by experimental fish and good water quality parameters that were within the recommended range for fish culture (Tucker and Robinson, 1990) may have contributed to the high SR mean. Uniform growth was observed irrespective of test diets subjected on experimental fish during the first three weeks of trials (figure 4.1).

The uniformity in growth may have been attributed to time taken for re-adjustment in digestion and nutrients uptake from experimental diets by *C. gariepinus* that were initially fed with wheat bran for a week before the trials. In support to our finding, (Kumar *et al.,* 2018) reported that the assimilation process of the digestive tract may be stimulated or impaired and therefore affect digestion on sudden change of diet and feeding regime in fish. It is during the initial stage of study that the slowest growth rates in *C. gariepinus* were observed in the entire trial duration. The trends observed during the initial stage of study were also obtained by (Maina *et al* 2020) on *C. gariepinus* fingerlings subjected to diets containing CP of 41% under the same FM replacement ratios as used in this study. The growth curves obtained by catfish subjected to diet containing (25%) BSFL inclusion for the present study was similar to the trend reported for catfish using the same inclusion rate by (Maina *et al.,* 2020). In the contrary, the growth curves obtained by catfish subjected to diets containing (50 and 75%) BSFL inclusion for the present study was not similar to the trend reported for catfish using the same inclusion rate by (Maina *et al.,* 2020). However, the weight of fish fed on control diet and fish fed on diet containing (25%) BSFL inclusion had similar trends as was reported on zebra fish fed on BSFL diets by (Zarantoniello *et al.,* 2019).

Fish fed on 50% BSFL had a better growth rate between week five and week eleven compared to fish fed on 75% BSFL which had the least growth rate for the first eleven weeks of the diets trial. After the eleventh week, the growth rate of 75% BSFL diet improved compared to 50% BSFL thus suggesting better growth performance as fish age on subjection of higher percentage of BSFL formulated diets. Final weight gain by fish fed on 50% BSFL diet was higher than fish fed on 75% BSFL diet corroborating results reported by (Steffens 1994 and Goda *et al.,* 2007) on rainbow trout and *C. gariepinus* having replaced FM by poultry by-products and BSFL respectively at 50% ratio. For present study, BSFL test diets were not supplemented with vitamins nor mineral premix that are essential for early-stage of fish development thus slow growth rate observed at the onset of the trial. The slow growth rate observed at fingerling stage may be hasten by inclusion of necessary supplements which are either unavailable or are deficient in BSFL that was used in the feed trials. In intensive culture system where fish depend entirely on

formulated feeds, the need for nutrients supplementation has been observed to increase with the variation between the standing fish biomass (De Silva 1992) and that of formulated food. Though the weight gains of fish fed on control diet was significantly higher than that for fish fed on BSFL diets, BSFL diets showed steady increase while control diet showed gradual decrease in fish growth towards the end of the experiment. The weight gains of fish reported for feed trial under the present study show that diets in which BSFL substituted FM, obtained similar growth. The substitution percentages used in the present studies were similar to those used by (Fawole *et al.,* 2019 and Adeworu *et al.,* 2010) with results from both trials indicating BSFL can replace FM up to 75% without impairing growth and affecting survival rates of *C. gariepinus*.

5.3 Effect of substrates on growth performance and survival of *C. gariepinus*

Fish under pumice substrate obtained highest final growth and FCR in comparison to fish subjected other substrate treatments under the current study. The growth rate and FCR obtained for the present study was not better than the growth rate and FCR obtained for catfish reared under polyester fiber, rockwool, zeolite and gravel substrates by (Yesiltas *et al.,* 2021). However, the difference in growth and FCR obtained from the two studies may be attributed to the size of fish that were used for the experiments. Under the present study, post fingerlings with initial average weight (14 97g) were used while (Yesiltas *et al.,* 2021), used catfish with initial weight of (48.32g). Better specific growth rates were reported for all the substrates used for this study in comparison specific growth rate of 0.65 obtained for catfish reared under aquaponic system using gravel substrates by (Palm *et al.*, 2014). The homogenous mixture of pumice and charcoal and charcoal substrates followed pumice in terms of obtaining better growth, FCR and SGR for catfish that were subjected to the substrates under this study. Control treatment without substrates was least in terms of growth, FCR and SGR of catfish subjected to the treatment. The outcome suggested that substrates had influence on growth performance of catfish that were reared under the aquaponic system. Survival rate obtained for present study was over 90% with none of the treatment attaining 100% survival rate contrary to 100% survival rate

reported on catfish cultured under polyester fiber, rockwool, zeolite and gravel substrates by (Yesiltas *et al.,* 2021).

5.4 Substrate influence on *S. oleracea* productivity

There was no significant statistical variation ($p<0.05$) for analyzed *S. oleracea* parameters between treatments except for the number of leaves harvested. Significantly higher values for leave breadth, leave length, leave numbers and number of plants harvested were obtained under the treatment that had a mixture of pumice and charcoal substrate. Thus, the observation was contrary to the outcome observed for fish in comparison to the reduction efficiency of pumice and the mixture of pumice and charcoal substrates in regards to growth of *S. oleracea*. Unlike fish that performed better under pumice treatment, *S. oleracea* performed better under the mixture of pumice and charcoal treatment. The outcome may be attributed to the environment created by the substrates having varied influence on roots aerobic respiration and other metabolic processes for the plants under different treatments affecting nutrients absorption and ultimately varying *S. oleracea* development. Anoxic environment decreases roots permeability to water which can subsequently reduce nutrients absorption and plant growth (Estim *et al.,* 2018) in aquaponic systems. Charcoal treatment had the lowest *Spinacia oleracea* growth parameters under the current study suggesting that charcoal alone is not appropriate substrate for *Spinacia oleracea* production under aquaponic culture systems.

5.5 Fatty acid profile in *C. gariepinus* tissues

BSFL has proven to have the potential of converting organic waste substrates into high quality larval protein suitable for fish feed formulation (Huis *et al.,* 2013). However, the insect ability to convert a variety of organic waste to produce larvae with varying nutritional composition (Matteo *et al.,* 2020) pose a challenge to nutritionists in formulating diets. The percentage inclusion of ingredients also influences nutrients composition in formulated diets (Weiss and St-Pierre 2009), thus the need for diet analysis to ensure nutritional requirement of fish is met. Statistical variation in fatty acid composition was observed for the formulated diets. Therefore, the fatty acids composition obtained by fish under different experimental diets was

attributed to the inclusion rates of ingredients while replacing fishmeal with BSFL in the diets. Studies by (Lin *et al*., 2016; Omolo *et al.,* 2017), using varying percentages of linseed oil in replacing fish oil and (Chepkurui *et al.,* 2021), using different percentages of water spinach in diets reported statistical variation in dietary fatty acid composition which influenced the composition of fatty acids in fish tissues. Inclusion of BSFL at different rates in diets also contributed to variations in fatty acids composition in both diets and tissues under current study. The study by (Zhou *et al.,* 2016) reported that saturated fatty acids on cyprinid increased while unsaturated fatty acids concentration decreased with increasing replacement of BSFL. In the present study, the highest concentration of saturated acid was observed for 75% BSFL while the least concentration was obtained for 25% BSFL.

Palmitic acid C 16:0 was the most dominant saturated fatty acid in fish tissues in the present study. Palmitic acid can be provided in the diet or synthesized endogenously through *de novo* lipogenesis (DNL), (Carta *et al.,* 2017), hence explaining the variation observed between diets and tissues for the fatty acid in this study. Higher retention of palmitic acid observed in muscles and the liver is associated with energy generation through oxidation process (Lim *et al.,* 2013) as the two tissues are known to store energy and facilitate metabolic functions in the body. During β-oxidation process, palmitic acid undergoes a series of sequential steps in the mitochondria to produce usable energy expended by organisms to perform various physiological functions. When in excess, saturated fatty acids can cause physio-pathological conditions resulting from imbalance between saturated and unsaturated fatty acids (St-Hilaire *et al.,* 2007). Under the present study, unsaturated fatty acids were more concentrated than saturated fatty acids in diets and tissues hence creating the required balance that prevent negative health issues associated with excess saturated fatty acids. The concentration of monounsaturated and polyunsaturated acids in tissues decreased with increasing concentration of saturated acid in accordance to results reported by (St-Hilaire *et al.,* 2007) on rainbow trout fed on diets containing BSFL and house fly larvae. Under monounsaturated fatty acids, oleic acid dominated in experimental diets and fish tissues. The concentration of oleic acid in tissues was higher than in diets corroborating results reported by (Omolo *et al.,* 2017, Mwanja *et al.,* 2010 and Olsen *et al.,* 1990). Oleic acid is a derivative of oxidative desaturation

process of stearic acid in animals thus, explaining the correlation between the tissue stearic acid and oleic acid composition in the present study. The observed high concentration oleic acid in fish tissues under the current study may have been attributed to the elevated levels of the fatty acid in the diets.

In the present study, concentration of polyunsaturated fatty acids obtained for the fish tissues; increased with decreasing BSFL levels in diets which was in accordance to study on *Jian carp* (Zhou *et al.,* 2016) and on rainbow trout (St-Hilaire *et al.,* 2007). On the contrary, studies by (Lin *et al.,* 2016) reported significant increase of polyunsaturated fatty acids with increase of linseed oil inclusion in fish tissues. The concentration of omega-3 fatty acids was lower in all the tissues compared to the concentration of omega-3 fatty acids in the diets. However, docosahexaenoic acid C22:6 retention and deposition in the fish tissues was significantly higher than that of the diets. Thus, the trend observed for retention of docosahexaenoic acid for the present study conform with the report by (Ng *et al.,* 2003) on African catfish that had higher retention of docosahexaenoic acid in fish tissues compared to diets. According to (Mourente *et al.,* 2005) higher retention of docosahexaenoic acid is attributed to high specificity of acyl transferase for docosahexaenoic acid and relative resistance of docosahexaenoic acid to beta-oxidation due to its complex metabolic pathway. Total omega-3 fatty acids were higher in liver tissue compared to both whole body and muscle tissues probably due to efficiency of the liver in converting polyunsaturated acids into highly unsaturated fatty acids. The observed difference of fatty acids in tissues may also be attributed to the difference in lipids storage capacity in different tissues and preference in selective storage and mobilization of specific fatty acids (Mourente *et al.,* 2005).

Under the current study, the amount of EPA obtained was less than DHA in three fish tissues analyzed. The low values of EPA observed in tissues could have been attributed to the ability of EPA being highly oxidized compared to DHA that require complex catabolism process (Madsen *et al.,* 1998). Additionally, EPA is an intermediate in the biosynthetic pathway of DHA that maintain LC-PUFAs in tissues (Chepkurui *et al.,* 2021) hence explaining the disparity observed of the fatty acids in fish tissues. The n-3 values obtained for the diets under the current study were higher

compared to n-6 values and showed statistical variation ($p<0.05$) between dietary treatments. In the contrary, n-3 values for fish tissues were lower compared to n-6 values and did not show statistical variation. The statistical variation observed for n-3 and n-6 values for the experimental diets may be attributed to the inclusion rates of BSFL in the diets. Highest value of n-3 was obtained by diet 2 which had the lowest percentage (25) % BSFL inclusion thus, suggesting fishmeal to be a superior source of n-3 fatty acid in comparison to BSFL. The ratio of n-3/n-6 obtained for diets had values more than 1 except for the commercial diets which obtained the lowest value of 0.5 among the diets used for the present study. The values obtained for n3/n6 for fish tissues were all less than 1 including the tissues of fish that were subjected on the commercial diet.

5.6 Water quality parameters under aquaponic system

Water quality monitoring is essential in achieving optimum growth for all organisms propagated under aquaponic system (Somerville *et al.,* 2014). For effective and efficient aquaponic system, maintaining water quality parameters within tolerable limits ensures optimal growth of fish, plants and bacteria (Goddek *et al.,* 2016). Dissolved oxygen (DO) levels were lower for all the treatments than the recommended range of 5-6 mgL^{-1} required for optimum growth of plants and most warm water fish in aquaponic systems (Wongkiew *et al.,* 2015). Studies carried out on channel catfish by (Boyd and Hanson 2010) showed better growth performance and feed conversion ratio (FCR) in culture systems where mean average DO concentration did not fall below 3.5 mgL^{-1}. The slow growth rate and higher FCR observed under the current study for control treatment may be attributed to low DO levels observed of 2.63 ± 0.42 mgL^{-1} in comparison to the rest of the treatments that had a mean average DO concentration of above 3.5 mgL^{-1}. Low DO levels in aquaponic systems are attributed to several biological processes that include plant roots and fish respiration, oxidation of ammonia by nitrifying bacteria and organic load production within the fish rearing tanks (Espinosa-moya *et al.,* 2018). Also, accumulation of nutrients and toxic gasses developed within fish tanks reduces affinity for DO in water as was observed in the control treatment of this study. Temperature and pH values observed under the current study, were within acceptable

range for catfish culture under intensive aquaculture system hence created a conducive environment for growth of all organisms within the aquaponic system. The range of values obtained for ammonia concentration for the present had no deleterious effect on fish growth and survival since the values obtained for temperature and pH were within the required limit and therefore, did not hinder catfish nor other organisms' productivity within the system.

5.7 Effects of nitrifiers on reduction of ammonia toxicity

In water, ammonia exist either as un-ionized (NH_3) or ionized ($NH_4^{+)}$ with the relative proportion of the two forms mainly affected by pH and temperature. Ammonia toxicity increases with rising pH and temperature levels in water. At pH below 8, only 10% of ammonia in water is considered toxic (Hargreaves and Tucker 2004) for fish. However, at any pH level, ammonia toxicity increases with rise in temperature due to reduction of dissolved oxygen in water as a result of temperature change. As such, the water quality parameters should be maintained at an equilibrium for healthy fish growth under aquaponic system. Nitrifiers are responsible for nutrients management in water under aquaponic system (Hu *et al.,* 2015), which explains the variation of ammonia, nitrites and nitrates observed under the current study. The action of *Nitrosomonas* and *Nitrobacter* majorly contribute to the oxidation of ammonium and nitrites ions in water creating a conducive environment for fish growth. Other mechanisms that are associated with ammonia removal include; uptake by plant roots and assimilation by microorganisms that convert nutrients back to organic matter. Although nitrites and nitrates are considerably less toxic than ammonia, it is important for the nutrients to be removed to prevent accumulation so as to enhance fish growth and development under favorable environment. The levels observed for the nutrients concentrations under the present study were within manageable ranges that would have not affected catfish growth negatively.

5.8 Effects of substrates on nutrients reduction

The essence of substrates under aquaponic system is to hold water, air and maintain optimal conditions for bacteria and plant development (Lin *et al.,* 2019). Substrates

have an influence on growth performance of fish and plant development as shown under the current study (tables 4.4 and 4.5) paralleling results obtained by (Roosta and Afsharipoor 2012), for straw berry growth and culture of Grass carp and Silver carp using perlite, cocopeat and a mixture of perlite and cocopeat mixed at different ratios. The nutrients reduction efficiency of the system was probably influenced by the substrate suitability in providing attachment, for microorganism that solubilizes nutrients for uptake by plant and conversion of toxic ammonia to nontoxic form, (Wongkiew *et al.*, 2015). The assessment on the suitability of the various substrates that were used for the present study indicated that pumice substrate contributed significantly in nutrients reduction efficiency within the aquaponic system compared to other substrates used for the trial. Charcoal substrate contributed significantly in restoration of water clarity through the filtration of suspended particles but did not match pumice and the interaction between charcoal and pumice substrates in nutrient removal.

The interaction between charcoal and pumice was expected to perform better in nutrients removal than the rest of the substrates, however it was second after pumice. The aeroponic was the least in reduction efficiency for all the nutrients analyzed under the current study because it lacked *S. oleracea* plants and substrates that were responsible in nutrients removal in the other treatments for the experiment. Though least in nutrient reduction efficiency, the control had the ability to reduce nutrients through other mechanisms such as nitrification and denitrification processes by microorganisms that may have developed in the control unit. The maximum reduction efficiency of ammonia for the current study was 28.33% observed under the pumice treatment which was significantly lower than 92.77% reported by (Endut *et al.*, 2016) for *Clarias gariepinus* culture and water spinach propagation under aquaponic system using gravel substrate. Though the reduction efficiency for ammonia was low under the current study, the ammonia levels observed were comparatively low in mean average concentration ranging between (1.88±0.67 - 2.64±0.42) mgL^{-1} across all the treatments than the recommended concentration of (3.0-6.7) mgL^{-1} for *C. gariepinus* under aquaponic system (Endut *et al.*, 2016). Phosphate reduction was highest reduced nutrient at 61% efficiency followed by nitrites at 41.38% and nitrates at 35% under pumice treatment. Higher percentage

reduction values were observed for the mixed pumice and charcoal treatment compared to charcoal treatment for most of the nutrients except for phosphates as shown in Fig 4.4. Study by (Lin *et al.,* 2019) showed carbon source biofilter operated under anoxic period in wastewater treatment had removal efficiency of 91.15% on phosphorus compared to nitrites (59.32) and nitrates (63.46) thus, explaining the high reduction efficiency for charcoal substrate obtained for the present study.

5.9 Conclusions

1. Although proximate analysis indicated a low CP value of 25.3% for BSFL used under the present study, the diets formulated were suitable for catfish production under intensive culture system.

2. This study established that dietary FM can be substituted by partially defatted BSFL ingredient of 25.3% CP at (25, 50 and 75) % rates without; variation in growth performance and negatively affecting survival of *C. gariepinus* reared under aquaponic system.

3. The study also showed that BSFL can replace fishmeal in catfish diets without affecting the fatty acid composition of catfish flesh. As such, BSFL can be a sustainable and economical protein ingredient for formulation of catfish diets.

4. Pumice substrate performed better in fish growth while the interaction between pumice and charcoal substrate performed better in spinach growth. The growth variation observed for *C. gariepinus* and *S. oleracea* is attributed to the substrate type and their ability of creating varying environmental conditions for the organisms to thrive in.

5.10 Recommendation

1. Use of fully defatted BSFL will contribute to increased CP content value of the insect larvae to over 40% which will be better for the formulation of catfish diets for enhanced catfish growth.

2. For enhancement of fatty acid composition of *C. gariepinus* under aquaponic system may be attained by use of BSFL with higher CP value above the 25.3 % used for the present study.

3. Cost benefit analysis for BSF production for commercial upscaling for production and incorporation in fish feeds.

4. Inclusion of biofilters, increasing plants density and use of plants with high affinity of nutrients absorption will also significantly reduce nutrients from the system to enhance fish and crops productivity.

REFERENCES

Adekele, B., Deborah, R., Moodley, G. and Taylor, S. (2020). Aquaculture in Africa. A comparative review of Egypt, Nigeria and Uganda Vis-à-vis South Africa *Aquaculture Research, 48*(5), 167-197 https://doi.org/10.1080/23308249.2020.1795615

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

Amisa, S., Oteng, M.A. and Ofori, J.K. (2009). Growth performance of the African catfish (*Clarias gariepinus*) fed varying inclusion levels of *Leucaena leucocephala* leaf meal. *Journal of Applied Sciences and Environment Management 13*(1) 21-26 www.bioline.org.br/ja

Al Mahmud N., Hasan M.D., Hossaain M.B. and Minar M.H. (2012). Proximate Composition of Fish Feed Ingredients Available in Lakshmipur Region, Bangladesh. *American-Eurasian Journal of Agriculture & Environmental Science 12*(5), 556-560.

AOAC (Association of Official Analytical Chemistry) (1995). *Official Methods of Analysis*. (17th Edition). Gaithersburg, MD, USA: AOAC,

Badiola, M., Basurko, O.C., Piedrahita, R.H., Hundley, P. and Mendiola, D. (2018). Energy Use in Recirculating Aquaculture Systems (RAS): *A Review*. *Journal*

of Aquacultural Engineering 81, 57 – 70. https://doi.org 10.1016/. aqueng.2018.03.003.

Barbu, M., Ceanga, E. and Caraman, S. (2016). *Water Quality Modelling and Control in Recirculating Aquaculture System*, London: Intech Open Limited, DIO: 10.5772/63202

Barragan-Fonseca, K.B., Dicke, M. and Van Loon, J.J.A. (2017). Nutritional value of the black soldier fly (*Hermetia Illucens L*.) and its suitability as animal feed-A review; *Journal of Insects as Food and Feed, 3*(2): 105-120.

Barroso, F.G., Sanchez-Muros, J.M., Segura, M., Morote, E., Ruiz, A.T., Bueno, R.R. and Guil-Guerrero, J.L., (2017). Insects as food: Enrichment of larvae of *Hermetia illucens* with Omega 3 fatty acids by means of dietary modifications. *Journal of Food Composition and Analysis. 62*, 8-13 DOI: 10.1016/j.jfca.2017.04.008

Bartley, D.M (2022). World Aquaculture 2020. A brief overview. FAO Fisheries and aquaculture circular No 1233. Rome, Italy: FAO. https//doi.org/10.4060/cb7669en

Bostock, J. Mc-Andrew, B., Richards, R., Kim, J., David, L. and Corner, R. (2010). *Aquaculture: Global status and trends*, Rome: FAO. https://doi.org/10. 1098/rstb. 2010.0170

Bostock, J., Mc-Andrew, B., Richards, R., Kim, J., David, L. and Corner, R. (2010). *Aquaculture: Global status and trends*, Rome: FAO https://doi.org/10.1098/rstb.2010.0170

Boxman, S., E, Nystrom, M., Capodice, J. C., Ergras, S. J., Main, K. L. and Trotz, M. A. (2017). Effect of support medium hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic system. *Aquaculture Research, 48*(5), 2463-2477

Boyd, C.E. and Hanson, T. (2010). Dissolved oxygen concentration in ponds. Global Aquaculture Alliance; pp 40-41. Retrieved from https://www.researchgate.net/ publication/281309202

Buxton, R. (2011). Nitrate and Nitrite Reduction Test Protocol, *American Society of Microbiology Journal, Washinton DC, USA. 38*(4), 1- 20.

Cai, J. and Leung, P.S. (2020). *Short term global fish demand and supply gaps. FAO Fisheries and Aquaculture Technical Paper No. 607, 2020.*: Rome: FAO.

Carpenter, S.R., Fisher, S.G., Grimm, N.B. and Kitchell, J.F. (2016). *Global Change and Freshwater Ecosystems: Annual Review of Ecology and Systematics, Vol 23, pp. (119-139)* Retrieved from http://www.stor.org/stable/2097284

Carta, G., Murru, E., Banni, S. and Manca ,C. (2017). Palmitic acid. Physiological role, metabolism and nutritional implication. *Frontiers in Physiology, 8,* 902 https://doi.org/103389/fphys.2017.00902

Chepkurui, M. Orina, P. S., Opiyo, M., Muendo, P. Mbogo K. and Omond,i R. (2021). Fatty acid composition of Nile tilapia (*Oreochromis niloticus*) fingerlings fed diets containing different levels of water spinach (*Ipomea*

aquatica). *Journal of Agriculture and Food Research, 5*(2021) https://doi.org/101016/j.jasr.2021.100156

Chepkurui-Boit, V., Ngugi, C.C., Bowman, J., Oyoo-Okoth E., Rasowo J., Bundi M.J. and Cherop, L. (2011). Growth performance, survival, feed utilization and nutrients utilization of African catfish (*Clarias gariepinus*) larvae co-fed artemia and a micro-diet containing freshwater atyid shrimps (*Caridina nilotica*) during weaning, *Aquaculture Journal,* 17(2), e82- e89 https://doi.org/10.1111/j.1365-2095.2009.00737.x

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig A., Arvonen, K. and Pedersen, P.B. (2013). Farming different Species in RAS in Nordic Countries: Current Status and Future Perspectives. *Aquaculture engineering 53,* 2-13

De Silva, S.S. (1992). *Supplementary feeding in semi-intensive aquaculture systems. In farm made aquafeeds. Proceeding of FAO/AADCP, Bangkok, Thailand, pp. 24-60,* Rome: FAO.

Devic, E., Leschen, W., Murray, F. and Little, D.C. (2017). Growth performance, feed utilization and body composition of advanced nursing nile tilapia (*Oreochromis niloticus*) fed diets containing black soldier fly (*Hermetia illucens*) larvae meal. *Aquaculture Nutrition.* 1-8 https//doi.org/10.1111/ anu.12573

Dunning R. D., Losordo T. M. and Hobbs A. O. (1998). The Economics of Recirculating Tank Systems: A Spreadsheet for Individual Analysis. *SRAC Publication No. 456*, 8.

Endut, A., Lananan, F., Juson, A., Nik W.N.W. and Ali, W. (2016). Aquaponic recirculation system: A sustainable food source for the future water conserves and resources. *Malaysian Journal of Applied Sciences. 1*(1), 1-12.

Espinal, C.A and Mutalic, D., (2019). Recirculating Aquaculture Technologies: *Aquaponic Food Production System,* 35-77, https://doi.org/10.1007/978-3-15943-6_3

Espinosa-moya, A., Alvarez-gonzalez, A. and Albertos-alpuche, P. (2018). Growth and development of herbaceous plants in aquaponic systems. *Acta Universitaria Multidisciplinary Scientific Journal. 28*(2), 1-8. DOI: 10.15174/au.2018.1387

Estim, A., Saufie, S. and Mustafa, S. (2018). Water quality remediation using aquaponics sub-systems as biological and mechanical filters in aquaculture. *Journal of Water Process Engineering; 25* (4), 45-57. 30.10056. https://doi.org/10.1016/j.jwpe.2018.02.001

FAO (2018). Impact of Climate Change on Fisheries and Aquaculture. *Fisheries and Aquaculture Technical Paper* 627 - 654.

FAO (2020). The state of world fisheries and aquaculture; Meeting the sustainable goal. Food and Agricultural Organization of the United Nations, Rome, Italy.Retrieved from http://www.fao.org/3/ca9229en/CA9229EN.pdf

FAO (2018). The state of world fisheries and aquaculture; Meeting the sustainable goal. Food and Agricultural Organization of the United Nations, Rome, Italy.

194-195 Retrieved from https://creativecommons.org/licenses/by-nc.sa/3.0/igo.

FAO (2016). *Coping with Water Scarcity in Agriculture: A Global Framework for Action in a Changing Climate*; 12pp, Retrieved from Water-Scarcity@fao.org

FAO (2014). Success Factors in Aquaculture Enterprises in the Pacific: Farm Assets and Farm Performances of Private Aquaculture Enterprises, 88pp.Retrieved from www.fao.org/contact-us/licence

FAO (2004). *The State of Food and Agriculture*: Editorial Production and Design Group Publishing Management Service; Chief Publishing Management Service, Information Division, FAO; Viale delle Terme di Caracalla Rome, 00100 Rome, Italy; Retrieved from @fao.org

Fawole, J.F., Adeoye, A.A., Tiamiyu, O.L., Ajala, I.K., Obadara, O.S. and Ganiyu, O.I. (2019). Substituting fishmeal with *hermetia illucens* in diets of african catfish (*Clarias gariepinus*): Effects on growth, nutrient utilization, haemato-physiological response, and oxidative stress biomarker. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2019.734849

Filbrum, J, Reynolds, C.A. and Culver, D.A. (2013). Effects of feeding rate on habitat quality in fish rearing ponds. *Journal of the World Aquaculture Society, 44*(2), 198-209.

FRDC (2001). *A Social-Economic Valuation of Resource Allocation Options between Recreational and Commercial Sectors: General Theoretical Framework. Economic Research Associates*, Perth, Western Australia: FRDC.

Gabriel, U.U., Akinrotimi, O.A., Bekibele, D.O., Onunkwo, D.N., Anyanwu, P.E. and Harcout, P. (2007). Locally produced fish feed: Potentials for aquaculture development in Sub- Saharan Africa. *Africa Journal of Agriculture, 2*, 287-295.

Geisenhoff, L O, Jordan, R A, Santos, F C, De Oliveira, F C and Gomes, E P (2016). Effects of different substrates in aquaponic lettuce production associated with intensive tilapia farming with water recirculation systems. *Journal of Agricultural Engineering, 36*(2), 291-299

Goda, A.M., El-Haroun, E.R. and Kabir, C. (2007). Effect of total or partial replacing fishmeal by alternative protein sources on growth of African catfish (*Clarias gariepinus*) (Burchell, 1822) reared in concrete tanks. *Aquaculture Resources, 38*(3), 279-287.

Goddek, S., Espinal, C.A., Delaide, B., Jijakli, M.H., Schmautz, Z., Wuertz, S. and Keesma, K.J. (2016). Navigating towards decoupled aquaponic systems. A system dynamics design approach. *Water, 8*(7), 303.

Hargreaves, J.A. and Tucker, C.S. (2004). Managing ammonia in fish ponds. Southern regional aquaculture center; SRAC publication No. 4603. Retrieved from: http://fisheries.tamu.edu/files/2013/09/SRAC-Pblication-No.-4603-Managing-Ammonia-In-Fish-ponds.pdf

Helena, L. F., Annette, L. W., Caroline, E. C., Graham, C. B. and Philip, C. C. (2014). The Use of Gas Chromatography to Analyze Compositional Changes of Fatty Acids in Rat Liver Tissue during Pregnancy: *Journal Listj Vis Exp* (85), 51445.

Helfrich, L.A. and Libey, G., (1991). Fish Farming in Recirculating Aquaculture Systems (RAS): Department of Fisheries and Wildlife Sciences, Virginia Tech pp.19

Hu, Z. Lee, J.W., Chandran, K., Brotto, A.C. and Khanal, S.K. (2015). Effects of plant species on nitrogen recovery in aquaponics. *Bioresource Technology Journal: 188*; 92-98

Huis, A. V., Itterbeek, J. V., Klunder, H. Mertens, E., Halloran A., Muir G. and Vantomme, P. (2013). *Future prospects for food and feed security*. Rome: FAO.

Huntington, T., Avarre J.C., Focken U., Osward M., Pucher, J. and Sorgeloos, P., (2017). Opportunities and Challenges for Aquaculture in Developing countries; Joint Report by; Agence Francaise De Development (AFD), European Commission (EC) and Deutsche Gesellschaft for Internationale Zusammenarbeit (GIZ), Retrieved from https://ec.europeaid/fisheries-and-aquaculture-european-development-cooperation-state-play-2015en

Ikehi, M.E. and Zimoghen, J., (2015). Impacts of Climate Change on Fishing and Fish Farming in Niger Delta Region of Nigeria, *Direct Research journal of Agriculture and Food Science (DRJAFS), 3*(1), 1-6.

KMFRI (2017*). Kenya Aquaculture Brief 2017: Status, trends, challenges and future outlook.* Kenya Marine and Fisheries Institute, Mombasa, Kenya. Nairobi: KMFRI Aquaculture Division Researchers

KNBS (Kenya National Bureau of Statistics) (2017). *Economic survey*, Nairobi, KNBS.

Koprucu, K. and Ozdemir, Y. (2005). Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 250,* 308-316

Kosemani, S E, Adewole, H, Olaleye, V and Azeez, O (2017). Assessment of the effects of fish feeding practices on the water quality of some fish ponds in Ekiti State fish farm, Ado, Ekiti, Nigeria. *Journal of the World Aquaculture Society, 5*(2), 357-364.

Kyule, D., Opiyo M. A., Ogello E., Obiero K., Maranga B., Orina P., Charo-Karisa H. and Munguti, J. (2016). Determination of fish value added product-Preferences among the residents of Wote town, Makueni County, Kenya, *Bulletin of Animal Health Production in Africa, Fish. (2016),* 69- 78

Kris-Etherton, P. M., Harris, W. S., &Appel, L. J. (2003). Fish Consumption, fish oil, Omega-3 Fatty Acids and Cardiovascular Disease. *Arteriosclerosis Thrombosis Vascular Biology,* 20-31.

Kumar, A., Pradhan, P.K., Chadha, N.K., Mohindra V., Tiwari V.K. and Sood, N. (2018). Effects of dietary regimes on development of digestive system of stinging catfish (*Heteropneustes fossilis,* Bloch) larvae. *International Journal of Current Microbiology and Applied Science. 7*(8), 413-421.

Lim, J. H., Hines, Z. G., Dominy, J. E., Lee Y., Kim S., Tabata M., Xiang Y. K. and Puigserve, P. (2013). Oleic acid stimulates complete oxidation of fatty acids through protein kinase A-dependent activation of SIRTI-PGCI α-complex.

Journal of Biological Chemistry, 288(10): 7117-7126 Doi: 10.1074/jbc.M112.415729

Lin, F. M, Lin, X, Lin, S. M, Chen Y. W and Guan, T (2016). Effects of dietary fish oil substitution with linseed oil on growth, muscle fatty acid and metabolism of tilapia (Oreochromis niloticus). *Aquaculture Nutrition, 22,* 499-508

Lin, Z., Wang, Y., Huang, W., Wang, J., Chen L., Zhou J. and He, Q. (2019). Single-stage denitrifying phosphorous removal biofilter utilizing intracellular carbon source for advanced nutrient removal and phosphorous recovery. *Bioresource Technology, 277*, 27-36. https://doi.org/10.1016/j.biortech.2019.01.025

Liti, D., Cherop, L., Munguti, J. and Chhorn, L., (2005). Growth and Economic Performance of Nile Tilapia (*Oreochromis niloticus* L.) Fed on Two Formulated Diets and Two Locally Available Feeds in Fertilized Ponds: *Aquaculture Research, 36*, 746-752. doi:10.1111/j.1365-2019.2005. 01265.x

Liti, D. M., Mugo, R. and Muchiri, M. (2002). Growth and Economic Performance of Nile tilapia Fed on Three Brans in Fertilized Ponds, *Aquaculture America, California USA, 2002*, 187.

Loan, D.K., Con, T.H., Hong, T.T. and Ly, L.T.M., (2013). Quick Determination of Ammonia Ions in Water Environment Based on Thymol Color Creating Reaction; *Environmental Sciences, 2013*(2), 83-92 http://dx.doi.org/10.12988/es.2013.31010

Madsen, L., Froyland, L, Dyroy, E., Helland, K. and Berge, R. K. (1998). Docosahexaenoic and eicosapentaenoic acids are differently metabolized in

rat liver during mitochondria and peroxisome proliferation, *Journal of Lipid Research. 39,* 583-593.

Maina, A.N., Osugo, I.M., Munga, L.K. and Munguti, J.M. (2020). *Growth performance and carcass characteristics of the African catfish (Clarias gariepinus) reared on diets containing black soldier fly (Hermetia illucens) larval meal.* Unpublished MSc Thesis, Nairobi: Kenyatta University.

Martins, C. I. M., Eding, E. H., Verdegem, M. C. J., Heinsbroek, L. T. N., Schneider, O. and Blancheton, J. P. (2010). New Developments in Recirculating Aquaculture Systems in Europe: A Perspective on Environmental Sustainability. *Aquaculture engineering, 43* (3), 89-93

Matteo, Z. Basilio, R., Giorgia, G., Christina, T., Elisabetta, G., Paola, R., Giorgin, G., … and Like, O. (2020). Zebra (*Danio rerio*) physiological and behavioural responses to insect-based diets. A multidisciplinary approach. Scientific reports, Nature Research https://doi.org/10.1038/s41598-020-67740-w.

Maucieri, C, Nicoletto, C, Junge, R, Schmautz, Z, Sambo, P and Borin, M (2018). Hydroponic systems for water management in aquaponics. A review, *Italian Journal of Agronomy, 13*(1), 1-11 https://doi.org/10.4081/ija.2017.1012

Mbogo, K. O., Onyango, A., Magoma, G., Munguti, J., Masinde ,W. P. and Ogila, K., (2017). Effect of Varying Dietary Proportion Of Linseed And Olive Oils On Fatty Acid Composition Of Nile Tilapia (*Oreochromis Niloticus*) And

African Catfish (*Clarias Gariepinus*) Under Aquaponic Culture System: *African Journal of Fisheries Science, 5* (3), 238-248

Milena, S.C.B., Graziele, A. C. and Pedro, A. P., (2020). Determination, Through Derivation and GC-MS Analysis, of Omega-3 and Omega-6 Fatty Acids in Fish Oil Capsules Sold in Salvado, Bahia; *Journal of the Brazelian Chemical Society, 31*(3), 89-95.

Mourente, G., Good, J. E. and Bell, J. G. (2005). Partial substitution of fish oil rapeseed, linseed and olive oils in the diets for European sea bass (Dicentrarchus labrax L.): Effects of flesh fatty acid composition plasma prostaglandins E2 and F2α immune function and effectiveness of a fish oil finishing diet. *Aquaculture Nutrition, 11*(1), 25-40.

Muin, H., Taufek, N.M., Kamarudin, M.S. and Razak, S.A (2017). Growth performance, feed utilization and body composition of nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fed with different levels of black soldier fly, *Hermetia illucens* (Linnaeus 1758) maggot meal diet. *Iranian Journal of Fisheries Science 16*(2), 567-577

Munguti, J.M., Kirimi, J.G., Obiero, K.O., Ogello E.O., Sabwa J.A., Kyule D.N., Liti D.M. and Musalia, L.M. (2021). Critical Aspect of Aquafeed Value Chain in Kenyan Aquaculture Sector: - *A Review: Sustainable Agriculture Research 10*(2), 87-97

Munguti, J.M., Kim, J.D. and Ogello, E.O. (2014) an Overview of Kenyan Aquaculture: current status, challenges and opportunities for future development, *Fisheries and Aquatic Sciences, 17*(1), 1-11

Munguti, J.M. Safina, M., Orina, P.S., Kyule, D.N., Opiyo, M.A., Karisa, H.C. and Ogello, E.O. (2014). An Overview of Current Status of Kenyan Fish Feed Industry and Feed Management Practices, Challenges and Opportunities: *International Journal of Fisheries and Aquatic studies. 1(*6), 128-137.

Mustapha, R., Bolajoko, O., and Akinola, O.O., (2014). Omega-3 and Omega-6 fatty acid potential of smoked and boiled catfish (*Clarias gariepinus*). *Current Research in Nutrition and Food Science, 2*(2), 94 – 97

Mwanja, Y., Nyende L., Kagoda, S., Munguti, M. and Mwangi, W. (2010). Characterization of fish oil of mukena (*Rastrioneobola argentae*) of Nile basin water- Lake Victoria, Lake Kyoga and the Victoria Nile River, *Tropical Freshwater Biology 19*(1), 49-58.

Nadaf, S.B, Bhilave, M.P. and Deshapande, V.Y. (2010). Growth performance and feed conversion ratio of freshwater fish fed on formulated feed. *Journal of Aquaculture Biology, 25*(1), 181-185

Nairuti, R.N., Munguti, J.M., Waidbacher, H. and Zollitsch, W. (2021). Growth performance and survival rate of Nile tilapia (*Oreochromis niloticus L.)* reared on diets containing black soldier fly (*Hermitia illucens L*.) larval meal. *Journal of Land Management, Food and Environment. 72*(1), 9-19. DOI: 10.2478/boku-2021-0002

Newton, L., Sheppard, C., Watson, D., Burtle, G. and Dove, R. (2005). *Using the black soldier fly Hermetia illucens, as a value-added tool for the management of swine manure.* Animal and poultry waste management center, Raleigh, NC 17: North Carolina State University.

Ng, W. K., Limand, P. K. and Boeuy, P. L. (2003). Dietary lipids and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish (*Clarias gariepinus*), *Aquaculture. 215*, 229-243.

Ngugi, C. C., Bowman, J. R. and Omolo, B. O., (2007). A New Guide to Fish Farming In Kenya. Aquaculture Collaborative Research Support Program (ACRSP) Aquaculture CRSP Management Office, Oregon State University, Oregon USA. 100pp. Retrieved from http://pdacrsp.oregonstate.edu/pubs/featured_titles/kenya_Manual.pdf

Nina, N. N., Zinaida A. N., Svetlana N. P., Victor P. V., Natalia S. S., Maria V. C. and Svetlanas, M. (2020). The effect of photoperiod on the fatty acids profile and weight in the hatchery-reared underlying and yearlings of Atlantic Salmon (*Salmon alar* L.), *Biomolecules 10*(6), 845-856

Nyonje B.M., Opiyo M.A., Orina P.S., Abwao J., Wainaina M. and Charo-karisa H. (2018). Current status of freshwater fish hatcheries, broodstock management and fingerling production in the Kenya Aquaculture sector. *Livestock Research for Rural Development, 30*(1), 1-5 https://www.lrrd.org/lrrd30/1/mary30006.html

Obiero, K.O., Waidbacher H., Nyawanda O.B., Munguti J.M., Manyala O. and Arara B.K. (2019). Predicting Uptake of Aquaculture Technologies among Small Holder Fish Farmers in Kenya; *Aquaculture international, 27*,1689-1707 https://doi.org/10.1007/s/0499-00423-0

OCHA (2010). Water Scarcity and Humanitarian, Policy Development and Studies Branch; Ocha UN Office of the Coordination of Humanitarian Affairs, *Occasional Policy Briefing Series No 4*, 15.

Oguz, T. and Mahmut, A. G. (2017). Fatty Acids, Angel Catala, Interch-Open, DOI: 105772/68048. Retrieved from: https://www.interchopen.com/books/fatty-acids-in-fish

Okechi, J K and Jennson, P. (2005). Profitability assessment. A case study of African catfish (*Clarias gariepinus*) Farming in the Lake Victoria basin, Kenya. p8

Olsen, R. E., Henderson, R. J. and McAndrew, B. J. (1990). The conversion of linoleic acid and linolenic acid to longer chain polyunsaturated fatty acids by Nile tilapia (Oreochromis niloticus) in vivo, *Fish Pathology and Biochemistry 8*, 261-270.

Omolo, K. Mbogo., Onyango, A., Magoma, G., Munguti, J., Masinde W. P. and Ogila, K. (2017). Effect of varying dietary proportion of linseed and olive oils on fatty acid composition of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) under aquaponic culture system: *African Journal of Fisheries Science*, *5*(3), 238-248.

Omondi, J.G; Gichuri, W.M and Veverica, K. (2001). A Partial Economic Analysis for Nile tilapia (*Oreochromis niloticus*) and Sharp Toothed Catfish (*Clarias gariepinus*) Polyculture in Central Kenya. *Blackwell Journal of Aquaculture Research, 32,* 693-700

Pattillo, D.A., (2017). An Overview of Aquaponic System; Hydroponic Components; United States Department of Agriculture, *National Institute of Food and Agriculture; Technical Bulletin series #13.* 10.

Prato, E., Biandoline, F., Parlapiano, I., Giandomenico, S., Denti, G., Calo, M., Spada L. and Di Leo A. (2019). Proximate, Fatty Acid and Metals in Edible Marine Bivalves from Italian Market; *Science of the Total Environment Journal. 648*, 153-163. https://doi.org/10.1016/j.scitotenv.2018.07.382

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, S., Malfatto, V., Prearo, M., … and Gasco L. (2017). Evaluation of the suitability of partially defatted (*Hermetia illucens L.)* Lavae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. *Journal of Animal Science and Biotechnology*,104, 017-0191-3

Rinehart, L. (2010). *Aquaponics-Integration of Hydroponics and Aquaculture; A Publication of ATTRA-National Sustainable Agriculture Information Service;* 1-800-346-9140 Retrieved from www.attra.ncat.org IP163 Slot 54 Version 033010

Roosta, H.R. and Afsharipoor S. (2012) Effects of different cultivation media on vegetative growth, eco-physiological traits and nutrients concentration in strawberry under hydroponic and aquaponic cultivation systems. *Advanced Environmental Biology. (6),* 543-555.

Rurangwa, E. and Verdegem, M.C.J., (2014). *Micro-organisms in Recirculating Aquaculture systems and their Management, Reviews in Aquacultue,* Karatina: INASP KENYA- Karatina university College.

Safran, P. (2009). Fisheries and Aquaculture; Fishing in the Tropics, Philippine Council for Aquatic and Marine Research and Development, *Los Banos Laguna, Philippines IV*, 134-154

Seneviratne, S. I., Nicholls, N., Easterling, D., Goodess C. M., Kanae S., Luo Y., Marengo J., … and Zhang X. (2012). Changes in climate extremes and their impacts on the natural physical environment. Managing the risks of extreme events and disasters to advance climate change adaptations, A special report of working groups of intergovernmental panels on climate change (IPCC) Cambridge: Cambridge University Press.

Sheppard, D.C., Newton, G.L., Thompson, S.A. and Savage, S. (1994). A value-added manure management system using black soldier fly. *Bioresource Technology, 502,* 275-279

Shumo, M., Osuga I. M., Khamisi F. M., Tanga C. M., Fiaboe K. K. M., Subramanian S., Ekesi S., Huis A. V. and Borgemeister C. (2019). The Nutritive Value of Black Soldier fly Larvae Reared on Common Orgarnic Waste Streams in Kenya. *Scientific Reports, 9*(2019): 10110

Somerville, C., Cohen, M., Pantanella, E., Stankus A. and Lovatelli A., (2014). Small-Scale Aquaponic Food Production System; Integrated Fish and Plant Farming; *FAO Fisheries and Aquaculture Technical Paper 589.*

Sorensons, R. (2009). Home hydroponics; Virginia State University and US Department of Agriculture; *Virginia Cooperative Extension, Publication* 426-084

Sprangers, T., Ottoboni, M., Klootwijk, C., Deboosere, S., Meulenaer, B., Michiels, J., Eeckhout M. Clerk P. and De-Smet S. (2017). Nutritional Composition of Black soldier fly (Hermetia illucens) Prepupae Reared on Different Organic Waste Substrates. *J. Sci. Food Agric., 97* (8), 2594-2600

Stamer, A., Wesselss, S., Neidigk, R. and Hoersten, G. (2014). Black soldier fly (*Hermetia illucens*) larval-meal as an example of a new feed ingredients' class in aquaculture diets. Rahmann G and Aksoy U (Eds). (2014) Proceedings of the 4th ISOFAR Scientist conference. 13-15 Oct, Istanbul, Turkey (e-print ID 24223)

St-Hilaire, S., Sheppard, C., Tomberline, J.K., Irving, S., Newton, L., McGuire, M. A., Mosley, E. E., Hardy, R. W. and Sealey, W. (2007). Fly prepupae as a feedstuff for rainbow trout (*Oncorhynchuss mykiss*). *Journal of the world Aquaculture Society, 38,* 59-67.

Steffens, W. (1994). Replacing fishmeal with poultry by-product meal in diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 124,* 27-34.

Tiamiyu, A.M., Adedeji, O.B. and Olatoye, I.O. (2017). Growth performance of the African catfish (*Clarias gariepinus*) fed varying inclusion of Allium sativa as feed additives. *American Journal of Biotechnology and Bioinformatics 1*(1), 1-7.

Tilami, S.K., Turek, J., Cervery, D., Lepic P,., Kozak P., Burkina, V., Sakallis S., Tocala, A., Sampels, S. and Mraz, J. (2020). Insect meal as a partial replacement for fishmeal in a formulated diet for perch (*Perca fluviatilis*), *Turkish Journal of Fisheries and Aquatic Sciences. 20*(12), 867-878.

Tran, G., Gnaedinger, C. and Merlin, C. (2015). Black soldier fly larvae (*Hermetia illucens*). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. Retrieved from htttps:/www.feedipedia.org/node/16388

Tucker, C.S and Robinson, E.H. (1990). *Water Quality Management in Ponds. In: channel catfish farming handbook.* Boston, MA: Springer. DOI https://doi.org/10.1007/978-1-4757-1376-3_10

United Nations (2019) World Population Prospects 2019; Department of Economic and Social Affairs Population Division, Retrieved from http://creative.commons.org/licenses/by/3.0/igol

USAID (2011). Harvest. Helping address rural vulnerability and ecosystem stability. *Technical Bulletin, 7,* 1-2, Retrieved from www.cambodiaHARVEST.org

Weiss W. and St-Pierre N. (2009). Impact and management of variability in feed and diet composition. Tri-State Dairy Nutritional Conference, 83-96 Retrieved from https://www.researchgate.net/publication/228486219

Wongkiew, S., Hu, Z., Chandran, K., Lee, J.K. and Khanal, S.K. (2015). Nitrogen transformation in aquaponics systems: A review. *Aquacultural Engineering Journal. (76)*; 9-19.

Xiao, X., Jin, P., Cai, M., Yu, Z., Yu, J. and Zheng, J. (2018). Effects of Black Soldier Fly (*Hermetia illucens*) Larvae Meal Protein as a Fishmeal Replacement on the Growth and Immune Index of Yellow Catfish (Pelteobargrus fulvidraco); *Aquaculture Research. 2018* 1-9 https://doi.org/10.1111/are.13611

Yesiltas M, Kocer M A T, Sevgili H and Koru E. (2020). Effects of different inorganic substrates on growth performance of African catfish (Clarias gariepinus, Burchell 1822) and lettuce (Lactuca sativa L.) *Turkish Journal of Agriculture-Food Science and Technology, 9*(4), 714-722 DOI: https://doi.org/10.24925/turjaf.v9i4.714-722.4024

Zarantoniello M., Randazzo B., Truzzic C., Giorgini E., Marcellucci C., Vargas-Abundaz J.A., Zimbelli A., Annibaldi A., Parisi G., Tulli F., Riolo P. and Olivotto I. (2019). A six-month study on black soldier fly (*Hermetia illucens*) based diets in zebra sci. Rep., 9(8598): 1-12 https://10.1038/s41598-019-45172-5p

Zhou, J. S., Liu, S. S. and Yu, H. B. (2016). Effects of replacing dietary fishmeal with black soldier fly acid composition of Jian Carp (Cyprinus carpio var. Jian). *Aquaculture Nutrition 24,* 424-433 https://doi.org/10.org/10.1111/anu.12574

APPENDICES

Appendix I: Abstracts of publications

Growth Performance of African Catfish (*Clarias Gariepinus*) Fed on Diets Containing Black Soldier Fly (*Hermetia Illucens*) Larvae Under Aquaponic System

Cost of fish production can be reduced by replacement of high-priced fishmeal (FM) with insects sourced ingredients. Four months feed experiment was conducted at a fish farm in Baringo County, Kenya to investigate effects of substituting fishmeal (FM) with black soldier fly larvae meal (BSFLM) on survival and growth performance of *C. gariepinus* under aquaponic system. Three test diets 35% crude protein content (CP) in which FM was substituted by BSFLM at 25%, 50% and 75% were formulated and experimented with commercial diet of 35% CP. Four weeks old *C. gariepinus* were stocked in 12 tanks at a density of 50 fish/tank and subjected to the diets. Fish were sampled every three weeks; water parameters were sampled weekly and mortality recorded on occurrence. Diet with 50% BSFLM obtained better FCR for formulated diets with no significance ($P<0.05$) for FCR and survival. Weight gain of control diet (97.07 g) was significant ($P<0.05$) compared to formulated diets 64.09g, 69.78g and 67.77g for 75%, 50% and 25% of BSFL replacement respectively. Growth performance and survival demonstrated that BSFLM has potential to substitute FM up to 75%. The fish productivity can be improved and feed cost reduced by incorporating fully defatted BSFLM with CP higher than 25.3% used for the diets.

Key words: Black soldier fly, Fishmeal, Catfish, Growth performance

Effect of growth substrates on water quality, catfish (*Clarias gariepinus*) culture and spinach (*Spinacia oleracea*) propagation under aquaponic system

Modern technologies such as aquaponic system should be embraced to capitalize on fish and crop production. pumice, charcoal and a homogeneous mixture of (pumice and charcoal) were used as substrates and assessed on a control (aeroponic) for their suitability in nutrients removal from water for *C. gariepinus* culture and *S. oleracea* propagation under aquaponic system. Specified water parameters in fish tanks; the inlets and outlets of the hydroponic units, were determined in-situ and in the laboratory. *C. gariepinus* post fingerlings weighing 14.97±0.5g and length 14.05±0.5cm were stocked at 50 fish/tank in 12, 1000L tanks under aquaponic system. Experimental fish were subjected to the same diet that was analyzed for its proximate composition and found to contain 35% crude proteins content (CP). Fish were sampled after every three weeks while mortality was recorded on occurrence. The outcome of the trials on water quality, *C. gariepinus* growth performance and survival rates revealed statistical variation ($p<0.05$) for the treatments used. The pumice substrate had better performance in percentage nutrients reduction for the nutrients analyzed, followed by the mixture of pumice charcoal substate. Charcoal substrate outperformed the other treatments in percentage reduction efficiency for phosphates. The control treatment was the least in percentage reduction efficiency. Mean weight gained by fish for the treatments differed statistically at ($p<0.05$) with weight gains of 93.81g, 77.57g, 69.79g and 55.37g for pumice, pumice charcoal, charcoal and control treatments respectively. The survival rates ranged between 92-98%. *S. oleracea* performed better under a mixture of pumice and charcoal treatment in comparison to the other treatments under the present study. Results from this study suggest that nutrient reduction efficiency can be increased by replicating the hydroponic units with appropriate substrate to increase retention time of water in the hydroponic for increased fish and crops production.

Key words: *aquaculture, growth performance, reduction efficiency, growth media, water management*

Appendix II: Statistical analysis for BSF formulated and control diets

Multiple Comparisons

Dependent Variable: Weight

Tukey HSD

(I) Diet		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-3.424126984126960	1.797564979322280	.226	-8.045215828582400	1.196961860328480
	Diet 3	-1.010952380952350	2.009738743868810	.958	-6.177486774086510	4.155582012181800
	Diet 4	-13.197261904761866*	1.681468070771840	.001	-17.519894707210700	-8.874629102313030
Diet 2	Diet 1	3.424126984126960	1.797564979322280	.226	-1.196961860328480	8.045215828582400
	Diet 3	2.413174603174600	2.009738743868810	.626	-2.753359789959550	7.579708996308760
	Diet 4	-9.773134920634910*	1.681468070771840	.001	-14.095767723083700	-5.450502118186070
Diet 3	Diet 1	1.010952380952350	2.009738743868810	.958	-4.155582012181800	6.177486774086510
	Diet 2	-2.413174603174600	2.009738743868810	.626	-7.579708996308760	2.753359789959550
	Diet 4	-12.186309523809513*	1.906605579753360	.001	-17.087714411379400	-7.284904636239590
Diet 4	Diet 1	13.197261904761866*	1.681468070771840	.001	8.874629102313030	17.519894707210700
	Diet 2	9.773134920634910*	1.681468070771840	.001	5.450502118186070	14.095767723083700
	Diet 3	12.186309523809513*	1.906605579753360	.001	7.284904636239590	17.087714411379400

Based on observed means.

*. The mean difference is significant at the .05 level.

Statistical analysis for BSF formulated

Tests of Between-Subjects Effects

Dependent Variable: Weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3848.104[a]	2	1924.052	2.400	.091
Intercept	1237483.870	1	1237483.870	1543.469	.000
Diet	3848.104	2	1924.052	2.400	.091
Error	1344542.899	1677	801.755		
Total	2637139.060	1680			
Corrected Total	1348391.003	1679			

a. R Squared = .003 (Adjusted R Squared = .002)