



Aquaponics productivity response for Niger seed cake (*Guizotia abyssinica*) inclusion and increased level of mineral supplementation in fish diet

Abebe Tadesse

A PhD Dissertation Submitted to the Department of Zoological Sciences

In Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy in Biology (Fisheries and Aquatic Sciences)

Addis Ababa University

Addis Ababa, Ethiopia

Advisors: Prof. Abebe Getahun

Prof. Beatrix Alsanus

2017

Declaration

I hereby declare that this dissertation has been composed entirely by myself and has not been previously submitted to any other degree of qualification.

All sources of information have been specifically acknowledged and confirm that I have done all the work.

Abebe Tadesse

Supervisors' approval

I hereby approve that Abebe Tadesse has composed this dissertation under my supervision.

Supervisor name

Signature

Prof. Abebe Getahun

Prof. Beatrix Alsanus

Signature Page

Addis Ababa University

Graduate Program

This is to certify that the dissertation prepared by Abebe Tadesse, entitled: *Aquaponics productivity response for Niger seed cake (Guizotia abyssinica) inclusion and increased level of mineral supplementation in fish diet* and submitted in fulfillment of the requirement for the Degree of Doctor of Philosophy (Fisheries and Aquatic Sciences) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining committee:

Examiner _____ Signature _____ Date _____

Examiner _____ Signature _____ Date _____

Advisor Prof. Abebe Getahun Signature _____ Date _____

Advisor Prof. Beatrix Alsanus Signature _____ Date _____

Chair of Department or Graduate Program Coordinator

Abstract

*This study was conducted at Addis Ababa University aquaponics research facility to determine level of Niger seed cake inclusion and mineral supplementation on aquaponics diet to improve Tilapia-Lettuce productivity in aquaponics system. Three main studies were conducted to determine the efficient level of Niger seed inclusion for tilapia (*Oreochromis niloticus* L.) growth, effective Niger seed dietary level on lettuce (*Lactuca sativa* L.) growth and effective mineral supplementation level for tilapia and lettuce growth. Mineral supplementation level was studied by taking the best Niger seed cake inclusion level for tilapia and lettuce growth. Five levels of Niger seed cake (NC) inclusions with different level of fish meal (FM) (NC:FM); 0 (Negative control diet; D₁), 0.29 (D₂), 0.83 (D₃), 2.14 (D₄), 3.4 (D₅) and Hydroponics system(H) (Control 2; Positive control for nutrients) were tested against tilapia and lettuce growth and water quality attributes for 28 days. After decision on the best Niger seed cake inclusion level five mineral supplementation levels 0% (D₁), 1.2% (D₂), 2.3% (D₃), 3.5% (D₄) and 4.6% (D₅) on fish diet were tested against aquaponics productivity taking the best diet from previous experiment as negative control. Impact of Niger seed cake inclusion level brought significant variation ($p < 0.05$) on water quality parameters but remain within the range suitable for growth and development of lettuce. Most tilapia and lettuce growth performance measures were significantly quadratic with NC:FM ratio in fish diet ($r^2 > 0.25$, $p < 0.1$). Highest Specific growth rate (SGR), Relative growth rate (RGR), Protein productive value (PPV), Crude protein content, Mineral retention (K, Ca, Fe and N) and feed efficiency response of tilapia were measured from D₄. Similarly highest lettuce; Yield, Fresh biomass per plant, Leaf fresh weight, Root fresh weight from experimental diets were recorded from D₄ that is statistically not different with H treatment ($p > 0.05$). Increased NC:FM composition resulted in quadratic decrease of Dry matter (DM) and mineral content. Mineral*

retained in lettuce was significantly correlated ($p < 0.05$) with diet mineral composition and resulted in increased mineral level recovered by lettuce and in water. Fish tank water mineral content response was also significantly quadratic with NC:FM ratios ($r^2 > 0.25$, $p < 0.1$). Mineral supplementation performance on tilapia and lettuce growth was found to be significantly comparable among treatments and numerically lowest and highest RGR, and Protein efficiency ratio (PER) were achieved from treatment D₁ and D₅, respectively. Increased mineral supplementation level on fish diet brought significantly linear RGR, FCR, PPV and PER response ($r^2 > 0.5$, $p < 0.05$). Among experimental diets significantly highest Absolute growth rate, Relative growth rate, Leaf weight ratio and lettuce yield were achieved from 2.3% mineral supplementation level ($p < 0.05$). Hydroponics mineral supplemented diet with a level of 2.3% resulted in significantly highest ($p < 0.05$) total (tilapia and lettuce) biomass yield and suitable growing environment for tilapia and lettuce. Aquaponics productivity can be improved through hydroponics mineral inclusion in fish diet which contains 2.14 NC:FM ratio supplemented with 2.3% hydroponics mineral.

Acknowledgements

I would like to thank my advisors Prof. Abebe Getahun and Prof. Beatrix Alsanus for their continuous and fruitful support and guidance during the development of this research. Many thanks to staff of the Department of Zoological Sciences, Addis Ababa University who made this work possible and their assistance in maintenance and construction of the research facility and the aquaponics green house.

My appreciation to my friends and colleagues at the Department of Zoological Sciences for their kind support.

I would like to extend my acknowledgement to Addis Ababa University, Food and Agriculture Organization of the United nations (FAO), Smart fish Project, Intergovernmental Authority on Development (IGAD) and Swedish University of Agricultural Sciences, Linnaeus Palme project, for the financial and technical assistance to the research.

My special thanks to my family, friends, and staff members of Debre Berhan University for their encouragement.

Table of contents

Contents

Declaration	i
Signature Page	ii
Abstract	iii
Acknowledgements	v
Table of contents	vi
List of tables	xii
List of figures	xiii
Chapter 1	1
General Introduction	1
1.1 Introduction	1
1.2 Objective of the research	7
1.3 Dissertation structure	8
Chapter 2	9
Literature review	9

2.1 Global food production	9
2.2 Fish contribution	9
2.3 Aquaculture	10
2.3.1 World Aquaculture	10
2.3.2 African aquaculture	11
2.3.3 Aquaculture in Ethiopia.....	12
2.4 Hydroponics	12
2.4.1 Hydroponics in Ethiopia.....	15
2.4.2 Hydroponics input	15
2.4.3 Hydroponics techniques and benefit.....	16
2.4.4 Challenges of hydroponics	17
2.5 Aquaponics.....	17
2.5.1 Challenges of aquaponics	21
2.5.2 Aquaponics input.....	25
2.6 Importance of Macro nutrients and micronutrients on growth, development and physiology of tilapia and lettuce	27

2.7 Feed ingredients	27
Chapter 3	31
Growth and physiological response of Nile tilapia (<i>Oreochromis niloticus</i> L.) to Niger seed cake inclusion in fish diet, as a major protein and mineral source in Tilapia -Lettuce based aquaponics system.	31
3.1 Introduction	31
3.2 Materials and methods	34
3.2.1 Description of the experiment setup.....	34
3.2.2 Feed preparation and preservation.....	37
3.2.3 Feed regime	37
3.2.4 Data collection.....	39
3.3 Data analysis	41
3.4 Results and discussion.....	41
3.4.1 Growing medium quality.....	41
3.4.2 Biomass production	42
3.4.3 Proximate composition.....	49

Chapter 4.....	61
Growth rate, quality and yield response of Lettuce (<i>Lactuca sativa</i> L.) for different Niger seed cake inclusion levels in fish diet in Tilapia-Lettuce based aquaponics system.....	61
4.1 Introduction.....	61
4.2 Materials and methods.....	64
4.3 Data analysis.....	68
4.4 Results and Discussion.....	69
4.4.1 Growing medium quality.....	69
4.4.2 Biomass production.....	71
4.4.3 Proximate composition.....	78
4.4.4 Pigment.....	79
4.4.5 Growth rate.....	81
4.4.6 Mineral in water.....	84
Chapter 5.....	89
Effect of mineral supplementation in fish diets in aquaponics system on growth rate, quality and yield response of Nile tilapia (<i>Oreochromis niloticus</i>) and Lettuce (<i>Lactuca sativa</i>).....	89

5.1 Introduction	89
5.2 Materials and methods	91
5.2.1 Experimental set-up	91
5.2.2 Diet preparation and preservation	91
5.2.3 Feed regime	92
5.3 Data collection	93
5.4 Results and Discussion	94
5.4.1 Growing medium quality	94
5.4.2 Fish growth	99
5.4.3 Proximate composition of fish	102
5.4.4 Plant biomass	105
5.4.5 Proximate composition of lettuce	109
5.4.6 Growth rate	110
5.4.7 Lettuce yield	112
5.4.8 Minerals in water	114
Chapter 6	119

General discussion	119
Chapter 7	122
General conclusion and recommendations	122
Chapter 8	124
References	124

List of tables

Table 1 Composition and percentage proximate composition of the experimental diet.....	38
Table 2 Physico-chemical measurements (Mean±SE) of fish tank water. Mean values sharing common letters are not significantly different ($p < 0.05$).....	42
Table 3 Growth response of Nile tilapia (Mean ±SE) for experimental diets.	44
Table 4 Proximate composition of Nile tilapia subjected to experimental diets. Mean values sharing common letters are not significantly different ($p < 0.05$).	48
Table 5 Mineral retention efficiency of fish from experimental diets.	54
Table 6 Macronutrient content (mgL^{-1}) of fish tank water. Mean values sharing common letters are not significantly different ($p < 0.05$).	56
Table 7 Micronutrient content (mgL^{-1}) of fish tank water. Mean values sharing common letters are not significantly different ($p < 0.05$).	58

List of figures

Figure 1 Dissertation structure.....	8
Figure 2 Schematic diagram of advanced aquaponics setup.	20
Figure 3 Schematic non-scale diagram of aquaponics unit used in the experiment; top view (above) and side view (below).	36
Figure 4 Mean TW (g/fish) of fishes with growth period. (Bar represent level of SE of the means)	45
Figure 5 Specific growth rate of fish as a function NC:FM ratio in diet.	45
Figure 6 Relative growth rate of fish as a function of NC:FM ratio in diet.	46
Figure 7 Mean (\pm SE) Weight gain (top), Protein Productive Value (middle), and Feed conversion ratio (bottom) with respect to dietary NC:FM ratios.	51
Figure 8 Correlation between mean RGR and mean PPV.....	52
Figure 9 Potassium retained in fish biomass from diet. Mean values sharing common letters are not significantly different ($p < 0.05$).	55
Figure 10 Calcium retained in fish from diet. Mean values sharing common letters are not significantly different ($p < 0.05$).	55
Figure 11 Correlation between mean K (mgL^{-1}) in fish tank water and K in fish diet.	59

Figure 12 Correlation between mean Ca (mgL^{-1}) in fish tank water and Ca in fish diet.....	59
Figure 13 Mean nitrate (mgL^{-1}) content in fish tank water as a function of NC inclusion in diet.	60
Figure 14 Dissolved Oxygen (DO) (mgL^{-1}) (Circles) and pH (rectangles) (Mean \pm SE) in Aquaponics and hydroponics growing media at different treatments.	70
Figure 15 Temperature ($^{\circ}\text{C}$) (Mean \pm SE) response in aquaponics and hydroponics growing media.	70
Figure 16 Electrical conductivity ($\mu\text{S}(\text{cm})^{-1}$) (Log transformed) (Mean \pm SE) response in growing media for experimental diets and hydroponics treatments.	71
Figure 17 Fresh total weight (Mean \pm SE) of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	73
Figure 18 Total dry weight (Mean \pm SE) of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	73
Figure 19 Leaf fresh weight of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	74
Figure 20 Root fresh weight of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	74

Figure 21 Leaf number (Mean±SE) of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	75
Figure 22 Root to shoot ratio (Mean±SE) of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	75
Figure 23 Total plant fresh weight (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.	76
Figure 24 Leaf number (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.....	76
Figure 25 Leaf dry weight (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.	77
Figure 26 Relative growth rate (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.	77
Figure 27 Leaf fresh weight (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.	78
Figure 28 Chlorophyll a, Chlorophyll b and Carotinoids (Mean±SE) of lettuce for different NC:FM ratio and hydroponics treatments.	80
Figure 29 Total chlorophyll content and Chlorophyll content per fresh weigh (Mean±SE) of lettuce for different NC:FM ratio and hydroponics treatments.	81

Figure 30 Relative growth rate, Absolute growth rate and Leaf weight ratio (Mean±SE) of lettuce for different NC:FM ratio and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$)..... 83

Figure 31 Yield per square meter growing area (Mean±SE) response of lettuce for different NC:FM ratio and hydroponics treatments..... 84

Figure 32 Dry matter content (Mean±SE) of lettuce as a response to different NC:FM ratio in fish diet and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$)..... 86

Figure 33 Log transformed Potassium, Calcium, Nitrate and Phosphate (mgL^{-1}) content (Mean±SE) of nutrient water as a response to different NC:FM ratio in fish diet and hydroponics treatments..... 87

Figure 34 Log transformed Potassium, Calcium and Iron content (Mean±SE) of Lettuce as a response to different NC:FM ratio in fish diet and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$). 88

Figure 35 Dissolved Oxygen (Mean±SE) concentration response in Aquaponics and hydroponics growing media for different mineral supplementation levels. 96

Figure 36 Electrical conductivity (Mean±SE) of growing medium in aquaponics treatments growing medium. 97

Figure 37 pH (Mean±SE) response in growing media of aquaponics and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	98
Figure 38 Temperature ($^{\circ}\text{C}$) (Mean±SE) response in growing media of aquaponics and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	98
Figure 39 Daily growth rate (Mean±SE) of fish in different levels of mineral supplementation in fish diet.....	100
Figure 40 Relative growth rate of fish in different levels of mineral supplementation in fish diet.	100
Figure 41 Feed conversion ratio (g/g) of fish for different levels of mineral supplementation on fish diet.....	102
Figure 42 Dry matter content response of whole fish body (Mean±SE) for mineral supplementation on fish diets.	104
Figure 43 Protein productive value (g/g) response of fish for mineral supplementation on fish diet.	104
Figure 44 Protein efficiency ratio (%) response of fish for mineral supplementation on fish diets.	105
Figure 45 Root fresh weight of lettuce (Mean±SE) subjected to experimental diet and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	107

Figure 46 Leaf number (Mean±SE) of lettuce as a function of different levels of mineral supplementation on fish diet.	107
Figure 47 Leaf fresh weight (Mean±SE) of lettuce as a function of different levels of mineral supplementation in fish diet.	108
Figure 48 Total fresh weight (Mean±SE) of lettuce as a function of different levels of mineral supplementation in fish diet.	108
Figure 49 Total dry weight (Mean±SE) of lettuce as a function of different levels of mineral supplementation on fish diet.	109
Figure 50 Absolute growth rate (g/day) of lettuce as a function of different levels of mineral supplementation on fish diet.	111
Figure 51 Absolute growth rate (Circle) and RGR (%) (Rectangle) of lettuce for different treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	111
Figure 52 Lettuce yield response as a function of different levels of mineral supplementation in fish diet.	112
Figure 53 Fish yield (kg), lettuce yield (kg) and total yield (kg per kg diet) of aquaponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).	113
Figure 54 Leaf weight ratio (Mean±SE) of lettuce for different treatments.	116

Figure 55 Dissolved phosphate (Mean±SE) concentration of growing medium. Mean values sharing common letters are not significantly different ($p < 0.05$). 116

Figure 56 Dissolved Nitrite (Mean±SE) concentration of growing medium. Mean values sharing common letters are not significantly different ($p < 0.05$). 117

Figure 57 Dissolved Nitrate (Mean±SE) concentration of growing medium. Mean values sharing common letters are not significantly different ($p < 0.05$). 117

Figure 58 Dissolved ammonia (Mean±SE) concentration of growing medium. Mean values sharing common letters are not significantly different ($p < 0.05$). 118

Chapter 1

General Introduction

1.1 Introduction

Aquaculture is rearing of aquatic organisms that extend from microorganisms to large aquatic animals and plants through human intervention (Carballo *et al.*, 2008). Global aquaculture fish production is increasing by 9.1% while capture fishery output is decreasing by 1.2% (Gutierrez-Wing and Malone, 2006), which indicates the necessity of aquaculture development to nourish global population. However, conventional aquaculture systems like intensive outdoor pond and hapa net production are not sustainable due to environmental impact and their limited provision of quality food in sufficient quantity (Ebeling and Timmons, 2012). But Recirculating indoor Aquaculture Systems (RAS) are more economically viable, scalable and can provide control over products' quality and quantity (Ebeling and Timmons, 2012). One of the critical driving forces for RAS is its 500-1000 times water use efficiency (Gutierrez-Wing and Malone, 2006). However, recently serious bottleneck issues were raised on RAS due to its nutrient rich waste water leakage to the environment and higher operational cost based on level of intensity (Gutierrez-Wing and Malone, 2006; Edwards, 2015).

Highest productive aquaculture system is expected to use high quality industrial diet with highest assimilation efficiency but lowest productive aquaculture systems use ecological diets which lead to lowest assimilation efficiency (FAO, 2009; Edwards, 2015). Increasing productivity per unit effort has economic background and hence looks for higher profit and economic viability. Recently, RAS technologies got sophisticated and focused on managing nutrients that hinder

and/or promote fish growth, but due to highest stocking density maximum nutrient loading to the system is expected which in turn resulted in high accumulation of some nutrients in the growing system (Bostock *et al.*, 2010; Edwards, 2015). It is estimated that 60% of fish diet and 85% of phosphorus, 80–88% of carbon, 52–95% of nitrogen in diet are not assimilated by fish and remain in water as solid waste, dissolved chemicals or gases (Gutierrez-Wing and Malone, 2006; Rakocy *et al.*, 2006). Therefore, improving fish diet efficiency is focusing on increasing productivity and decreasing environmental stress. Pelleted feed global production has increased by four fold within 13 years and reached 27.1 million tons in 2007 and with estimated average growth rate of 11.1%, it is expected to reach 70.9 million tons by 2020 (Tacon *et al.*, 2006; Edwards, 2015).

Aquaculture systems can be land-based, fresh water based or marine-based and due to the nature of their site, they can be either flow-through or recirculating. Land-based recirculating systems are commonly manifested as ponds, tanks, raceways, cages (in pond), and pens (in pond) fish culture systems. System design and choice depend on production intensity (extensive/traditional, semi-intensive, intensive), on environmental demand (water quality, temperature, oxygen) and on trophic requirements (carnivorous, omnivorous, herbivorous) of stocked fish (Edwards, 2013) and can be open, semi-closed or closed (Tidwell, 2012b). Aquaculture systems have recently been comprehensively described by various authors (Ebeling and Timmons, 2012; Fornshell *et al.*, 2012; Masser, 2012a; Masser, 2012b; Mims and Onders, 2012; Tidwell, 2012b; Tidwell, 2012a; Tucker and Hargreaves, 2012). In pond culture, fish grow in large water bodies formed by cut or modified land masses with ground, surface or wastewater as a water source (Tucker and Hargreaves, 2012). Fish reared in cage culture, on the other hand are contained in a mesh confinement (all side) suspended or submersed in open water bodies (large pond, lake, ocean) with volume of 1-4 m³ (Masser, 2012a). Also in pen culture systems, fish are held in the mesh

confinement, which has a lower edge attached to bottom surface. Raceway systems are defined as shallow, narrow and long canals (30:3:1; L:W:D) with optimum water flow rate warranting removal of solid waste (Stickney, 2005). Recirculating aquaculture systems (RAS) are tank based and 90% of water is reused and it is most intensive fish rearing system (Gutierrez-Wing and Malone, 2006).

Productivity of pond fish production system is lowest as compared with raceway and tank production systems. Pond size ranges from 0.5–10 ha with a depth of 1.5-1.75 m (Tucker and Hargreaves, 2012). The average production of fish per year with continuous supplemental feeding and aeration is 10,000 kg ha^{-1} with 3-10 m³ of water exchange for 1 kg of produced fish (Ebeling and Timmons, 2012). However, raceways aquaculture systems (common for salmonids) can produce 27 kgm⁻³ (270,000 kg ha^{-1}) of fish with 98 m³ water exchange for 1 kg fish production (Tucker and Hargreaves, 2012). Well-managed tank production (flow through or recirculating system) can carry 120 kgm⁻³ fish with only 0.1 m³ water use for 1 kg fish production. Raceway, pond, and recirculating systems produce 0.16, 0.0034, and 0.00021 L effluent per minute respectively (Tidwell, 2012a). As recirculating aquaculture systems have the highest carrying capacity and employ biofilters for conversion of organic nitrogen, their effluent water occurs to be the most appropriate to replace hydroponic nutrient solution for soilless plant production as compared to pond and raceway aquaculture systems. In intensive recirculating aquaculture systems, 5-10% of total water volume needs to be exchanged daily due to high nutrient load (Ebeling and Timmons, 2012), unless a biological and chemical treatment units for nitrogen and phosphorous removal are incorporated for biofiltration and denitrification processes. Nutrient removal component of RAS can bring economic return through production of plants if carefully considered. Connecting the biofiltration of recirculating aquaculture system to hydroponic

production unit is found to be one alternative. Aquaponics is harmonized fish and plant production system developed based on the mutual beneficiaries of two main crops through microbial intervention (Tyson *et al.*, 2011). In addition to aquaponics (integration with hydroponics) high tech aquaculture systems like recirculating aquaculture systems can be integrated to other agriculture production systems (Carballo *et al.*, 2008).

Hydroponics is a technology of plant growing without soil on inert supporting media through addition of nutrient solution containing all nutrients required for plant healthy growth and development (Rush, 2012). Hydroponic production get intensified and complex through time due to increased economic demand for producing high value crops in more stable situations (Rush, 2012).

Hydroponics utilizes different types of inorganic fertilizers in solution form for plant growth and development. These fertilizers are produced from earth's limited resource by immense energy consumption, which directly affect the global climate. Furthermore, excess nutrients and unutilized nutrients are expected to be removed from the system; hence, high likelihood to create environmental pollution. Limited access and high cost of hydroponic technologies remain the bottlenecks for its expansion in developing countries, despite its potential for food and nutrition security.

Among several vegetables produced hydroponically, lettuce is the most cultivated one. Among the many varieties; bibb, iceberg, and romaine lettuce are the most common candidates (Licamele, 2009). Romaine lettuce (*Lactuca sativa* L.) is the most cultivated in hydroponics and aquaponics due to its hardy nature for environmental variability, rapid growth rate, and high nutrient use efficiency (Licamele, 2009). Lettuce is the prior global vegetable consumption scale and global

commercial production level due to its nutritional value and easy access as response to fast growth rate (3-4 weeks). Lettuce's ability to bio-load much nutrient in short time makes it preferable for aquaponics despite the presence of biotic (genetic, disease and pest) and abiotic (temperature, light, pH, water potential and nutrient mode of existence) factors that hinder its growth performance (Licamele, 2009).

Mitigating mother technologies (Aquaculture and Hydroponics) associated problems has cost implication and hence developing multi-trophic culture system, which is aquaponics, would be considered as most parsimonious. Aquaponics integrates aquaculture and hydroponics with the median biological action of microorganisms to enable simultaneous production of both fish and plant in a single effort (Rakocy, 1997; Timmons *et al.*, 2002). Aquaponics provides several advantages including environmental, economical, societal and health through its potential of reducing water consumption and waste discharge to the environment, and highest productivity potential (Dediu *et al.*, 2012; Buzby *et al.*, 2016).

Among several organisms produced in aquaponics, Nile tilapia and Romanie Lettuce are the most common and widely produced candidates and are also most studied (Seawright *et al.*, 1998; Dediu *et al.*, 2012; Liang and Chien, 2013; Love *et al.*, 2015a).

Currently, all aquaponics operations utilize fish diet primarily prepared by considering physiological demand of fish and minimal possible nutrient loss to the environment despite the need to balance the plant demand to earn good profit from the whole setup. Hence, in many aquaponics operations nutrient imbalance or nutrient shortage for plants is observed through prolonged aquaponics operation using commercial fish diet (Ogunji *et al.*, 2008). Limited plant growth and development is reported in aquaponics due to the possibility of mineral imbalances in

the system (Rakocy, 1997; Seawright *et al.*, 1998; Endut *et al.*, 2010); hence, one possible mitigation measure can be diet quality adjustment. In addition, plants have their own nutrient requirements, which vary based on plant type; hence, depending only on conventional fish diet as nutrient source in aquaponics make the plant component suffer. Therefore, new insight should be advocated about aquaponics input (fish diet) formulation scenarios giving mutual emphasis for fish, plant, and microorganisms physiological requirements. Except multi-element ionic nutrients like nitrate and phosphate, other macro and micronutrients might be adjusted by simple addition on fish diet with critical fish and plant tolerance limit consideration. The major cost in fish diet preparation (50-60%) is protein and focusing on its source might bring economic benefit in the whole system. Fishmeal is the major protein source in conventional fish diet formulation. Fishmeal global production shows sharp increase in price and also inaccessible in most land locked countries like Ethiopia (FAO, 2012). Hence, formulating new aquaponics diets using locally available ingredients considering both fish and plant demand is one critical step forward for aquaponics development.

Among several plant ingredients available to use as protein source in Ethiopia, Niger seed cake is the cheapest and widely available ingredient. Niger seed cake is a remnant cake after oil extraction from an oil seed called Niger (“Nuge”). Niger seed (*Guizotia abyssinica*) is an oil seed which is produced in immense quantity in Ethiopia usually used for feed for ruminants (Getinet and Sharma, 1996) and has shown promising potential for aquaponics as nitrogen and other mineral main source (Little *et al.*, 1986; Getinet and Sharma, 1996; Feedipedia., 2013).

1.2 Objective of the research

The general objective of this research is to determine level of Niger seed cake inclusion and mineral supplementation for improved productivity in Tilapia-Lettuce Aquaponics system.

Specific objectives

The following are specific objectives;

- to determine the level of Niger seed cake inclusion in fish diet for aquaponics for highest fish productivity;
 - to analyze the potential of Niger seed cake in promoting growth, yield, and quality of Lettuce in aquaponics; and
- to identify the effective level of hydroponic mineral supplementation on fish diet for increased aquaponics productivity.

1.3 Dissertation structure

Eight chapters make up this dissertation as shown in Figure 1. Chapter 1 and 2 focus on general introduction and literature review respectively while long term aquaponics experiment trials are included in chapter 3 (fish response to Niger seed cake inclusion), chapter 4 (lettuce response to Niger seed cake inclusion), and chapter 5 (Fish and lettuce response for mineral supplementation). General discussion, general conclusion and recommendation, as well as references are included in chapter 6, chapter 7, and chapter 8 respectively.

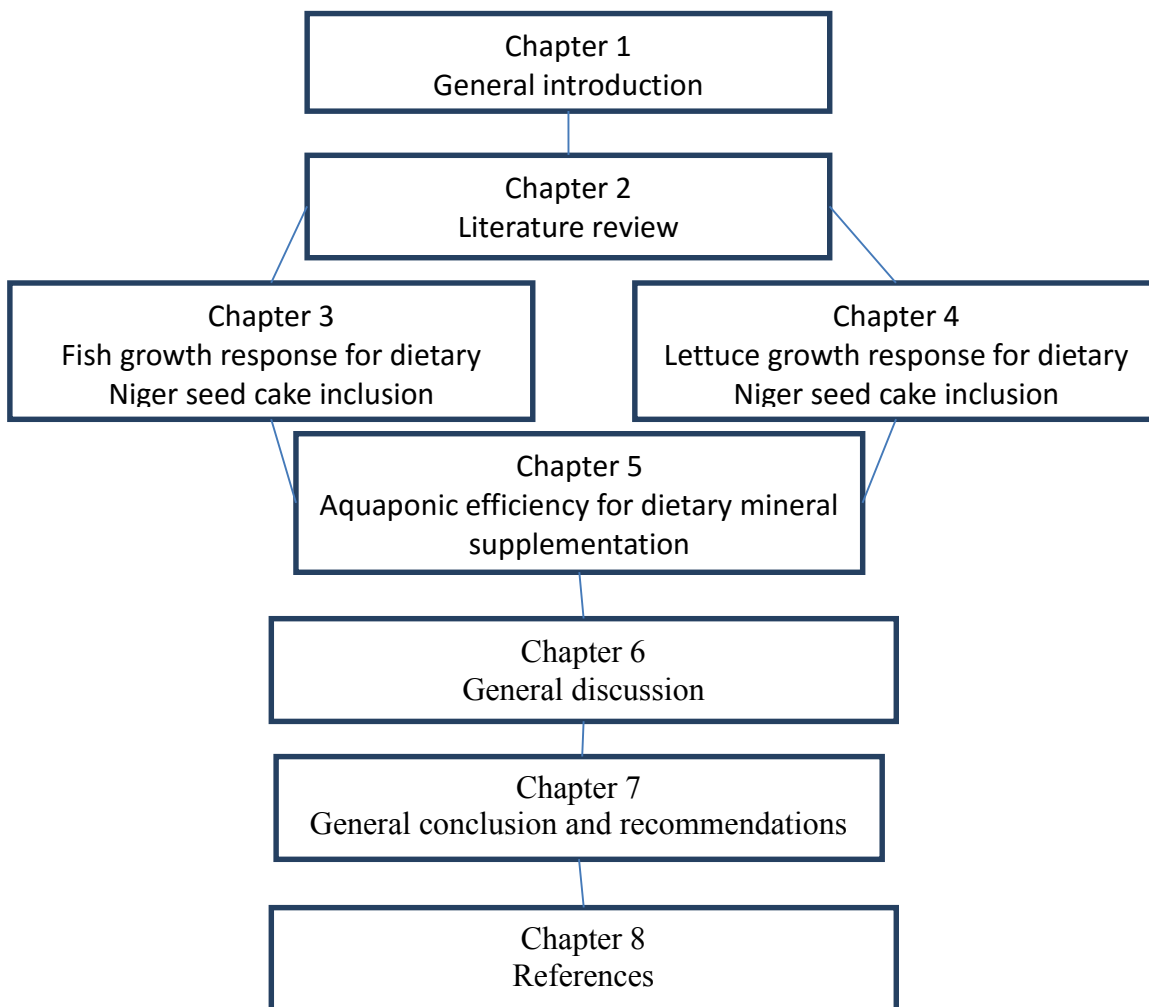


Figure 1 Dissertation structure

Chapter 2

Literature review

2.1 Global food production

Global population size is expanding through time despite the limitation of agricultural productivity due to land shortage and climate change. Global population size is expected to reach 9.3 billion in 2050 (FAO, 2012); hence, providing healthy diet for such population size from conventional food production systems is difficult (FAO, 2012). Due to the ever-increasing global population, equitable food provision is a great challenge (FAO, 2009; FAO, 2010; FAO, 2012). The problem associated with conventional food production on soil has been exacerbated due to climate change, decline in soil productivity, pollution, arable land shrinkage, urbanization etc.... Therefore, dynamic paradigm shift on food production is required and started globally (FAO, 2012).

2.2 Fish contribution

Land shortage for agriculture and consumers' perception about increased utilization of immense fertilizers and their impacts on health and the environmental push the world to shift consumption from land-based products to water-based products. Protein consumption increases with time and reaches 15% in 2009 due to increased consumers demand on healthy and protein rich diet like milk, meat, egg and fish (FAO, 2010; FAO, 2012); hence, *per-capita* protein consumption increased dramatically (Dediu *et al.*, 2012; Buzby *et al.*, 2016). Fish contributes more than 6.5% of global protein consumption and 16.6% of global animal protein consumption in 2011 (FAO, 2012). Global *per-capita* fish consumption for 1970-1998 increased by 24% (Gutierrez-Wing and Malone, 2006). In addition, *per-capita* fish consumption in 2009 reaches 18.4 kg in live weight

but *per-capita* consumption in Africa remains lower (9.1 kg) that elaborates the differences influenced by economy (FAO, 2012). Increased fish consumption is associated with increased public awareness about nutritional quality. Fish has polyunsaturated fatty acids, fat soluble vitamins (A, D and E), low digestible energy, water soluble vitamins and minerals (Calcium, Phosphorus, Iron, Iodine and Selenium) (Tacon *et al.*, 2006).

Global fish food production from both capture and culture fisheries increased by 3.2% overpassing population increase rate (1.7%) and has reached 131 million tons from global fish production of 85.1% (154 million tons) used as food source in 2011 (FAO, 2012). From the total global fish production; capture fisheries accounts 90 million tons, of which inland waters contributed 11.2 million tons in 2010 which increased by 30% since 2004 (Tacon *et al.*, 2006). However, most of the freshwaters are reported to be overexploited and degraded by natural and artificial pressures. Hence, freshwater productivity is expected to decrease in the near future unless critical measures are taken worldwide. One of the basic alternatives is shifting fish production mode from capture based to culture based; hence, aquaculture stands as a mitigation technology to fill the gap (Ebeling and Timmons, 2012).

2.3 Aquaculture

2.3.1 World Aquaculture

The global aquaculture industry has grown radically and developed as a major industry in the last half century. Economic and food security interest of global population push farmers to consider production systems with highest intensities that initiated the development of modern intensive

aquaculture technologies which are able to use less water, provide highest return and minimize waste release to the Environment (Edwards, 2013; Edwards, 2015).

Fish production from aquaculture industry is expected to rise in order to maintain fish supplies to the rising human population. Global fish production has been increasing steadily since 1950's. In 2004, global fish production had reached 140 million tons with aquaculture contributing 45.5 million tons (FAO, 2010). Inland aquaculture (fresh water and brackish water) contributed 27.2 million tons of fish and marine aquaculture contributed 18.3 million tons (FAO, 2010). Aquaculture production in developing nations contributed more than 80% of global aquaculture output in 2004 (FAO, 2010).

In 2010, global aquaculture food fish production increased with an annual growth rate of 8.8% and reached 60 million tons, increased by 7.5% from 2009 (FAO, 2012). Global aquaculture expansion statistics show imbalance between continents (FAO, 2010); Asia is the largest producer (46 million tons in 2008) followed by America (2.4 million tons in 2008); the lowest aquaculture production is from Africa which is 0.94 million tons in 2008 (FAO, 2010).

2.3.2 African aquaculture

African aquaculture practice is still in its infancy compared with Asian experience. Total aquaculture practice in Africa in 2012 was estimated to be 1.5 million tons per year, which showed a rapid increase in the last two decades (FAO, 2010). Among top African countries in aquaculture production, Egypt takes the leading position by 1.02 million tons (67%); however, aquaculture production in Sub Saharan African countries increased by 96.2% and reached to 454,691 tons from 1990 to 2012 (FAO, 2010; FAO, 2014b). African aquaculture is mostly dominated by small scale

extensive production systems but the highest production came from limited commercial enterprises (Hecht, 2007).

2.3.3 Aquaculture in Ethiopia

Aquaculture activities in Ethiopia have started around 1970`s with establishing pond cultures for different indigenous and non-indigenous fish for experimental purposes by the Ethiopian National Fisheries and Other Aquatic Resources Research Center (FAO, 2003). Despite the potential of aquaculture to alleviate food security and other economic issues, the sector in Ethiopia remains at its early stage (Bostock *et al.*, 2010). Aquaculture practices started in different parts of the country after the development of “National Aquaculture Development Framework of Ethiopia” by the Ministry of Agriculture and Rural Development. Different cage culture, pond culture and hatchery, fish diet, integrated aquaculture and aquaponics activities have been started in some places with the motives of regional and national fish research centers, universities and other supporting organizations. However, the contribution of aquaculture for the total fish production remains insignificant.

2.4 Hydroponics

Hydroponics can be defined as the technology of plant production without soil through inert media (gravel, sand, vermiculite, pumice, perlite, coco coir, sawdust, rice hulls, or other substrates) used to support plant structure and with nutrient solution provision (containing all the essential elements needed by a plant for its normal growth and development) (Rush, 2012). Hydroponics usually termed as water culture or soilless culture with that plants grown in water enriched with water soluble fertilizers with continuous adjustment of pH, electrical conductivity level and nutrients

concentration upon the crop's demands. Hydroponic technology expansion depends on its productivity efficiency which claimed to provide 10 times more yield with quality and with minimal effort than soil based plant production (Rush, 2012).

Currently 17 elements are considered essential for most plants; these are Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sulfur, Iron, Copper, Zinc, Manganese, Molybdenum, Boron, Chlorine and Nickel (Trejo-Téllez and Gómez-Merino, 2012). In hydroponics, crops are anchored physically on medium on which root grows and keep in continuous contact with these nutrients in usable form from nutrient solution.

Global hydroponic production increased by four fold from 1980 to 2001 reaching 20,000 to 35,000 hectares (Rush, 2012). Currently hydroponics is practiced at small scale to large commercial scale worldwide (Jones, 2005; Rush, 2012). Hydroponic vegetable production is evident everywhere in the world and 83 countries reported to have commercial hydroponic vegetable production (Rush, 2012). Hydroponics, whether indoor or outdoor, can be operated in all places, which are very unsuitable for conventional plant production. Plants are irrigated either by drip, capillary, and sub-irrigation, or with shallow and continuous water flow (Nutrient Film Technique, NFT) or with larger continuously moved water volumes (Deep Flow Technique, DFT) (Jones, 2005). The highest specialization is observed on aeroponic systems; where the plant root is suspended in root chamber without growing medium or steady supply with water and nutrients. Instead, water and nutrients are sprayed in pulses on roots and excess nutrient solution is drained from root chamber (Rush, 2012).

The choice of system design will be determined based on plant type to be grown and other associated factors. Urban horticulture solutions (green walls, extensive green roofs, roof

gardening) are based on hydroponic principles. In intensive horticulture with vegetable, the single water/nutrient loop considers only one crop species; crop diversification within the same greenhouse may be realized by several parallel water/nutrient loops. In intensive production of ornamentals and herbs as well as in urban settings, several crop species are served by the same water/nutrient loop (Rush, 2012).

Hydroponic systems are organized either in open systems or in closed ones with recirculation of nutrient solution. Closing of nutrient and water loop prevents run-off of nutrient rich irrigation water into soil and catchment and reduce eutrophication. However, a risk for dispersal of root diseases is a matter of concern in closed systems (Stanghellini and Rasmussen, 1994). System design, therefore, needs to consider the grown crop, its disease profile, its economic value, and economic impact of crop losses. During the last 20 years, solutions to mitigate risk for dispersal of plant pathogens by recirculating nutrient solution have been developed (Stanghellini and Rasmussen, 1994). Two principles may be discriminated, (i) prevention of pathogen transmission by recirculating nutrient solution and (ii) prevention of pathogen transmission between plant roots (Stanghellini and Rasmussen, 1994). Spot disinfection encompasses measures that either kill or remove the target pathogen at one specific place within the system and comprises thermal (pasteurization), irradiation (UV-treatment), oxidation (O_3 , H_2O_2 , photocatalysis), halogens (Cl, B, I) as well as heavy metal (Cu, Ag) and filtration (slow filtration, sedimentation filtration, membrane filtration) approaches (Jones, 2005; Rush, 2012). These measures may be target specific or affect the entire microbiota inhabiting the nutrient solution. Biological control agents and biosurfactants may counteract dispersal of root pathogens between plants that share the same volume of growing medium in hydroponics.

2.4.1 Hydroponics in Ethiopia

In Ethiopia, hydroponic activities are very limited to the research centers like Ethiopian institute of agriculture research and there are no any documents about commercial hydroponic farms. However, there are some activities in flower farms and strawberry farms but their intensity and impacts are not well recorded. The most limitations for hydroponic expansion in Ethiopia are non-availability of hydroponic farm structures, recipe, service providers in market and very expensive price of inorganic salts to prepare hydroponic solutions that ranges from 5-10% of total production cost in real hydroponics (Tyson *et al.*, 2011), which will be 100 times more expensive in Ethiopia. Despite these limitations, most Ethiopians prefer to get quality and fresh food products with sufficient quantity and affordable price; hence, there is a prospect for hydroponic farm expansion.

2.4.2 Hydroponics input

Hydroponics inputs are inorganic salts with a property of high solubility in water and provision of at least two important nutrients. Fertilizers can be grouped based on the nutrient of interest as macronutrient fertilizers and micronutrient fertilizers (Rush, 2012). Chemicals used to formulate macronutrients include but not limited to Magnesium sulfate, Calcium chloride, Calcium sulfate, Phosphoric acid, Triple supper phosphate, Monocalcium phosphate, Potassium sulfate, Potassium chloride, Monopotassium phosphate, Amonium monohydrogen phosphate, Ammonium nitrate, Ammonium dihydrogen phosphate, Calcium nitrate, and Potassium nitrate (Trejo-Téllez and Gómez-Merino, 2012). Micronutrient fertilizers include Ferrous sulfate, Ferric chloride, Iron Chelate, Boric acid, Disodium octaborate, Sodium tetraborate, Copper sulfate, Manganese sulfate, Manganese chloride, Zinc sulfate, Zinc chloride, Ammonium molybdate, Sodium molybdate, Zinc chelate and Manganese chelate (Rush, 2012).

2.4.3 Hydroponics techniques and benefit

Hydroponics technical advancement is increasing through time; however, the basic plant component design remains in limited types like water culture and solid medium culture. Water culture hydroponic system is without using any solid root supporting media and it includes aeroponics, Nutrient film techniques, and floating raft system. Solid culture uses solid substrate to provide physical root support for the plant growth (Jones, 2005). Despite the principal operational and technical similarities between the two system types; variation on productivity, nutrient management, crop type and economic return are observed (Jones, 2005). Water culture in hydroponic production system is the type in which plant roots are kept in nutrient solution without using any solid root supporting media. Aeroponics system is based on the suspension of plant roots in closed dark chamber in which nutrient solution forcedly make a fog through high-pressure pump to create 100% relative humidity (Rush, 2012). Plant roots are periodically washed by jets of nutrient solution or stay moistened by the nutrient fog. Solid supporting media is fixed on the top part of nutrient solution to support plants. Floating raft system consists of relatively deep nutrient solution container, which is simultaneously used as growing bed (Rush, 2012). Plant growing structure is situated floating on nutrient solution and plant roots have direct contact with nutrient solution, which is relatively static. Ebb and flow method is scheduled irrigation and draining system in which nutrients pumped to the growing media are contained for some times and drained back to the nutrient container (Rush, 2012). This technique claimed to increase the aeration system. Ebb and flow system is relatively robust hydroponic system type that can host wide range of growing media (Rush, 2012). In Bucket systems plants grow on inert growing medium in bucket and nutrient solution supplied by dripping system but the used nutrients may or may not be recirculated. Nutrient film techniques (NFT) is sometimes referred to as nutrient flow technique

due to the need for continuous nutrient flow through the system. It is a water culture in which plant roots are kept in pipes with a very thin layer of nutrients hence the root is subjected to thin nutrient layer and also air (Rush, 2012).

2.4.4 Challenges of hydroponics

Hydroponics global developments are faced with different challenges including environmental, technological and societal (Wortman, 2015). Environmental problems arise from the issue of eutrophication due to nutrient leach to environment. Technological challenges are related to the need to understand the detail nutrient and growing condition demand of each crop to increased productivity and which will possibly take long time. The modulation system for growing condition and also growing facilities are relatively expensive and limits the expansion of the technology to developing world. Production enhancement to maximize economic return demands use of too much pesticides and hormone, which together with complete use of inorganic nutrients for plant growth, makes hydroponic products mostly not organic. These in turn negatively trigger societal acceptance issue on the product since health awareness of the consumers is going increasing. Other bottlenecks of hydroponics expansion are extended payback period of initial investment due to expensive price of production facilities (Jones, 2005).

2.5 Aquaponics

Aquaponics is integration of recirculating aquaculture and hydroponic (Rakocy, 2012; Ebeling and Timmons, 2012; Rush, 2012; Wortman, 2015). Aquaponics history goes back to ancient Aztec people but the term “aquaponics” has been used repeatedly among scientific communities more recently (Rakocy, 2012). Aquaponics technical advancement starts to be extended by integrating

technical advancements in recirculating aquaculture and hydroponic systems without compromising the productivity of independent systems (Johanna *et al.*, 2016). All technical advancements including fish tank design, biofilter design, sterilization modalities, hydraulic loading rate, hydraulic retention time, nutrient quality and quantity monitoring and even biosafety issues should be considered in advanced aquaponics. Recently commercial size aquaponics setups started in different places including in Africa following technical advancements in the sector (Love *et al.*, 2015a).

Aquaponics technical advancement increased through time with a scale from backyard hobbyist system to large automated greenhouse based commercial setups. Aquaponics systems can be categorized to conventional and commercial aquaponics systems based on advancement and production intensity. Conventional aquaponics systems comprise different basic design, structural components of which fish compartment, plant compartment, biofilters, and sump containers are the basic components (Johanna *et al.*, 2016). In conventional aquaponics basic optimizing modalities between fish and plant component ratio is based on simple rule of thumb calculation from plant growing area and goes back to fish density (Rakocy, 2012). Hence, the size of the system remains small and productivity per unit effort remains lower (Seawright *et al.*, 1998).

Commercial aquaponics depends on advanced technology to earn maximum return from minimum input through effective and efficient production processes. High caliber aquaponics system is expected to employ high tech recirculating aquaculture (Jegatheesan *et al.*, 2006) and hydroponic practices (Jones, 2005). Despite the biological and environmental specific demand of culture species (fish and plant); general aquaponics management requires nutrient, energy, and economic pool consideration.

Advanced aquaponics system should contain additional system component from basic ones to increase the ultimate aquaponics economic, health and environmental return. Advanced aquaponics system components will go beyond the basic one based on the need to manage aforementioned pools efficiently (Johanna *et al.*, 2016). System component for advanced aquaponics can be fish component, plant component, biological component, clarifier, nutrient management, disinfection, nutrient thermostat, acidity management, biosafety, hydraulic, aeration and energy units. Advanced aquaponics system should consider advanced recirculating aquaculture system and advanced hydroponic system without compromising the productivity of each technology (Johanna *et al.*, 2016) (Figure 2).

Despite its potential to be important protein and vitamin production system with very limited environmental problem and low water use efficiency, aquaponics face several technical and economic challenges (Goddek *et al.*, 2015). Currently aquaponics is considered as suboptimal agriculture alternative as compared to aquaculture and hydroponics due to technological demand. In addition, securing land and infrastructure might be expensive to start urban aquaponics farm.

Limited or absence of data about economic dynamics of aquaponics hinders expansion of technology through improving its market and value chains. In addition, public awareness about health potential of aquaponics products is limited and hinders the market of aquaponically produced products. Aquaponics technical advancement mostly is a joint result of hydroponics and aquaculture technological advancement; hence, it requires absolute knowledge and skill of both advanced aquaculture and advanced hydroponic operations under a more organic mode of production.

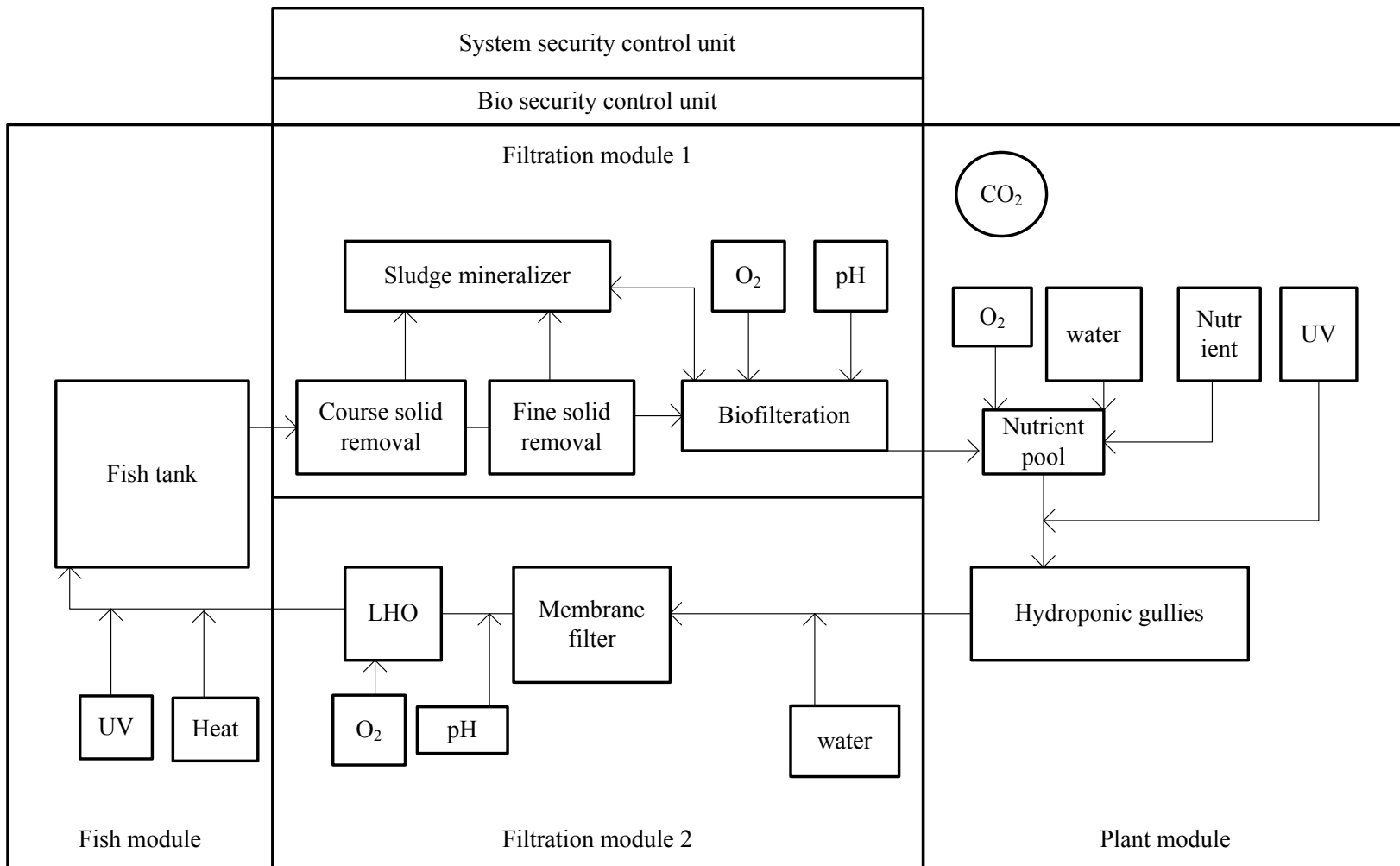


Figure 2 Schematic diagram of advanced aquaponics setup.

2.5.1 Challenges of aquaponics

2.5.1.1 Nutrient imbalance

With prolonged aquaponics operation, nutrient deficiency or salt accumulation will be evident (Seawright *et al.*, 1998; Roostaa and Hamidpour, 2011; Roosta and Mohsenian, 2012). Mineral availability in diets and its integrity among each constituent determine nutrient accessibility to fish and plants. Fish diet mineral metabolism is regulated with health, age, density, diet quality, diet quantity, and growing medium condition (Lovell, 1991). Hence, minerals availability for plant nutrition in aquaponics is highly dependent on fish growth and conditions. Despite the existence of several diet alternatives for each mineral required for fish and plant growth; some minerals like Boron condition is not yet started in fish diet while it is important for plant growth (NRC, 1999; Jones, 2005). In addition, minerals, which are not necessary for plant growth, are included in fish nutrition like Selenium, its biohazard and bio benefits for plants should be stated in clear, and no reliable study is still available until now.

The principle of aquaponics is to minimize waste discharge to the environment through increasing productivity of fish and plant component. Hence, mineral from fish diet is expected to be assimilated in the system to bring zero discharge to environment. Mineral availability and use efficiency by aquaponics crops is dependent on biological (assimilation, excretion, and microbial consumption), chemical (mineralization of uneaten diet, oxidation reduction reaction) and physical (solubility and dissociation) fate of delivered fish diet which is principal nutrient source to the system (Jatoba *et al.*, 2011). Despite its critical contribution of mineral in the system solid excreta and uneaten feed mineralization has got less attention than soluble fish excreta in previous works than (Rakocy and Hargreaves, 1993; Seawright *et al.*, 1998). Mineral availability in fish waste

water for plant is not correlated with mineral level in fish diet rather it depends on integrated biological, chemical and physical agent activities in the system but little information is available in this aspect (Rakocy and Hargreaves, 1993; Seawright *et al.*, 1998; Cometti *et al.*, 2011). Mineralization is not identical for all feed ingredients and for minerals in ingredients. Hence, it is difficult to model the nutrient dynamics in aquaponics system unless standard aquaponics diet formula is developed and optimized for different fish and plant production modalities (Cometti *et al.*, 2011; Seawright *et al.*, 1998; Rakocy and Hargreaves, 1993). However, with detail future research to transform all minerals in fish diet to fish and plant biomass, basic aquaponics principle which is zero discharge system may be achievable (Neori *et al.*, 2004).

For some minerals diet mineral content affect fish excreta mineral contents, but the majority of mineral sources come from uneaten diet mineralization since fish consume only 30-35% of administered diet (Rakocy, 1997). Therefore, managing feed quality is observed as key actinig point to improve aquaponics mineral use efficiency. Existing fish diet is produced focusing on economic return of fish farm with low mineral release to environment. This demands thinking about the plant component as a biofilter rather than a mutual economic interest of aquaponics operators. Searching for feed quality that leads to sufficient available nutrient loading in the system in optimum proportion for healthy growth and functioning of plant component is a critical point to act to optimize aquaponics system for highst economic return. Mineral loading to the system and use efficiency depends on diet quality, quantity, growth medium charactertics and plant and fish type, size and age. Formulation of fish diet for aquaponics should consider availabilitiy, quality, cost and environmental issues of the ingredient.

Mineral concentration in aquaponics is lower than the concentration in hydroponics, which indicates the need to revise the input for aquaponics system. Nutrients in aquaponics are directly influenced by biological and chemical mineralization process by microorganisms or earth worms (Bajsa *et al.*, 2003) which requires building up of the biomass of the acting agents, which is directly influenced by system carbon to nitrogen ratio. High carbon to nitrogen ratio (N:C) increases the bioflock level in the system which are beneficial or harmful for aquaponics crop (Azim and Little, 2008). For instance, due to the presence of high carbon in the system plant root pathogens can easily flourish and hinder the plant growth performance and will increase biological oxygen demand (BOD) and chemical oxygen demand (COD) in the system which creates negative impact on system oxygen investment. Even if there is a need for future investigation on the impact of organic compounds on plant growth in aquaponics, there are some reports that mention the possibility of beneficial impact of organic carbon on plant growth (Azim and Little, 2008). Some researches even report more yield in aquaponics than hydroponics despite mineral deficiency (Lennard and Leonard, 2004; Pantanella *et al.*, 2012). Therefore, developing new diet should consider optimum carbon to nitrogen ratio which in turn should be investigated for aquaponics system specifically.

2.5.1.2 Growth conditions

Aquaponics system design, construction, and operation are highly complex and require multidisciplinary engagement from fishery, environment, engineering, water works, chemistry, horticulture, and others. Advancement in aquaponics requires technical advancement in computer based system monitoring, and the involvement of Information and Communication Technology (ICT). Therefore, for commercial aquaponics operation multidisciplinary expertise on technical,

biological, chemical, physical, economical, and managerial knowledge, attitude, and skills are required to optimize the setup and to maximize the return.

Among several challenges the critical one in conventional aquaponics operation is pH requirement of the organisms; microorganisms, plant and fish. The pH requirement of aquaponics components is variable; for optimum growth fish, in general, require a neutral pH but selection of fish species might help. For instance, Tilapia tolerate a pH range of 3.7-11 with best growth between 7 and 9 (Alder, 2001). However, despite variations with variety, plants require a pH range between 5.5 and 6.5 for optimum nutrient uptake (Goddek *et al.*, 2015). Nitrifying organisms' pH requirement is higher than plants and fish and a pH of 7.8 is ideal level for efficient nitrification process. Common nitrifiers pH requirement is reported to be 7.5 (*Nitrobacter*), 7-7.5 (*Nitrosomonas*) and 8-8.3 (*Nitrospira*) (Goddek *et al.*, 2015). Ammonia oxidation increases by 13% by each pH increment. Therefore, searching for alternatives to balance the pH is still an ongoing scientific challenge. Maintaining pH range from 6.8-7 is reported to be good range for aquaponics (Licamele, 2009). However, efficiency of plant and microorganisms might be compromised. Nitrification process release proton to the system and tend to decrease pH which in turn inhibit the efficiency of nitrifying microorganism, and carbonates and hydroxide supplementation in fish diet is used as a mitigation measure but the efficiency on overall system function is still in question (Goddek *et al.*, 2015). Another mitigation measure is implementation of fluidized lime bed to balance the acid water; however, apparent knowledge on pH fluctuation in the system or fixed automated pH regulator device must be available to determine the lime dose. However, these methods consider only nitrification process while in aquaponics plenty of activities have been determined by pH (Goddek *et al.*, 2015). In advanced high tech aquaponics system, modular pH management units can be implemented to enable maintaining each module pH demand optimum (Figure 2).

2.5.2 Aquaponics input

Aquaponics basic inputs are fish feed, water, fish, plant seedling, oxygen, and carbon dioxide. Aquaponics diets produced for fish production varies widely in their mineral content as per ingredients used and species of interest. Diet quality attributes for tilapia studied for long time to reach to a formula with proper nutritional value which reduce environmental stress, disease stress and growth periods. Tilapia nutritional dietary requirements for energy, protein, lipids, vitamins and minerals were established after several studies (NRC, 1999). To increase nutritional efficiency of tilapia diet in aquaponics, diet formulation should consider physiological demand of fish and plants. Mineral contents in fish waste, which resulted from fish diet mostly, face nutrient deficiency as compared to hydroponic solutions. Lettuce needs essential minerals in proper amount, type, and integrity for its best growth. Diet quality management for aquaponics should consider fulfilling the need for plant growth without compromising fish growth. Several investigations indicate the possibility of integrating plant ingredients in fish diet to replace fishmeal, which is usually unavailable in most places and expensive. Most studies consider replacing the major protein source which is believed to take 60% of fish diet cost (Ng and Romano, 2013). However, fish diet for aquaponics should be developed using feed ingredients with highest mineral content and availability based on return of investment consideration rather than decreasing the price of the diet. Ingredients including mineral supplements quality and quantity for fish diets should be revised considering increasing aquaponics efficiency.

Physical characteristics to describe quality of tilapia diet include water stability, palatability, digestibility, and fecal stability (FAO, 2014a). These quality attributes need revision for aquaponics diets. In contrary to conventional fish diets, aquaponics diets should have low water

stability to enable mineral in diet to be released to water medium from uneaten diets and to prevent solid waste accumulation in the system. Diet water stability attributes can be compromised with increased feeding frequency to increase the likelihood of administered diet consumption by fish and to minimize non consumed diet level in the system. Low water stability of feed is directly correlated with ingredients' water stability. Therefore, selection of feed ingredients with quality of fast mineral release or dissociation should get preference. Aquaponics diets should also consider increasing mineral solubility and less fecal stability to increase the possibility of maximum mineral release to the plant of interest.

Tilapia dietary macronutrients (crude protein, fat, energy and mineral content) demand varied with their growth so as fry (< 0.5 g), juvenile (0.5-10 g), fingerlings (10-30 g) and adult (> 30 g) require protein level 40-45%, 35-40%, 30-40% and 20-30% respectively (El-Sayed, 2006). Tilapia fry and fingerlings require higher level of protein, fat, and minerals and lower level of carbohydrates to maintain their rapid muscle and skeletal development (El-Sayed, 2006). Through time, their metabolic energy demand increases to maintain their increased basal metabolism while protein demands decrease (Ng and Romano, 2013). Varying the level of dietary protein indicates the possibility of getting various level of nitrogenous waste from fish, based on diet quality and quantity; but it simultaneously fluctuate the proportion of other minerals in fish diet.

Tilapia dietary lipid content is expected to be within the range of 5-12% (Ng and Romano, 2013). Major source of dietary lipids is fish oil but recently studies confirm that tilapia can consume plant based oil more efficiently than fish oil; therefore, soybean and rape seed oil can be used in fish diet (El-Sayed, 2006; Ng and Romano, 2013). Tilapia dietary fiber content ranged from 6-8% (El-Sayed, 2006). Carbohydrate content in tilapia diet ranges from 25-30% (El-Sayed, 2006; Ng and

Romano, 2013). These organic compounds contain carbon and increase carbon to nitrogen ratio in the system. Therefore, optimizing the system bioflock dynamics and functioning should be clearly stated for aquaponics setup (Azim and Little, 2008; David *et al.*, 2009).

2.6 Importance of Macro nutrients and micronutrients on growth, development and physiology of tilapia and lettuce

Inorganic minerals are important for life functioning of fish and lettuce. With the exception of organically bound nutrients, there exist more than 20 essential inorganic minerals categorized as macronutrients and micronutrients based on the level needed for the healthy functioning of the organisms. Calcium, Magnesium Sodium, Potassium, Phosphorus, Chlorine, Sulfur, Iron, Zinc, Manganese, Copper, Iodine¹, Cobalt, Nickel, Fluorine, Vanadium, Chromium, Molybdenum, Selenium, Silicon, and Boron² are the frequently reported essential nutrients. These minerals are important to make structural hard and soft tissues, nerve impulse transmission, muscle constriction, regulate pH of body fluids, essential component and coenzymes of many vitamins, enzymes, hormones and osmotic balance regulation (Fageria *et al.*, 2002; Trushenski *et al.*, 2006).

2.7 Feed ingredients

Fishmeal is known for its role as major feed ingredient for tilapia farm due to its highest contribution for protein content (El-Sayed, 1998; Khan *et al.*, 2013). Fishmeal primarily provides protein with optimum amino acid composition, all minerals in good proportion, lipid and energy for fish growth and development (Olsen and Hasan, 2012; Ng and Romano, 2013). Fishmeal use proportion in commercial tilapia feed ingredients shows decreasing pattern with time reaching 3%

¹ Needed only by fish

² Needed only by plants

in 2010 and expected to drop to 1% in 2020 through repeated testing of replacing it with terrestrial based ingredients (Olsen and Hasan, 2012; Ng and Romano, 2013).

Fish feed production from fishmeal becomes challenging due to fishmeal production decline, fishmeal use for terrestrial animal diet formulation and direct human consumption of pelagic species that were used to fishmeal production before (El-Sayed, 1998; Fontainhas-Fernandes *et al.*, 1999; El-Saidy and Gaber, 2003; David *et al.*, 2009; Brinker and Reiter, 2011; Khan *et al.*, 2013; Anderson *et al.*, 2016; Koch *et al.*, 2016; Moutinho *et al.*, 2017). Increasing inclusion of fish waste after processing to whole fish proportion in fishmeal become a problem through time and leads to variation of fishmeal quality with places and type of resources used (Olsen and Hasan, 2012). Use of fishmeal as major protein source in fish diet increased the diet price and brought profit consequences (El-Saidy and Gaber, 2003; Kyeong-Jun *et al.*, 2010). In global aquaponics activities, the major protein source is expected to be fishmeal and the aforementioned challenges are also evident for this sector too. Aquaponics diet takes the major input expense from the operation and protein is the expensive component in fish diet because of the high cost of primary protein source, which is fishmeal.

Aquaculture including aquaponics development in Ethiopia remains at its infancy due to lack of information and investment opportunities with regard to fish fingerlings, infrastructure, and feed. Therefore, providing the information on availability and accessibility of technologies and resources on which aquaponics is dependent is mandatory. Developing new feed formulation to fill the existing gap in relation to nutrition and also economics should be the research arena to African countries so as to shape their national action plan on aquaculture (Hecht, 2007) including aquaponics. Therefore, future aquaculture development including aquaponics in Ethiopia depends

on the availability of quality feed that considers demands of fish, plant, and beneficial microorganisms in sufficient quantity and affordable price.

Despite its quality as fish feed ingredients replacing fishmeal by plant ingredients would have enormous advantages on access and also price (Lieberta and Portz, 2005). Several plant ingredients have been tested as major protein ingredients in fish diet (Khan *et al.*, 2013); for instance Soybean (Shiau *et al.*, 1987; Lin and Luo, 2011; Khan *et al.*, 2013; Suloma *et al.*, 2014; Mohamed *et al.*, 2015), Sunflower (Sanz *et al.*, 1994; Mérida *et al.*, 2010), Cotton seed (Abdel-Fattah, 1990; Anderson *et al.*, 2016), Canola (Collins *et al.*, 2012; Ngo *et al.*, 2016), linseed (Hossain and Jauncey, 1989; Regost *et al.*, 2003), Peanut (Garduno-Lugo and Olvera-Novoa, 2008; Cai *et al.*, 2013). Despite the increased use of plant ingredients in fish diet managing the mineral content and availability of the feed for aquaponics production system gets little or no attention.

Animal-based dietary ingredient alternatives for fishmeal have been tested for long time. The most commonly used include feather meal, meat bone meal, poultry byproduct (El-Sayed, 1998). However, the quality of such ingredients varies based on place of origin, animal condition, and environmental factors hence developing standard feed formula with such ingredients will remain challenging especially for aquaponics system but the research field is open for any researcher. Among repeatedly tested animal based ingredients blood meal brought reportedly best growth and feed utilization efficiency (Ng and Romano, 2013).

Diet ingredient selection should consider availability, quality, cost, environmental issue, and sustainability. The conventional fish diet optimization experiments mostly emphasize on reducing price while maintaining diet quality for best fish growth. However, in aquaponics diet formulation, fish and plant requirement should be considered equally to earn highest economical return per unit

effort. Therefore, selection of ingredients should take mineral demand of fish, plants, and microorganisms into consideration.

Minerals in fish diet exist in two forms as an integral part of ingredients mostly organically bound and as a mineral premix usually formed from inorganic salts. The organically bound minerals need to pass through long and complex biological and chemical processes to be available for fish and to be released to the water for plant and microbial consumption (NRC, 1999). Mineral from inorganic supplements can be relatively easily released to fish and to the water from which fish can absorb through gill and skin directly (NRC, 1999). Therefore, increasing inorganic mineral supplementation in fish diet or independent inorganic fertilizer supplementation in the system has positive impact on the productivity of the system by considering the toxic level of the element on fish and plant.

For aquaponics, diet selection criteria of ingredients mainly should consider mineral dissolution quality to the water; this can be affected by multiple factors including digestibility of ingredients. Commonly used inorganic salts for feed and hydroponic solution formulation are similar except for boron, molybdenum, selenium, sodium, chlorine, and iodine sources (Rush, 2012; Ng and Romano, 2013). So far, there is no detail investigation to optimize these inorganic salts for use in aquaponics through dietary modulation.

In order to meet mineral requirement of aquaponics crops (fish and plant), dietary mineral source must be available. Dietary mineral sources can be organic feed ingredients and inorganic salts. Selection of mineral source based on digestibility, availability, and affordability might seem to be an alternative to optimize fish diet, however, availability of minerals for aquaponics crops depend on plenty of factors. Fish ability to convert organically bound minerals to readily available form

is limited due to gastric acidic condition. Minerals in ionic form usually make complexes with other ions and might be unavailable for aquaponics crop (Ng and Romano, 2013). The basic problem to use plant ingredients as fish diet component is associated with the presence of phytic acid, which makes calcium, phosphorus, and zinc unavailable (Portz and Liebert, 2004). Phytase enzyme supplementation or inoculation of phytase producing microorganisms might ameliorate the problem but there is no evidence reported for aquaponics. In addition, in simple ionic form a high concentration of calcium and phosphorus react with magnesium and zinc to form insoluble precipitate. Dietary supplementation of copper, iron, manganese, selenium, and zinc in fish diet is evident due to low level of ions in feed or due to interaction with other ions (Portz and Liebert, 2004). This indicates the presence of minimum amount of such minerals in aquaponics system while the system mineral demand increases beyond fish demand due to incorporation of plant in the system. Therefore, revising the mineral supplementation levels and mineral salt types might need further investigation but seems to be the sustainable alternative to balance minerals in the system for highest system performance.

Chapter 3

Growth and physiological response of Nile tilapia (*Oreochromis niloticus* L.) to Niger seed cake inclusion in fish diet, as a major protein and mineral source in Tilapia -Lettuce based aquaponics system.

3.1 Introduction

Nile tilapia (*Oreochromis niloticus* L. 1978) is the third globally cultured fish with 4.48% contribution to the global fish production (El-Sayed, 1999; El-Sayed, 2006). Nile tilapia is widely

cultivated in closed aquaculture systems including aquaponics due to its hardy nature, wide feeding spectrum, fast growth rate and good seed availability (Lovell, 1991). Increased Nile tilapia aquaculture production per unit effort in closed recirculating production system is a function of scientific achievements on strain selection, efficient system engineering, and diet formulation.

Aquaponics is believed to have a tremendous potential to maximize production benefit of existing aquaculture and hydroponic technologies (Lennard and Leonard, 2004; El-Sayed, 2006). Several achievements have been recorded for the successful cultivation of different fish and plants in aquaponics of which Nile tilapia is the repeatedly tested fish (Rakocy and Hargreaves, 1993; Rakocy, 1997; Klinger and Naylor, 2012; Roosta, 2014a; Love *et al.*, 2015a). However, low yield of fish and plant in aquaponics (Klinger and Naylor, 2012) remains a bottleneck for the technology expansion and advancement. Different scholars present the deficiency of P, K, Fe, Mn, and S in plants in aquaponics systems that totally depend on commercial fish diet (Seawright *et al.*, 1998; Tyson *et al.*, 2011). Aquaponics diet (fish feed) is the major input which determines the productivity potential of the system.

Aquaponics production efficiency correlates with system input (feed) quality and quantity with regard to growth and body functioning requirements of plants, fish, and microorganisms. Low production might be associated with lack of distinct feed formula specific to aquaponics system that considers the physiological demand of the three organisms: fish, plant and microbes.

The existing commercial Nile tilapia feed considers only the fish component and using it in aquaponics creates a nutrient shortage for plant growth. According to previous study (Kassahun *et al.*, 2012) 60% of the existing commercial diet cost goes to Nitrogen (protein) of which 20-25% is assimilated in fish (Turcios and Papenbrock, 2014). In conventional thinking of aquaponics the

remaining nitrogen will be assimilated into plant biomass (Turcios and Papenbrock, 2014). However, other needs of plants and microorganisms should be addressed to achieve higher yield (Savidov *et al.*, 2007; Roosta, 2014b). Hence, Rakocy (2012) recommends supplementation of the system with mineral salts to achieve higher yield but this deviates from the basic principle of aquaponics and this shows the need for adjusting feed quality specific for Aquaponics system. Some aquaponics operators use nutrient supplement to the system in addition to fish feed; however, fish and plant needs should possibly be optimized in one input formula. To formulate new diet ingredients in the diet, selection should be based on availability, cost, and efficiency for plant and fish biomass.

The current major nitrogen source in fish feed is fishmeal, but the decline of its global production, increased cost, inaccessibility and its high water stability with regard to macro and micro nutrients for plant triggers the need to look for an alternative, efficient, sustainable protein and mineral source for aquaponics (Tran-Duy *et al.*, 2008). Several researchers have tested different plant ingredients as major protein source in Nile tilapia feed, some of which are moringa extract (Portz and Liebert, 2004; Astuti *et al.*, 2009), linseed cake (Bell *et al.*, 2004), mixture of plant sources (El-Saidy and Gaber, 2003) and caraway seed (Ahmad and Abdel-Tawwab, 2011). Currently, there is no distinct diet formula for Aquaponics and hence all experiments use recommendations given for recirculating aquaculture systems. Dealing with two crops (fish and plant) with single input (fish feed) makes aquaponics optimization more complex. Hence, searching for best feed formula which addresses an optimal yield of aquaponics with available, cheap and sustainable feed resources is found essential. Among various alternative potential nitrogen sources Niger seed cake is a potential candidate for fish feed formulation. Niger seed (*Guizotia abyssinica*) is an oil seed which constitutes 50% of Ethiopian oil seed production (Getinet and Sharma, 1996). The

remaining of oil extract (Niger seed cake) is widely used as animal feed in Ethiopia, it contains 30-34% crude protein, 8.3-9.4% ether extract, 21.7-27.3% crude fiber and 10.1-11.3% ash (Getinet and Sharma, 1996; Chadd *et al.*, 2002; Kassahun *et al.*, 2012; Feedipedia., 2013). Niger seed cake is found to replace (100%) linseed cake and groundnut cake as protein source for calf and White Yorkshire pigs, respectively (Getinet and Sharma, 1996). Its nutritional composition and its being relatively free from antinutritional factors dictate its potential to be used in fish feed (Chadd *et al.*, 2002). In addition, its amino acid profile (Isoluecine, 4.66%; Leucine, 6.99%; Lysine, 4.74%, Methionine, 2.06%; Phenylealanine, 4.8%; Threonine, 3.73%; Valine, 5.76%; Arginine, 9.36%; and Histidine, 0%) is comparable with the recommended amino acid amount in Nile tilapia feed (Isoluecine, 3.11%; Leucine, 3.39%; Lysine, 5.12%, Methionine, 2.68%; Phenylealanine, 3.75%; Threonine, 3.75%; Valine, 2.8%; Arginine, 4.2%; and Histidine, 1.72%) (Getinet and Sharma, 1996; FAO, 2014a). With respect to plant need; Niger seed cake is reported to have 6.89% (N), 1.18% (P), 0.7% (Ca), 62% (Mg), 0.1% (Na), 1.16% (K), 73 ppm Cu, 90 ppm Zn and 169 ppm Fe (Little *et al.*, 1986). Therefore, this experiment tests the performance of Niger seed cake inclusion in fish diet in different proportion as a function of fish growth and nutrient dynamics in aquaponics.

3.2 Materials and methods

3.2.1 Description of the experiment setup

The experiment was conducted at Addis Ababa University aquaponics research facility. The experimental setup constituted 10 fish tanks (circular, plastic) with 250 L water holding capacity. Each tank was attached to 80 L biofilter tank, which contains 0.25 Kg bioballs and attached to 20 L clarifier (Figure 3). The plant component was nutrient film techniques (NFT) with 44 planting pots attached to each fish tank. Each system was autonomous in every aspect and identical.

Throughout the experiment, nutrient water flow from fish tank to clarifier then to biofilter and pumped up to hydroponics gullies and back to fish tank by gravity maintaining similar hydrological characteristics recommended for aquaponics by previous trials including hydraulic loading rate and hydraulic retention time (Endut *et al.*, 2010; Rakocy, 2012; Rush, 2012). Hydraulic loading rate (1.28 m(d)^{-1}) and hydraulic retention time (2.3 h) were maintained identical following the recommendation of Endut *et al.* (2010).

All units utilized municipal tap water and received identical system management procedures to minimize experimental errors between treatments. The pH, temperature, and dissolved oxygen of the growing medium were regulated using acid-base addition, submersible thermostat, and aerators, respectively.

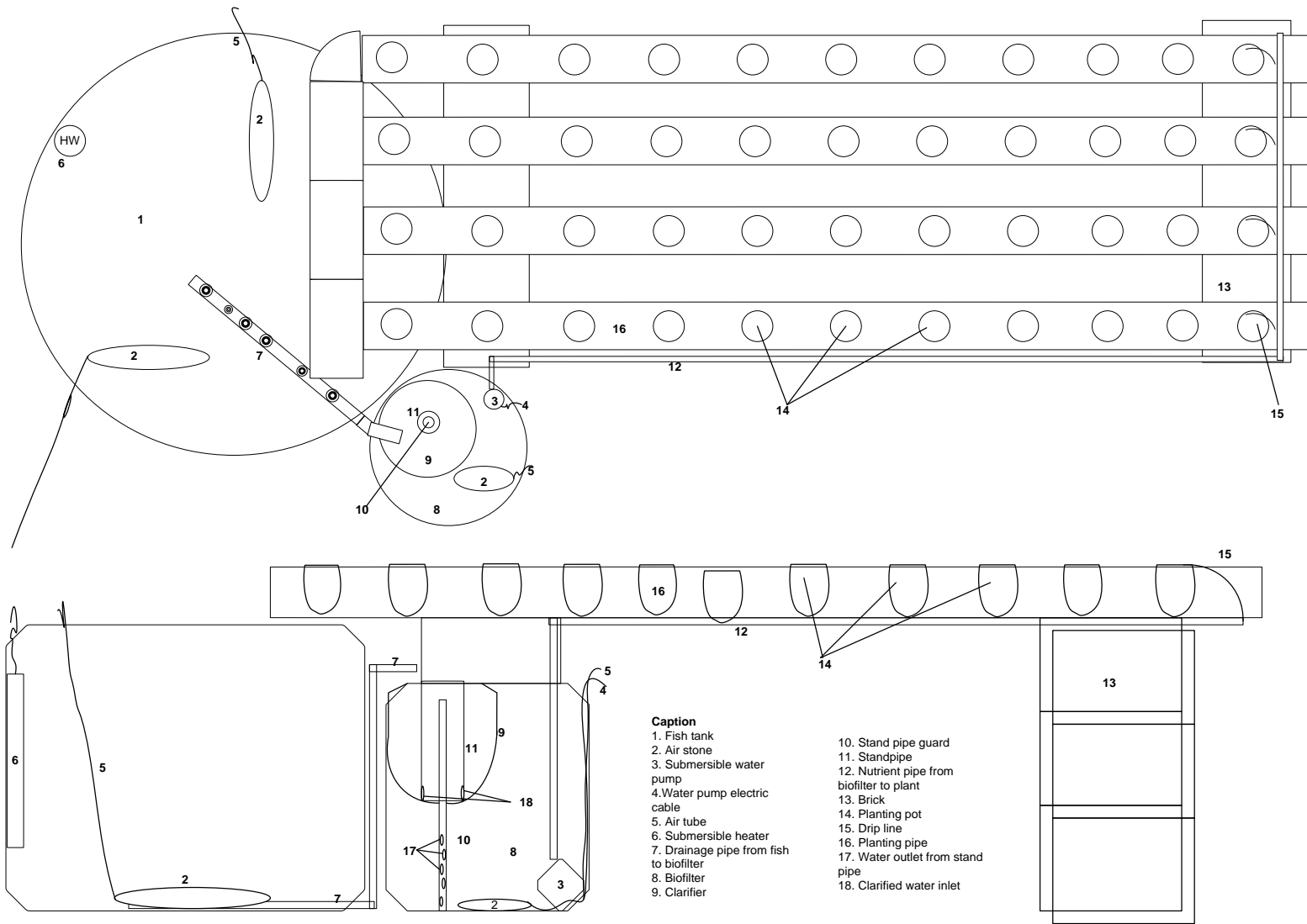


Figure 3 Schematic non-scale diagram of aquaponics unit used in the experiment; top view (above) and side view (below).

3.2.2 Feed preparation and preservation

Five experimental diets were formulated containing five different Niger seed cake inclusion (percentages) with fishmeal cost (NC:FM ratio): 0 (D₁), 0.29 (D₂), 0.83 (D₃), 2.14 (D₄) and 3.4 (D₅) (Table 1). Ingredients were homogenized for 40 minutes in juice mixer to get all ingredients mixed equally then the experimental diets were prepared by passing the hot paste of ingredients through 2 mm diameter size meat mill to get pelletized (Abdel-Tawwab *et al.*, 2010). Pellets were dried in shadow and preserved in deep freeze at -18 °C till consumption (Al-Souti *et al.*, 2012).

Fingerlings (31.47± 0.783 g (mean ± SE)) were obtained from Ziway Fishery Research Center and were stocked at a density of 20 kgm⁻³ in each tank. Fingerlings were acclimated for two weeks prior to the experiment and all cleaned with sterilized saline solution, anesthetized by clove oil solution for 20 Sec and weighed and distributed to each experimental tank in equal biomass (Simões *et al.*, 2011). All treatments were done in two replicates.

3.2.3 Feed regime

Fish were fed experimental diet with 3% of body weight per day (FAO, 2014a) administered twice a day (Abdel-Tawwab *et al.*, 2010) at 07:30 and 17:30 throughout the experiment for a month. Feed ration adjustment was made based on mean weight increment of fish at each sampling week.

Table 1 Composition and percentage proximate composition of the experimental diet.

Ingredients (gKg⁻¹)	D₁	D₂	D₃	D₄	D₅
Fishmeal	550	425	300	175	125
Meat bone meal	150	150	150	150	150
Niger seed cake	0	125	250	375	425
Wheat grain	150	150	150	150	150
Wheat bran	100	100	100	100	100
Limestone	7	7	7	7	7
DiCalcium phosphate	10	10	10	10	10
Vitamin-mineral premix ¹	3	3	3	3	3
Fish oil (mlkg ⁻¹)	2	2	2	2	2
Soyabean oil (mlkg ⁻¹)	20	20	20	20	20
Percentage composition (%) ²					
Dry matter	93.8	92.9	92.2	92.17	92.16
Crude protein	32.2	30.6	29.2	28.9	28.8
Fat	5	5.3	5.9	6.7	7.2
Ash	15.96	13.57	11.32	10.61	9.97
K	0.297	0.388	0.389	0.391	0.483
Ca	6.47	6.95	5.6	4.4	3.2
Fe	0.0019	0.0016	0.0014	0.0013	0.0018
NC:FM ratios	-	0.29	0.83	2.14	3.4

¹ Vitamin-mineral premix (mgkg⁻¹); Vitamin A (retinol) 2100, Vitamin D₃ (Chole-calciferol) 50, Vitamin E 10000IU, Vitamin k₃ 2000, Thiamine 1000, Riboflavin 4000, Niacin 10000, Panthothenic acid 5000, Pyridoxine 750, Folic acid 250, Vitamin B12 8, Vitamin H as Biotin 30, Betain 100000IU, Antioxidant 125000IU, Manganese 80000, Zinc 50000, Iron 20000, Copper 5000, Iodine 1200, Cobalt 200, Selenium 200.

² Proximate composition calculated as dry matter basis

3.2.4 Data collection

Data on water quality parameters; pH (Hanna HI9124), Dissolved Oxygen (DO) (Hanna HI9142), Temperature (T) (°C) (Thermometer) and Electrical Conductivity (EC) (Jenway pH and EC meter) were collected in three days interval for the entire experimental period.

Total wet weight measurements of fish were taken weekly from each treatment. Six fish from each treatment were sacrificed and preserved at -18 °C for proximate composition analysis. Proximate composition (moisture content, crude protein, crude fat and ash content) analysis of feed and fish were done according to standard procedure (AOAC, 2000); Moisture content by drying at 80 °C for 48 h, crude protein using Kjeldahl nitrogen and total lipid by di-ethyl ether extraction with Soxhlet and ash by incineration in muffle furnace at 550 °C for 6 h. Mineral proximate content of fish were analyzed according to standard procedures of AOAC (2000) using Atomic absorption spectrophotometer for Calcium and Iron, and flame photometer for Potassium.

Fish tank water, feed and fish mineral content analysis were performed in Lennart Mansson International Laboratory (Sweden) (using Inductively coupled plasma spectrophotometer), Addis Ababa University, and Ethiopian Institute of Health and Nutrition (using Atomic absorption spectrophotometer, flame photometer and Ultraviolet visible spectrophotometer), respectively.

Growth performances of fish were calculated based on wet total weight as a function of treatments. The efficiencies of administered diets were calculated with respect to fish growth performance and diet use efficiency by standard mathematical equations (El-Saidy and Gaber, 2003; Abdel-Tawwab *et al.*, 2010; Gullian-Klanian and Arámburu-Adame, 2013). We calculated the following parameters:

Specific growth rate (SGR_{1-2} ; %/day) = $100 (\ln W_2 - \ln W_1) / (T_2 - T_1)$; where W_1 and W_2 are initial and final fish weight, respectively, and T_1 and T_2 are initial and final sampling days, respectively;

Daily growth rate (DGR, g/day) = $(W_2 - W_1) / t$; where W_1 and W_2 are the initial and final fish weight in gram, respectively, and t is the number of fish growing days;

Relative growth rate (RGR, %/day) = $100 (W_2 - W_1) / W_1$; where W_1 and W_2 are the initial and final fish weight, respectively;

Feed conversion ratio (FCR) = feed intake (g)/weight gain (g);

Protein efficiency ratio (PER) = weight gain (g)/protein intake (g);

Protein productive value (PPV; %) = $100 \times (\text{protein gain (g)} / \text{protein intake (g)})$.

Food intake (F_i) = $(DFI \times 100) / ((W_{tf} + W_{ti}) / 2)$

where DFI is mean daily dry feed intake per fish (t_i , t_f) and W_{tf} , W_{ti} are the averaged wet weights at the start (t_i) and end (t_f) of the experimental period (El-Saidy and Gaber, 2003),

Nutrient deposition (retention) (%) = $100 \times [(W_f \times N_f) - (W_i \times N_i)] / (F_i \times N_{fe})$

where W_f is final body weight, N_f is final body nutrient content, W_i is initial body weight, N_i is initial body nutrient, F_i is feed intake and N_{fe} is nutrient in feed (Portz and Liebert, 2004).

3.3 Data analysis

Data on fish growth performance, feed use efficiency and growing medium quality were tested for statistical difference and similarity using analysis of variance (one way ANOVA) with the statistical package SPSS (v.16.0 for Windows) (Al-Souti *et al.*, 2012; Gullian-Klanian and Arámburu-Adame, 2013). Data reaching significance were further subjected to least square deviation (LSD) analysis. Growth and physiological efficiency impacts of test diets were subjected to linear and quadratic contrasts based on two basic comparisons; a) if growth and efficiency response of control diet has variation from test diets and b) if the response for the Niger seed cake to Fishmeal ratio in test diets is either linear or quadratic. Treatment effects were considered significant at $p < 0.05$ for mean difference analysis. However, tests for fixed effects considered significant at $p < 0.1$ and all non-linear contrasts are considered significant at $p \leq 0.1$ and $r^2 \geq 0.25$ (Koch *et al.*, 2016).

3.4 Results and discussion

3.4.1 Growing medium quality

Physical and chemical characteristics of rearing units were maintained in equilibrium between treatments during the initiation of the experiment ($p \geq 0.05$). During the experiment DO remained from 0.67 to 2.58 mgL⁻¹. The decrease in DO may be associated with the high oxygen consumption of microorganisms for ammonia oxidation process in addition to fish and plant oxygen consumption (Tyson *et al.*, 2011). The pH of the fish tank water ranged between 6.9 and 7.5, which is an acceptable range for healthy growth and functioning of tilapia (Tyson *et al.*, 2011; Bhavimani and Puttaiah, 2014) (Table 2).

Table 2 Physico-chemical measurements (Mean±SE) of fish tank water. Mean values sharing common letters are not significantly different (p < 0.05)

Treatm ent	Dissolved Oxygen (mgL⁻¹)	pH	Temperature (°C)	Electrical conductivity (µS(cm)⁻¹)
D ₁	0.67±0.08 ^a	7.46±0.02 ^a	24.5±0.51 ^a	339±11.64 ^a
D ₂	2.29±0.22 ^b	6.96±0.07 ^b	23.3±1.36 ^a	323±10.04 ^a
D ₃	2.58±0.16 ^b	6.92±0.03 ^b	25.36±0.53 ^{abd}	263±10.94 ^b
D ₄	1.9±0.13 ^b	7.16±0.02 ^c	23.18±0.42 ^{abe}	207±5.60 ^c
D ₅	1.33±0.16 ^c	7.14±0.06 ^c	26.88±0.40 ^{ac}	271±10.07 ^b

Proximate composition of the experimental diets is in a standard range for Nile tilapia nutrient need for good growth and development. The presence of nutrients in the feed might not mean that all the nutrients will go to fish body but it, at least, indicates the maximum available proportion of nutrients in the input (feed) (Table 1). Mineral composition of the experimental diet is with-in the range of minerals in fish diet composed of different ingredients as reported by previous studies (Rafieea and Saad, 2005; Goddard *et al.*, 2010).

3.4.2 Biomass production

Effects of Niger seed cake (NC) inclusion in fish diet in aquaponics were expressed with growth response parameters. Final weight, weight gain, and growth rates increased with increased NC inclusion up to 37.5% (D₄). Hence, D₄ showed significantly highest weight gain (WG), final weight (FW), DGR, SGR and RGR as compared to other experimental treatments (p < 0.05) and significantly comparable with growth response of D₁ (0%) (Table 3, Figure 4, Figure 5, Figure 6).

Fish biomass production and growth rate response (FW, WG, DGR, SGR and RGR) were quadratically related to dietary NC:FM ratio (Figure 4, Figure 5, Figure 6). For example, measures of FW ($y = 38.71 + 17.17 * x + -4.34 * x^2$; $r^2 = 0.991$; $p = 0.09$), WG ($y = -4.65 * x^2 + 17.86 * x + 9.32$; $r^2 = 0.772$, $p=0.08$) and DGR ($y = 0.49 + 0.36 * x + -0.099 * x^2$; $r^2 = 0.989$; $p = 0.09$) were quadratically correlated with dietary NC:FM ratio. Quadratic fish growth response for different inclusion levels of plant based ingredients reported by different researchers have indicated that intermediate combination of plant based diet ingredients to FM brought better growth than extreme combinations (El-Saidy and Gaber, 2003; Koch *et al.*, 2016). Results on SGR of this experiment were in agreement with other experiments conducted using different plant materials as feed ingredient for Nile tilapia in aquaculture: jatropha 5 (Kumar *et al.*, 2012), canola 2.39 (Plaipetch and Yakupitiyage, 2014), soybean 2.29 (Abdel-Warith *et al.*, 2013), plant protein 2.64 (Fontainhas-Fernandes *et al.*, 1999). An increment of growth rate through Niger seed cake inclusion might be associated with the possible high C:N ratio in diet which possibly triggers the biofloc development in the system and as a result fish might have taken advantage for their physiological demand (De Schryver *et al.*, 2008).

Table 3 Growth response of Nile tilapia (Mean ±SE) for experimental diets.

Treatment	NC:FM Ratio ¹		Response ²								
			IW ³	Wf ⁴	WG ⁵	DGR ⁶	SGR ⁷	RGR ⁸	FCR ⁹	PPV ¹⁰	PER ¹¹
D ₁	00	Mean	32.5	41.927	22.817	0.815	1.892	71.149	1.619	29.259	2.000
		SE	1.43	1.419	6.089	0.217	0.475	23.359	0.351	6.551	0.446
D ₂	0.29	Mean	31.33	39.127	16.850	0.602	1.544	56.208	2.135	24.572	1.653
		SE	1.48	1.375	7.109	0.254	0.628	30.187	0.533	9.627	0.609
D ₃	0.83	Mean	30.83	39.660	19.867	0.710	1.781	67.106	1.843	30.332	2.025
		SE	2.21	1.951	7.566	0.270	0.657	33.182	0.535	12.101	0.710
D ₄	2.14	Mean	32	44.177	23.217	0.829	1.934	73.286	1.753	34.368	2.181
		SE	2.02	1.240	7.231	0.258	0.493	24.877	0.608	11.800	0.735
D ₅	3.4	Mean	30.67	39.070	16.383	0.585	1.514	54.380	2.626	24.722	1.702
		SE	2.03	1.037	7.291	0.260	0.579	23.290	1.878	9.926	0.683
	Pooled SEM				1.31	0.047	0.102	4.84	0.175	1.86	0.2
Fixed effect	Control vs. TA-TD Diet			Pr>F	0.58*	0.361**	0.046*	0.075*	0.001**	0.4**	0.007*
	TA-TD, NC:FM, quadratic			Pr<F	0.11	0.11	0.002	0.019	0.02	0.074	0.086
	TA-TD, NC:FM, quadratic Goodness of fit ¹²			R ²	0.986	0.986	1	1	0.99	1	0.987

contrast is linear ** The contrast is quadratic

¹ Niger seed cake to Fish meal ratio

² % fresh weight basis

³Initial weight (g)

⁴Final weight (g)

⁵Weight gain (g)

⁶Daily growth rate (g/day)

⁷Specific growth rate (%/day)

⁸Relative growth rate

⁹Feed conversion ratio (%)

¹⁰Protein productive value (%)

¹¹Protein efficiency ratio (%)

¹² R² represents how well the trend fits the treatments mean

* The

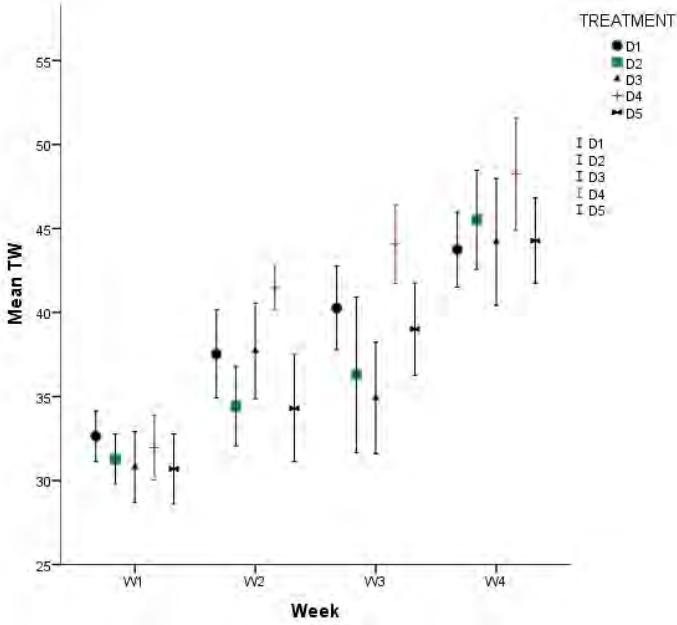


Figure 4 Mean TW (g/fish) of fishes with growth period. (Bar represent level of SE of the means)

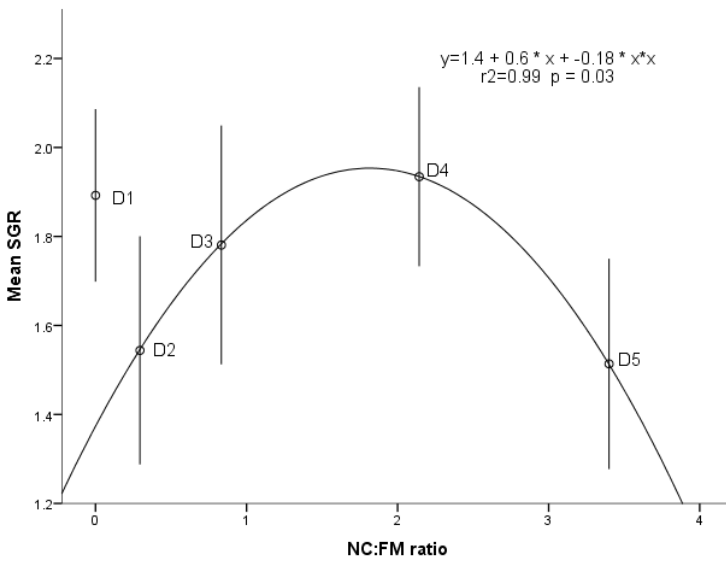


Figure 5 Specific growth rate of fish as a function NC:FM ratio in diet.

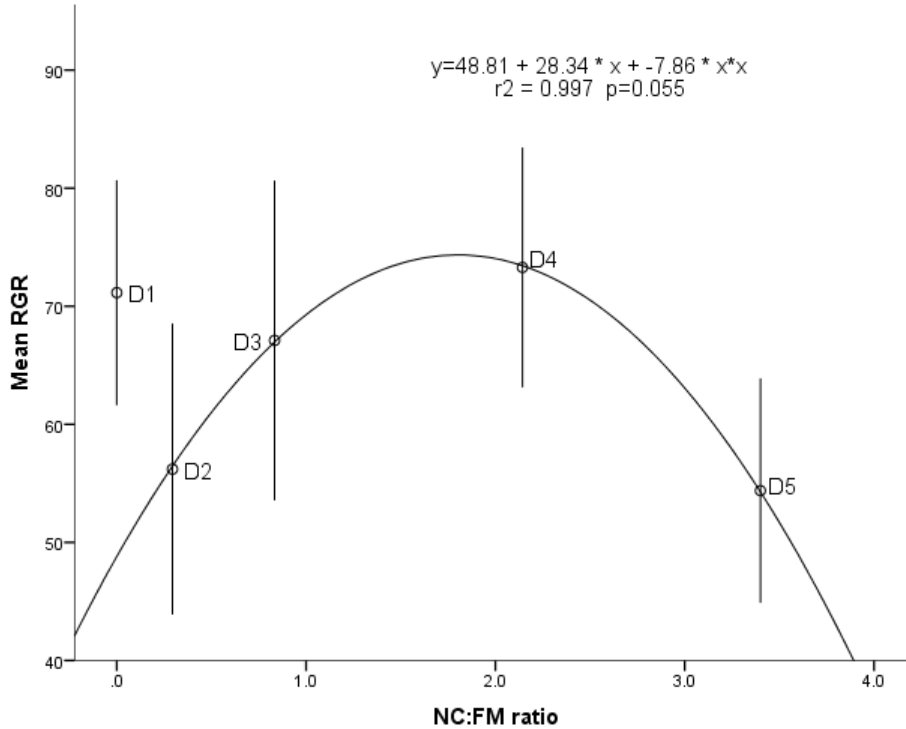


Figure 6 Relative growth rate of fish as a function of NC:FM ratio in diet.

Feed conversion efficiency of tilapia increased with increased proportion of Niger seed cake in fish diet. Increased dietary NC:FM ratio brought quadratic response ($y = 2.39 + -0.91 * x + 0.29 * x^2$; $r^2 = 0.999$; $p = 0.026$) to FCR and the lowest calculated value was obtained from D₄ (Table 3, Table 5, Figure 7). This might be related to the additional carbon sources that come to D₄ due to higher percentage of Niger seed cake, which will trigger the development of biofloc in the system. Niger seed cake efficiency in aquaponics for Nile tilapia was comparable with other plant based protein sources in aquaculture. Feed conversion ratio found in this experiment was comparable with results of studies done on soybean 1.09 - 1.4 (Abdel-Warith *et al.*, 2013; Plaipetch and Yakupitiyage, 2014), micronized wheat, full fat toasted soybean, defatted soybean meal, extruded pea seed meal, pea seed meal, lupin and faba bean meal 1.2-1.56 (Fontainhas-Fernandes *et al.*, 1999; Azaza *et al.*, 2009b) and soybean and jatropha 1.7 (Kumar *et al.*, 2012). In contrary, some

previous experiments found a decrease of FCR with decreasing dietary crude protein content (Siddiqui *et al.*, 1988). However, FCR from this study is higher than FCR reported by previous study 1.1-1.21 (Dediu *et al.*, 2012) and some studies report highest feed conversion efficiency 0.9-0.97 (Goddard *et al.*, 2010; Shete *et al.*, 2016). Tilapia adaptation for Niger seed cake based diet increases with culture period (Figure 4). This is manifested by increased feed efficiency through culture periods. The variation between culture period is significantly different ($p < 0.05$) and final sampling week showed significantly higher FCR ($p < 0.5$) than other culture periods. Mineral composition of diet with respect to K ($y = 0.6 + 8.64 * x^2$; $r^2 = 0.81$, $p = 0.1$), and Fe ($y = 6.01 + -6861.97 * x + 2759646.04 * x^2$; $r^2 = 0.99$, $p = 0.02$) brought quadratically significant impact on FCR response.

Table 4 Proximate composition of Nile tilapia subjected to experimental diets. Mean values sharing common letters are not significantly different ($p < 0.05$).

Treatment	NC:FM Ratio		Response ¹					
			DM ²	Ash ^{3%}	Protein	K ⁴	Ca ⁵	Fe ⁶
D ₁	00	Mean±SE	25.24±0.04 ^a	4.95±0.09 ^a	14.63±0.06 ^a	2.72±0.0083	51.75	0.16 ^a
D ₂	0.29	Mean±SE	25.68± 0.04 ^b	4.84±0.11 ^a	14.79±0.44 ^a	2.883±0.004	52.96	0.155 ^b
D ₃	0.83	Mean±SE	26.67±0.01 ^c	5.95±0.34 ^b	14.81±0.85 ^{bd}	4.02±0.477	68.9	0.139 ^{ac}
D ₄	2.14	Mean±SE	27.57±0.01 ^e	4.57±0.21 ^a	15.74±0.23 ^d	3.73±0.188	60.1	0.123 ^d
D ₅	3.4	Mean±SE	28.06±0.001 ^e	5.79±0.03 ^b	14.53±0.6 ^c	3.06±0.006	53.32	0.143 ^{ae}
Pooled SEM			0.173	0.13	0.11	0.093	1.19	0.02
Contrast, Fixed effect								
D ₁ vs. D ₂ -D ₅		Pr>F	0.00	0.049	-	0.652	-	0.008
D ₂ -D ₅ , NC:FM, quadratic		Pr<F	0.04	0.184	0.49	0.541	0.524	0.069
D ₂ -D ₅ , NC:FM, quadratic		R ²	0.919	0.816	0.867	0.708	0.69	0.868
Goodness of fit								

¹ % Dry weight basis for minerals (K, Ca and Fe) and % wet weight basis for crude protein

² % Dry mater of tilapia whole body

³ % Ash of tilapia whole body

⁴ % Potassium of tilapia whole body as DW basis

⁵ % Calcium of tilapia whole body as DW basis

⁶ Total Iron (mg/100g) of tilapia whole body as DW basis

3.4.3 Proximate composition

Whole fish body proximate composition has responded to the level of Niger seed cake in diet (Table 4). Moisture content of whole fish has significantly decreased with increasing Niger seed cake proportion in fish diet and the lowest obtained was at D₅ ($p < 0.05$). The relationship between dry matter and dietary NC:FM ratio was significantly linear ($y = -3.86 * x + 47.82$; $r^2 = 0.989$, $p = 0.005$). Crude protein content significantly increased with increased Niger seed cake inclusion in diet up to 37.5% (D₄) then decreased ($p < 0.05$). Significantly, higher ash content was found on D₃ and D₅ ($p < 0.05$). Crude protein composition of tilapia showed significantly linear response for dietary NC:FM ratio ($y = 0.72 * x + 25.79$; $r^2 = 0.919$, $p = 0.04$). Dry matter and crude protein were significantly affected linearly by dietary total nitrogen content ($p < 0.05$) (Table 4) and similar correlation was observed in previous study (Siddiqui *et al.*, 1988). However, crude protein content of fish increased with decreased diet protein content, which is contrary to the study of Siddiqui *et al.*, (1988), and this might have been caused by biofloc development or impact of the carbon load in aquaponics system from root exudates. Crude protein of fish in this experiment was found to be in agreement with the results obtained from different studies on tilapia fed by soybean 14.4-16.7 (Abdel-Warith *et al.*, 2013) faba bean 15.6-16.2 (Azaza *et al.*, 2009a) , fishmeal and pea nut leaf meal 14.9-17.3 (Garduno-Lugo and Olvera-Novoa, 2008), sesame meal 16.05-16.9 (Guo *et al.*, 2011) and caraway seed meal 16.7-17 (Ahmad and Abdel-Tawwab, 2011).

Protein productive value ($y = 20.43 + 15.48 * x + -4.1 * x^2$, $r^2 = 0.999$, $p = 0.032$) and PER ($y = 1.45 + 0.83 * x + -0.22 * x^2$, $r^2 = 0.987$, $p = 0.1$) of Nile tilapia were significantly increased quadratically with increased dietary NC:FM ratio. However, the mean difference between different diets was not significant ($p > 0.01$). PPV in this study was significantly linearly correlated with fish RGR

value ($y = 1.88 * x + 9.06$, $r^2 = 0.99$, $p = 0.007$) (Figure 8). PPV in this experiment was within the range of other findings 24.3-32.4 (Kumar *et al.*, 2012; Krome *et al.*, 2014). Niger seed cake protein efficiency in aquaponics was comparable with soybean meal 1.39-2.5 (Thompson *et al.*, 2012; Plaipetch and Yakupitiyage, 2014), faba bean meal 2.04-2.34 (Azaza *et al.*, 2009b) and canola diet 2.1-2.46 (Plaipetch and Yakupitiyage 2014) in aquaculture. According to Azim and Little (2008), tilapia can perform good in higher C:N ratio at lowest protein concentration and they found increased feed utilization performance with increasing C:N ratio and decreasing protein content (De Schryver *et al.*, 2008; Crab *et al.*, 2009; Li *et al.*, 2016). Some investigations associated low growth performance of fish fed with plant based ingredients with the presence of phytic acid which might be present in NC. However, result of this study showed increased growth and physiological performance of fish with increased NC inclusion and this might be associated with the presence of microbial biota in the system which might produce phytase enzyme (Lieberta and Portz, 2005).

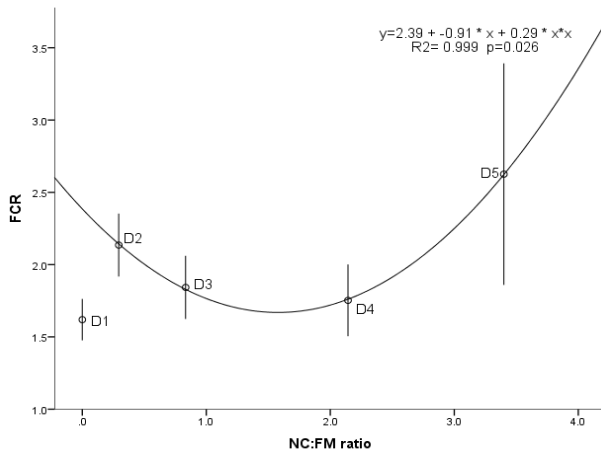
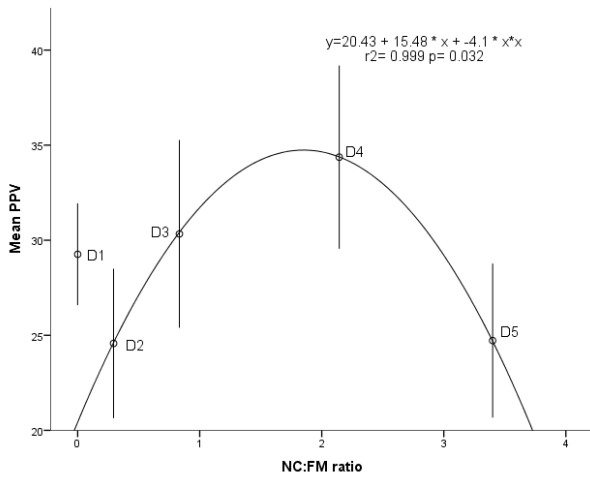
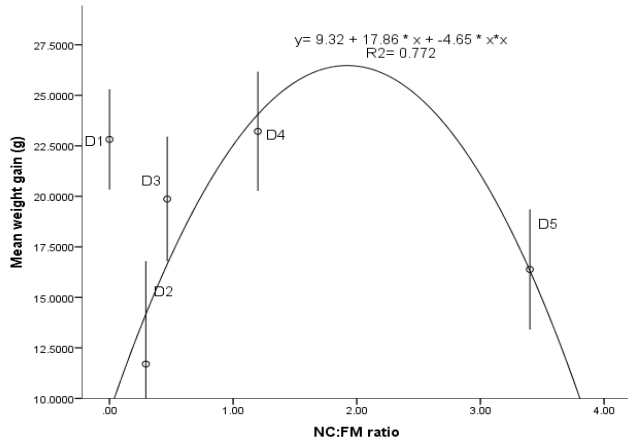


Figure 7 Mean (\pm SE) Weight gain (top), Protein Productive Value (middle), and Feed conversion ratio (bottom) with respect to dietary NC:FM ratios.

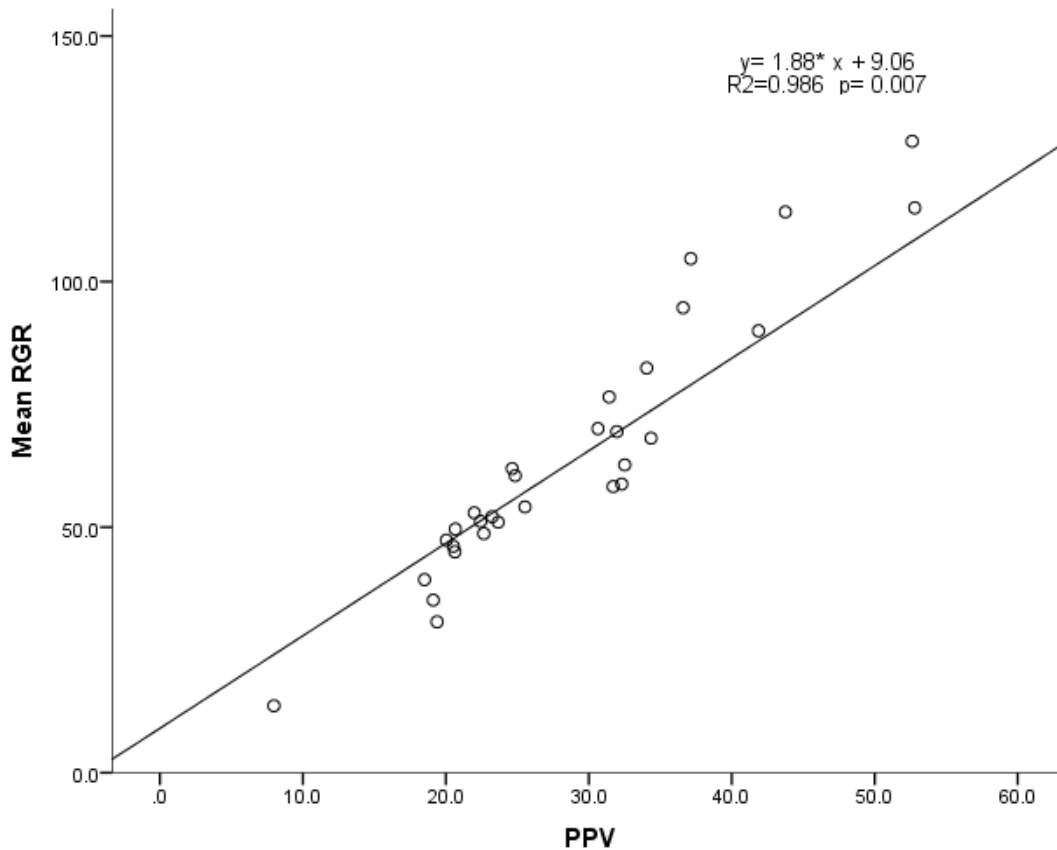


Figure 8 Correlation between mean RGR and mean PPV.

Mineral composition of whole fish was significantly affected by level of NC dietary inclusion ($p < 0.05$). Among treatments significantly highest and lowest Potassium content of fish resulted from D₃ (25%) and D₁ (0%) treatments, respectively ($p < 0.05$). Calcium and Potassium proximate composition of fish were found significantly higher in D₃ (25%) ($p < 0.05$) and Iron composition is higher in D₂ (12.5%) treatments (Table 4). The result of this study on mineral proportion of fish was found to be within the range reported by previous studies (Liang and Chien, 2013).

Mineral retention efficiency was quadratically significant with dietary NC:FM ratio ($p < 0.05$) (Table 5). Potassium, Calcium and Iron retention efficiency was significantly higher in D₄ (37.5%) ($p < 0.05$) (Table 5, Figure 9, Figure 10). An intermediate combination of Niger seed cake to fishmeal provided highest mineral retention efficiency than the two extreme combinations and this finding was in agreement with previous findings (Gonzales and Brown, 2007; Crab *et al.*, 2009). Mineral assimilation efficiency increased while mineral content in fish tank water decreased and this might be associated with the efficiency of fish to assimilate minerals with increased NC proportion up to 37.5% (D₄).

Table 5 Mineral retention efficiency of fish from experimental diets.

Treatment	NC:FM ratio	FCR ¹	FI ²	KR ³	CaR ⁴	FeR ⁵
D ₁	00	1.62±0.14	2.09±0.07	21.20±3.07	29.62±4.51	0.36±0.03
D ₂	0.29	1.96±1.77	2.39±0.18	19.67±5.32	23.89±5.64	0.26±0.07
D ₃	0.83	1.84±0.22	2.11±0.10	40.01±6.67	55.44±7.24	0.31±0.09
D ₄	2.14	1.75±0.25	2.05±0.07	40.83±5.59	68.79±7.90	0.47±0.07
D ₅	3.4	2.63±0.77	2.19±0.09	16.48±2.57	60.18±8.34	0.16±0.03
Pooled SEM		0.37	0.05	2.93	4.53	0.03
D ₁ vs. D ₂ -D ₅ Diet	Pr<F	0.199	1.478	6.682	9.493	3.839
D ₂ -D ₅ , quadratic	NC:FM, Pr<F	0.098	0.009	0.029	0.015	0.036
D ₂ -D ₅ , quadratic of fit	NC:FM, R ² Goodness of fit	0.99	1	0.971	0.935	0.964

¹ Feed conversion ratio

² % Feed intake

³ % Potassium retained

⁴ % Calcium retained

⁵ % Iron retained

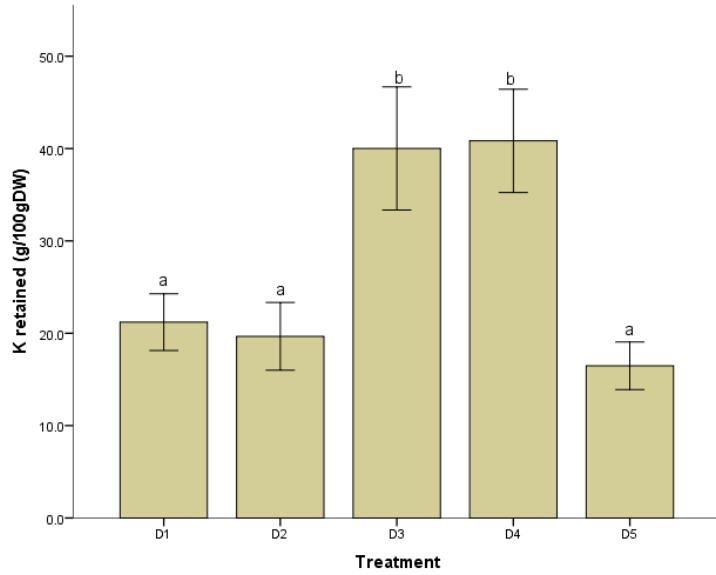


Figure 9 Potassium retained in fish biomass from diet. Mean values sharing common letters are not significantly different ($p < 0.05$).

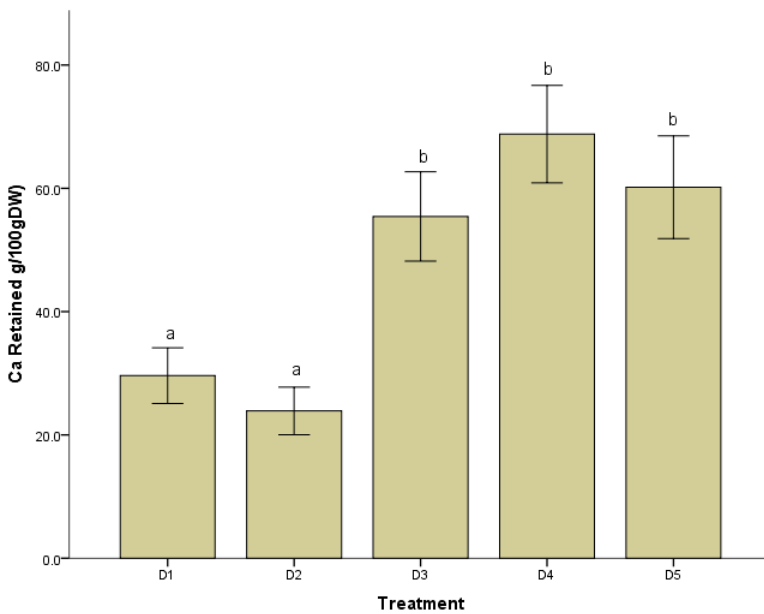


Figure 10 Calcium retained in fish from diet. Mean values sharing common letters are not significantly different ($p < 0.05$).

Table 6 Macronutrient content (mgL⁻¹) of fish tank water. Mean values sharing common letters are not significantly different (p < 0.05).

Treatment	NC:FM ratio		NO ₃	PO ₄	K	Mg	S	Ca	NH ₄
D ₁	0	Mean±SE	7.2±1.62 ^a	10.51±0.11 ^a	14.77±3.94	15.05±1.28 ^a	5.56±2.27 ^a	33.78±3.12 ^a	5.85±1.72 ^a
D ₂	0.29	Mean±SE	8.28±1.32 ^a	13.38±0.14 ^b	13.33±5.22	12.77±0.6 ^b	3.88±0.42	31.93±1.67	0.2±0.00 ^b
D ₃	0.83	Mean±SE	2.33±0.98 ^b	10.25±0.02 ^c	10.85±4.11 ^a	12.52±0.63 ^b	5.32±2.14	29.52±2.45 ^b	1.33±1.67
D ₄	2.14	Mean±SE	2.26±0.93 ^b	12.6±0.02 ^d	14.15±6.07	12.41±2.83 ^b	3.27±0.73 ^b	29.09±4.02 ^b	6.59±5.83 ^{ac}
D ₅	3.4	Mean±SE	5.03±1.05	13.07±0.13 ^c	19.06±7.82 ^b	13.15±1.04 ^b	4.52±2.33	32.61±3.73	0.31±0.28 ^{abd}

Nutrient composition of fish water showed the presence of all essential macro and micronutrients for plant growth and development (Table 6, Table 7). Potassium concentration of fish tank water has significantly linear correlation with dietary Potassium content ($r^2 = 0.86$, $p = 0.072$) (Figure 11). This correlation indicates the possibility to manage Potassium content of fish tank water through manipulation of dietary Potassium content. Calcium concentration of fish tank water showed significant quadratic correlation with dietary Calcium content ($r^2 = 0.994$, $p = 0.074$) achieving the lowest concentration in D₃ (Figure 12). The result of this study is in agreement with the study on the use of catfish pond water for the production of lettuce ($12 \text{ mgL}^{-1} \text{ K}$ and $10 \text{ mgL}^{-1} \text{ Ca}$) in aquaponics (Sikawa and Yakupitiyage, 2010).

All experimental treatments showed lower nitrate content in comparison with the control diet. Measurement of PO₄, K, Mg, Ca, and NH₄ showed quadratic response for dietary NC:FM ratio and lowest response was from D₄, D₃, D₃, D₄, and D₄, respectively. Nitrate content showed significantly quadratic response for Niger seed cake proportion in fish diet providing lowest concentration at D₃ (37.5%) ($y = 9.56 - 8.38 * x + 2.099 * x^2$, $r^2 = 0.432$, $p = 0.004$) (Figure 13).

The control diets relatively respond to higher macronutrient concentration than all experimental treatments. Mineral concentration in this experiment was found to be higher than the results obtained by other researcher (Rafieea and Saad, 2005; Shete *et al.*, 2016). Water nutrient concentrations depend on the growth and development of plants integrated with the system; hence, the decrease in nutrient concentration in fish water subjected to experimental diets might be associated with the increased biomass development of lettuce plant.

Table 7 Micronutrient content (mgL⁻¹) of fish tank water. Mean values sharing common letters are not significantly different (p < 0.05).

Treatment	Na	Cl	Mn	B	Cu	Fe	Zn	Mo	Al
D ₁	-	-	-	-	-	0.05±0.005 ^a	-	-	-
D ₂	25.32±1.51	8.99±2.68	0.0073±0.01	0.045±0.01	0.004±0.002	0.06±0.003	0.015±0.00502	0.006	0.19±0.011
D ₃	17.54±0.52	4.31±0.69	0.0073±0.01	0.029±0.003	0.0037±0.0020	0.05±0.003 ^a	0.01±0.0049	0.006	1.23±0.18
D ₄	16.45±1.89	5.46±2.35	0.0063	0.035±0.01	0.0063±0.0056	0.07±0.01 ^b	0.0167±0.0184	0.006	0.42±0.01

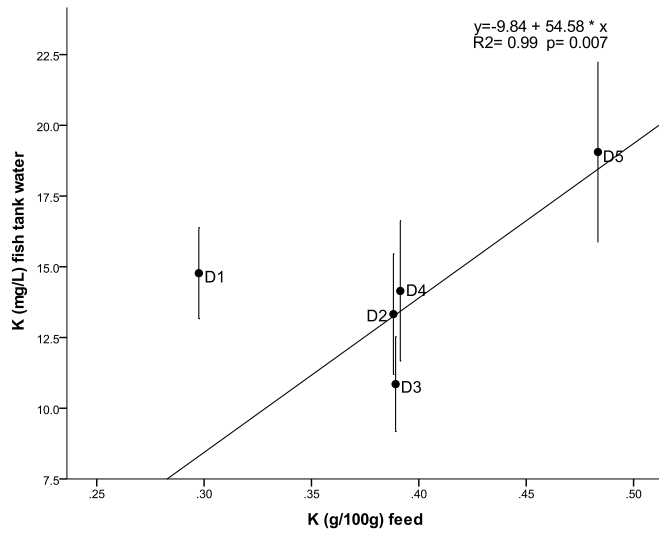


Figure 11 Correlation between mean K (mgL⁻¹) in fish tank water and K in fish diet.

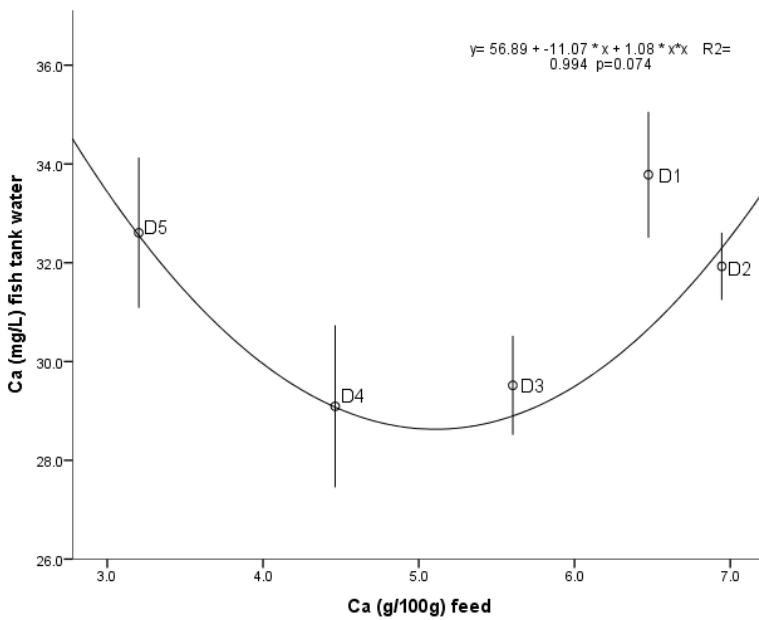


Figure 12 Correlation between mean Ca (mgL⁻¹) in fish tank water and Ca in fish diet.

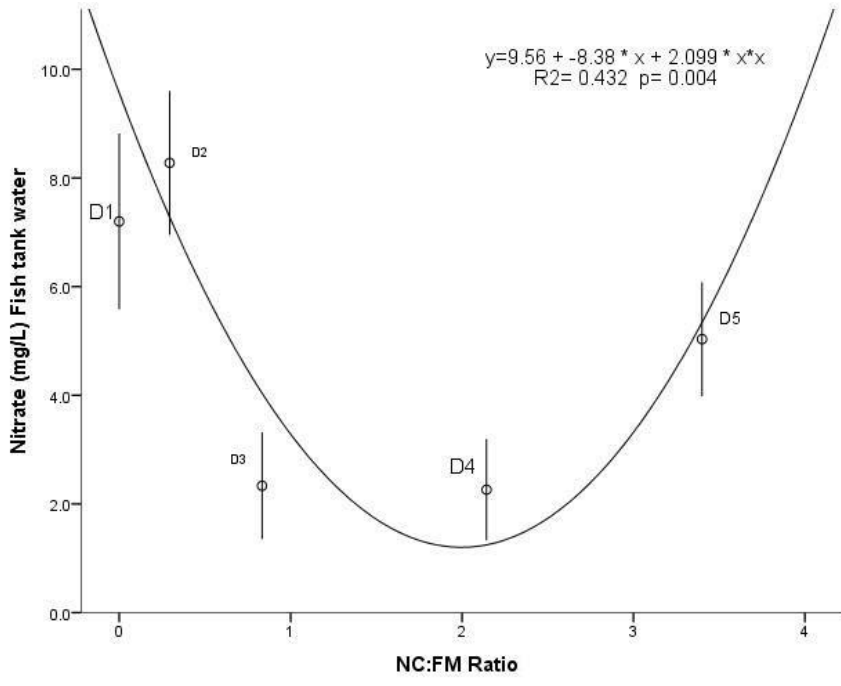


Figure 13 Mean nitrate (mgL⁻¹) content in fish tank water as a function of NC inclusion in diet.

Chapter 4

Growth rate, quality and yield response of Lettuce (*Lactuca sativa* L.) for different Niger seed cake inclusion levels in fish diet in Tilapia-Lettuce based aquaponics system.

4.1 Introduction

Lettuce (*Lactuca sativa* L.) is one of the most globally cultivated vegetable (Cometti *et al.*, 2013) with a production estimate of 22 million tons in 2008 covering 1 million hectares of land (Gladys and Aba, 2015). However, intent of increasing productivity through high nutrient input modalities face critical environmental and economic problems due to the rise in cost of fertilizers (Kerbiouiou *et al.*, 2013).

Hydroponic production of lettuce is widely accepted worldwide for several reasons including nutrient balance, higher productivity and precise growth environment management. (Assimakopoulou *et al.*, 2013). Furthermore, green house based hydroponic lettuce production is evident in all climates (Samarakoon *et al.*, 2006). Despite maximizing lettuce production, lettuce quality improvement or maintaining its basic nutritional value including vitamins A, K and C, β -carotene and other health beneficial entities remains a challenge for hydroponic systems (Gladys and Aba, 2015). However, hydroponically produced lettuce are reported for low occurrence of human bacterial pathogen compared to conventionally produced ones (Monge *et al.*, 2011). Balancing the benefits of hydroponics mainly should be based on maximizing the product quantity and quality and minimizing environmental stress; hence, all nutrient demand of lettuce needs to be maintained at optimum level, and this will take higher percentage of greenhouse lettuce production operational cost. Macronutrients and micronutrients concentration of hydroponic recipe for lettuce

is expected to be N 165 ppm, P 50 ppm, Ca 180 ppm, Mg 50 ppm, K 210 ppm, Cu 0.1 ppm, Zn 0.1 ppm, Fe 5 ppm (Rush, 2012). Lettuce productivity in hydroponics is determined by nutrient management based on phenological stage and environmental parameters (Rush, 2012). One cause for critiques on hydroponics is the possibility of nutrient rich waste release to the environment which has both cost and ecological issues, despite recently applied reuse modalities (Chen *et al.*, 1997). One moderate size green house is expected to release 1,000 - 4,000 L liquid fertilizer per year of which nitrate is the dominant ion (Chen *et al.*, 1997). Mitigating such challenges using sustainable food production system is one critical alternative; hence, researchers design different integrated technologies including aquaponics. Producing lettuce through aquaponics system is one alternative to mitigate the highest nutrient cost in hydroponics and also the risk of nutrient leach to the environment (Dediu *et al.*, 2012; Rakocy, 2012).

Several researchers claim that macronutrients and micronutrients in fish waste in intensive aquaculture system have the potential to replace hydroponic solutions (Endut *et al.*, 2010; Rakocy, 2012) despite the unpredictable nutrient balance shift through operation. However, researchers acknowledge deficiency of some nutrients for plant growth in aquaponics and suggested a possibility of balancing nutrient in aquaponics by changing the proportion of fish to plant (Rakocy *et al.*, 2006). Moreover, possibility of managing nutrient balance through fish diet quality management scheme has also been hypothesized by other researchers (Seawright *et al.*, 1998).

In perfectly designed and managed aquaponics, major nutrient input is fish feed. The existing commercial fish diets are produced considering only fish growth and health conditions. Fish diet quality is expressed in increased assimilation efficiency and reduced nutrient leakage to the water. Nutrient deficiency, imbalance, and salt accumulation cause yield reduction in different trials and

dictate the difficulty of maintaining proper nutrient concentration and preventing salt accumulation in aquaponics system if commercial diet is used for prolonged time (Seawright *et al.*, 1998; Endut *et al.*, 2010). Due to lack of distinct diet prepared for aquaponics, all aquaponics operations use fish feed produced for other fish production systems despite of the claim that aquaponics is more profitable and sustainable farm than the conventional one (Fitzsimmons, 1991; Rakocy and Hargreaves, 1993). Hence, limited growth was observed in different aquaponically produced plants unless supplementary nutrients were added to the system (Rakocy, 1997; Seawright *et al.*, 1998). Managing nutrient balance for highest and sustainable aquaponics productivity through feed quality manipulation seems sustainable alternative but needs clear understanding of the physiological demand of all organisms (fish, plant, and microbes). In addition, inaccessibility of commercial diet in most developing countries due to access and/or high cost remains a bottleneck for attaining the principal benefit of aquaponics despite its highest potential on food security, and poverty reduction.

Searching for new feed formula using locally available ingredients, among the different alternatives, for production of tilapia and lettuce in aquaponics is critical. In conventional thinking, the major cost of fish diet is Nitrogen; hence, replacing the source with locally available low cost ingredient is decisive together with improving the mineral composition and feed characteristics suitable for fish and plant need. Niger seed cake (*Guizotia abyssinica*) is an oil seed cake which is produced in large quantity in Ethiopia and is usually used for feed for ruminants (Getinet and Sharma, 1996). It has currently shown promising potential as source of Nitrogen and other minerals for aquaponics (Getinet and Sharma, 1996; Feedipedia., 2013). With respect to plant need, Niger seed cake is reported to have 6.89% N, 1.18% P, 0.7% Ca, 0.62% Mg, 0.1% Na, 1.16% K, 73 ppm Cu, 90 ppm Zn and 169 ppm Fe (Little *et al.*, 1986). Therefore, this experiment focuses

on testing the potential of Niger seed cake on promoting growth, yield, and quality of lettuce in aquaponics.

4.2 Materials and methods

This experiment was conducted at the aquaponics research facility, Addis Ababa University. All experimental setups were within a greenhouse and photoperiod for entire experimental period was 12 h day and 12 h night. The experimental set up design and installation was maintained as stated in Section 3.2.1 of this document. Aquaponics and hydroponics operational characteristics were maintained based on the recommendation of Rakocy (2012) and Rush (2012), respectively as stated in Section 3.2.1 of this document.

Nile tilapia (*Oreochromis niloticus* L.) fingerlings of size $31.47 \text{ g} \pm 0.783$ (mean \pm SE), obtained from Ziway Fisheries Research Center, were stocked at a density of 20 kg(m)^{-3} and Lettuce seedlings were germinated in Addis Ababa University aquaponics research facility and 44 Lettuce (*Lactuca sativa* L.) seedlings of a phenological level five (5 leaf stage) (BBCH 5) were transplanted to aquaponics and hydroponics units after repeated washings of roots.

Experimental diets were prepared at Fisheries and Aquatic Sciences Laboratory, Addis Ababa University, based on standard fish feed preparation protocol to test five NC inclusion levels in fish diet towards impacts on growth and quality response of lettuce. Experimental diets had NC:FM ratio of 0 (Negative control diet; D₁), 0.29 (D₂), 0.83 (D₃), 2.14 (D₄), 3.4 (D₅) and Hydroponic system (Control 2; Positive control for biomass development). Detail ingredients proportion and formulated diet composition is stated in section 3.2.2 of this document. Proximate nutrient composition of test diets was within the range for tilapia and lettuce requirement (Table 1).

Hydroponic solution was formulated to contain macronutrients and micronutrients in mgL^{-1} as N (NO_3^-) (163.77), K (93.272), P (36.939), Mg (25), Ca (234.332), S (34.295), Fe (4.9), Zn (0.25), B (0.7), Cu (0.07), Mo (0.05), Na (0.024), and Mn (1.97) using analytical grade fertilizers. Electrical conductivity of hydroponic solution was 1.3 mS (cm)^{-1} .

Fish in aquaponics system were fed 3% of body weight per day (FAO, 2014a) administered twice a day (Abdel-Tawwab *et al.*, 2010) at 07:30 and 17:30 throughout the experiment. Feed ration adjustment was made based on mean weight increment of fish in each sampling week. For hydroponic unit nutrient recharging was done in a week interval to minimize risk of nutrient depletion (Rafiee and Roos, 2006).

Table 2 Lettuce sample was collected in a weekly basis starting from the time of transplantation. During each sampling period six lettuces were collected from each unit and root washed with deionized water and each lettuce was divided into root and shoot (stem and leaf), non-destructive measurements taken and preserved for further analysis at -18°C (Sumanta *et al.*, 2014). Meristic measurements including shoot fresh weight (SFW), leaf fresh weight (LFW) and root fresh weight (RFW) (Kerbiriou *et al.*, 2013) were collected with digital balance sensitive to 0.001 g.

Each leaf was plotted on a graph paper and leaf area was calculated from the drawings. Representative fresh leaf sample was collected and washed with distilled water and dried with bolt and kept in -18°C and analyzed for chlorophyll a, and b and carotinoids. Then 0.5 g of fresh leaf weighed and homogenized with 95% Ethanol and centrifuged by 10000 rpm for 15 min at 4°C then 0.5 ml of supernatant was taken and diluted by 4.5 ml of Ethanol (95%) and absorbance was measured by Spectrophotometer at wave length of 664 nm (A_{664}), 649nm (A_{649}) and 470 nm

(A₄₇₀). Pigment concentrations (mgL⁻¹) were calculated based on the following formula (Cai *et al.*, 2011; Sumanta *et al.*, 2014)

$$C_a = 13.36A_{664} - 5.19 A_{649}$$

$$C_b = 27.43A_{649} - 8.12 A_{664}$$

$$C_{x.c} = (1000A_{470} - 2.13C_a - 97.63C_b)/209.$$

Chlorophyll content on wet weight basis (FW) was calculated as

$$\text{Chlorophyll content (gkg}^{-1}\text{)} = (\text{pigment concentration} \times 20 \text{ mL}/1000)/\text{gFW}$$

Total chlorophyll concentration = sum of chlorophyll a and chlorophyll b concentrations (Cai *et al.*, 2011);

where C_a = chlorophyll a concentration (mgL⁻¹); C_b = chlorophyll b concentration (mgL⁻¹); C_{xc} = carotenoid concentration (mgL⁻¹).

At each sampling period root dry weight (RDW), shoot dry weight (SDW) and leaf dry weight (LDW) were measured after samples were separated to root, shoot and leaf and dried in dry oven for 48 h at 70 °C (Chen *et al.*, 1997; Petridis *et al.*, 2013).

Growth response of lettuce was calculated based on standard mathematical equations (Hunt, 2003)

$$\text{Absolute growth rate in size (AGR)} = (W_2 - W_1)/(t_2 - t_1)$$

$$\text{Efficiency measurement [Relative growth rate (RGR)]} = (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

Balance of payments;

$$\text{Leaf Area Ratio (LAR)} = \text{LA}/\text{W}$$

$$\text{Leafiness of the leaf Specific leaf area (SLA)} = \text{LA}(\text{LW})^{-1}$$

$$\text{Leafiness of the plant or productive investment Leaf weight ratio (LWR)} = \text{LDW}/\text{TDW}$$

Proximate nutritional (Moisture, protein, ash), and mineral (K, Ca, and Fe) compositions of lettuce were analyzed following standard procedures (Chen *et al.*, 1997; AOAC, 2000). Moisture content was determined by oven drying at 70 °C for 48 h, Crude protein was determined using Kjeldhal method (Chen *et al.*, 1997; AOAC, 2000). Total mineral (ash content) was determined by incineration in muffle furnace at 550 °C for 6 h (AOAC, 2000). Following dissolution of lettuce; Ca and Fe concentrations were determined using atomic absorption spectrophotometer, and K by flame photometer (Chen *et al.*, 1997).

Water samples (600 ml) were collected from hydroponics gullies, filtered with glass fiber filter paper with 0.7 µm pore size, and preserved at -18 °C for nutrient analysis. Water, feed and lettuce mineral content analyses were performed in Lennart Mansson International Laboratory (Sweden) (using Inductively coupled plasma spectrophotometer), Addis Ababa University, and Ethiopian Institute of Health and Nutrition (using Atomic absorption spectrophotometer, flame photometer and Ultraviolet visible spectrophotometer),

Assimilation efficiency of lettuce for Ca, K, N, and Fe were determined using Specific absorption rate (A) for each mineral on total dry weight bases.

$$A = (1/R_x)((M_2 - M_1)/(t_2 - t_1))$$

where R_x was dry total weight, M_2 and M_1 were mineral (Ca, K, N or Fe) concentration at time 2 and 1, respectively (Hunt, 2003).

4.3 Data analysis

Data were analyzed using statistical software SPSS version 16 (Al-Souti *et al.*, 2012; Gullian-Klanian and Arámburu-Adame, 2013). Growth and quality performance response of lettuce for experimental diets and hydroponic solution and change in growing medium quality were analyzed using analysis of variance (one-way ANOVA). Data, which had significant tests, was further subjected to least significant difference (LSD) analysis. Growth and physiological efficiency impacts of experimental diets were subjected to linear and quadratic contrasts based on two basic comparisons; a) if growth and quality response of control diet has variation from test diets and hydroponic solution and b) if the response for the Niger seed cake to Fishmeal ratio (NC:FM) in test diets is either linear or quadratic. To determine the limiting factor for biomass development throughout the experiment data were subjected to stepwise regression as biomass is dependent variable and physico-chemical and nutrient concentrations as deriving factors, then the best model was taken to estimate the biomass development based on independent factors identified. Treatment effects were considered significant at $p < 0.05$ for mean difference analysis. However, tests for fixed effects were considered significant at $p < 0.1$ and all non-linear contrasts were considered significant at $p \leq 0.1$ and $r^2 \geq 0.25$ (Koch *et al.*, 2016).

4.4 Results and Discussion

4.4.1 Growing medium quality

Physicochemical parameters in this experiment remained within the range suitable for growth and development of lettuce (Rush, 2012). Despite the risk of nutrient depletion through precipitation (Ca, P, Mn and Mg) and increasing ammonia toxicity with increased pH; nitrifying bacteria's activity increased with higher pH (8.5), although previous work suggest that the optimum pH for leafy vegetables lies slightly in the acidic range (Tyson *et al.*, 2011). This showed that the impact of NC:FM ratio in fish diet had limited effect on changing DO, pH, temperature and EC of the growing environment with respect to lettuce growth and microbial wellbeing (Figure 14, Figure 15, Figure 16). Water quality parameters showed variation between treatments as a function of NC inclusion level but agreed with other findings (Endut *et al.*, 2010; Sikawa and Yakupitiyage, 2010; Roosta, 2011; Dediu *et al.*, 2012; Roosta and Mohsenian, 2012; Lam *et al.*, 2015); this indicates the effect of Niger seed cake in water quality for lettuce growth is bound under the scope of lettuce physiological demand for good growth.

Dissolved oxygen response between treatments was found to be significantly different and significantly highest DO was recorded from the hydroponics treatment (H) ($p < 0.05$). Impact of Niger seed cake inclusion in fish diet was significantly quadratic for DO content ($y = 5.08 + -0.11 * x + -0.06 * x^2$; $r^2 = 0.410$ $p = 0.004$) and EC ($y = 364.55 + -151.41 * x + 36.28 * x^2$; $r^2 = 0.858$, $p < 0.05$) in nutrient water. Even if critical DO concentration for root transpiration was reported in the range of 1.6 - 2.5 mgL⁻¹ (Goto *et al.*, 1996); NC inclusion in fish diet did not decrease DO level below this critical line and remains within the range reported by different scholars (Goto *et al.*, 2000; Yoshida *et al.*, 2014).

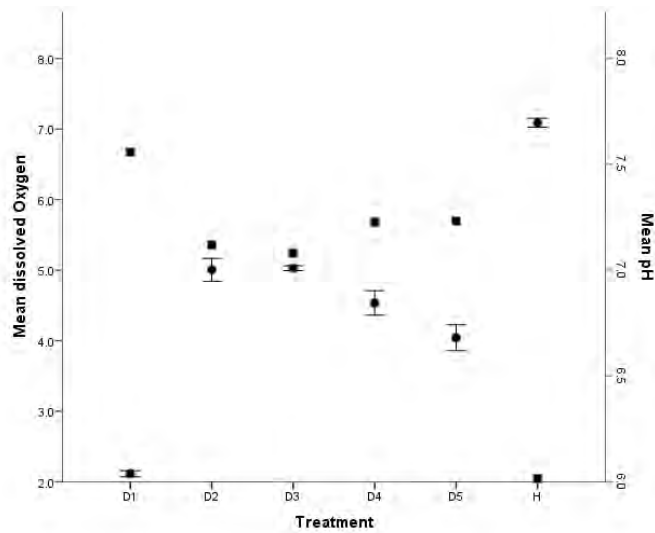


Figure 14 Dissolved Oxygen (DO) (mgL⁻¹) (Circles) and pH (rectangles) (Mean±SE) in Aquaponics and hydroponics growing media at different treatments.

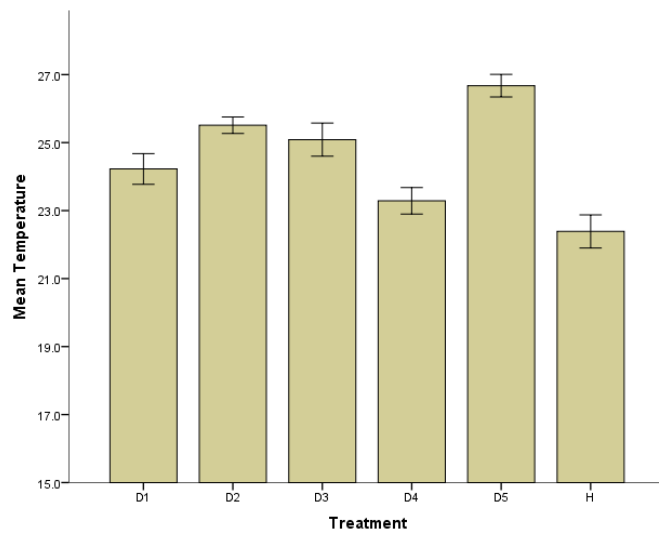


Figure 15 Temperature (°C) (Mean±SE) response in aquaponics and hydroponics growing media.

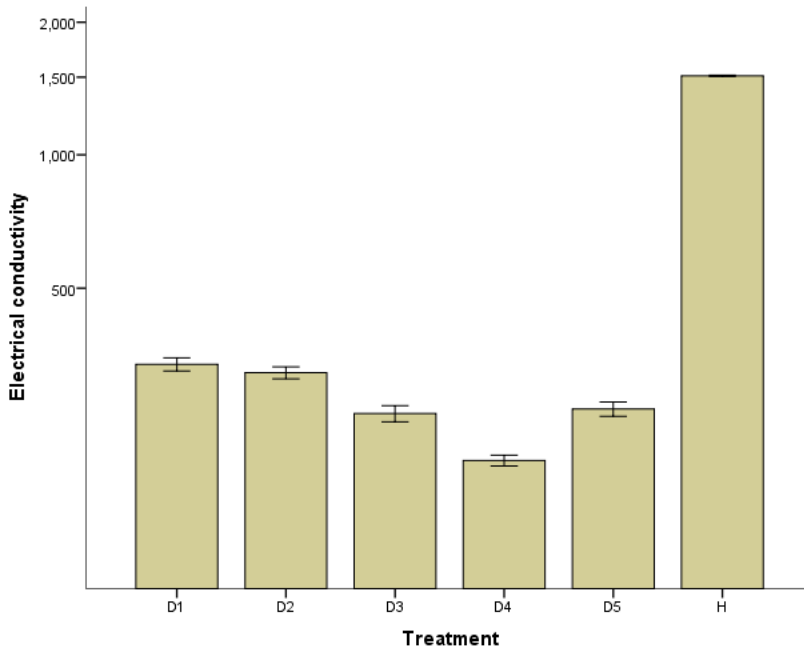


Figure 16 Electrical conductivity ($\mu\text{S}(\text{cm})^{-1}$) (Log transformed) (Mean \pm SE) response in growing media for experimental diets and hydroponics treatments.

4.4.2 Biomass production

Biomass production of lettuce increased with increased dietary NC level up to 37.5% (D₄) and showed decline at 43% (D₅). Highest biomass production was achieved with D₄, which is significantly higher than the negative control treatment D₁ ($p < 0.05$). Increasing Niger seed cake inclusion in diet brought significantly higher total dry weight production in D₄ than other treatments except H treatment (Figure 18). All biomass production measures are found significantly quadratic with dietary NC:FM ratio ($p < 0.05$). For example D₁ and D₅ are numerically inferior from D₄ (Figure 17, Figure 18, Figure 19, Figure 20) and all biomass measures of H were higher than experimental diets. Fresh total weight ($y = 60.66 + 35.65 * x + -9.08 * x^2$; r^2

= 0.978, $p = 0.008$) (Figure 23), leaf fresh weight ($y = 48.26 + 26.58 * x + -6.93 * x^2$; $r^2 = 0.959$, $p < 0.05$) (Figure 19), shoot fresh weight ($y = 8.35 + 3.48 * x + -0.87 * x^2$; $r^2 = 0.982$), root fresh weight ($y = 5.99 + 2.65 * x + -0.62 * x^2$, $r^2 = 1$, $p < 0.05$) (Figure 20), leaf dry weight ($y = 4.37 + 3.21 * x + -0.83 * x^2$; $r^2 = 0.491$, $p = 0.001$) (Figure 25), and leaf number ($y = 15.41 + 6.05 * x + -1.49 * x^2$; $r^2 = 0.484$, $p = 0.001$) (Figure 24) increased quadratically with increased level of inclusion of NC in diets.

The existing variation of biomass produced in this study was within the range of biomass production obtained by different scholars in aquaponics and hydroponic setups (Sikawa & Yakupitiyage, 2010, Cometti *et al.*, 2013; Dediu *et al.*, 2012; Gent, 2012a). In all experimental treatments, lettuce fresh weight (g/plant) was grown to the size reported by some studies 84 (Dediu *et al.*, 2012), 151 (Cometti *et al.*, 2013), 116-147 (Gent, 2012a) conducted under similar growing conditions. In all treatments lettuce dry matter content remained higher than the range that normal dry matter content of lettuce could be obtained in good growth condition (4-6%) (Dediu *et al.*, 2012). It is within the range reported for lettuce grown in hydroponics by some researchers 4-5 (Sikawa and Yakupitiyage, 2010; Gent, 2012b; Cometti *et al.*, 2013) and root dry weight response of lettuce for Niger seed cake inclusion was found within the range of results obtained by Cometti *et al.* (2013).

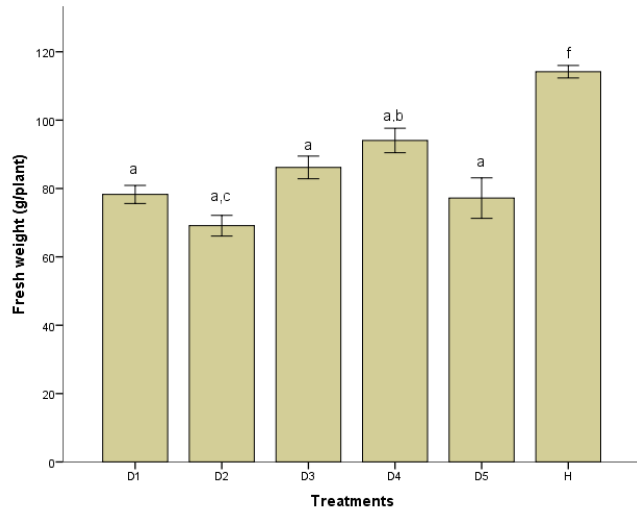


Figure 17 Fresh total weight (Mean±SE) of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

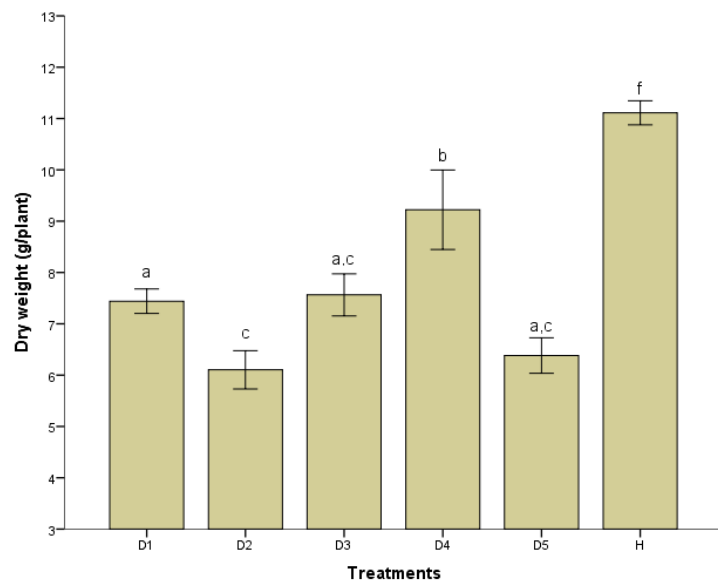


Figure 18 Total dry weight (Mean±SE) of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

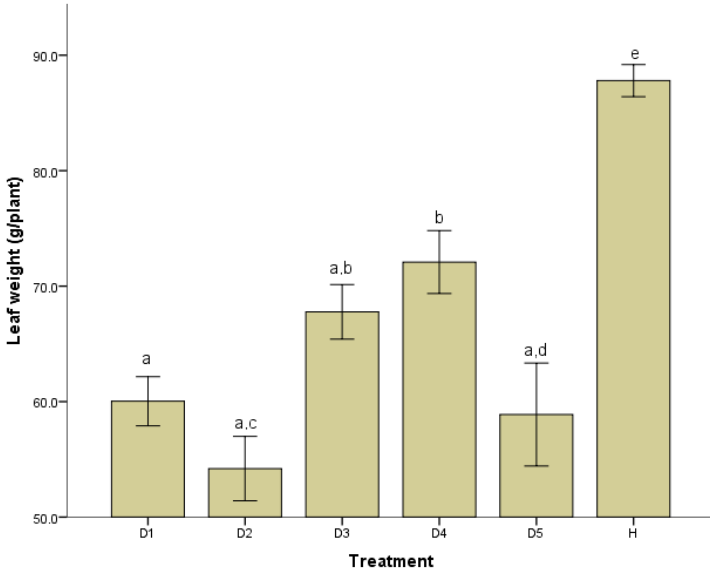


Figure 19 Leaf fresh weight of lettuce from experimental diets and hydroponics treatments.

Mean values sharing common letters are not significantly different ($p < 0.05$).

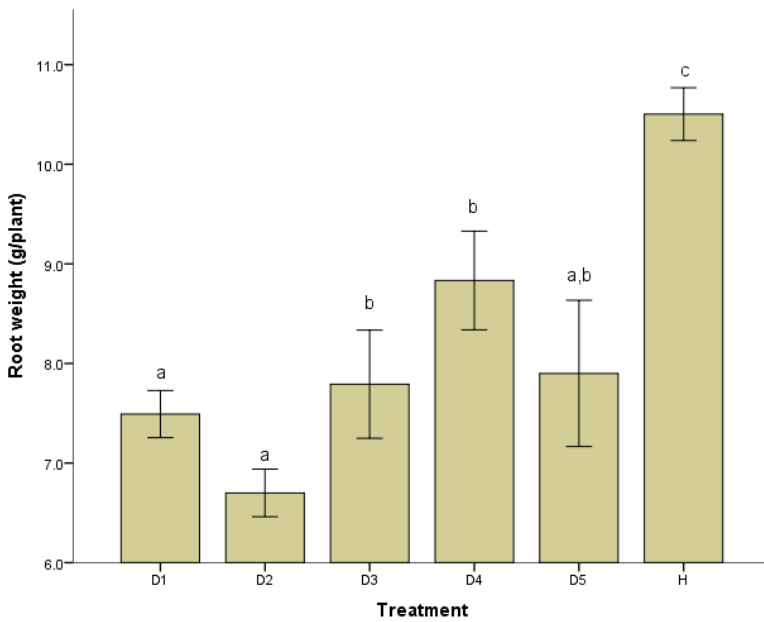


Figure 20 Root fresh weight of lettuce from experimental diets and hydroponics treatments.

Mean values sharing common letters are not significantly different ($p < 0.05$).

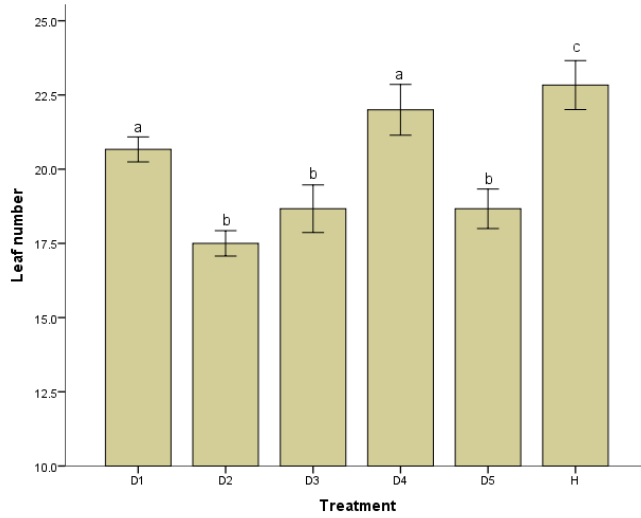


Figure 21 Leaf number (Mean±SE) of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

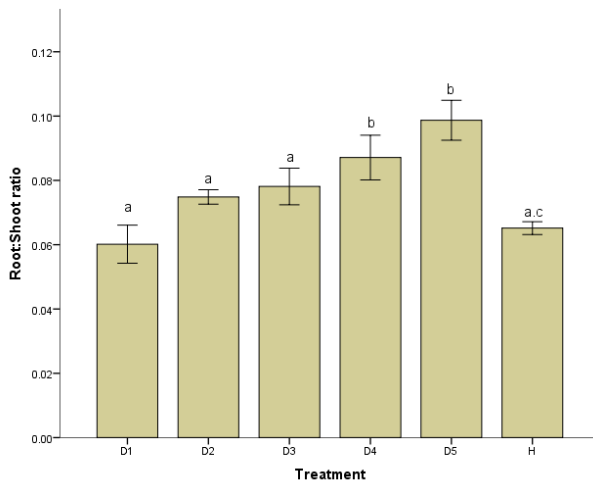


Figure 22 Root to shoot ratio (Mean±SE) of lettuce from experimental diets and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

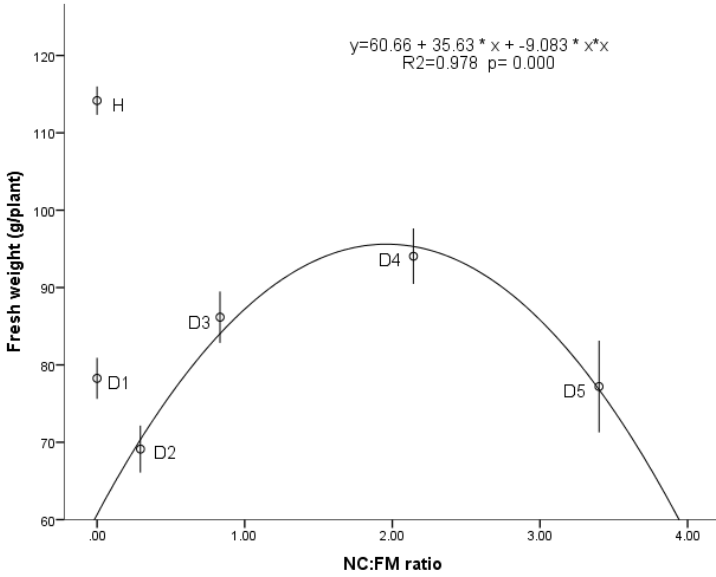


Figure 23 Total plant fresh weight (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.

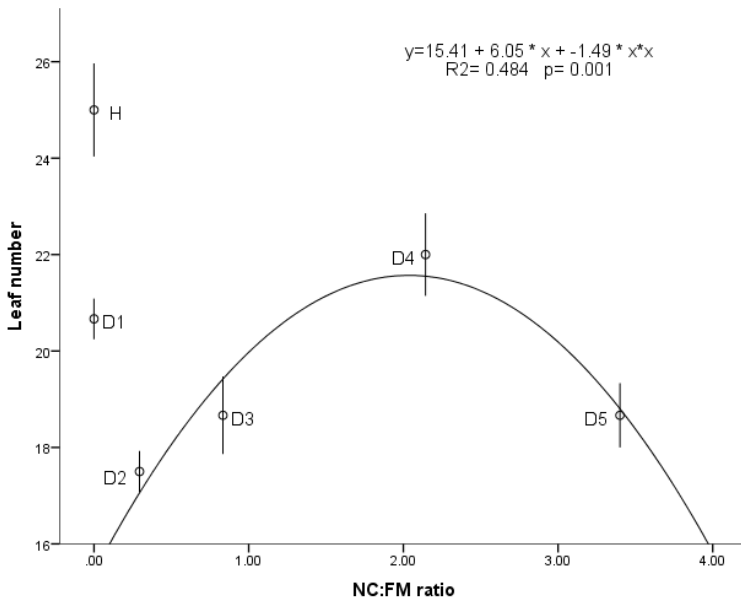


Figure 24 Leaf number (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.

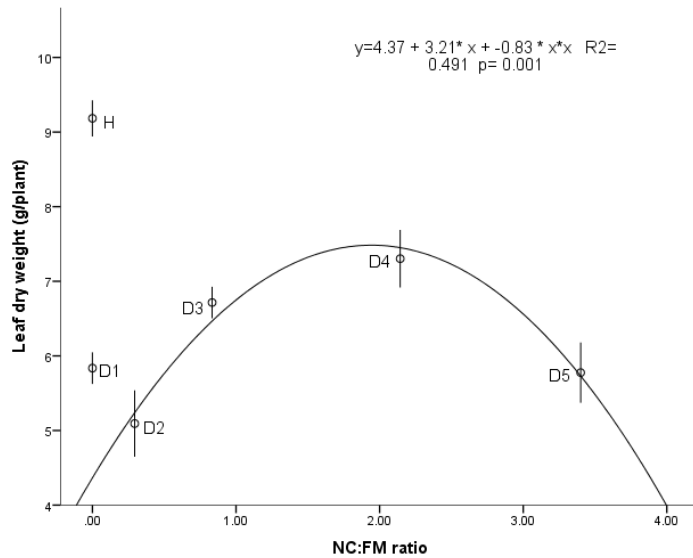


Figure 25 Leaf dry weight (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.

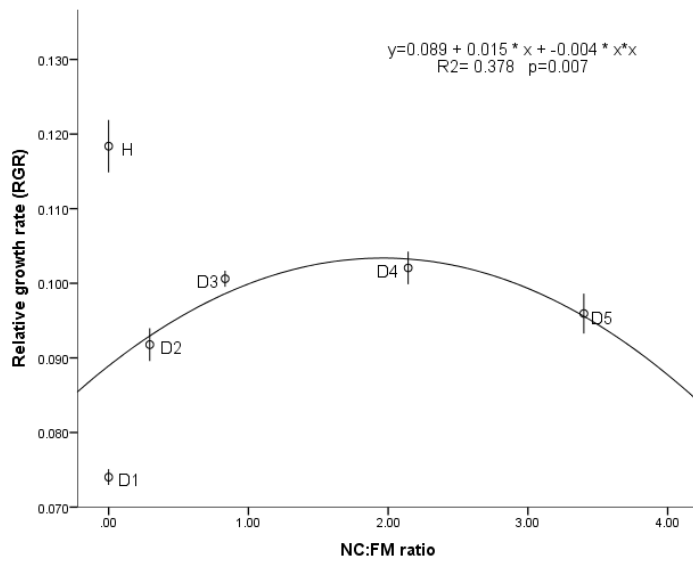


Figure 26 Relative growth rate (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.

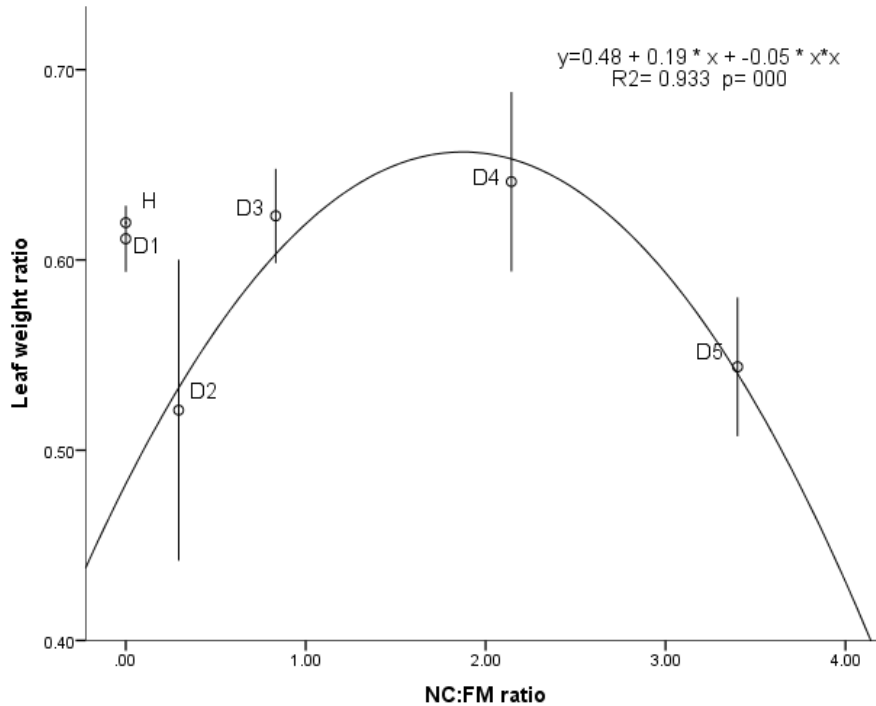


Figure 27 Leaf fresh weight (Mean±SE) of lettuce as a function of different levels of NC:FM ratio in fish diet.

4.4.3 Proximate composition

Proximate composition of lettuce showed significant correlation with level of Niger seed cake inclusion in fish diet. Moisture content of D₃ was higher than other treatments despite no significance difference ($p > 0.05$). However, organic matter content of H was significantly higher than D₂ but D₄ had significantly higher organic matter content of other experimental treatments ($p < 0.05$) (Figure 32). Dry matter content found in this experiment was within a range of previous findings 4-8 (Sikawa and Yakupitiyage, 2010; Gent, 2012a; Cometti *et al.*, 2013); this indicates the possibility of modulating fish diet in aquaponics to attain positive impact on lettuce proximate compositions. Dry matter content of lettuce grown under optimal condition ranged between 4-6%

(Seginer, 2004) and result of this experiment was above the range but it was lower than dry matter level reported in other findings 16.22-12.83 (Dediu *et al.*, 2012).

Mineral composition in lettuce tissue respond significantly quadratic for Potassium (K) ($y = 0.012 + -0.007 * x + 0.0021 * x^2$, $r^2 = 0.64$, $p = 0.00$) and significantly cubic for Calcium ($y = -0.004 + 0.023 * x + -0.015 * x^2 + 0.0026 * x^3$; $r^2 = 0.949$, $p < 0.05$) and Iron ($y = -0.0001 + 0.006 * x + -0.004 * x^2 + 0.001 * x^3$; $r^2 = 0.93$, $p < 0.05$) for Niger seed cake inclusion level.

Potassium content of D₂ was significantly higher than the remaining treatments ($p < 0.05$) and D₃ had the lowest content. In addition, Niger seed cake inclusion in diet brought quadratic increase in Calcium concentration between treatments. Significantly, highest and lowest Calcium concentration was achieved in D₃ and H ($p < 0.05$) respectively (Figure 34).

Bivivariate correlation analysis demonstrated strong correlation between retained Potassium and Iron in lettuce and dietary Potassium and Iron content respectively ($p < 0.05$). In addition, Potassium, Iron, and Calcium recovered from water had strong correlation with dietary Potassium, Iron, and Calcium content ($p < 0.05$). Mineral content recovered in experimental treatments by lettuce was found with in the range reported by different scholars (Rafiee and Roos, 2006; Fallovo *et al.*, 2009; Sikawa and Yakupitiyage, 2010).

4.4.4 Pigment

Response of pigment development was significantly linear with NC:FM ratio; Chlorophyll a ($y = 0.39 * x + 3.22$; $r^2 = 0.932$, $p = 0.00$), chlorophyll b ($y = 0.2 * x + 1.805$ $r^2 = 0.955$, $p = 0.00$), carotenoids ($y = 0.082 * x + 1.164$; $r^2 = 0.43$, $p = 0.01$) and total chlorophyll ($y = 0.595 * x + 5.029$ $r^2 = 0.94$, $p = 0.00$). All pigment composition was statistically insignificant with hydroponics

treatment ($p > 0.05$) (Figure 28, Figure 29). The result obtained in this experiment was in agreement with finding of previous researchers 3.6 (chlorophyll a), 1.1 (chlorophyll b) (Heo *et al.*, 2012). Bivariate correlation indicated the presence of strong correlation between total Potassium and total Calcium concentration in water and carotinoid pigment.

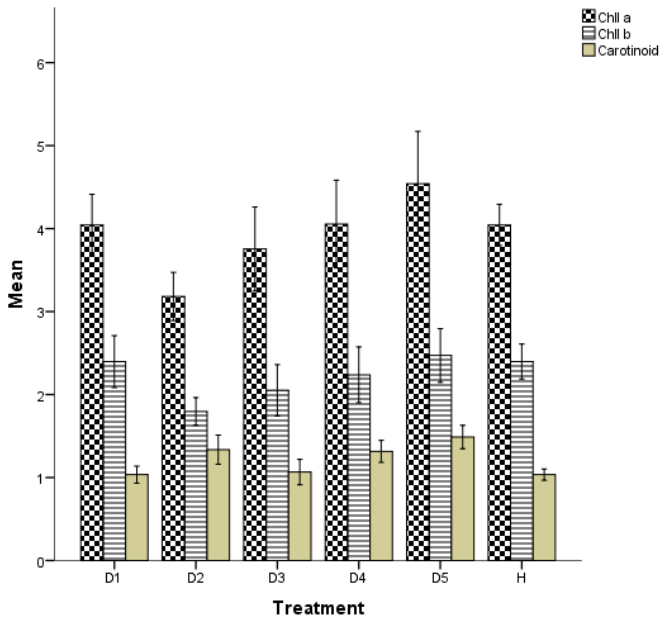


Figure 28 Chlorophyll a, Chlorophyll b and Carotinoids (Mean±SE) of lettuce for different NC:FM ratio and hydroponics treatments.

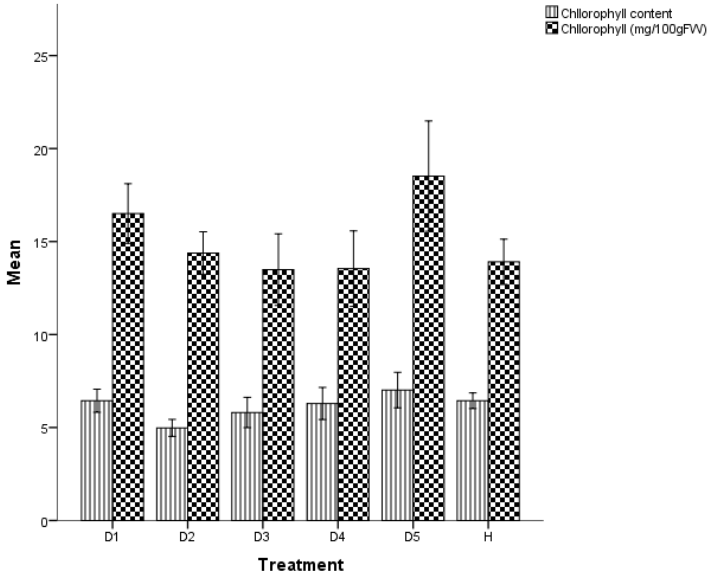


Figure 29 Total chlorophyll content and Chlorophyll content per fresh weigh (Mean±SE) of lettuce for different NC:FM ratio and hydroponics treatments.

4.4.5 Growth rate

Growth response of lettuce as a function of experimental diets was strongly correlated with Niger seed cake inclusion levels in diet (Figure 30). Increasing dietary NC:FM ratio resulted in increase of RGR in quadratic manner ($y = 0.089 + 0.015 * x + -0.004 * x^2$; $r^2 = 0.378$, $p = 0.007$) and significantly highest RGR obtained from D₄ ($p < 0.05$) (Figure 26, Figure 30). Lettuce RGR between experimental treatments and hydroponics treatment showed significant variation in gross ($p < 0.05$); however, RGR of D₄ is significantly comparable with H ($p < 0.05$) (Figure 30). The D₁ exhibited significantly lowest RGR ($p < 0.05$) from other treatments (Figure 30). Similar pattern was observed on AGR pattern and highest AGR was recorded from D₄ with insignificant variation with H ($p > 0.05$). Lettuce RGR was significantly correlated with K, Ca, and Fe content of diet, DO, pH, Temperature, EC, Fe and Ca value of growth medium (water) ($p < 0.05$). However,

stepwise regression indicates that lettuce RGR was strongly influenced by the combined effect of Fe (feed), Fe (water), pH, temperature, Ca (water) and K (water) ($r^2 = 0.994$, $P = 0.005$) of which the most limiting factor was Fe (water) ($r^2 = 0.969$, $p < 0.05$). Lettuce AGR obtained in this experiment was within the range reported by previous study 3.14-3.54 (Dediu *et al.*, 2012).

Leafiness of the leaf of lettuce was quadratically affected by dietary NC:FM ratio and highest SLA measured from D₁ and H treatments ($p < 0.05$). In addition, LWR was positively affected by Niger seed cake inclusion ($p < 0.05$) hence LWR quadratically increased with a function of dietary NC:FM ratio and the achievement of D₄ was higher than all treatments and comparable with the controls ($y = 0.48 + 0.19 * x + -0.05 * x^2$; $r^2 = 0.933$, $p < 0.05$). Hence, lettuce from D₄ was leafier than other experimental treatments and comparable with control treatments. Furthermore, the productive investment of D₄ was significantly higher than other experimental diets and comparable with control diet ($p < 0.05$) (Figure 27).

Root to shoot ratio indicates the level of resource partitioning between root and other parts of lettuce. Increasing Niger seed cake inclusion in diet brought significantly linear increase in root to shoot ratio (Figure 22) ($y = 0.0076 * x + 0.0719$, $r^2 = 0.993$, $p < 0.05$). Among several factors level of Potassium in fish diet significantly affected ($r^2 = 0.795$; $p < 0.05$) root to shoot ratio.

The highest yield of lettuce was obtained from H (5023 g(m)⁻²) and the lowest was obtained from D₂ (3041.13 g(m)⁻²) (Figure 31). Niger seed cake inclusion brought quadratic growth in yield ($y = 2669.13 + 1567.84 * x + -399.65 * x^2$; $r^2 = 0.495$, $p = 0.001$). Even if it was significantly lower than H ($p < 0.05$), yield from D₄ (4.14 kg(m)⁻²) was higher than reported by previous works: 3.45 kg(m)⁻² (Dediu *et al.*, 2012), 2 kg(m)⁻² (Seawright *et al.*, 1998) 4.13 kg(m)⁻² (Lennard and Leonard, 2004) 2 kg(m)⁻² (Rafiee and Roos, 2006) 3.5 kg(m)⁻² (Fallovo *et al.*, 2009). Yield variation between

aquaponics and hydroponics might be associated with nutrient balance variation between the systems.

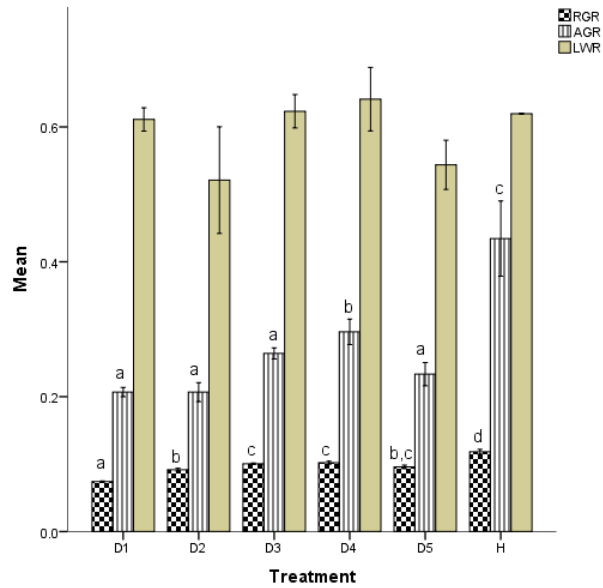


Figure 30 Relative growth rate, Absolute growth rate and Leaf weight ratio (Mean±SE) of lettuce for different NC:FM ratio and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

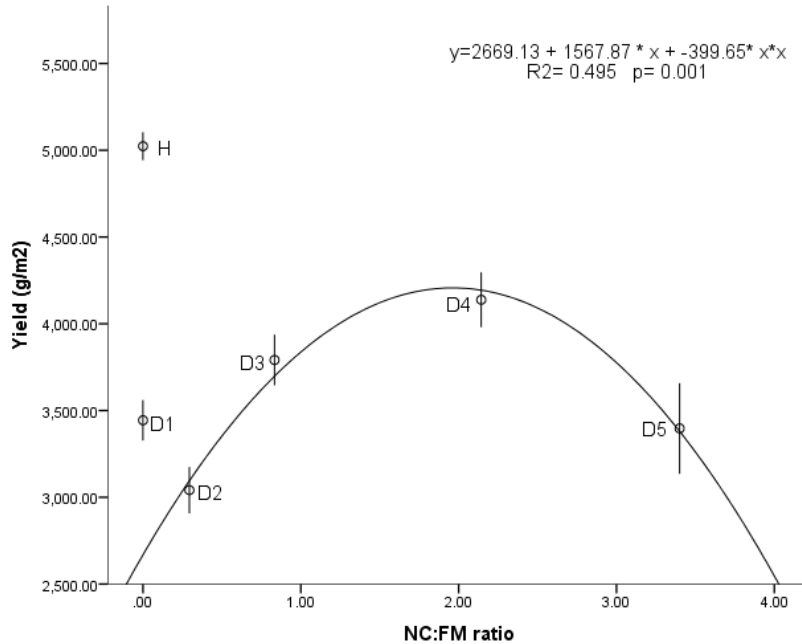


Figure 31 Yield per square meter growing area (Mean±SE) response of lettuce for different NC:FM ratio and hydroponics treatments.

4.4.6 Mineral in water

Nutrients recovered from aquaponics system were highly dependent on the number, type, and size of crop grown in the system. In perfectly designed aquaponics system low nutrient concentration in water appeared in highest vegetable component productivity (Tyson *et al.*, 2011). Macronutrient concentration in nutrient water showed significantly quadratic response for the dietary NC:FM ratio; NO_3 ($y = 0.84 + 0.54 * x + 0.0018 * x^2$; $r^2 = 0.919$, $p < 0.05$), PO_4 ($y = 3.57 + -1.64 * x + 0.54 * x^2$; $r^2 = 0.773$, $p < 0.05$), K ($y = 28.91 + -14.36 * x + 4.21 * x^2$; $r^2 = 0.392$, $p = 0.005$), Mg ($y = 6.36 + -1.52 * x + 0.44 * x^2$; $r^2 = 0.572$, $p = 0.000$), S ($y = 2.005 + 0.29 * x + -0.078 * x^2$; $r^2 = 0.791$, $p < 0.05$), Ca ($y = 16.325 + -10.41 * x + 2.66 * x^2$; $r^2 = 0.795$, $p < 0.05$).

Hydroponics treatment showed significantly highest concentration of NO_3 , PO_4 , K and Ca than all experimental treatments ($p < 0.05$) (Figure 33). Nitrate concentration in production systems showed no significant correlation with fish diet protein content ($p > 0.05$). Among experimental treatments significantly highest nitrate content was observed from D_1 ($p < 0.05$) and lowest from D_3 . Stepwise multiple regression showed high correlation between plant fresh biomass and nitrate concentration in the system ($r^2 = 0.47$, $p < 0.05$). Bivariant correlation indicates significant correlation ($p < 0.05$) between nitrate content and RGR ($r^2 = 0.59$), leaf number ($r^2 = 0.67$), total dry weight ($r^2 = 0.69$) and fresh weight ($r^2 = 0.7$) of lettuce. This indicates that the increased absorption rate to build biomass resulted in decreased nitrate content in the system.

Phosphate and Potassium concentration in nutrient water quadratically responded to Niger seed cake inclusion and lowest concentration was recorded from D_4 and highest from D_5 ; however, compared to H treatment all experimental treatments showed significantly lower concentrations ($p < 0.05$) (Figure 33). Phosphate and Potassium concentrations were inversely correlated with plant growth and biomass development; this indicate higher phosphate utilization efficiency in D_4 than other experimental treatments but the condition is different for the hydroponics treatment. Therefore, in the presence of surplus nutrient concentration excess nutrient remained in the nutrient solution. Relative addition of nutrients in the system brought variation in the growth of lettuce and resource partitioning of lettuce up to the critical level. The concentration of Mg, S, Ca and NH_4 showed variation between treatments but not patterned. Significantly, highest Ca concentration was achieved in H treatment ($p < 0.05$).

Nitrate, phosphate, Potassium and Calcium concentrations in this experiment were found to be lower as compared to some previous studies (Seawright *et al.*, 1998; Sikawa and Yakupitiyage,

2010) but were above the critical concentration level for efficient nutrient absorption by lettuce reported by previous study (Sikawa and Yakupitiyage, 2010). Calcium concentration was above the reported level of previous studies (Falovo *et al.*, 2009; Sikawa and Yakupitiyage, 2010) and within the range reported by Rafiee and Roos, (2006) but lower than Ca concentration in some hydroponics studies (Seawright *et al.*, 1998; Sikawa and Yakupitiyage, 2010).

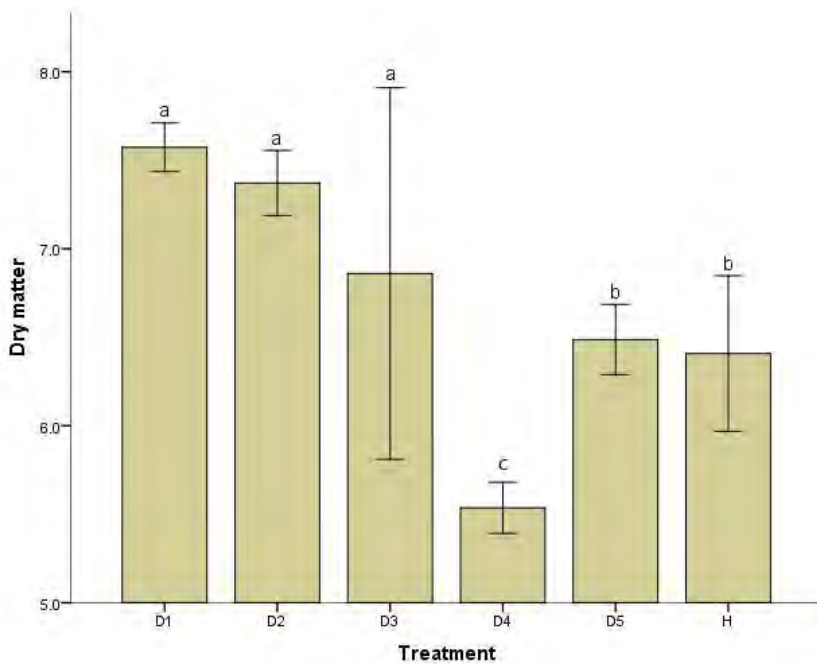


Figure 32 Dry matter content (Mean±SE) of lettuce as a response to different NC:FM ratio in fish diet and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

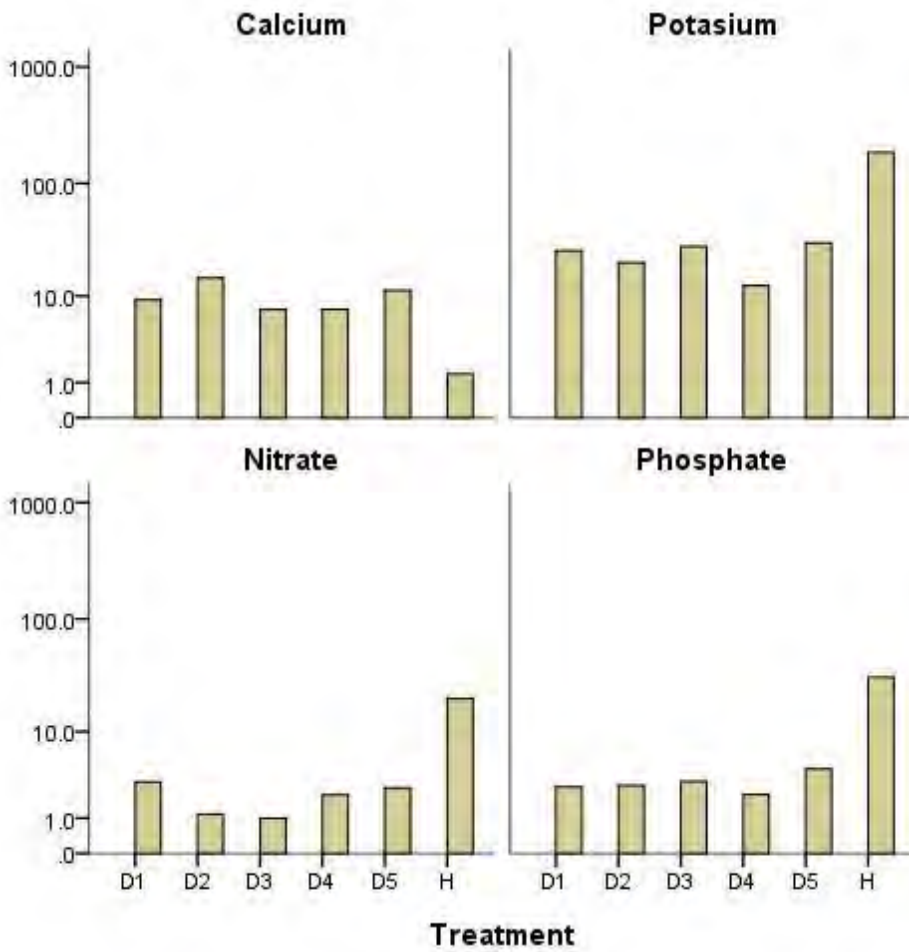


Figure 33 Log transformed Potassium, Calcium, Nitrate and Phosphate (mgL⁻¹) content (Mean±SE) of nutrient water as a response to different NC:FM ratio in fish diet and hydroponics treatments.

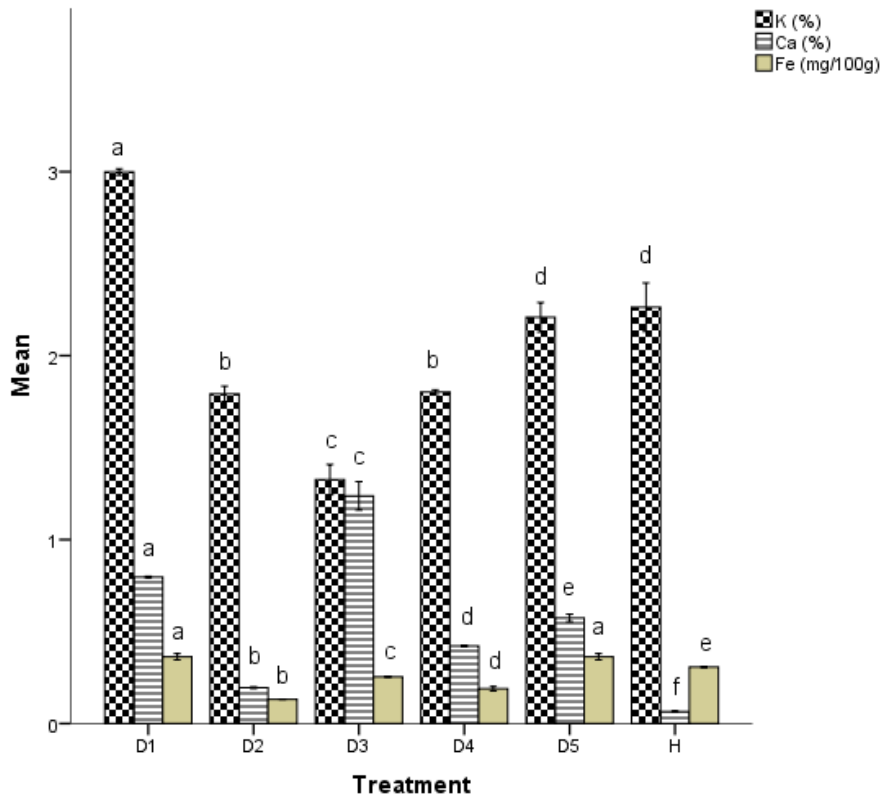


Figure 34 Log transformed Potassium, Calcium and Iron content (Mean±SE) of Lettuce as a response to different NC:FM ratio in fish diet and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

Chapter 5

Effect of mineral supplementation in fish diets in aquaponics system on growth rate, quality and yield response of Nile tilapia (*Oreochromis niloticus*) and Lettuce (*Lactuca sativa*).

5.1 Introduction

Aquaponics means integration of recirculating aquaculture and hydroponic systems to have simultaneous fish and plant production in one system using fish feed as main nutrient input (Alder, 2001; Diver, 2006). Aquaponics primary potential to increase productivity, to decrease environmental stress and to improve nutrition security makes its expansion rapid up to 1998, however, its economic contribution remains stagnated (Seawright *et al.*, 1998). Productivity of aquaponics has been tested for different fish and plant species (Rakocy and Hargreaves, 1993; Rakocy, 1997; Klinger and Naylor, 2012; Liang and Chien, 2013; Roosta, 2014a; Love *et al.*, 2015b). However, its expansion to the world faces several challenges specially in developing countries due to missing technical advancement of the system and also its need to deal with the physiological demand of three different organisms; fish, plants and microorganisms.

Likewise, expansion of commercial aquaponics is limited as compared to aquaculture and hydroponics. This might be associated with the presence of critical gaps with system optimization for commercially valuable crops. Principally, current aquaponics operators utilize commercial fish diets as main nutrient source for all type of plants exercised in aquaponics but each plant species has its own demands regarding nutrient as well as physical and chemical property of the growing medium.

Characterizations of fish waste have been done for different systems but the diets used were commercially produced and thus aim on efficient growth of fish and with low amount of nutrient release to the environment (Seawright *et al.*, 1998). Hence, nutrient concentration in fish waste from fish fed commercial diets has not the target to produce plants; therefore, plants in aquaponics exhibit limited growth, as overtime nutrient depletion and imbalances happen in fish water (Rakocy *et al.*, 2006; Wortman, 2015). Macronutrients and micro nutrients essential for healthy growth of fish and lettuce are Calcium, Magnesium, Potassium, Phosphorus, Sulfur, Nitrogen, Chlorine, Iron, Zinc, Manganese, Copper, Iodine, Cobalt, Nickel, Florine, Chromium, Molybdenum, Selenium, Tin, Silicon and Boron (plant only) (Stickney, 2005; Rush, 2012). Fish diets are expected to satisfy mineral demand of fish; however, fish waste mineral usable for plants show depletion over time depending on system intensity, fish to plant ratio, fish species, plant type, diet quality and quantity, and environmental conditions (Fitzsimmons, 1991; Seawright *et al.*, 1998; Rakocy *et al.*, 2006). Most reported depleted minerals are Iron, Zinc, Copper, and Manganese but not limited to these. Therefore, intervention on diet quality management might be one critical point to mitigate such problems (Rafieea and Saad, 2005). From several points of intervention on diet quality modulation, one alternative is adjusting mineral supplementation level by incorporating adequate amount of minerals to support good plant growth, while remaining under negative impact threshold levels for fish.

The objective of this study is to analyze the performance of mineral supplementation in fish diets in aquaponics productivity.

5.2 Materials and methods

5.2.1 Experimental set-up

The study was conducted within the aquaponics facility at Addis Ababa University, Ethiopia. The experimental set-up design, installation and operation were maintained similar between treatments as stated in section 3.2.1 in this document. The pH, temperature, and dissolved oxygen of the growing medium were regulated using acid-base addition, submersible thermostat, and aerators, respectively.

5.2.2 Diet preparation and preservation

Five isonitrogenous experimental diets were prepared using basal ingredients and five different mineral supplementation rates: 0 (Control diet, D₁); 1.15% (D₂), 2.3% (D₃), 3.454% (D₄) and 4.6% (D₅).

Ingredients used to prepare basal diets were Niger seed (*Guizotia abyssinica*) cake (375 gkg⁻¹), Meat bone meal (150 gkg⁻¹), fish meal (175 gkg⁻¹), wheat grain (150 gkg⁻¹), wheat bran (100 gkg⁻¹), vitamin-mineral premix¹ (3 gkg⁻¹), lime stone (7 gkg⁻¹), dicalcium phosphate (10 gkg⁻¹), fish oil (2 mlkg⁻¹) and soya-bean oil (20 mlkg⁻¹). Experimental diets were formulated and managed based on standard fish feed formulation protocols as described in section 3.2.2 in this document. Dry matter (DM), Crude protein (CP) and Crude fat (CF) contents were 92.2%, 28.9%, and 6.7%, respectively in the experimental diets.

¹ Vitamin-mineral premix (mgkg⁻¹); Vitamin A (retinol) 2100, Vitamin D₃ (Chole-calciferol) 50, Vitamin E 10000IU, Vitamin k₃ 2000, Thiamine 1000, Riboflavin 4000, Niacin 10000, Panthothenic acid 5000, Pyridoxine 750, Folic acid 250, Vitamin B12 8, Vitamin H as Biotin 30, Betain 100000IU, Antioxidant 125000IU, Manganese 80000, Zinc 50000, Iron 20000, Copper 5000, Iodine 1200, Cobalt 200, Selenium 200.

Hydroponic nutrient solution (H)¹ was prepared using analytical grade inorganic fertilizers following the recommendation by Rush (2012). For hydroponics control treatment full strength solution was prepared using analytical grade inorganic fertilizers to contain macronutrients and micronutrients in mgL⁻¹ as N (NO₃⁻) (163.77), K (93.272), P(PO₄) (36.939), Mg (25), Ca (234.332), S (34.295), Fe (4.9), Zn (0.25), B (0.7), Cu (0.07), Mo (0.05), Na (0.024), and Mn (1.97). In addition, mineral solution² for dietary supplementation was formulated to contain macronutrients and micronutrients in mgL⁻¹ as K (103.5), P(PO₄)(70.4), Mg (45), Ca (190), S (60), Fe (4), Zn (0.1), B (0.5), Cu (0.1), Mo (0.05), Na (0.024), and Mn (0.5) using analytical grade fertilizers. Electrical conductivity of hydroponic solution was 1.3 mS(cm)⁻¹.

Nile tilapia (*Oreochromis niloticus* L.) fingerlings of 41.1±1.3g (mean±SE) size were stocked at a density of 20 kgm⁻³ in each tank. All experimental treatments were organized in duplicate. Lettuce (*Lactuca sativa* var longifolia) seedlings with a phenological level BBCH 7 were root washed and transplanted in all units.

5.2.3 Feed regime

Fish diet administered twice a day as stated in section 3.2.3 in this document. Feed ration adjustment was made based on mean weight increment of fish each sampling week. Hydroponic solution was recharged weekly to avoid nutrient deficiency.

¹ Hydroponic mineral premix for hydroponic (H) control treatment: Ca(NO₃)₂, KNO₃, H₃BO₃, CuSO₄.5H₂O, MgSO₄.7H₂O, MnSO₄.H₂O, K₂HPO₄, Na₂MoO₄.2H₂O, ZnSO₄.2H₂O, Ca(H₂PO₄)₂.H₂O and Fe(EDTA)

² Hydroponic mineral premix for dietary supplementation: H₃BO₃, CuSO₄.5H₂O, MgSO₄.7H₂O, MnSO₄.H₂O, K₂HPO₄, Na₂MoO₄.2H₂O, ZnSO₄.2H₂O, Ca(H₂PO₄)₂.H₂O and Fe(EDTA)

5.3 Data collection

Water quality parameters; pH (Hanna HI9124), dissolved Oxygen (DO) (Hanna HI9142), temperature (°C) (Thermometer) and electrical conductivity (Jenway pH and EC meter) were collected weekly for the entire experimental period from fish tank, hydroponic gullies and biofilter.

Total fresh biomass of fish and plant data were taken weekly from each treatment. Three fish and lettuce samples were sacrificed each week and preserved at -18 °C for destructive measurements (proximate compositions). Fish total weigh, fish total length, leaf number, leaf weight, root weight, shoot weight and leaf area were measured weekly (Sumanta *et al.*, 2014). Meristic measurements including shoot fresh weight (SFW), leaf fresh weight (LFW) and root fresh weight (RFW) (Kerbiriou *et al.*, 2013) were collected by digital balance sensitive to 0.001 g.

At each sampling period Root dry weight (RDW), shoot dry weight (SDW) and leaf dry weight (LDW) were measured after samples were separated to root, shoot and leaf and dried in dry oven for 48 h at 70 °C (Chen *et al.*, 1997; Petridis *et al.*, 2013).

Dry matter, crude protein, crude Fat and ash proximate content of feed, fish and lettuce were analyzed according to standard procedures (AOAC, 2000). Moisture content was analyzed by drying at 70 °C for 48 h, Crude protein by using Kjeldahl nitrogen (Chen *et al.*, 1997) and total lipid by di-ethyl ether extraction with Soxlet and ash by incineration on muffle furnace at 550 °C for 6 h.

Administered diet efficiency with respect to tilapia and lettuce growth and development performance (DGR, RGR, PPV, AGR_L, RGR_L and LWR) were compared against control treatments. Diet efficiency indicators and diet use efficiency (FCR and PER) were calculated

based on standard mathematical equations (Chen *et al.*, 1997; El-Saidy and Gaber, 2003; Hunt, 2003; Abdel-Tawwab *et al.*, 2010; Gullian-Klanian and Arámburu-Adame, 2013) as stated in sections 3.2.4 for fish and 4.2 for plant in this document.

Water samples were collected from hydroponic gullies, filtered with glass fiber filter paper with 0.7µm pore size, and preserved at -18 °C for nutrient analysis. Water nutrient quality analysis for ammonia, nitrite, nitrate, and phosphate were performed at Addis Ababa University using Ultraviolet visible spectrophotometer. Ammonia was determined by using phenate method, nitrate by salicylate method, and phosphate by Ascorbic acid method (APHA, 1999).

5.3.4.1 Data analysis

Growth performances of fish and lettuce was tested for statistical difference and similarity using analysis of variance (one way ANOVA) with the statistical package SPSS (v.16.0 for Windows) (Al-Souti *et al.*, 2012; Gullian-Klanian and Arámburu-Adame, 2013). Data reaching significance was further subjected to least square deviation (LSD) analysis. Treatment effects were considered significant at $p < 0.05$ for mean difference analysis. However, tests for fixed effects were considered significant at $p < 0.1$ and all non-linear contrasts were considered significant at $p \leq 0.1$ and $r^2 \geq 0.25$.

5.4 Results and Discussion

5.4.1 Growing medium quality

Growing medium quality including dissolved oxygen (DO) (Figure 35), electrical conductivity (EC) (Figure 36), acidity (pH) (Figure 37) and temperature (Figure 38) were found within the

range suitable for tilapia, lettuce, and bacteria (Rakocy and Hargreaves, 1993). However, pH level of the growing media increased above the suitable condition of lettuce except in hydroponics treatments. Furthermore, growing medium (water) quality varied as a response to the experimental diets but remained within the range reported by previous investigations (Rakocy and Hargreaves, 1993).

The response of the growing medium to DO was significantly quadratic for the experimental diets ($y = 1.73 + 3.92 * x + -0.83 * x^2$; $r^2 = 0.701$ $p = 0.004$) (Figure 35). Dissolved Oxygen increased with increasing mineral supplementation up to 2.3% (D₃) and then decreased. However, significantly lowest DO was recorded for mineral supplementation of 4.6% (D₅) which is significantly comparable with the control diet (D₁) ($p > 0.05$). However, as compared to Hydroponic (H) control treatments; D₁ (0%) and D₅ (4.6%) showed significantly lower DO level ($p < 0.05$) (Figure 35). In all treatments except D₁ and D₅, DO concentration (mgL^{-1}) was found within the range suitable for tilapia and lettuce growth and development 3.5 (Soderberg, 2006), 2.1 (Goto *et al.*, 2000), 6.1-7.3 (Lam *et al.*, 2015), 6.11 (Roosta and Mohsenian, 2012).

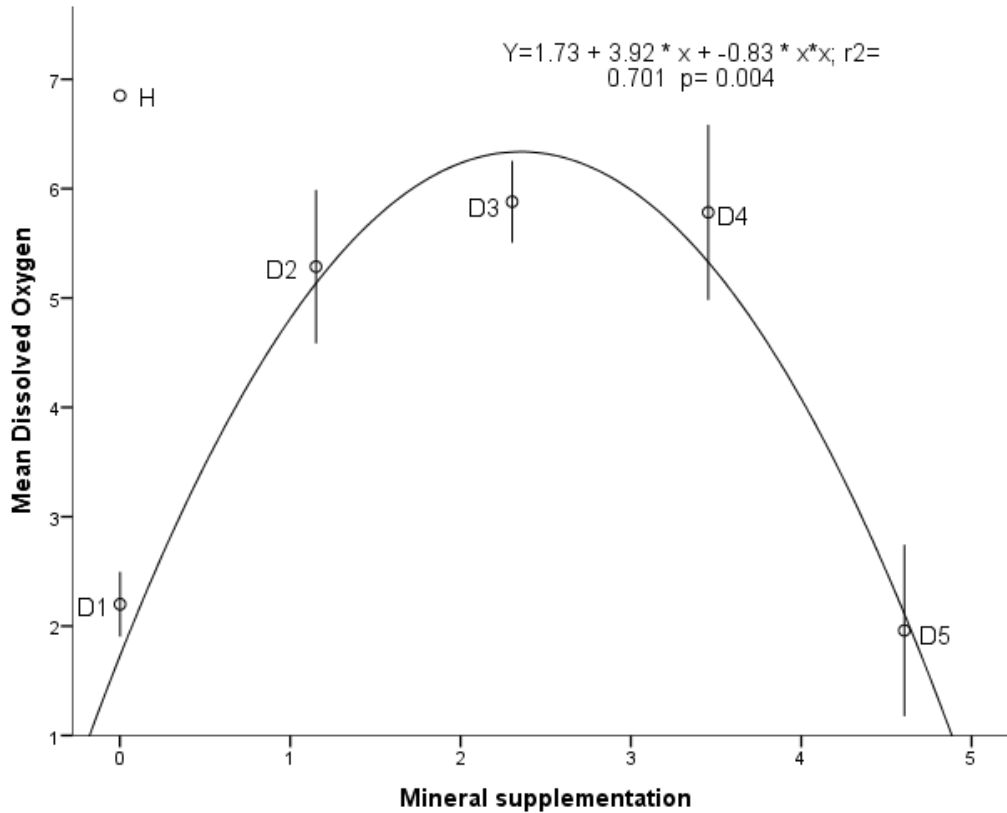


Figure 35 Dissolved Oxygen (Mean±SE) concentration response in Aquaponics and hydroponics growing media for different mineral supplementation levels.

Electrical conductivity in the growing media of aquaponics remained significantly lower ($p < 0.05$) than in the hydroponics treatments. Among mineral supplementation treatments, the response of EC was significantly quadratic with mineral supplementation level ($y = 251 + -47.79 * x + 7.17 * x^2$; $r^2 = 0.819$, $p < 0.05$) (Figure 36). Electrical conductivity ($\mu\text{sim}(\text{cm})^{-1}$) values were below the level reported in previous studies 540-1103 (Graber and Junge, 2009; Roosta, 2011; Roosta and Mohsenian, 2012).

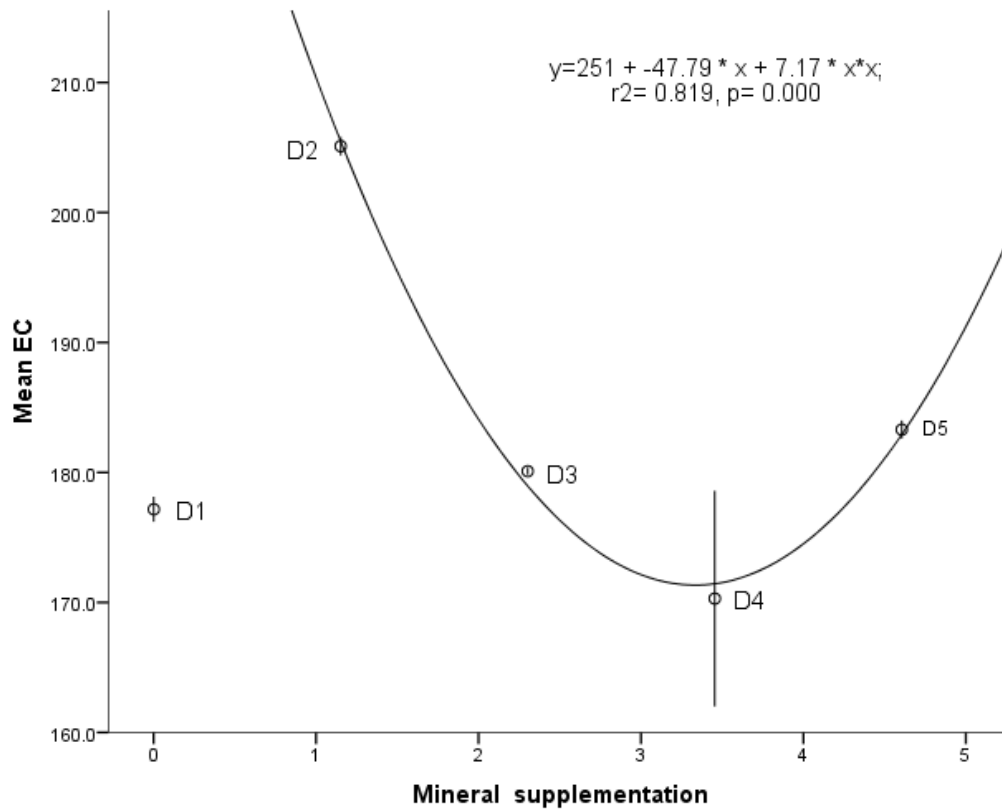


Figure 36 Electrical conductivity (Mean±SE) of growing medium in aquaponics treatments growing medium.

All mineral supplementation treatments resulted in a pH value between 7.57 ± 0.73 (Mean±SE) and 7.66 ± 0.60 (Mean±SE), and all aquaponics treatments were found to be significantly higher than the hydroponics treatment (6.23 ± 0.23) ($p < 0.05$) (Figure 37). All except the hydroponics treatment responded a pH value within a range suitable for tilapia and microbial growth 7-8 (Stickney, 2005; El-Sayed, 2006; Sikawa and Yakupitiyage, 2010; Tyson *et al.*, 2011; Rush, 2012) and above the optimum level for lettuce growth and development 5.5-6.5 (Tyson *et al.*, 2011; Rush, 2012). However, there was no significant correlation between pH values and level of mineral supplementation on fish diet ($p > 0.05$).

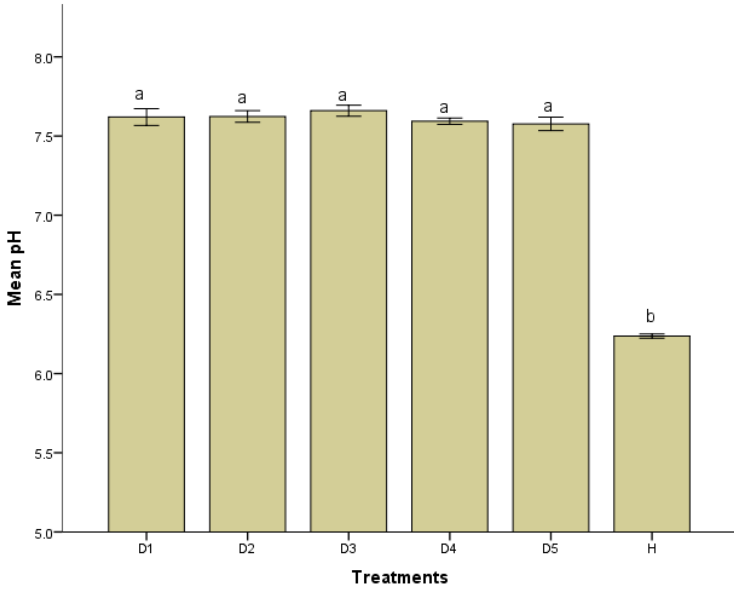


Figure 37 pH (Mean±SE) response in growing media of aquaponics and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

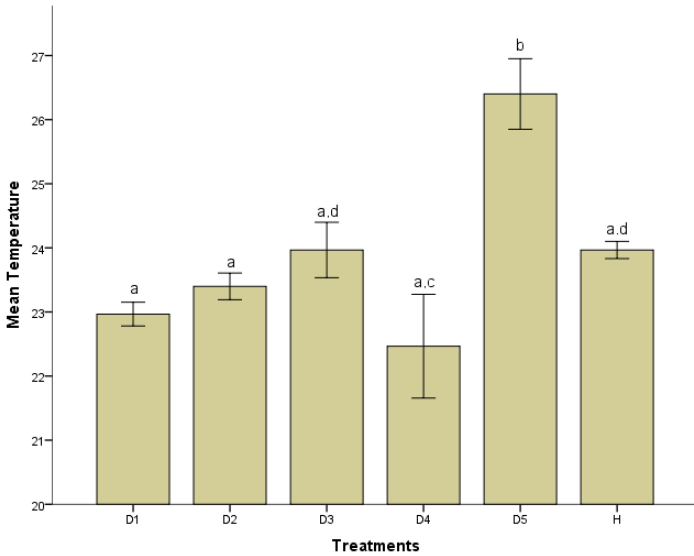


Figure 38 Temperature (°C) (Mean±SE) response in growing media of aquaponics and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

5.4.2 Fish growth

Hydroponic mineral supplementation on fish diet has brought impact on fish biomass development and growth rates. Fish total weight, Daily growth rate (DGR) (Figure 39), Relative growth rate (RGR) (Figure 40) and yield decreased with increased level of mineral supplementation. However, the mean difference of fresh total weight (TW), DGR, and yield was not significantly different among treatments ($p > 0.05$).

Highest and lowest DGR were observed from control (D_1) and D_5 treatments, respectively. In this experiment, impact of mineral supplementation brought significantly linear response for DGR ($y = -0.075 * x + 0.82$; $r^2 = 0.93$, $p = 0.035$) (Figure 39) and RGR ($y = -0.079 * x + 0.68$; $r^2 = 0.935$, $p = 0.033$) (Figure 40). Significantly lowest RGR was recorded from D_5 (4.6%) ($p < 0.05$). Growth rate (g/day) response observed in this experiment was found within the range reported by a previous study 0.5-1.2 (Soderberg, 2006).

Growth inhibition was observed with mineral supplementation on fish diet up to 4.6% (D_5). This indicates that supplementing hydroponic mineral in fish diet up to D_5 will have no significant fish growth consequences hence based on the value of minerals for plant growth and development improving diet mineral composition will be beneficial.

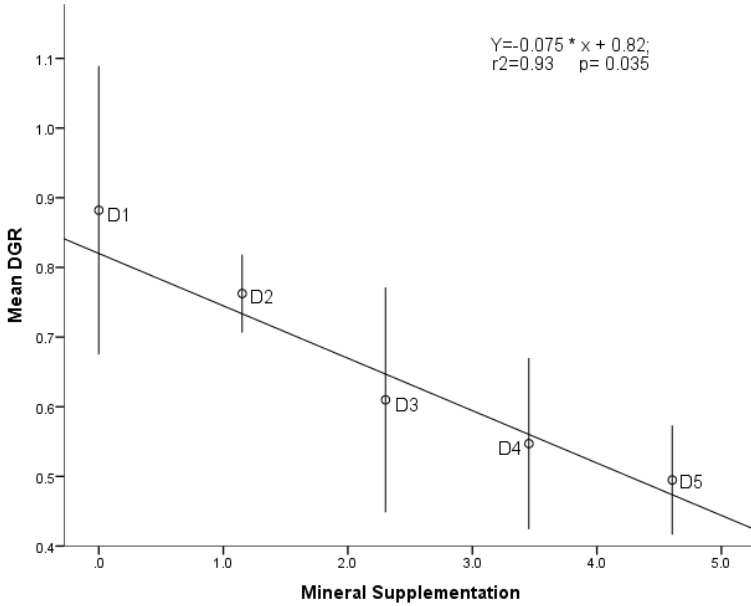


Figure 39 Daily growth rate (Mean±SE) of fish in different levels of mineral supplementation in fish diet.

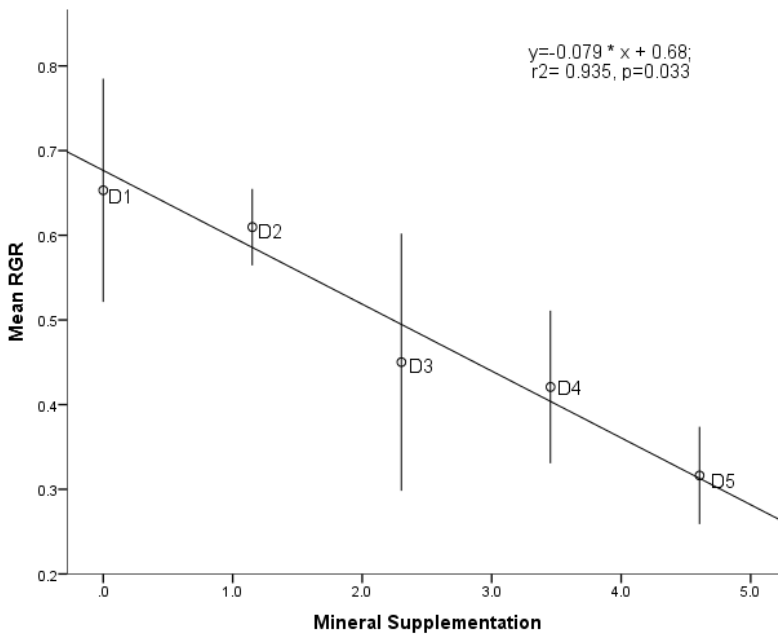


Figure 40 Relative growth rate of fish in different levels of mineral supplementation in fish diet.

Feed conversion ratio increased with increased level of mineral supplementation in fish diet (Figure 41) and showed decline on D₅. Highest FCR was obtained from D₄ treatment. However, the variation between treatments was not significant ($p > 0.05$). Mineral supplementation on fish diet brought significantly quadratic response on FCR ($y = 1.04 + 0.8 * x + -0.12 * x^2$; $r^2 = 0.998$, $p = 0.042$) (Figure 41). FCR values obtained were in agreement with other findings 1.56 (Al-Souti *et al.*, 2012), 1.38 (Azaza *et al.*, 2013), 0.72-1.4 (Abdel-Tawwab and Wafeek, 2014), 1.61-2.03 (El-Saidy and Gaber, 2003), 1.8-2.57 (El-Sayed, 1998), 1.5 (Lam *et al.*, 2015), 1.7 (Portz and Liebert, 2004). However, feed efficiency in this experiment showed slightly inferior value from the result obtained by some investigations 1.23-1.39 (Endut *et al.*, 2010), 1.04-1.11 (Jascha *et al.*, 2007), 1.12-1.45 (Ogunji *et al.*, 2008), 0.95-1.3 (Koch *et al.*, 2016), 1.09-1.37 (Lieberta and Portz, 2005), 1.14-2.8 (Suloma *et al.*, 2014). Feed efficiency was slightly affected by mineral supplementation and this might be associated with the organoleptic characteristic change of fish diet.

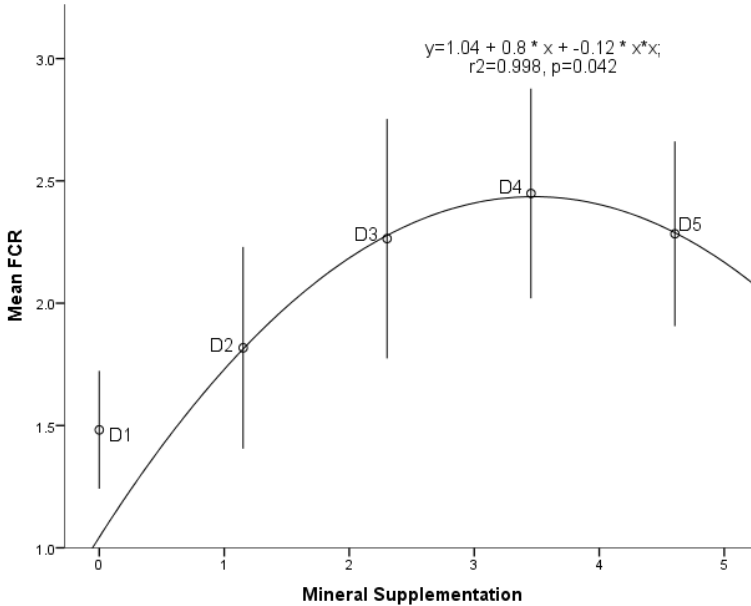


Figure 41 Feed conversion ratio (g/g) of fish for different levels of mineral supplementation on fish diet.

5.4.3 Proximate composition of fish

Proximate composition of fish showed different response for mineral supplementation in DM, CP and ash. Dry matter content of fish showed significantly quadratic response for mineral supplementation level ($y = 25.99 + -3.79 * x + 0.63 * x^2$; $r^2 = 0.871$, $p < 0.05$) (Figure 42). Control treatment (D₁) and D₄ showed highest and lowest DM response, respectively (Figure 42) but mineral supplemented diets brought no significant difference between treatments ($p > 0.05$). Fish DM composition from all treatments were found within the range of DM content reported by different researches 22.42 – 29.31 (Dongmeza *et al.*, 2006; Ogunji *et al.*, 2008; Abdel-Tawwab and Wafeek, 2014; Koch *et al.*, 2016).

Mineral supplementation on fish diet brought numerical variation on fish crude protein content, but the variation was not significant ($p > 0.05$). Highest and lowest CP contents were found from D₁ and D₃, respectively. Even if it was numerically lowest from other treatments, CP value from D₃ was in agreement with other findings 15.6% (Dongmeza *et al.*, 2006), 13.9-15.58% (Ogunji *et al.*, 2008) and 13.97-14.8% (Koch *et al.*, 2016).

All treatments except D₅ showed comparable PPV and PER response of fish. The highest mineral supplementation level 4.6% (D₅) brought significantly lower PPV (Figure 43) and PER (Figure 44) as compared to D₁ ($p < 0.05$); however, protein efficiency ratio values obtained from all experimental diets were in agreement with other studies 1.9-2.9 (Lieberta and Portz, 2005; Dongmeza *et al.*, 2006; Abdel-Tawwab and Wafeek, 2014). Both PPV ($y = -4.26 * x + 35.44$; $r^2 = 0.386$, $p = 0.013$) (Figure 43) and PER ($y = -0.27 * x + 2.35$; $r^2 = 0.394$, $p = 0.012$) (Figure 44) responded significantly linearly for level of mineral supplementation.

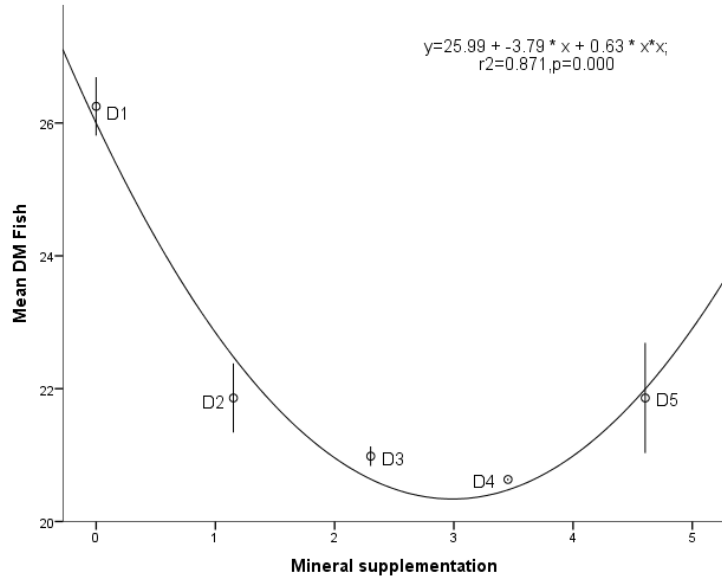


Figure 42 Dry matter content response of whole fish body (Mean±SE) for mineral supplementation on fish diets.

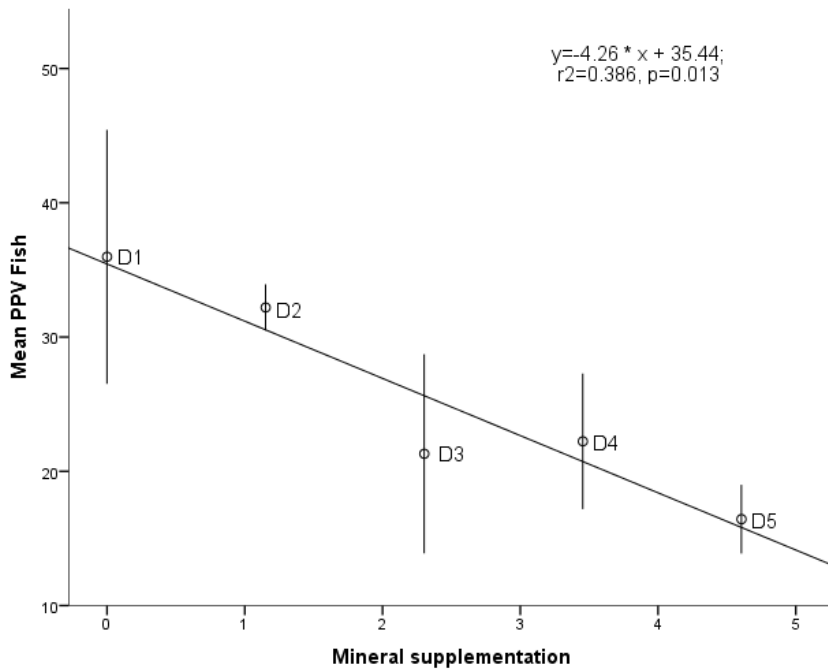


Figure 43 Protein productive value (g/g) response of fish for mineral supplementation on fish diet.

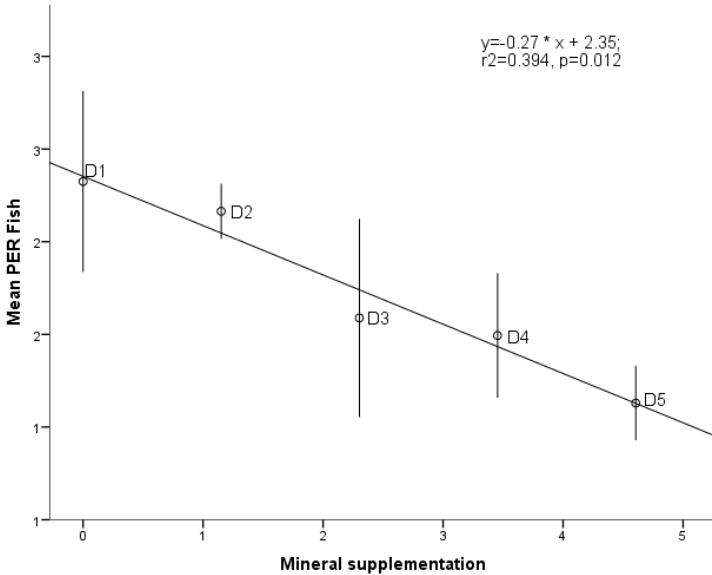


Figure 44 Protein efficiency ratio (%) response of fish for mineral supplementation on fish diets.

5.4.4 Plant biomass

Plant biomass production showed parabolic response for different levels of hydroponic mineral supplementation on fish diet (Figure 48). Significantly highest biomass production was achieved from Hydroponics (H) treatment; however, among experimental diets, D₃ mineral supplementation level resulted in significantly higher biomass production ($p < 0.05$) (Figure 48). Attributes of increased lettuce biomass as a result of increased mineral supplementation in diet include PW ($y = 100.344 + 21.125 * x + -4.281 * x^2$; $r^2 = 0.635$, $p = 0.002$) (Figure 48), LN ($y = 20.33 + 4.49 * x + -0.88 * x^2$; $r^2 = 0.657$, $p = 0.008$) (Figure 46), LW ($y = 76.7 + 22.23 * x + -4.51 * x^2$; $r^2 = 0.695$, $p = 0.005$) (Figure 47), and DW ($y = 6.36 + 4.56 * x + -0.84 * x^2$; $r^2 = 0.697$, $p = 0.005$) (Figure 49). Lettuce biomass responses of all treatments were in agreement with the biomass reported by previous researchers (Cometti *et al.*, 2011; Gent, 2012a; Cometti *et al.*, 2013).

Among the experimental and control diets significantly highest plant fresh biomass (Figure 48), leaf weight (Figure 47) and leaf number (Figure 46) was obtained from D₃ mineral supplementation ($p < 0.05$) despite significantly inferior value as compared to hydroponics treatment (H) ($p < 0.05$). This indicates the possibility of improving aquaponics lettuce fresh weight, leaf weight and leaf number by supplementing Niger seed cake based diet with 2.3% minerals but increasing mineral level inclusion in fish diet beyond may not increase lettuce productivity. In addition, the result of this experiment will not guarantee equivalent fresh weight production, leaf weight and leaf number between aquaponics and hydroponics treatments. The cause for decreasing fresh weight and leaf number with increasing mineral supplementation might be associated with low dietary acceptability by fish which is manifested by higher FCR which is possibly due to organoleptic value change of fish diet due to mineral supplementation.

Root growth increased with increasing dietary mineral supplement level but hydroponics treatment showed significantly highest root growth ($p < 0.05$) (Figure 45). Among the experimental diets significantly highest root biomass was achieved from D₂ ($p < 0.05$) but root biomass showed decreasing pattern with increased mineral supplementation.

Response value for root biomass development for mineral supplementation was higher than the root biomass achieved in different researches 2.8-6 (Cometti *et al.*, 2011; Cometti *et al.*, 2013).

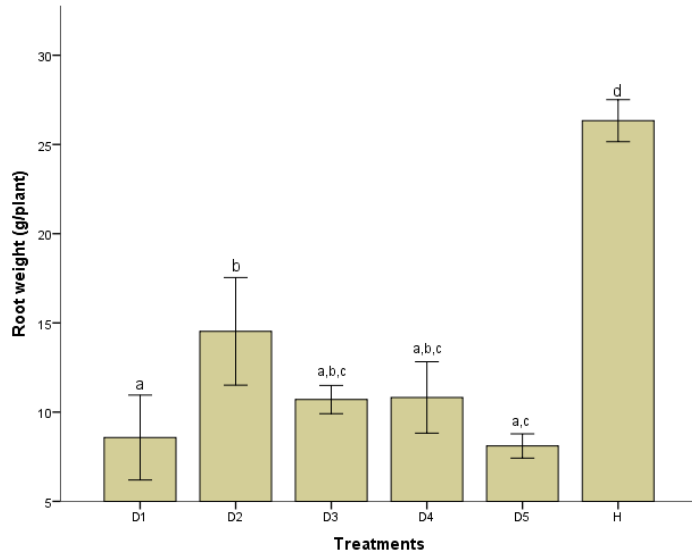


Figure 45 Root fresh weight of lettuce (Mean±SE) subjected to experimental diet and hydroponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

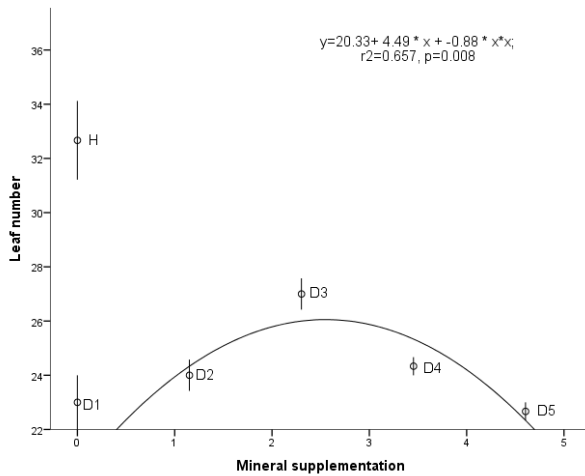


Figure 46 Leaf number (Mean±SE) of lettuce as a function of different levels of mineral supplementation on fish diet.

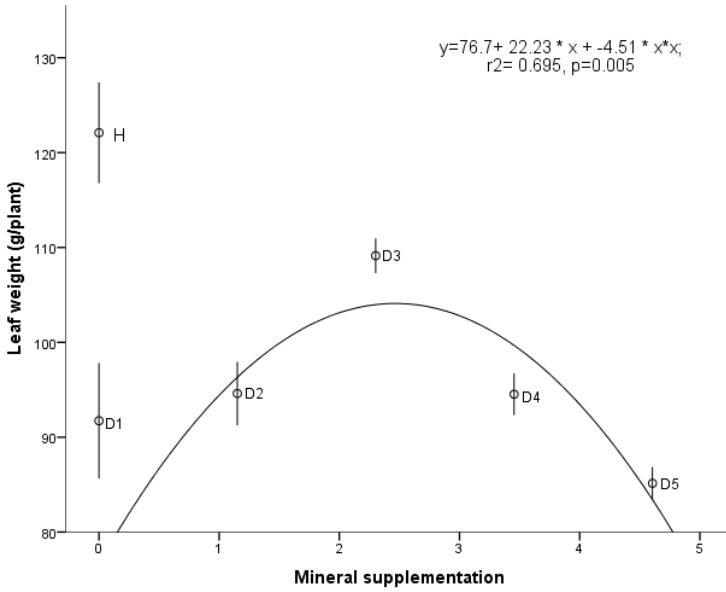


Figure 47 Leaf fresh weight (Mean±SE) of lettuce as a function of different levels of mineral supplementation in fish diet.

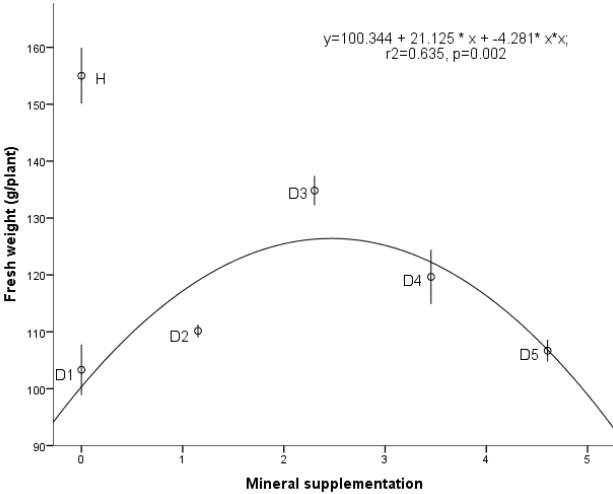


Figure 48 Total fresh weight (Mean±SE) of lettuce as a function of different levels of mineral supplementation in fish diet.

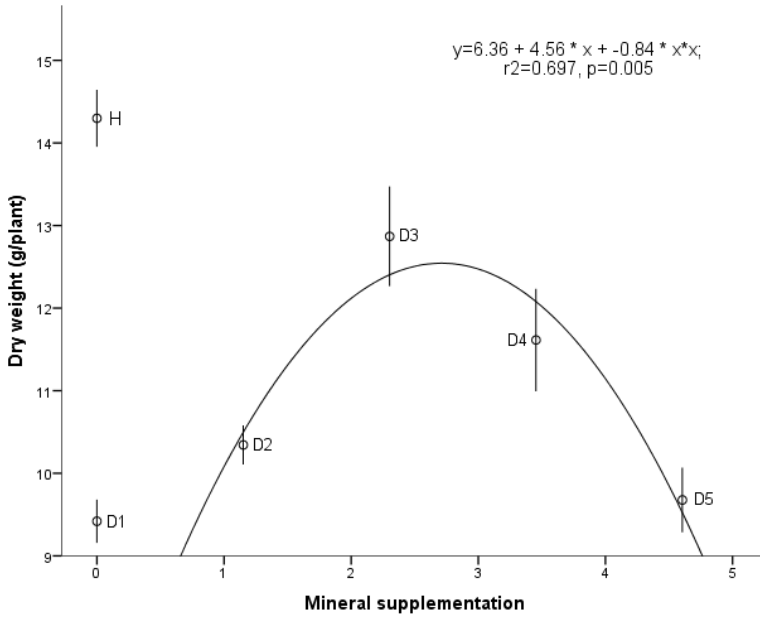


Figure 49 Total dry weight (Mean±SE) of lettuce as a function of different levels of mineral supplementation on fish diet.

5.4.5 Proximate composition of lettuce

Proximate composition of lettuce showed no significant correlation with level of mineral supplementation ($p > 0.05$). However, treatment H showed significantly higher total mineral composition than all experimental diets ($p < 0.05$). All mineral supplementations showed higher proximate composition as compared to control treatment but higher value for DM, CP and ash were recorded from D₃, D₄, and H treatments, respectively. Despite the absence of significant variations, mineral supplementations showed possibility on increasing proximate composition on lettuce.

5.4.6 Growth rate

Lettuce growth rate (AGR and RGR) was significantly correlated with level of mineral supplementation on fish diet. Increasing mineral supplementation level on fish diet brought significantly quadratic AGR ($y = 0.14 + 0.15 * x + -0.027 * x^2$; $r^2 = 0.711$, $p = 0.004$) (Figure 50) response (Figure 51) and significantly highest AGR was found in hydroponics ($p < 0.05$), while among experimental diets D₃ showed significantly highest AGR ($p < 0.05$) (Figure 51).

Among the experimental diets, RGR achieved in D₃ mineral supplementation was significantly higher than the control treatment ($p < 0.05$) and significantly comparable with treatment H ($p > 0.05$) (Figure 51). RGR obtained from this experiment was found to be in agreement with the findings of previous study (Dediu *et al.*, 2012).

Leafiness of the plant (LWR) showed a quadratic response for mineral supplementation and lowest LWR was achieved from D₄. Lettuce plant from D₁, D₂, D₄ and H showed significantly comparable leafiness. Hence, mineral supplementation in fish diet impact on lettuce leafiness was found to be non-significant and inversely quadratic (Figure 54).

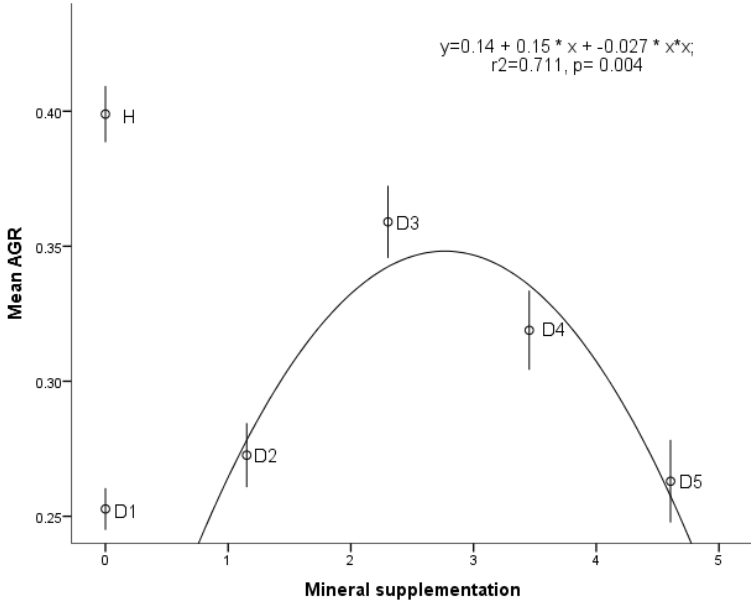


Figure 50 Absolute growth rate (g/day) of lettuce as a function of different levels of mineral supplementation on fish diet.

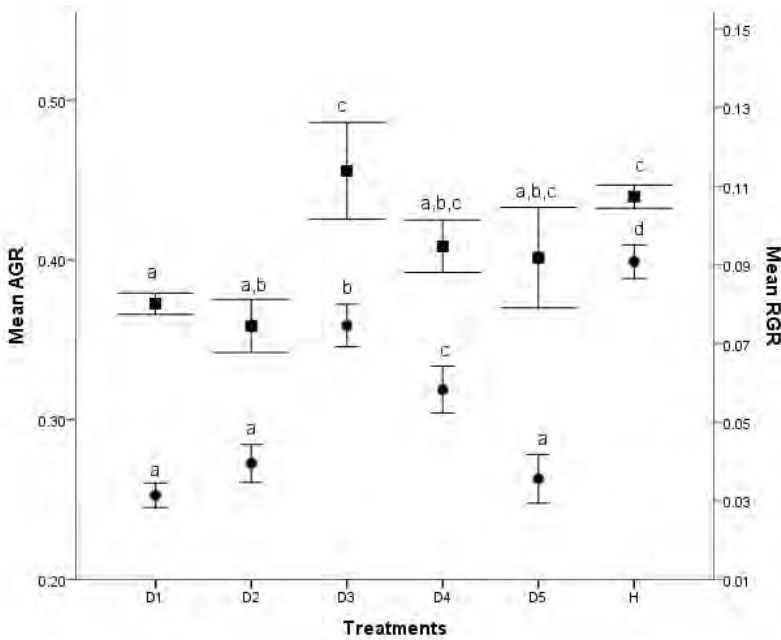


Figure 51 Absolute growth rate (Circle) and RGR (%) (Rectangle) of lettuce for different treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

5.4.7 Lettuce yield

Among the experimental diets significantly highest yield was achieved from D₃ but treatment H resulted in significantly highest yield among all treatments ($p < 0.05$) (Figure 53). Mineral supplementation on fish diet has brought significantly quadratic response of lettuce yield ($y = 4415.126 + 929.506 * x + -188.37 * x^2$; $r^2 = 0.635$, $p = 0.002$) (Figure 52).

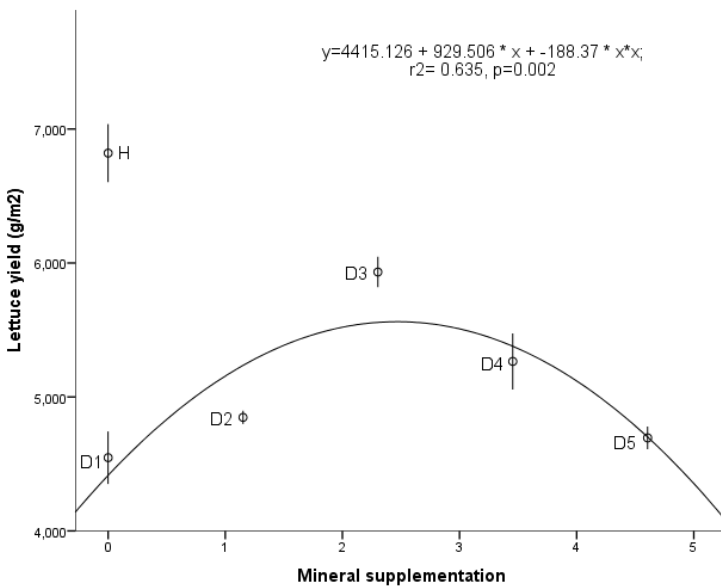


Figure 52 Lettuce yield response as a function of different levels of mineral supplementation in fish diet.

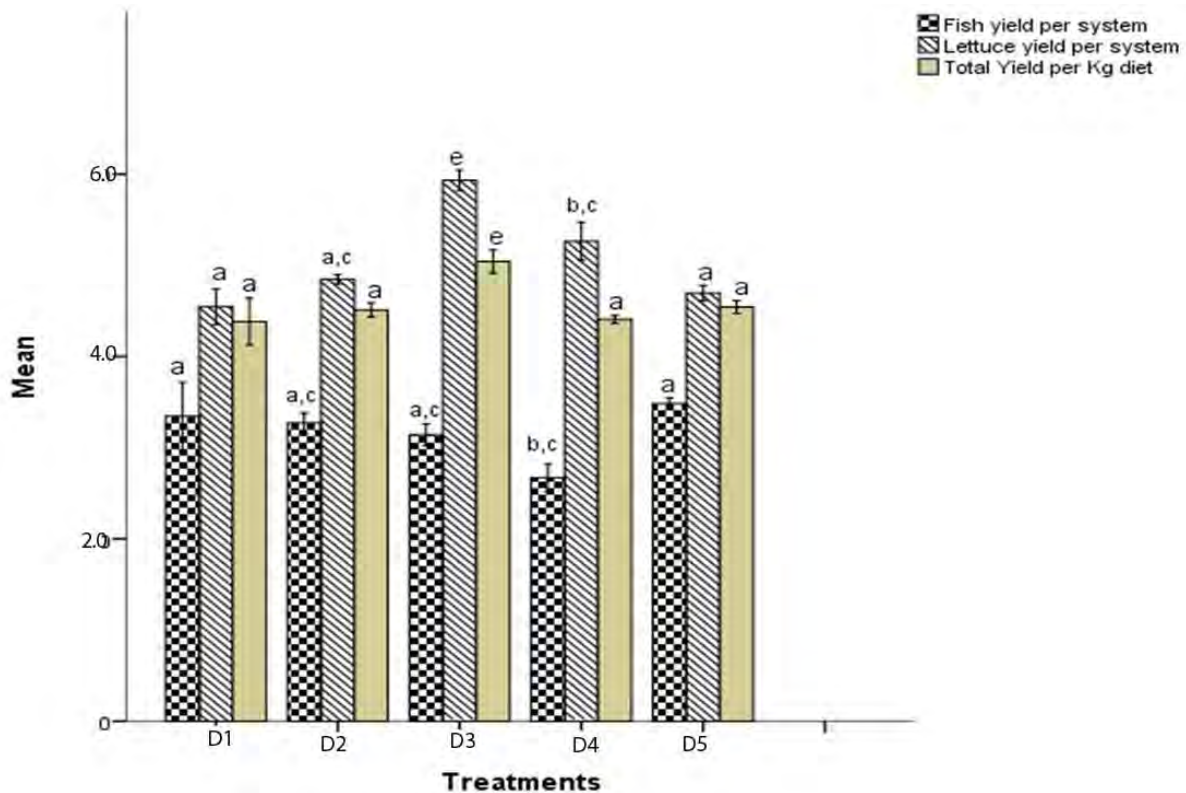


Figure 53 Fish yield (kg), lettuce yield (kg) and total yield (kg per kg diet) of aquaponics treatments. Mean values sharing common letters are not significantly different ($p < 0.05$).

Total yield (fish and lettuce) per kg diet administered to the system showed statistical variation between treatments and significantly highest total yield recorded from D₃ ($p < 0.05$). However, lettuce and fish yield per system showed opposite response for experimental diets and significantly lowest fish yield was achieved from D₄ and significantly highest lettuce yield was obtained from D₃ ($p < 0.05$) (Figure 53).

Root to shoot ratio of lettuce was significantly correlated ($p < 0.05$) with dissolved ammonia concentration: increasing ammonia concentration had a tendency to increase root to shoot ratio. In addition, stepwise regression showed combined effects of physical and chemical qualities of the

growing medium ($p < 0.05$) significantly affected lettuce biomass. Among all growing medium qualities, total nitrogen (sum of ammonia, nitrite, and nitrate) significantly affected fresh lettuce biomass development ($p < 0.05$). Total nitrogen and phosphate concentration were significantly correlated with fresh biomass and fresh leaf weight ($p < 0.05$).

5.4.8 Minerals in water

Among experimental diets, significantly highest and lowest dissolved ammonia concentration was recorded from D₅ and H, respectively ($p < 0.05$). Ammonia concentration linearly increased with level of mineral supplementation in fish diet (Figure 58). Ammonia concentration from the experimental diets was below the control diet; hence, mineral supplementation brought lowest accumulation of toxic ammonia as compared to the control diet.

Increasing mineral supplementation resulted in ammonia buildup which was significantly correlated with DO concentration ($p < 0.05$, $r^2 = 0.499$). Simultaneous increased pH and ammonia concentration resulted in the decrease of fish growth rate due to the increased ammonium ion conversion to ammonia, which is a gill poison for the fish that will lead to low feed intake. Dissolved ammonia concentration was significantly correlated with increased pH value ($p < 0.05$, $r^2 = 0.526$).

Nitrite concentration decreased with increased mineral supplementation on fish diet and significantly highest and lowest nitrite concentration was recorded from D₁ and D₅, respectively ($p < 0.05$) (Figure 56). However, nitrite concentration of all treatments except D₁ was statistically not different with nitrite concentration in D₁ and H. This indicates the condition in which the

impact of mineral supplementation with respect to nitrite accumulation on fish and lettuce would not be negative.

All experimental diets resulted in significantly lowest nitrate accumulation ($p < 0.05$) as compared to treatment H (Figure 57). Nitrate and total dissolved nitrogen levels were found significantly comparable among experimental diets ($p > 0.05$) where numerically highest Nitrate and total nitrogen content were observed from D₃. Mineral supplementation in fish diet resulted in significantly quadratic total dissolved nitrogen response ($r^2 = 0.354$, $p = 0.072$). Total dissolved nitrogen was significantly correlated with AGR ($r^2 = 0.535$, $p = 0.004$) and LWR ($r^2 = 0.52$, $p = 0.042$). Increased total dissolved nitrogen content had resulted in increased AGR but with reduced LWR. However, no significant correlation was observed between total yield and fish growth rate and total dissolved nitrogen ($p > 0.05$).

Mineral supplementation in fish diet resulted in significantly quadratic response for dissolved phosphate concentration ($r^2 = 0.723$, $p < 0.01$). Significantly highest phosphate concentration came from D₅ and H treatments ($p < 0.05$). Phosphate concentration from D₃ was found numerically higher than other experimental diets (Figure 55).

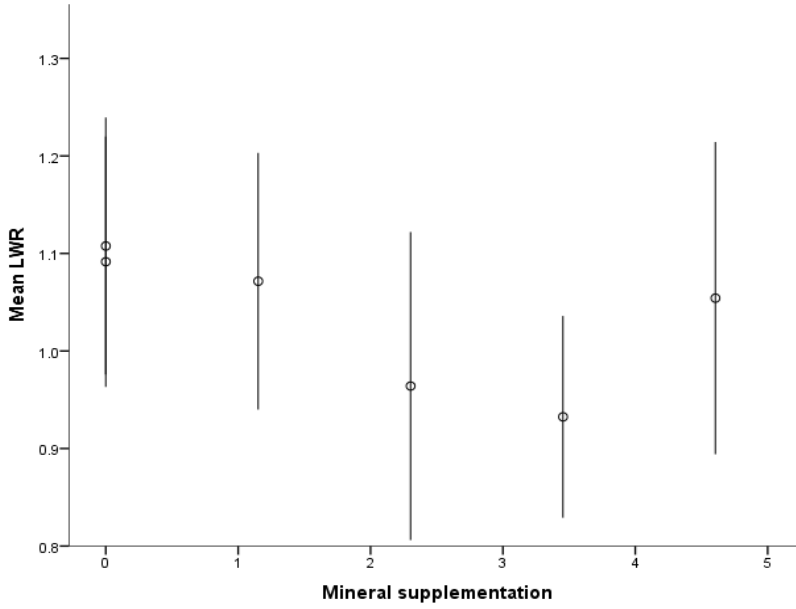


Figure 54 Leaf weight ratio (Mean±SE) of lettuce for different treatments.

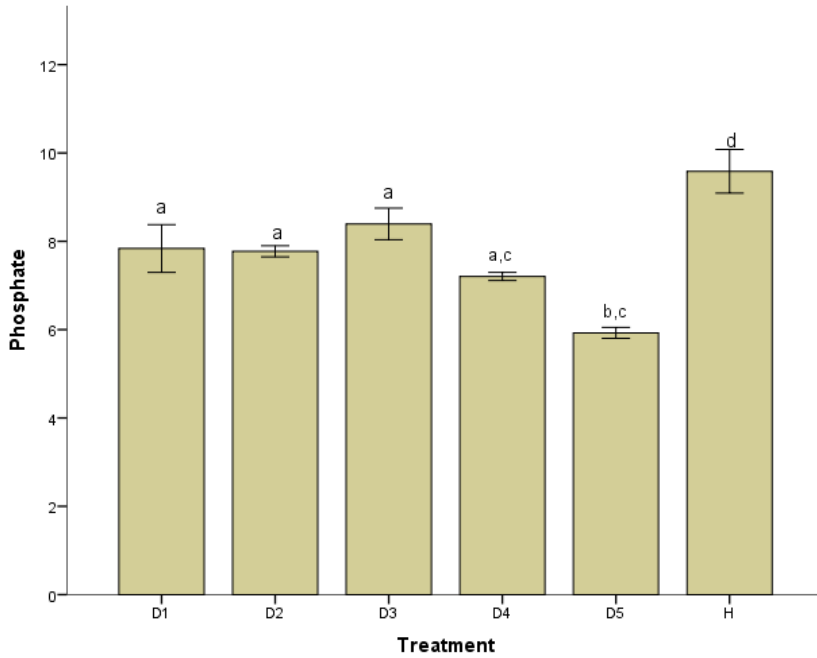


Figure 55 Dissolved phosphate (Mean±SE) concentration of growing medium. Mean values sharing common letters are not significantly different ($p < 0.05$).

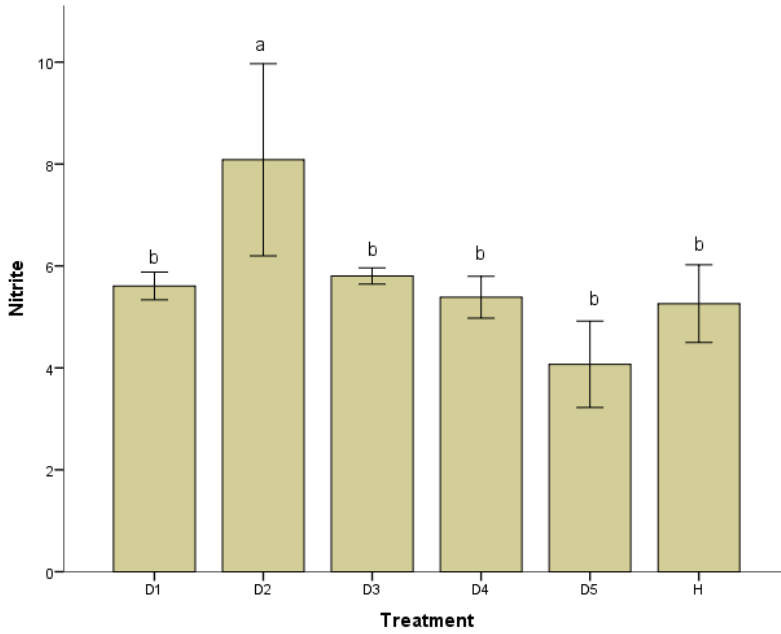


Figure 56 Dissolved Nitrite (Mean±SE) concentration of growing medium. Mean values sharing common letters are not significantly different ($p < 0.05$).

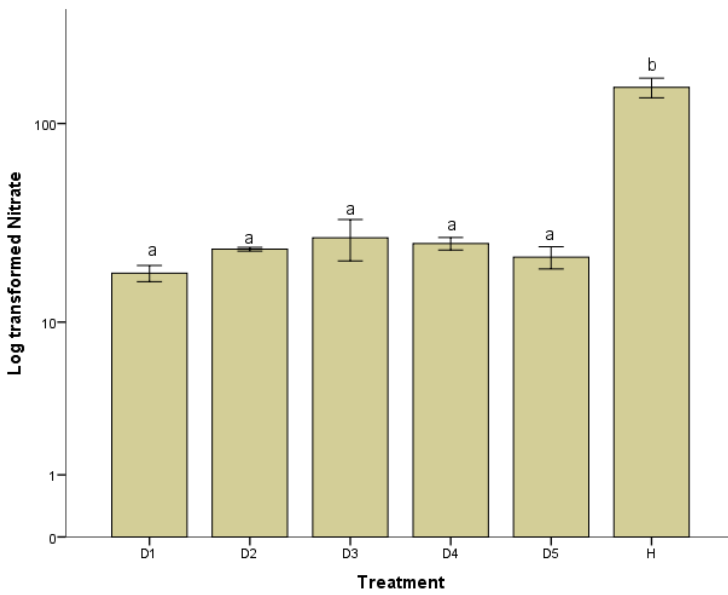


Figure 57 Dissolved Nitrate (Mean±SE) concentration of growing medium. Mean values sharing common letters are not significantly different ($p < 0.05$).

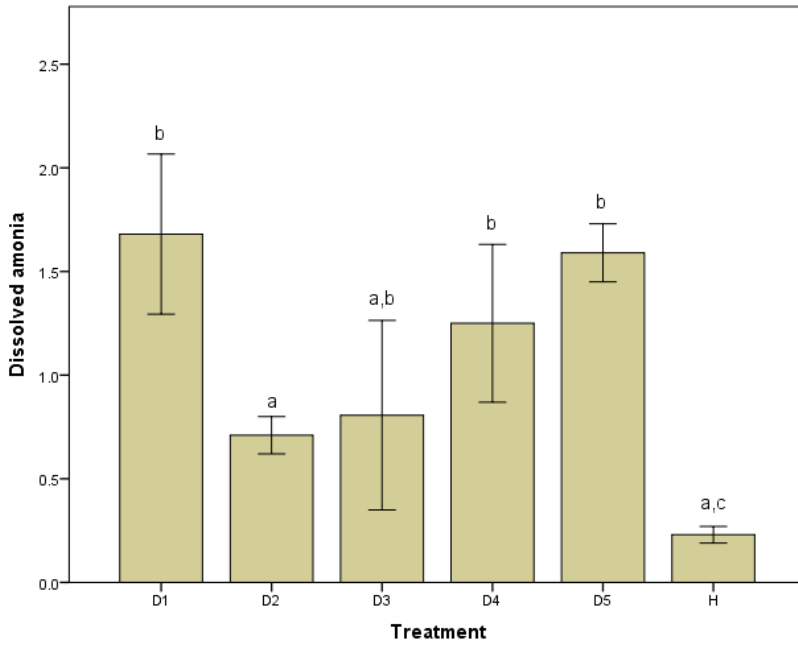


Figure 58 Dissolved ammonia (Mean±SE) concentration of growing medium. Mean values sharing common letters are not significantly different ($p < 0.05$).

Chapter 6

General discussion

Niger seed cake dietary inclusion level brought fish and lettuce growth difference among treatments. Experimental diets brought quadratic response with respect to fish and plant biomass development and resource use efficiency and highest growth performance of fish and lettuce observed from D₄ (37.5%). Other researches also mentioned the negative effect of extreme plant to fish meal ratio in fish diet on fish growth (El-Saidy and Gaber, 2003; Koch *et al.*, 2016). Niger seed cake effect on SGR of fish was quadratic but the value obtained from this experiment were comparable with SGR values obtained using different plant materials as feed ingredient for Nile tilapia in aquaculture: canola 2.39 (Plaipetch and Yakupitiyage, 2014), jatropha 5 (Kumar *et al.*, 2012), soybean 2.29 (Abdel-Warith *et al.*, 2013), plant protein 2.64 (Fontainhas-Fernandes *et al.*, 1999). Niger seed cake effect on FCR was quadratic and the value obtained was comparable with value obtained from studies done on aquaculture using soybean 1.09 - 1.4 (Abdel-Warith *et al.*, 2013; Plaipetch and Yakupitiyage, 2014), and soybean and jatropha 1.7 (Kumar *et al.*, 2012).

Niger seed cake dietary inclusion brought increased protein use efficiency up to D₄ and the value obtained was in agreement with PER value reported in different aquaculture researches using soybean meal 1.39-2.5 (Thompson *et al.*, 2012; Plaipetch and Yakupitiyage, 2014), faba bean meal 2.04-2.34 (Azaza *et al.*, 2009b) and canola diet 2.1-2.46 (Plaipetch and Yakupitiyage 2014).

All aquaponics treatments with Niger seed cake dietary inclusion resulted in low concentration of minerals in fish water than control diet. Continuous use of fish diet in aquaponics as a sole nutrient source resulted in nutrient deficiency in fish water for plant growth (Rakocy, 1997; Seawright *et*

al., 1998; Endut *et al.*, 2010). This was manifested by the low value of minerals and lettuce biomass attained from all aquaponics treatments as compared to hydroponic treatment.

Despite the low response of lettuce biomass for aquaponics diet with Niger seed cake as compared to hydroponics: the values obtained from this experiment were within the range of biomass production obtained by different scholars in aquaponics and hydroponics setups (Sikawa & Yakupitiyage, 2010, Cometti *et al.*, 2013; Dediu *et al.*, 2012; Gent, 2012a). In these studies significant effect of Niger seed cake effect on lettuce dry mater content were not observed and lettuce dry mater value remained within the normal range under good growth condition (4-6%) (Dediu *et al.*, 2012) and were comparable with values by other researchers 4-5 (Sikawa and Yakupitiyage, 2010; Gent, 2012b; Cometti *et al.*, 2013). Lettuce biomass and growth rates response were significantly affected by mineral level in fish water ($p < 0.05$) and the most limiting mineral from this experiment was Fe which is also deficient in other aquaponics trial (Seawright *et al.*, 1998). Despite the mineral limitation lettuce growth rate response for Niger seed cake were quadratic and the value obtained from this study were in agreement with growth rates value obtained from previous researches (Dediu *et al.*, 2012). Lettuce AGR obtained in this experiment was within the range reported by previous study 3.14-3.54 (Dediu *et al.*, 2012).

Therefore, to alleviate the challenges of mineral deficiency in the system dietary mineral supplementation was taken as alternative and tested for efficiency. From aforementioned experiments fish diet with 37.5% Niger seed cake resulted in good growth of fish and lettuce. Supplementing this diet with different level of hydroponic minerals brought different response for fish and lettuce. Increased dietary mineral content resulted in decreased fish growth and increased plant growth. Increased mineral supplementation resulted in decreased FCR but the value remained

within the range reported in other aquaculture researches 1.56 (Al-Souti *et al.*, 2012), 1.38 (Azaza *et al.*, 2013), 0.72 (Abdel-Tawwab and Wafeek, 2014), 1.61 (El-Saidy and Gaber, 2003), (El-Sayed, 1998), 1.5 (Lam *et al.*, 2015), 1.7 (Portz and Liebert, 2004). Even if the value of PPV is significantly lowest in D₅ ($p < 0.05$), the PER value obtained were in agreement with other studies 1.9-2.9 (Lieberta and Portz, 2005; Dongmeza *et al.*, 2006; Abdel-Tawwab and Wafeek, 2014). Lettuce growth and biomass response for dietary mineral supplementation were significantly quadratic ($p < 0.05$) and D₃ resulted highest lettuce performance. The value obtained in this study were in agreement with the biomass reported by previous researchers (Cometti *et al.*, 2011; Gent, 2012a; Cometti *et al.*, 2013). Aquaponics diet formulated from 37.5% Niger seed cake inclusion provide highest lettuce yield when supplemented with 2.3% hydroponic mineral as dietary supplement. Significantly highest lettuce biomass obtained from hydroponic treatment 6.82 kg(m)⁻² followed by D₃ 5.93 kg(m)⁻². Lettuce yield from D₃ was higher than reported by previous works: 3.45 kg(m)⁻² (Dediu *et al.*, 2012), 2 kg(m)⁻² (Seawright *et al.*, 1998), 4.13 kg(m)⁻² (Lennard and Leonard, 2004), 2 kg(m)⁻² (Rafiee and Roos, 2006), and 3.5 kg(m)⁻² (Fallovo *et al.*, 2009). In this experiment significantly highest total yield (fish and lettuce) per kg diet delivered to the system was obtained from D₃ (37.5 NC and 2.3% dietary mineral supplement) 5.041 kg(kg diet)⁻¹ ($p < 0.05$).

Chapter 7

General conclusion and recommendations

The existing natural, social, and economical environment favors aquaponics development as business venture in Ethiopia. However, the development of the sector is limited or non-existent in Ethiopia due to several factors including lack of quality input like fish feed for the sector. Despite the potential of producing fish and vegetable with a single input (fish diet), aquaponics low productivity is evident in many places due to the use of commercial fish diet which is produced considering only the fish physiological demand and ignoring the plant demand. Therefore, optimizing aquaponics productivity based on the input (fish diet) quality produced from locally available ingredients by considering both fish and plant physiological demand is found to be the critical research issue to be addressed for the development of aquaponics in Ethiopia. Therefore, this research analyzed the potential of Niger seed cake to be used as basic fish diet ingredient and the optimum level of mineral supplementation in fish diet for increased aquaponics productivity. Niger seed cake was selected for the study due to cost implication, availability, and nutritional quality. Studying level of dietary mineral supplementation is selected due to lack of plant demand considerations on existing fish diet used to produce both fish and plant together in aquaponics to increase productivity.

Major conclusions drawn from this research are as follows:

1. Nile tilapia perform good growth in higher Niger seed cake inclusion in aquaponics diet and therefore we recommend Niger seed cake which is relatively accessible and cheaper in

Ethiopia to be used in aquaponics diet as a major Nitrogen (protein) source. NC:FM ratio of 2.14 is the best combination for growth and physiological response of tilapia;

2. Result from this experiment showed possibility of using Niger seed cake in fish diet for aquaponics systems. Highest lettuce growth performance was obtained from NC:FM ratio of 2.14. Therefore, it has been concluded that Niger seed cake dietary inclusion to the level of 37.5% resulted good growth of lettuce in aquaponics; and
3. Hydroponic mineral salts can be incorporated in fish diet to increase aquaponics productivity. Hydroponic mineral supplementation up to 2.3% brought highest biomass production without negative impact on tilapia and lettuce growth conditions. Therefore, it can be concluded that hydroponic mineral supplementation on fish diet is beneficial to increase productivity of the system.

From the research the three major recommendations are:

- Detailed analysis on aminoacid contribution of NC is recommended for future investigations;
- Refining the diet quality using mineral supplementation to increase productivity above hydroponic production system can be achieved hence detail mineral composition investigation should be done with respect to diet mineral composition for efficient growth and development of both fish and lettuce; and
- Detailed analysis on mineral interaction with the microorganisms and sensory value of products is recommended for future aquaponics studies.

Chapter 8

References

- Abdel-Fattah, E. (1990). Long-term evaluation of cotton seed meal as a protein source for Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture* **84**(3–4): 315-320.
- Abdel-Tawwab, M., Ahmad, M., Khattab, Y. & Shalaby, A. (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.) *Aquaculture* **298**: 267-274.
- Abdel-Tawwab, M. & Wafeek, M. (2014). Influence of water temperature and waterborne cadmium toxicity on growth performance and metallothionein-cadmium distribution in different organs of Nile tilapia, *Oreochromis niloticus* (L.). *J Therm Biol* **45**: 157-162.
- Abdel-Warith, A., Younis, E., Al-Asgah & Abdulla, N. (2013). Influence of Dietary Inclusion of Full-Fat Soybean Meal and Amino Acids Supplementation on Growth and Digestive Enzymes Activity of Nile Tilapia, *Oreochromis niloticus*. *Turkish Journal of Fisheries and Aquatic Sciences* **13**(1): 69-77.
- Ahmad, M. & Abdel-Tawwab, M. (2011). The use of caraway seed meal as a feed additive in fish diets: Growth performance, feed utilization, and whole-body composition of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. *Aquaculture* **314**: 110-114.

- Al-Souti, A., Al-Sabahi, J., Soussi, B. & Goddard, S. (2012). The effects of fish oil-enriched diets on growth, feed conversion and fatty acid content of red hybrid tilapia, *Oreochromis sp.* *Food Chem* **133**: 723–727.
- Alder, P. (2001). Overview of economic evaluation of phosphorus removal by plants. *Aquaponics Journal* **5**: 15-18.
- Anderson, A., Alam, M., Watanabe, W., Carroll, P., Wedegaertner, T. & Dowd, M. (2016). Full replacement of menhaden fish meal protein by low-gossypol cottonseed flour protein in the diet of juvenile black sea bass *Centropristis striata*. *Aquaculture* **464**: 618-628.
- AOAC (2000). *Official methods of analysis*. Arlington, Virginia, USA, : AOAC.
- APHA (1999). Standard Methods for the Examination of Water and Wastewater In *American Public Health Association: American Water Works Association, Water Environment Federation*.
- Assimakopoulou, A., Kotsiras, A. & Nifakos, K. (2013). Incidence of Lettuce Tipburn as Related to Hydroponic System and Cultivar. *Journal of Plant Nutrition* **36**(9): 1383-1400.
- Astuti, D., Becker, K. & Richter, N. (2009). Utilization of methanol extracted of moringa and mulberry leaves to evaluate energy and protein balance of Nile tilapia. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*: 70-82.

- Azaza, M., Assad, A., Maghrbi, W. & El-Cafsi, M. (2013). The effects of rearing density on growth, size heterogeneity and inter-individual variation of feed intake in monosex male Nile tilapia *Oreochromis niloticus* L. *Animal* **7**(11): 1865-1874.
- Azaza, M., Kammoun, W., Abdelmouleh, A. & Kraiem, M. (2009a). Growth performance, feed utilization, and body composition of Nile tilapia (*Oreochromis niloticus* L.) fed with differently heated soybean-meal-based diets. *Aquaculture International* **17**(6): 507-521.
- Azaza, M., Wassim, K., Mensi, F., Abdelmouleh, A., Brini, B. & Kraiem, M. (2009b). Evaluation of faba beans (*Vicia faba* L. var. *minuta*) as a replacement for soybean meal in practical diets of juvenile Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **287**(1-2): 174-179.
- Azim, M. & Little, D. (2008). The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **283**(1-4): 29-35.
- Bajsa, O., Nair, J., Mathew, K. & Ge, H. (2003). Vermiculture as a tool for domestic wastewater management. *Water Sci Technol.* **48**(11): 1245-1132.
- Bell, J., Henderson, R., Tocher, D. & Sargent, J. (2004). Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. *Lipids* **39**(3): 223-232.

- Bhavimani, H. & Puttaiah, E. (2014). Fish Culture and Physico-chemical Characteristics of Madikoppa Pond, Dharwad Tq/Dist, Karnatak *Hydrology Current Research* **5**(1): 1-3.
- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I. & Corner, R. (2010). Aquaculture: global status and trends. *Philos Trans R Soc Lond B Biol Sci* **365**: 2897-2912.
- Brinker, A. & Reiter, R. (2011). Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part I: Effects on feed utilization and fish quality. *Aquaculture* **310**(3-4): 350-360.
- Buzby, K., Waterland, N., Semmens, K. & Lin, L. (2016). Evaluating aquaponic crops in a freshwater flow-through fish culture system. *Aquaculture* **460**: 15-24.
- Cai, C., Song, L., Wang, Y., Wu, P., Ye, Y., Zhang, Z. & Yang, C. (2013). Assessment of the feasibility of including high levels of rapeseed meal and peanut meal in diets of juvenile crucian carp (*Carassius auratus gibelio*♀ × *Cyprinus carpio*♂): Growth, immunity, intestinal morphology, and microflora. *Aquaculture* **410**: 203-215.
- Cai, H., You, M., Ryall, K., Li, S. & Wang, H. (2011). Physiological Response of Chinese Cabbage to Intercropping Systems. *Agronomy Journal* **103**(2): 331-336.
- Carballo, E., Eer, A., Schie, T. & Hilbrands, A. (2008). *Small-scale freshwater fish farming*. Wageningen, the Netherlands: Agromisa Foundation and CTA.

- Chadd, A., Davies, P. & Koivisto, J. (2002). Protein sources for the animal feed industry. In *Protein sources for the animal feed industry, Expert consultation and workshop, 25* Bangkok: Food and Agriculture Organization.
- Chen, X., Gastaldi, C., Siddiqi, M. & Glass, A. (1997). Growth of a lettuce crop at low ambient nutrient concentrations: A strategy designed to limit the potential for eutrophication. *Journal of Plant Nutrition* **20**(10): 1403-1417.
- Collins, S., Desai, A., Mansfield, G., Hill, J., Kessel, V. & Drew, M. (2012). The effect of increasing inclusion rates of soybean, pea and canola meals and their protein concentrates on the growth of rainbow trout: Concepts in diet formulation and experimental design for ingredient evaluation. *Aquaculture* **344**: 90-99.
- Cometti, N., Bremenkamp, D., Galon, K., Hell, L. & Zanotelli, M. (2013). Cooling and concentration of nutrient solution in hydroponic lettuce crop. *Horticultura Brasileira* **31**: 287-292.
- Cometti, N., Martins, M., Bremenkamp, C. & Nunes, J. (2011). Nitrate concentration in lettuce leaves depending on photosynthetic photon flux and nitrate concentration in the nutrient solution. *Horticultura Brasileira* **29**: 548-553.
- Crab, R., Kochvac, M., Verstraete, W. & Avnimelech, Y. (2009). Bio-flocs technology application in over-wintering of tilapia. *Aquacultural Engineering* **40**: 105–112.

- David, D., Boardman, G., Lawrence, A., Marsh, L. & Flick, G. (2009). Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. *Aquaculture* **296**: 51-57.
- De Schryver, P., Crab, R., Defoirdt, T., Boon, N. & Verstraete, W. (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture* **277**: 125–137.
- Dediu, L., Cristea, V. & Xiaoshuan, Z. (2012). Waste production and valorization in an integrated aquaponic system with baster and lettuce. *African Journal of Biotechnology* **11**(9): 2349-2358.
- Diver, S. (2006). Aquaponics-Integration of hydroponics and aquaculture In *Appropriate Technology for Rural Areas: Horticulture system guide* 21 Fayetteville.
- Dongmeza, E., Siddhuraju, P., Francis, G. & Becker, K. (2006). Effects of dehydrated methanol extracts of moringa (*Moringa oleifera* Lam.) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (*Oreochromis niloticus* (L.)). *Aquaculture* **261**: 15.
- Ebeling, J. & Timmons, M. (2012). Recirculating aquaculture system. In *Aquaculture production systems*, 421 (Ed J. Tidwell). Iowa, USA: John Willey & Sons, Ltd.

- Edwards, P. (2013). *Philosophy, principles and concepts of integrated Agri-Aquaculture systems*.
In: Integrated Agri-Aquaculture system. Canberra, Australia: Rural Industries research and
development corporation.
- Edwards, P. (2015). Aquaculture environment interactions: Past, present and likely future trends.
Aquaculture **447**: 2–14.
- El-Saidy, D. & Gaber, M. (2003). Replacement of fish meal with a mixture of different plant
protein sources in juvenile Nile tilapia *Oreochromis niloticus* (L.) diets. *Aquaculture
Research* **34** (3): 297-305.
- El-Sayed, A. (1998). Total replacement of fish meal with animal protein sources in Nile tilapia,
Oreochromis niloticus (L.), feeds. *Aquaculture Research* **29**: 275-280.
- El-Sayed, A. (1999). Alternative dietary protein sources for farmed tilapia, *Oreochromis spp.*
Aquaculture **179** 149–168.
- El-Sayed, A. (2006). *Tilapia Culture*. USA: CABI Publishing.
- Endut, A., Jusoh, A., Ali, N., Nik, W. & Hassan, A. (2010). A study on the optimal hydraulic
loading rate and plant ratios in recirculation aquaponic system. *Bioresour Technol* **101**(5):
1511-1517.

- Fageria, N., Baligar, V. & Clark, R. (2002). Micronutrients in Crop Production. In *Advances in Agronomy*, Vol. **77**, 185-268 (Ed L. Donald). Academic Press.
- Falovo, C., Roupael, Y., Rea, E., Battistellid, A. & Collaa, G. (2009). Nutrient solution concentration and growing season affect yield and quality of *Lactuca sativa* L. var. acephala in floating raft culture. *J. Sci. Food Agric.* **89**: 1682–1689.
- FAO (2003). *Information on fisheries management in the Federal Democratic Republic of Ethiopia*. Rome, Italy: food and Agriculture Organization of the United Nations.
- FAO (2009). *Study and analysis of feeds and fertilizers for sustainable aquaculture development*. Rome, Italy: Food and Agriculture Organization of the United Nations.
- FAO (2010). *Fishery and Aquaculture Statistics*. Rome, Italy: Food and agriculture organization of the United Nations.
- FAO (2012). *The state of world fisheries and aquaculture*. Rome, Italy: Food and Agriculture Organization of the United Nations.
- FAO (2014a). Nile tilapia - Feed formulation. In *Aquaculture Feed and Fertilizer Resources Information System* Rome, Italy: Food and agriculture organization.
- FAO (2014b). *The State of Fisheries and Aquaculture- Opportunities and Challenges*. . Rome, Italy: Food and Agriculture Organization of the United Nations.

- Feedipedia. (2013).Niger (*Guizotia abyssinica*) oil meal, expeller extraction. In *Animal Feed Resources Information System* INRA CIRAD AFZ and FAO.
- Fitzsimmons, K. (1991).Intensive tilapia production integrated with field crops in Southwest U.S. In *3rd International Symposium on tilapia aquaculture*. Manilla, Philipines: ICLARM. .
- Fontainhas-Fernandes, A., Gomes, E., Reis-Henriques, M. & Coimbra, J. (1999). Replacement of fish meal by plant proteins in the diet of Nile tilapia: digestibility and growth performance. *Aquaculture International* **7**(1): 57-67.
- Fornshell, G., Hinshaw, J. & Tidwell, J. (2012).Flow-through raceways. In *Aquaculture production systems*, 421 (Ed J. Tidwell). Iowa, USA: John Willey & Sons, Ltd.
- Garduno-Lugo, M. & Olvera-Novoa, M. (2008). Potential of the use of peanut (*Arachis hypogaea*) leaf meal as a partial replacement for fish meal in diets for Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research* **39**(12): 1299-1306.
- Gent, M. (2012a). Composition of hydroponic lettuce: effect of time of day, plant size, and season. *J Sci Food Agric* **92**(3): 542-550.
- Gent, M. (2012b). Rate of change of composition of lettuce in response to nitrogen depletion or re-supply. *J Sci Food Agric* **92**(15): 3007-3015.

- Getinet, A. & Sharma, S. (1996). *Niger: Guizotia abyssinica (L. f.) Cass. Promoting the conservation and use of underutilized and neglected crops. 5.* Rome, Italy: Institute of Plant Genetics and Crop Plant Research, Gatersleben, International Plant Genetic Resources Institute.
- Gladys, N. & Aba, I. (2015). Effects of Maturity on the Proximate Composition of Lettuce (*Lactuca sativa*). *Academic Research International* **6**(1): 1-8.
- Goddard, S., Al-Busaidi, A. & Al-Kendi, U. (2010). Fish Culture and Hydroponics at Low Salinities: A Monograph on Management of Salt-Affected Soils and Water for Sustainable Agriculture. Vol. Masters, 89-94 Sultanate of Oman: Sultan Qaboos University.
- Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K., Jijakli, H. & Thorarinsdottir, R. (2015). Challenges of Sustainable and Commercial Aquaponics. *Sustainability* **7**(4): 4199-4224.
- Gonzales, J. M. & Brown, P. B. (2007). Nutrient retention capabilities of Nile tilapia (*Oreochromis niloticus*) fed bio-regenerative life support system (BLSS) waste residues. *Advances in Space Research* **40**(11): 1725-1734.
- Goto, E., Both, A., Albright, L., Langhans, R. & Leed, A. (1996). Effect of dissolved oxygen concentration on lettuce growth in floating hydroponics. *Acta Horti* **440**: 205-210.

- Goto, F., Yoshihara, T. & Saiki, H. (2000). Iron accumulation and enhanced growth in transgenic lettuce plants expressing the iron-binding protein ferritin. *Theoretical and Applied Genetics* **100**(5): 658-664.
- Graber, A. & Junge, R. (2009). Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. *Desalination* **246**: 147–156.
- Gullian-Klanian, M. & Arámburu-Adame, C. (2013). Performance of Nile tilapia (*Oreochromis niloticus*) fingerlings in a hyper-intensive recirculating aquaculture system with low water exchange. *Lat. Am. J. Aquat. Res.*, **41**(1): 150-162.
- Guo, Y., Dong, X., Tan, B., Chi, S., Yang, Q., Chen, G. & Zhang, L. (2011). Partial replacement of soybean meal by sesame meal in diets of juvenile Nile tilapia, *Oreochromis niloticus* L. *Aquaculture Research* **42**(9): 1298-1307.
- Gutierrez-Wing, M. & Malone, R. (2006). Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. *Aquacultural Engineering* **34**(3): 163-171.
- Hecht, T. (2007). *Review of feeds and fertilizers for sustainable aquaculture development in sub-Saharan Africa*. . Rome, Italy: Food and Agriculture Organization of the United Nations.

- Heo, J., Kang, D., Bang, H., Hong, S., Chun, C. & Kang, K. (2012). Early Growth, Pigmentation, Protein Content, and Phenylalanine Ammonia-lyase Activity of Red Curled Lettuces Grown under Different Lighting Conditions. *Kor. J. Hort. Sci. Technol.* **30**(1): 6-12.
- Hossain, M. & Jauncey, K. (1989). Studies on the protein, energy and amino acid digestibility of fish meal, mustard oilcake, linseed and sesame meal for common carp (*Cyprinus carpio* L.). *Aquaculture* **83**(1–2): 59-72.
- Hunt, R. (2003). Growth and development. In *Growth analysis of Individual plants*(Ed L. Benjamin). Sheffield, UK: Elsavier
- Jascha, I., Ortega, R., Verreth, J. & Schrama, J. (2007). Digesta characteristics in relation to nutrient digestibility and mineral absorption in Nile tilapia (*Oreochromis niloticus* L.) fed cereal grains of increasing viscosity. *Aquaculture* **273**: 556–565.
- Jatoba, A., Vieira Fdo, N., Buglione-Neto, C., Mourino, J., Silva, B., Seiffter, W. & Andreatta, E. (2011). Diet supplemented with probiotic for Nile tilapia in polyculture system with marine shrimp. *Fish Physiol Biochem* **37**(4): 725-732.
- Jegatheesan, V., Zeng, C., Shu, L., Manicom, C. & Steicke, C. (2006). Technological advances in aquaculture farms for minimal effluent discharge to oceans. *Journal of Cleaner Production* **xx**: 1-10.

- Johanna, S., Dennis, D., Werner, K., Daniela, B., Sebastian, J., Günther, S. & Uwe, S. (2016). Advanced aquaponics: Evaluation of intensive tomato production in aquaponics vs. conventional hydroponics. *Agricultural Water Management* **178**: 335-344.
- Jones, B. (2005). *Hydroponics - A Practical Guide for the Soilless Grower* Washington, USA: CRC Press.
- Kassahun, A., Waidbacher, H. & Zollitsch, W. (2012). Effects of dietary starch and energy levels on maximum feed intake, growth and metabolism of Nile tilapia, *Oreochromis niloticus*. *Livestock Research for Rural Development* **24**(6): 1-18.
- Kerbiriou, P., Stomph, T., Van Der Putten, P., Van Bueren, E. & Struik, P. (2013). Shoot growth, root growth and resource capture under limiting water and N supply for two cultivars of lettuce (*Lactuca sativa* L.). *Plant and Soil* **371**(1-2): 281-297.
- Khan, K., Siddique, M. & Zamal, H. (2013). Replacement of fish meal by plant protein sources in Nile tilapia (*Oreochromis niloticus*) diet: growth performance and utilization. *Iranian Journal of Fisheries Sciences* **12**(4): 864- 872.
- Klinger, D. & Naylor, R. (2012). Searching for Solutions in Aquaculture: Charting a Sustainable Course. *Annual Review of Environment and Resources* **37**: 247-255.

- Koch, J., Rawles, S., Webster, C., Cummins, V., Kobayashi, Y., Thompson, K., Gannamd, A., Twibell, R. & Hyde, N. (2016). Optimizing fish meal-free commercial diets for Nile tilapia, *Oreochromis niloticus*. *Aquaculture* **452**: 357–366.
- Krome, C., Jauncey, K., Fedderke, S. & Focken, U. (2014). Effect of replacing different levels of dietary fishmeal with *Jatropha curcas* kernel meal on the development of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). *Journal of Applied Ichthyology* **30**(3): 507-512.
- Kumar, V., Akinleye, A., Makkar, H., Angulo-Escalante, M. & Becker, K. (2012). Growth performance and metabolic efficiency in Nile tilapia (*Oreochromis niloticus* L.) fed on a diet containing *Jatropha platyphylla* kernel meal as a protein source. *J Anim Physiol Anim Nutr (Berl)* **96**(1): 37-46.
- Kyeong-Jun, L., Madison, P., Frederick, B., Scott, S., Peter, B. & Ronald, H. (2010). Evaluation of supplemental fish bone meal made from Alaska seafood processing byproducts and dicalcium phosphate in plant protein based diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **302**(3–4): 248-255.
- Lam, S., Ma, N., Jusoh, A. & Ambak, M. (2015). Biological nutrient removal by recirculating aquaponic system: Optimization of the dimension ratio between the hydroponic & rearing tank components. *International Biodeterioration & Biodegradation* **102**: 107-115.

- Lennard, W. & Leonard, B. (2004). A comparison of reciprocating flow versus constant flow in an integrated, gravel bed, aquaponic test system. *Aquaculture International* **12**(6): 539-553.
- Li, H., Tanb, F., Keb, L., Xia, D., Wangb, Y., Heb, N., Zheng, Y. & Li, Q. (2016). Mass balances and distributions of C, N, and P in the anaerobic digestion of different substrates and relationships between products and substrates. *Chemical Engineering Journal* **287**: 329–336.
- Liang, J. & Chien, Y. (2013). Effects of feeding frequency and photoperiod on water quality and crop production in a tilapia-water spinach raft aquaponics system. *International Biodeterioration & Biodegradation* **85**: 693-700.
- Licamele, J. (2009). Biomass production and nutrient dynamics in an aquaponics system. In *Department of agriculture and biosystems engineering*, Vol. Doctor of Philosophy University of Arizona.
- Lieberta, F. & Portz, L. (2005). Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. *Aquaculture* **248**: 111–119.
- Lin, S. & Luo, L. (2011). Effects of different levels of soybean meal inclusion in replacement for fish meal on growth, digestive enzymes and transaminase activities in practical diets for

- juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. *Animal Feed Science and Technology* **168**(1–2): 80–87.
- Little, D., Anderson, F. & Durkin, J. (1986). A note on the effect of supplementation with noug cake (*Guizotia abyssinica*) on the milk production of crossbred cows. In *Utilization of agricultural by-products as livestock feeds in Africa* (Eds D. Little and A. Said). Rome: Food and Agriculture Organization of the United Nations.
- Love, D., Fry, J., Li, X., Hill, E., Genello, L., Semmens, K. & Thompson, R. (2015a). Commercial aquaponics production and profitability: Findings from an international survey. *Aquaculture* **435**: 67–74.
- Love, D., Uhlc, M. & Genello, L. (2015b). Energy and water use of a small-scale raft aquaponics system in Baltimore, Maryland, United States. *Aquacultural Engineering* **68**: 19–27.
- Lovell, R. T. (1991). Nutrition of aquaculture species. *J Anim Sci* **69**(10): 4193–4200.
- Masser, M. (2012a). Cage culture in freshwater and protected marine areas In *Aquaculture production systems*, 421 (Ed J. Tidwell). Iowa, USA: John Willey & Sons, Ltd.
- Masser, M. (2012b). In-pond raceways. In *Aquaculture production systems*, 421 (Ed J. Tidwell). Iowa, USA: John Willey & Sons, Ltd.

- Mérida, N., Tomás-Vidal, A., Martínez-Llorens, S. & Jover Cerdá, M. (2010). Sunflower meal as a partial substitute in juvenile sharpsnout sea bream (*Diplodus puntazzo*) diets: Amino acid retention, gut and liver histology. *Aquaculture* **298**(3–4): 275-281.
- Mims, S. & Onders, R. (2012). Reservoir ranching. In *Aquaculture production systems*, 421 (Ed J. Tidwell). Iowa, USA: John Willey & Sons, Ltd.
- Mohamed, H., Magdy, S. & Ahmed, A. (2015). Nutritive value of soybean meal after solid state fermentation with *Saccharomyces cerevisiae* for Nile tilapia, *Oreochromis niloticus*. *Animal Feed Science and Technology* **201**: 89-98.
- Monge, C., Chaves, C. & Arias, M. (2011). Bacteriological quality of traditional, organic and hydroponic cultured lettuce in Costa Rica. *Arch Latinoam Nutr* **61**(1): 69-73.
- Moutinho, S., Martínez-Llorens, S., Tomás-Vidal, A., Jover-Cerdá, M., Oliva-Teles, A. & Peres, H. (2017). Meat and bone meal as partial replacement for fish meal in diets for gilthead seabream (*Sparus aurata*) juveniles: Growth, feed efficiency, amino acid utilization, and economic efficiency. *Aquaculture* **468**(1): 271-277.
- Neori, A., Chopin, T., Troell, M., Buschmann, A., Kraemer, G., Halling, C., Shpigel, M. & Yarish, C. (2004). Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture* **231**(1–4): 361-391.

- Ng, W. & Romano, N. (2013). A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Reviews in Aquaculture* **5**: 220–254.
- Ngo, D., Wade, N., Pirozzi, I. & Glencross, B. (2016). Effects of canola meal on growth, feed utilisation, plasma biochemistry, histology of digestive organs and hepatic gene expression of barramundi (Asian seabass; *Lates calcarifer*). *Aquaculture* **464**: 95-105.
- NRC (National Research Council) (1999). *Nutrient Requirements of Fish*. Washington, USA: National academy press.
- Ogunji, J., Toor, R., Schulz, C. & Kloas, W. (2008). Growth Performance, Nutrient Utilization of Nile Tilapia *Oreochromis niloticus* Fed Housefly Maggot Meal (Magmeal) Diets. *Turkish Journal of Fisheries and Aquatic Sciences* **8**: 141-147
- Olsen, R. & Hasan, M. (2012). A limited supply of fishmeal: Impact on future increases in global aquaculture production. *Trends in Food Science & Technology* **27**(2): 120-128.
- Pantanella, E., Cardarelli, M., Colla, G., Rea, E. & Marcucci, A. (2012). Aquaponics vs. Hydroponics: Production and Quality of Lettuce Crop. *Xxviii International Horticultural Congress on Science and Horticulture for People (Ihc2010): International Symposium on Greenhouse 2010 and Soilless Cultivation* **927**: 887-893.

- Petridis, A., Gasparatos, D., Haidouti, C., Paschalidis, C. & Zamanidis, P. (2013). Effects of Nitrogen and Boron Fertilization on Lettuce Mineral Nutrition in a Calcareous Soil. *Communications in Soil Science and Plant Analysis* **44**(1-4): 733-740.
- Plaipetch, P. & Yakupitiyage, A. (2014). Effect of replacing soybean meal with yeast-fermented canola meal on growth and nutrient retention of Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758). *Aquaculture Research* **45**(11): 1744-1753.
- Portz, L. & Liebert, F. (2004). Growth, nutrient utilization and parameters of mineral metabolism in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) fed plant-based diets with graded levels of microbial phytase. *J Anim Physiol Anim Nutr (Berl)* **88**(9-10): 311-320.
- Rafiee, G. & Roos, C. (2006). The effect of natural zeolite (Clinoptiolite) on aquaponic production of Red Tilapia (*Oreochromis* sp.) and Lettuce (*Lactuca sativa* var. longifolia), and improvement of water quality. *Journal of Agricultural Science Technology* **8**: 313-322.
- Rafieea, G. & Saad, C. (2005). Nutrient cycle and sludge production during different stages of red tilapia (*Oreochromis* sp.) growth in a recirculating aquaculture system. *Aquaculture* **244**: 109–118.
- Rakocy, J. (1997). *Integrating Tilapia culture with vegetable hydroponics in recirculating systems*. Baton Rouge, Louisiana: World Aquaculture Society.

- Rakocy, J. (2012). Aquaponics. In *Aquaculture production systems*, 415 (Ed J. Tidwell). Iowa, USA: John Willey & Sons, Ltd.
- Rakocy, J. & Hargreaves, J. (1993). *Integration of vegetable hydroponics with fish culture*. USA: American Society of Agriculture Engineers.
- Rakocy, J., Masser, M. & Losordo, T. (2006). Recirculating Aquaculture Tank Production Systems: Aquaponics - Integrating Fish and Plant Culture. *Southern Regional Aquaculture Center (SRAC) 454*: 1-16.
- Regost, C., Arzel, J., Robin, J., Rosenlund, G. & Kaushik, S. (2003). Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*): 1. Growth performance, flesh fatty acid profile, and lipid metabolism. *Aquaculture 217*(1-4): 465-482.
- Roosta, H. (2011). Interaction between Water Alkalinity and Nutrient Solution Ph on the Vegetative Growth, Chlorophyll Fluorescence and Leaf Magnesium, Iron, Manganese, and Zinc Concentrations in Lettuce. *Journal of Plant Nutrition 34*(5): 717-731.
- Roosta, H. (2014a). Comparison of the Vegetative Growth, Eco-Physiological Characteristics and Mineral Nutrient Content of Basil Plants in Different Irrigation Ratios of Hydroponic: Aquaponic Solutions. *Journal of Plant Nutrition 37*(11): 1782-1803.

- Roosta, H. (2014b). Effects of Foliar Spray of K on Mint, Radish, Parsley and Coriander Plants in Aquaponic System. *Journal of Plant Nutrition* **37**(14): 2236-2254.
- Roosta, H. & Mohsenian, Y. (2012). Effects of foliar spray of different Fe sources on pepper (*Capsicum annum* L.) plants in aquaponic system. *Scientia Horticulturae* **146**: 182–191.
- Roostaa, H. & Hamidpour, M. (2011). Effects of foliar application of some macro- and micro-nutrients on tomato plants in aquaponic and hydroponic systems. *Scientia Horticulturae* **129**: 396–402.
- Rush, H. (2012). *Hydroponic Food Production: A definitive guidebook for the advanced home gardener and the commercial hydroponic grower*. New work, USA: CRC press.
- Samarakoon, U., Weerasinghe, P. & Weerakkody, W. (2006). Effect of Electrical Conductivity (EC) of the Nutrient Solution on Nutrient Uptake, Growth and yield of Leaf Lettuce (*Lactuca sativa* L.) in Stationary Culture. *Tropical agriculture research* **18**: 13-21.
- Sanz, A., Morales, A., de la Higuera, M. & Gardenete, G. (1994). Sunflower meal compared with soybean meals as partial substitutes for fish meal in rainbow trout (*Oncorhynchus mykiss*) diets: protein and energy utilization. *Aquaculture* **128**(3–4): 287-300.
- Savidov, N., Hutchings, E. & Rakocy, J. (2007). Fish and plant production in a recirculating aquaponic system: a new approach to sustainable agriculture in Canada. *Proceedings of the International Conference & Exhibition on Soilless Culture* 7(42): 209-221.

- Seawright, D., Stickney, R. & Walker, R. (1998). Nutrient dynamics in integrated aquaculture–hydroponics systems. *Aquaculture* **160**(3-4): 215-237.
- Seginer, I. (2004). Equilibrium and balanced growth of a vegetative crop. *Ann Bot* **93**(2): 127-139.
- Shete, A., Verma, A., Chadha, N., Prakash, C., Peter, R., Ahmad, I. & Nuwansi, K. (2016). Optimization of hydraulic loading rate in aquaponic system with Common carp (*Cyprinus carpio*) and Mint (*Mentha arvensis*). *Aquacultural Engineering* **72**: 53–57.
- Shiau, S., Chuang, J. & Sun, C. (1987). Inclusion of soybean meal in tilapia (*Oreochromis niloticus* × *O. aureus*) diets at two protein levels. *Aquaculture* **65**(3–4): 251-261.
- Siddiqui, A., Howlader, M. & Adam, A. (1988). Effects of Dietary Protein Levels on Growth, Feed Conversion and Protein Utilization in Fry and Young Nile Tilapia, *Oreochromis niloticus*. *Aquaculture* **70**: 63-73.
- Sikawa, D. & Yakupitiyage, A. (2010). The hydroponic production of lettuce (*Lactuca sativa* L) by using hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) pond water: Potentials and constraints. *Agricultural Water Management* **97**: 1317–1325.
- Simões, L., Lombardi, D., Gomide, A. & Gomes, L. (2011). Efficacy of clove oil as anesthetic in handling and transportation of Nile tilapia, *Oreochromis niloticus* (Actinopterygii: Cichlidae) juveniles. *ZOOLOGIA* **28**(3): 285-290.

- Soderberg, R. (2006). A linear growth model for Nile tilapia in intensive aquaculture. *North American Journal of Aquaculture* **68**(3): 245-248.
- Stanghellini, M. & Rasmussen, S. (1994). Hydroponics - a solution for zoosporic pathogens. *Plant Disease* **78**: 1128-1138.
- Stickney, R. (2005). *Aquaculture: An introductory text*. USA: CABI Publishing.
- Suloma, A., El-Husseiny, M., Hassane, I., Mabroke, S. & El-Haroun, E. (2014). Complementary responses between hydrolyzed feather meal, fish meal and soybean meal without amino acid supplementation in Nile tilapia *Oreochromis niloticus* diets. *Aquacult International* **22**: 1377-1390.
- Sumanta, N., Haque, H., Nishika, J. & Suprakash, R. (2014). Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents. *Research Journal of Chemical Sciences* **4**(9): 63-69.
- Tacon, A., Hasan, M. & Subasinghe, R. (2006). *Use of fishery resources as feed inputs to aquaculture development: Trends and policy implications*. Rome, Italy: Food and Agriculture Organization of the United Nations.
- Thompson, K., Velasquez, A., Patterson, J., Metts, L., Webster, C., Brady, Y., Gannam, A., Twibell, R. & Ostrand, S. (2012). Evaluation of plant and animal protein sources as partial

- or total replacement of fish meal in diets for Nile tilapia fry and juvenile stages. *North American Journal of Aquaculture* **74**(3): 365-375.
- Tidwell, J. (2012a). Characterization and categories of aquaculture production systems. In *Aquaculture production systems*, 421 (Ed J. Tidwell). Iowa, USA: John Wiley & Sons, Ltd.
- Tidwell, J. (2012b). Function and characteristics of all aquaculture systems. In *Aquaculture production systems*, 421 (Ed J. Tidwell). Iowa, USA: John Wiley & Sons, Ltd.
- Timmons, M., Ebling, J., Wheaton, F., Summerfelt, S. & Vinci, B. (2002). Recirculating Aquaculture Systems. *Northern Regional Aquaculture Center Publication* **2**: 1-2.
- Tran-Duy, A., Smit, B., Dam, A. & Schrama, W. (2008). Effects of dietary starch and energy levels on maximum feed intake, growth and metabolism of Nile tilapia, *Oreochromis niloticus*. *Aquaculture* **277**: 213-219.
- Trejo-Téllez, L. & Gómez-Merino, F. (2012). Nutrient Solutions for Hydroponic Systems. In *Standard Methodology for Plant Biological Researches*, 23 (Ed T. Asao). China: InTech.
- Trushenski, J., Kasper, C. & Kohler, C. (2006). Challenges and Opportunities in Finfish Nutrition. *North American Journal of Aquaculture* **68**: 122–140.

- Tucker, C. & Hargreaves, J. (2012). Ponds. In *Aquaculture production system*, 421 (Ed J. Tidwell).
Iowa, USA: John Willey & Sons, Ltd.
- Turcios, A. & Papenbrock, J. (2014). Sustainable Treatment of Aquaculture Effluents-What Can We Learn from the Past for the Future? *Sustainability* **6**(2): 836-856.
- Tyson, R., Treadwell, D. & Simonne, E. (2011). Opportunities and Challenges to Sustainability in Aquaponic Systems. *Hortitechnology* **21**(1): 6-13.
- Wortman, S. (2015). Crop physiological response to nutrient solution electrical conductivity and pH in an ebb-and-flow hydroponic system. *Scientia Horticulturae* **194** 34–42.
- Yoshida, T., Sakuma, K. & Kumagai, H. (2014). Nutritional and taste characteristics of low-potassium lettuce developed for patients with chronic kidney diseases. *Hong Kong Journal of Nephrology* **16**: 42-45.