EFFECT OF IRON AMINO ACID CHELATE SUPPLEMENT IN FISH FEEDS ON GROWTH PERFOMANCE OF NILE TILAPIA (*Oreochromis niloticus* Linnaeus, 1758) AND SPINACH (*Spinacia oleracea* Linnaeus, 1552*)* IN AN AQUAPONIC SYSTEM

BY

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DECLARATION

Declaration by the candidate

This research thesis is my original work and has not been presented in any other University. No part of this thesis may be reproduced without the prior written permission of the authors and/or the University of Eldoret.

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ABSTRACT

Aquaponic is the combination of aquaculture and hydroponics. It is an environmentally friendly production system involving reuse of waste and nutrients in production of fish and vegetables. The research was conducted to investigate the effect of iron amino acid chelate supplementation in fish feeds on the growth of Nile tilapia and spinach in aquaponic system. The study was conducted at the University of Eldoret for 119 days from August-December 2016. A complete randomized design was used. The supplementation rates in fish diets constituted 30g, 20g, 10g and 0g Fe kg^{-1} respectively. Nile tilapia fry with a mean weight of 0.475 ± 0.025g and spinach of height (3 ± 0.131cm) were stocked in 12 aquaria in an aquaponic system. On the fish growth significantly different ($p < 0.05$) was recorded in all the treatments where 30g Fe kg^{-1} treatment resulted in the highest fish growth performance with final weights of 11.61 ± 0.55g, and SGR of 2.52 ± 0.01 and a good FCR of 1.10 ± 0.107 compared to the other three treatment. In the carcass composition analysis, 30g Fe kg^{-1} treatment exhibited a significant difference ($p < 0.05$) for higher ash content, crude protein and lower crude lipids (16.350 ± 0.03%, 65.607 ± 0.74% and 12.20 ± 0.256%, respectively) while treatment 0g Fe kg^{-1} showed lowest ash content, crude protein and crude lipids (10.59 ± 2.12%, 59.671 ± 0.676% and 18.20 ± 0.465% respectively). The hemoglobin and hematocrit levels were higher at 30g Fe kg^{-1} (45.090 ± 0.704 % and 15.630 ± 0.935g dL^{-1}) and lower at 0g Fe kg^{-1} treatment (29.773 ± 0.213% and 9.9244 ± 0.071g dL^{-1}). A decrease in glucose levels in fish carcasses was recorded with increased supplementation of iron amino acids chelates level in diets. The 0g Fe kg^{-1} demonstrated higher glucose levels (26.8 ± 0.0289mg dL^{-1}) and lower levels at 30g Fe kg^{-1} (13.433 ± 0.169 mg dL^{-1}). At 30g Fe kg^{-1} treatments spinach indicated a significant growth at ($p < 0.05$) than other treatments with final mean height (52.44 ± 0.798cm) and 19 leaves. The least growth of spinach was at 0g Fe kg^{-1}treatments with final mean height, (25.36 ± 0.72cm, 10 ± 0.225) leaves. 30g Fe kg^{-1} treatment exhibited higher minerals content than other treatments with Phosphorus 67.51 ± 2.42 mgL^{-1}, Zinc 9.06 8 ± 0.45 mgL^{-1}, Iron 5.2 ± 0.218 mgL^{-1}, Manganese 7.655 ± 0.344 mgL^{-1}, Total Nitrogen 11.248 ± 0.141mgL^{-1} and Sodium 7.218 ± 0.028 mgL^{-1}. Additionally 30g Fe kg^{-1} treatment demonstrated higher levels of chlorophyll a (10.283 ± 0.22) and b (11.665 ± 0.250) as compared to other treatments. There was improved water quality at 30g Fe kg^{-1} compared to other treatments. These results revealed that 30g Fe kg^{-1} iron amino acid chelate supplementation had better nutritional attributes as feedstuff for *O. niloticus* growth than the two other dietary treatments. The study recommends the incorporation of iron amino acid chelate in on-farm formulated diets where complete diets are not easily accessible for small scale farmers.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

DO	Dissolved Oxygen
FAO	Food Agricultural organization
K	Potassium
Mn	Manganese
N	Nitrogen
P	Phosphorus
Zn	Zinc
PRAS	Partial Reuse Aquaculture systems
Fe	Iron
Ca	Calcium
B	Boron
Cu	Copper
Mg	Magnesium
S	Sulphur
Cl	Chlorine
Mo	Molybdenum
Co	Cobalt
Cr	Chromic
CP	Crude protein
SGR	Specific growth rate
FCR	Feed conversion ratio
MCV	Mean Corpuscular volume
MCH	Mean corpuscular haemoglobin

AOAC	Association of Official Analytical Chemist
NFT	Nutrient filter technique
NFE	Nitrogen free extract
RAS	Recirculating aquaculture system

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

Aquaponic is the combination of aquaculture (the raising of fish either in aquariums or in synthetic tanks) and hydroponics (the growing of plants without a soil medium) (Connolly and Trebic, 2010). The plants are grown in grow beds above the soil, which reduces the surface area required to grow vegetable crops. Toxic waste products from fish are removed by vegetables and aerobic micro-organism. This allows the recirculating aquaponic system to raise large quantities of fish in relatively small volumes (Ezekiel, 2015). Plants have the potential to grow very quickly when they use the dissolved nutrients from fish excretions, and from the nutrients generated from the microbial breakdown of fish wastes. Fish excrete waste nitrogen through their gills, in the form of ammonia, directly into the water. The bacteria in the water and in the growing medium then convert ammonia to nitrite and then to nitrate. Nitrate is relatively harmless to fish, while ammonia and nitrite are toxic; therefore nitrate is the preferred form of nitrogen for growing higher plants such as fruiting vegetables (Rakocy *et al.*, 2015).

The main goals of aquaculture industry are to optimize growth and to produce high quality fish (Bello *et al*., 2012). Aquaculture has evolved as the fastest growing food producing sector and developed as important component in food security (Ibrahim *et al*., 2010). Fish is a high quality food containing first class protein and nutrients, important for human health and growth (Olaifa *et al*., 2010). In fish culture, supplementary feeding plays a major role in determining the nutritional and economic success of aquaculture.

Feed formulations account for more than 50% of the total production cost in modern intensive aquaculture (Ibrahim *et al*., 2010). Increasing feed efficiency especially by improving the metabolic assimilation of dietary nutrients, is of high priority in contemporary animal production (Ibrahim *et al*., 2010). The aquaculture feed industry relies on the fishmeal, which is the most preferred protein source for fish feed owing to excellent amino acid and fatty acid and minerals traces elements profile. Limited supply, high cost and stagnant production level restrict use of fish meal for sustainable fish farming (Baruah *et al*., 2004). It is known that fish feed ingredient cannot provide all the essential macro and micro- nutrients to plants in an aquaponic system(Sarker *et al*., 2007) but there are a large number of micro- nutrient supplement with suitable amino acid profiles used to improve fish growth performance as well as the plants growth. According to Apines-Amaret *et al.* (2004) chelate amino acids can be used as a growth promoter but the mechanism of action of amino acid chelates with traces of micronutrients as a growth promoter is not yet adequately researched. Chelated amino acid iron could have the ability to increase bioavailability of nutrients in plant (Apines-Amaret *et al.*, 2004).

This study was therefore conducted to assess the effect of iron amino acid chelate supplement in fish feeds on growth performance of Nile tilapia (O*reochromis niloticus*) and spinach (*Spinacia oleracea)* in a recirculating aquaponic system at the University of Eldoret Fish Farm. The Experiments were used to elaborate on the theoretical, practical and application potential of gravel based aquaponic systems that can be adopted by local farmers.

1.2 Problem Statement

The word population is growing fast as from statistic estimate 6.8 and more billion thus increasing environmental pollution and high food demand. Water and land resources for agriculture are diminishing and world fisheries are at or past their maximum sustainable yields. To feed humanity for the next coming years it is speculated that more food must be produced than all the food produced since the beginning of recorded history**.** The intensive development of the aquaculture industry has been accompanied by an increase in environmental impact. Discharges from aquaculture into the aquatic environment generate substantial amounts of polluted effluent, containing uneaten feed and feces. Wastewater from aquaculture contains nutrients, various organic and inorganic compounds such as ammonium, phosphorus, dissolved organic carbon and organic matter. The high levels of nutrients cause environmental deterioration of the receiving water sources. The total nutrients from aquaculture form effluents which can contribute to significant environmental degradation. Aquaponic system is thought to be the solution to the effluent released from the aquaculture, but one unique challenge is the maintenances of micronutrient specifically iron, magnesium and potassium that are suitable for the plants growth and also the pH balance for both plants and fish component. The ideal water pH for hydroponic plants is 5.5–6.5, while the ideal pH for fish is between 7.0 and 9.0 (Seawright *et al.,* 1998). Plants may experience deficiency of some micronutrients at pH greater than 6.5 because many micronutrients form compounds that do not dissolve in water at these pH levels. Also biofilter bacteria are responsible for changing ammonia (NH_3), which is quite toxic to fish, to relatively harmless nitrate (NO_3). These bacteria can survive at the 5.5–6.5 pH range, but do not convert ammonia to nitrate as quickly, putting the fish at risk of suffering ammonia toxicity.

Application of micronutrient directly into the system interferes with water quality and it can also accumulate in the fish tissues which is not good for human consumption. New technologies like intensive recirculating Aquaculture with hydroponic practices must be coupled with sound environmental, food security, water utilization and fisheries policies to yield a productive and sustainable management system

1.3 Justification

The concept of aquaponic that involves integrating aquaculture and hydroponics is gaining increased attention as a bio- integrated food production systems. Reuse of the nutrient released by fish to grow crop plants is the primary goal of aquaponic. Aquaponic systems are more environmentally sustainable than most traditional farming practices, which have resulted in widespread soil erosion, desertification and pollution in many countries (Endut *et al.,* 2011). Thus, aquaponic system is vital and efficient in the use of resources, reduction in risk of total crop failure, additional sources of food, extra income and reduction of operation costs for farmers than fish culture alone.

Emerging use of chelated amino acid is suitable to solve micronutrients deficiency (iron, potassium and magnesium) and improved water quality for the cultured species. Synthetic chelated and inorganic minerals are the most appropriate sources of micronutrients for aquaponic because they allow the amount of each nutrient to be controlled independently.

Amino acid chelates are especially suitable for greenhouse and hydroponics systems because they are usually certified organic, readily available for uptake by plants by both roots and foliage, and are generally not phytotoxic. For example, in aquaponic systems where fish are integrated into the hydroponics system it is important that

nothing synthetic enter the tissues or meat of the fish. In aquaponic systems tilapia are fed feed that does not contain iron. Consequently the feces do not contain iron. Therefore, the water coming from the fish tanks does not contain iron and the plants downstream will exhibit iron deficiency, especially in the leafy vegetables like lettuce and basil. Therefore, the use of organic materials and the amino acid chelates can be applied directly to the foliage or to the nutrient solution for immediate correction of nutrient deficiencies.

Iron (Fe) is used in chloroplasts for chlorophyll biosynthesis and the electron transport chain, and is also critical for proper photosynthesis. Iron has to be added as chelated iron, otherwise known as sequestered iron or Fe*EDTA, because iron is apt to precipitate at pH greater than 7. The suggested addition is 5 ml m^{-2} of grow bed whenever deficiencies are suspected; a larger quantity does not harm the system.

The reason chelated iron is important is because free-floating iron is difficult for plant cells to absorb, though not impossible. If it is attached to an amino acid or other chelating agent, plant cell walls absorb it readily. Once inside, the chelating agent and iron atom disassociate and the iron can be used for cellular processes. It is especially important for the formation of chlorophyll (itself a chelate), which is responsible for photosynthesis and gives plants their green color. The application of Amino Acids chelate before, during and after the stress conditions supplies the plants with Amino Acids which are directly related to stress physiology and thus has a preventing and recovering effect. Chelated amino acid iron plays an important role in the production of hemoglobin with protein and copper and oxygenation of red blood cells and lymphocytes. Iron improves the function of enzymes in protein metabolism and enhances the functions of calcium and copper. It is absorbed in the small intestine and

stored in the liver, spleen, bone marrow, and blood, also is needed to metabolize B vitamins. Chelated amino acid is defined as feeds supplemented with specific ingredient(s) to achieve desirable efficiency of metabolic transformation, growth performance, health, and or compositional traits of aquaculture animals at various developmental stages. They are environmentally oriented aqua-feeds which are defined as feeds modified to minimize negative impacts of environmental changes (including salinity, ammonia, extreme pH, and stressors) on growth, health, and reproduction of aquaculture animals. Chelated amino acid iron contain supplements of Calcium and potassium which buffering the water to the correct the pH, as nitrification is an acidifying process. These are added as calcium hydroxide or potassium hydroxide, or as calcium carbonate and potassium carbonate. The choice of the buffer can be chosen based on the plant type being cultivated, as leafy vegetables may need more calcium, and fruiting plants more potassium. Aquaponic systems with the supplementation of chelated amino acid iron will yield sustainable and environmentally sound farming methods.

1.4 Objectives

1.4.1 General objective

To investigate the effect of iron amino acid chelate supplementation in fish feeds on the growth of Nile tilapia (*Oreochromis niloticus*) and spinach (*Spinacia oleracea*) in a small aquaponic system

1.4.2 Specific Objectives

i) To determine growth rate of monosex male *O. niloticus* at different levels of iron amino acid chelate supplement in aquaponic system.

ii) To evaluate the growth of *Spinacia oleracea* at different levels of iron amino acid chelate supplement in an aquaponic system

iii) To determine proximate composition in the carcass of monosex male *O. niloticus* at different levels of iron amino acid chelate supplement in an aquaponic system

iv) To determine chlorophyll levels of *Spinacia oleracea* at different levels of iron amino acid chelate supplement in an aquaponic system.

v) To determine nutrient levels in *Spinacia oleracea* at different levels of iron amino acid chelate supplement in an aquaponic system

vi) To determine water quality parameters at different levels of iron amino acid chelate supplement in an aquaponic system.

vii) To determine physiological response at different levels of iron amino acid chelate supplement in an aquaponic system.

1.5 Hypothesis

i) H_o There no significant differences on the growth rate of monosex male *O. niloticus* at different levels of iron amino acid chelate supplement in the aquaponic system

ii) H_o There no significant differences on the growth of *Spinacia oleracea* at different levels of iron amino acid chelate supplement in the aquaponic system

iii) H_o There no significant differences on the proximate composition in the carcass of male *O. niloticus* at different levels of iron amino acid chelate supplement in the aquaponic system

iv) H_o There is no significant differences on chlorophyll level at different levels of iron amino acid chelate supplement in an aquaponic system.

v) H_o There no significant differences in micro and macro- nutrient at different levels of iron amino acid chelate supplement in an aquaponic system

vi) H_o There no significant differences in water quality parameters at different levels of iron amino acid chelate supplement in an aquaponic system.

vii) H_o: There no significant differences on the physiological response in monosex male *O. niloticus* at different levels of iron amino acid chelate supplement in an aquaponic system.

CHAPTER TWO

LITERATURE REVIEW

2.1 The global context of Modern Aquaculture

Aquaculture includes the cultivation of fish, crustaceans, mollusks, and aquatic plants. Since the 1980s it has emerged as the fastest growing form of agriculture worldwide. Global aquaculture production of fish and other aquatic animals grew at an average of 6.3% per year from 34.6 million tons in 2001 to 59.9 million tons in 2010, while capture production stagnated at around 90 million tons per year over the same period. Asia consistently leads aquaculture production, with 53.3 million tons representing 89.0% of global production in 2010. Global production of aquatic plants was 19.9 million tons in 2010, with 95.5% coming from aquaculture (FAO, 2008). Aquaculture operations are primarily categorized by the waters in which they occur. Marine cultivation occurs within net pens in coastal or open ocean waters. Inland cultivation occurs within pens in freshwater ecosystems (lakes and rivers) or in artificial ponds, raceways, or tanks (Lovelace, 2009; Pillay, 2004). In 2010, inland cultivation accounted for the bulk of global aquaculture production (69.6%) with 41.7 million tons (FAO, 2008).

Aquaculture operations are also categorized by the intensity of management, namely, extensive, semi-intensive, or intensive. Under extensive cultivation, fish receive nutrition from naturally occurring food sources such as detritus and plankton; management efforts focus on protection from predators and competition (Bunting, 2013). Semi-intensive cultivation involves some supplementation of the natural food supply, or fertilization to increase the natural food supply. Under intensive cultivation,

fish receive nutrition exclusively from formulated, high-protein aquafeeds. Greater intensity implies higher stocking densities, concentrated waste, greater risk of disease outbreak, and higher yield per unit of area (Beveridge and Little, 2002; Naylor *et al*., 2000). Within these categories like a diversity of practices, but the global trend is toward intensification with formulated aqua feeds rapidly increasing in importance (Tacon *et al*., 2011). Aquafeed production was 29.2 million tons in 2008 and is expected to grow to 71.0 million tons by 2020 (FAO, 2008).

2.2 Culture Systems and Species

Aquaculture systems range from very extensive, through semi-intensive and highly intensive to hyper-intensive. When using this terminology the specific characterization of each system must be defined, as there are no clear distinctions and levels of intensification represent a continuum (Devillers *et al.,* 2009). Farming systems are also diverse for example such as water-based systems (cages and pens, inshore/offshore), land-based systems (rain fed ponds, irrigated or flow-through systems, tanks and raceways), recycling systems (high control enclosed systems, more open pond based recirculation), integrated farming systems (e.g. livestock-fish, agriculture and fish dual use aquaculture and irrigation ponds.

2.3 Improved Technologies

2.3.1 Recirculating system

Recirculating aquaculture systems are indoor, tank-based systems in which fish are grown at high density under controlled environmental conditions (Martins *et al.,* 2009). Its incorporate additional treatment technologies beyond those used in Partial Reuse Aquaculture Systems (PRAS), allowing for significantly greater quantities of water to be reused (Roque *et al.*, 2009). Recirculation systems afford a level of

control well beyond any other technology application in aquaculture and provide significant production and economic benefits (Zohar *et al.*, 2005).

Recirculating Aquaculture Systems (RAS) Recirculation systems are usually used where new water supplies are limited or expensive to achieve due to high pumping costs (Chen *et al*., 1997). When using RAS, the risk of introducing pathogens or contaminants into the system with influent water is high, effluent disposal capacity is limited, or where operators want to practice strict control over the water quality and temperature within the fish culture system (Eding *et al.*, 2006). Such systems are characterized by increased technical complexity, capital costs, and in some applications, operating costs. However, because RAS allow optimum culture conditions to be maintained year round, independent of fluctuations in water supply quality and ambient temperatures, fish growth rates may be accelerated allowing more fish or larger fish to be produced in the same amount of time (Zohar *et al.*, 2005). In a well-designed system, the production benefits will outweigh the additional costs resulting in a net lower cost of production.

It maximizes on water re-use by employing a comprehensive water treatment system. Water treatment processes typically include solids removal, bio-filtration, gas balancing, oxygenation, and disinfection (Ramadori *et al.*, 2010). By addressing each of the key water quality concerns through treatment, rather than flushing as is used in flow-through and partial reuse systems, ultimate control over culture conditions and water quality is provided (Zohar *et al.*, 2005).

Recycling has become an economic imperative in many industries and aquaculture is no exception. Recirculation technology has allowed aquaculture facilities to evolve to meet the growing need for economic and environmental sustainability. In simple

recirculation systems, water may be treated by two processes: mechanical filtration (to remove solids such as faecal matter, uneaten feeds, etc.) and biological filtration to remove dissolved toxic wastes (Eding *et al.,* 2006). Also other facilities like temperature control and water managements test kits are important to facilitate success of recirculating aquaculture.

The first limiting factor to production in recirculating aquaculture systems is dissolved oxygen (Timmons *et al.,* 2002). Keeping fish at a high density and feeding them feeds that contain high levels of protein will reduce the amount of oxygen available in the water for fish to breathe. There are two critical reasons for aerating: 1) Adding dissolved oxygen to the system to replace that consumed by fish and the breakdown of wastes, 2) to degas other dissolved gases that, in high concentrations, can be harmful to the fish such as CO_2, which can lower the pH (Timmons *et al*., 2002). Several methods are used to aerate systems, the most common being the use of oxygen injection. Other types of aeration systems include packed column aerators, air lifts, and air diffusers. In packed column aerators, the low oxygen water is sprayed in at the top of the column which is packed with a plastic medium, and the flow rate is kept low to keep the column from flooding. Air lifts add oxygen by injecting air into the water through a vertical pipe, agitating and circulating the water. This serves to both increase the dissolved oxygen level and degas the CO_2. Air diffusers or air stones are commonly used in stagnant systems and in the home aquarium industry; however, in commercial production of fish, air stones are limited in efficiency. While fine-bubble air stones are more efficient, these types of aerators require high-pressure sources of oxygen and easily become blocked by growth of bacteria and algae (Timmons *et al.,* 2002).

2.3.2 Aquaponic system

Aquaponic is a bio-integrated system that links recirculating aquaculture with hydroponic vegetable, flower, and/or herb production. In aquaponic, nutrient rich effluent from fish tanks is used to enrich hydroponic production beds (Diver, 2006). This is good for the fish because plant roots and rhizobacteria remove nutrients from the water. These nutrients generated from fish manure, algae, and decomposing fish feed are contaminants that would otherwise build up to toxic levels in the fish tanks, but instead serve as liquid fertilizer to hydroponically grown plants (Steve, 2007). In turn, the hydroponic beds function as a biofilter stripping off ammonia, nitrates, nitrites, and phosphorus so the freshly cleansed water can then be recirculated back into the fish tanks (Rakocy *et al*., 2015). The nitrifying bacteria living in the gravel and in association with the plant roots play a critical role in nutrient cycling; without these microorganisms the whole system would stop functioning (Connolly and Trebic, 2010).

2.3.3 Fish component

Fish are the power house of an aquaponic system; they provide the nutrients for the plants and are the protein source when harvested. Keeping fish in an aquaponic system is simpler than keeping aquarium fish (James *et al.*, 2004). There are many different species of fish that can be used in an aquaponic system, depending on the local climates and available supplies. Any fresh water fish or crustacean can be used in an aquaponic system, but environmental factors will, to a large extent, determine which species are feasible (Nelson, 2008). Factors affecting species selection include: Maximum and minimum ambient temperatures, daylight hours, water quality, size of

ponds and volume of water, market for the fish in your area, hardiness of the fish, availability of fry, legal issues, personal preference (Diver, 2006).

Fish selections differ from region to region but the basic principles remain the same. Fish can be raised for consumption or ornamental, example tilapia for consumption and gold fish for ornamental (Rakocy *et al.,* 2015). Fish species that differ from aquaponics systems designed for an entire community or for commercial use. These are some of the common fish species culture in an aquaponics system: Tilapia, Goldfish, Marron, Catfish, Trout, Perch (Diver, 2006), and Koi, Freshwater mussels, Freshwater prawns and Barramundi. Others beyond this list include warm-water fish that are hardy and can adapt to commercial fish feed and high levels of crowding (Nelson, 2008); including some ornamental fish (Rakocy, 1988). The hybrid striped bass is one species that reportedly does not perform well in aquaponic systems as it cannot tolerate high potassium levels a common supplement used for plant growth (Losordo *et al.,* 2006).

2.3.4 Hydroponic system

Hydroponics is the production of plants in a soilless medium whereby all of the nutrients supplied to the crop are dissolved in water. Plant roots grow in a nutrient solution with or without an artificial medium for mechanical support (Savidor *et al.*, 2005). Most fruits and vegetables are grown in field soil. Soil serves two basic purposes: it acts as a reservoir for essential elements and water and it provides physical support for the plant (Proietti *et al.,* 2004). Soilless culture (hydroponics) is an artificial means of providing plants with support and a reservoir for nutrients and water. The growing medium can be perlite, vermiculite, Rockwool, peat moss, coir, composted pine bark, sawdust, sand or gravel. Water only systems such as the nutrient

flow technique and the floating raft system utilize artificial means of support for the plant. Many hydroponic systems have been developed and the technology is rapidly changing (Resh, 2004), but they have only been used commercially for the last 50 years.

2.3.5 Different types of aquaponic systems

There are different types of systems; these are the nutrient film technique (NFT), flood and drain technique, deep water culture technique and raft technique.

Media filled systems

The hydroponic component is first distinguished by whether it employs a media or not. This becomes very important in aquaponic systems because the presence of a media that plant roots are grown in can possibly eliminate the need for a separate settling tank and biofilters. Sludge and solid from the fish tank get caught in the media and are processed by bacterial communities that develop in the media, thereby acting as a biofilters and eliminating the need to remove the solids in a separate system. If the system does not employ a media and plant roots are exposed directly to the water, then a settling tank and biofilters are necessary to return the water quality to sufficient levels in which fish can live (Connolly and Trebic, 2010).

Flood and drain (also known as ebb and flow)

In flood and drain systems, plant roots are exposed to a static nutrient solution for hours at a time before the solution is drained away, which could happen several times a day. The technique can be used regardless of whether a media is used in the system, and plant roots could either be completely submerged, or partially submerged, leaving

a portion exposed to the atmosphere. Flood and drain systems are noted for their simplicity, reliability and user-friendliness (Rakocy *et al.,* 2015).

Nutrient film technique (NFT)

Nutrient film technique consists of the plant roots being exposed to a thin layer of nutrient water than runs through gravels. The idea is that the shallow flow of water only reaches the bottom of the thick layer of roots that develops in the gravel trough while the top of the root mass is exposed to the air, thereby receiving an adequate oxygen supply. Channel slope, length, and flow rate must all be calculated to make sure the plants receive sufficient water, oxygen, and nutrients. If properly constructed, NFT can sustain very high plant densities. In aquaponic NFT systems, the biofilter becomes crucial as there is no large surface area whereby bacteria communities can develop (Nelson, 2008).

Floating raft system

Another system that has great potential for commercial use is the floating raft system. In this system plants are grown on floating Styrofoam rafts. The rafts have small holes cut in them where plants are placed into net pots. The roots hang free in the water where nutrient uptake occurs (Losordo *et al.,* 2006). A major difference between the raft systems and the NFT and media based systems is the amount of water used. The water level beneath the rafts is anywhere from 10 to 20 inches deep and as a result the volume of water is approximately four times greater than other systems. This higher volume of water results in lower nutrient concentrations and as a result higher feeding rate ratios are used. Bacteria form on the bottom surface of the rafts but generally, a separate biofilters is needed. Also, the plant roots are exposed to some harmful organisms that reside in the water, which can affect plant growth (Diver, 2006).

2.3.6 Plants adapted to aquaponic system

Most vegetables and herbs adapt well in an aquaponic system. Media filled beds seem to be the most successful for growing a large range of plants, and you can grow just about anything. In a true aquaponic system, both the fish and the plants are equally as important, but the ratio of revenues from each will vary (Savidov *et al.,* 2005). The selection of plants to grow in the aquaponic system will, just like the fish, depend on numerous factors. Such as temperature, daylight hours, water quality, type and size of grow beds and volume of grow media, market, hardness of the plant, suitability of plant environment, availability of seed, legal issues, personal preference (Savidov, 2006). Some plants thrive in aquaponic systems, others which are less tolerant to a damp environment don't. Below are some of the firm 14 favorites that have been tried and tested on aquaponic systems. Peas, Cucumber, Eggplant, Cabbage, Broccoli, Celery, Onion, Basil, Mint, Tomatoes, Lettuce, Spinach, Strawberries, Peppers, Chillies, Squash, Melons, Carrots, Beetroot, Garlic, ,etc. Aquaponic plants are subject to many of the same pests and diseases that affect field crops, although they seem to be less susceptible to attack from soil borne pests and diseases. Because plants may absorb and concentrate therapeutic agents used to treat parasites and infectious diseases of fish, these products cannot be used in aquaponic systems. Instead, non-chemical methods are used, i.e., biological control (Graber and Junge, 2009).

It also seems that plants in aquaponic systems may be more resistant to diseases. This resistance may be due to the presence of some organic matter in the water, creating a stable, ecologically balanced growing environment with a wide diversity of microorganisms, some of which are antagonistic to pathogens that affect the roots of plants (Connolly and Trebic, 2010).

2.3.7 Nutritional requirements in aquaponic system

All plants may have different nutritional requirements; for instance leafy green vegetable require more nitrates than fruiting plants. However all plants in aquaponic systems need 16 essential nutrients for maximum growth (Sharad *et al.,* 2015). These come in the form of macronutrients, which in addition to carbon, hydrogen, and oxygen, which are supplied by water, carbon dioxide, and atmospheric air, include nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), phosphorous (P) and sulphur (S). There are seven micronutrients necessary as well and they are chlorine (Cl), iron (Fe), magnesium (Mn), boron (B), zinc (Zn), copper (Cu), and molybdenum (Mo) (Stefan *et al.,* 2013).

Chelated minerals are minerals that have been combined chemically with amino acids to form "complexes. Minerals, like magnesium and iron, are metal ions which like to attach to other compounds. The compounds to which they attach can affect how well they are absorbed and how available they are for use in the body (Pardossi *et al.,* 2002). An amino acid chelated mineral is one in which a mineral has been chemically attached to an amino acid, and there is evidence that some, but not necessarily all, of these types of compounds may improve mineral absorption (Ashmead *et al*., 1974). For information about specific chelated amino acids, such as iron bis-glycine, magnesium diglycinate, zinc bis-glycinate, and selenomethionine are used for supporting normal growth, stabilizing bipolar disorder, building strong muscles and bones, and improving immune system function and overall health, chelated minerals act as dietary supplements that are superior to other mineral supplements, claiming chelated minerals are used more easily by the body (more bioavailable) than non-chelated minerals (Allen, 2002).

The present invention comprises compositions and methods of manufacturing electrically neutral amino acid chelate free of interfering ions. These amino acid chelates are prepared by reacting calcium oxide and/or hydroxide, an amino acid, and a soluble metal sulfate salt in an aqueous solution at a ratio sufficient to allow substantially all of the ions present in solution to react (Marschner, 1995). Thus, a metal amino acid chelate, calcium sulfate, and Water are formed without the presence of any significantly amounts of interfering ions. The metal amino acid chelate produced will have a ligand to metal molar ratio from about 2:1 to 3:1, depending on the valency of the metal, e.g., Fe (II) forms 2:1 and Fe(III) forms 3:1 (Allen, 2002).

The amino acid to be used in the present invention is preferably one or more of the naturally occurring amino acid asparagine, aspartic acid, cysteine, cystine, glutamine, glutamic acid, glycine, histidine, hydroxyl proline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, and combinations thereof. However, dipeptides, tripeptides, and tetrapeptides formed by any combination of the naturally occurring amino acids can also be used (Ashmead, 2001). The metal should be more soluble as a sulfate salt than calcium sulfate. Exemplary metals include those selected from the group consisting of Cu, Zn, Fe, Cr, Co, Mg, Mn, and combinations thereof. Therefore, the metal reactant is preferably provided as a sulfate salt selected from the group consisting of copper sulfate ($CuSO_4$), Zinc sulfate ($ZnSO_4$), ferrous sulfate ($FeSO_4$), manganese sulfate (MnSO4), cobalt sulfate (COSO4), magnesium sulfate (MgSO4), ferric sulfate ($Fe_2(SO4)_3$) and chromic sulfate ($Cr_2\ (SO_4)_3$) (Ashmead, 2001).

2.3.8 Technical Challenges in aquaponic system

Aquaponic system design and application can be considered a highly multidisciplinary approach drawing from environmental, mechanical and civil engineering design concepts as well as aquatic and plant related biology, biochemistry, and biotechnology (Vermeulen and Kamstra, 2013). System specific measurements and control technologies also require knowledge of subjects related to the field of computer science for automatic control systems. This high level of complexity necessarily demands in-depth knowledge and expertise of all involved fields. The biggest challenge in commercial aquaponic is its multi-disciplinary nature, requiring further expertise in economics, finance and marketing. Thus, a high degree of field-specific insight in terms of both practical and in-depth theoretical knowledge is required. This leads to an increasing level of complexity, which directly affects the efficiency factors of the running system. In the interest of highest efficiency and productivity, some numerical trade-offs are recommended and are outlined below. They include pH stabilization, nutrient balance, phosphorus, and pest management (Bunting, 2013).

Stabilization of the pH

A crucial point in aquaponic systems is the pH stabilization, as it is critical to all living organisms within a cycling system that includes fish, plants and bacteria. The optimal pH for each living component is different. Most plants require a pH value between 6 and 6.5 in order to enhance the uptake of nutrients. The fish species Tilapia (*Oreochromis niloticus*) is known to be disease resistant and tolerant to large fluctuations in pH value with a tolerance between pH 3.7 and 11, but achieves best growth performance between pH 7.0 and 9.0. The nitrifying bacteria have a higher optimum pH, which is above 7. (Seawright *et al.*, 1998) observed that nitrification

efficiency increased linearly by 13% per pH unit within a pH range between 5.0 and 9.0 with the highest activity of ammonium oxidizers at 8.2. Similar observations were made by (Ezekiel, 2015), who report the overall nitrification pH of approximately 7.8. There are three major bacteria, for which optimal pH conditions are as follows: (1) *Nitrobacter*: 7.5 (Keen and Prosser, 1987); (2) *Nitrosomonas*: 7.0–7.5 (Hatayama *et al.,* 2000); and (3) *Nitrospira*: 8.0–8.3 (Blackburne *et al.,* 2007).

Based on these data, the highest possible pH value should be consistent with the prevention of ammonia accumulation in the system. Then, the ideal pH value for the system is between 6.8 and 7.0. Although root uptake of nitrate raises pH as bicarbonate ions are released in exchange, the acidity producing nitrification process has a higher impact on the overall system pH, leading to a constant and slight decrease in the pH value.

Nutrient balance

As an innovative sustainable food production system, the challenge in aquaponic is to use the nutrient input efficiently, minimizing its discard and tending to a zero-discharge recirculating system. Fish feed, the main nutrient input; can be divided into assimilated feed, uneaten feed, and soluble and solid fish excreta. Soluble excreta are mainly ammonia and are the most available mineral until it is successively transformed into nitrite and nitrate by nitrifying bacteria (Lekang and Kleppe, 2000). Both uneaten feed and solid faeces need to be solubilized from organic material to ionic mineral forms that are easily assimilated by plants. Minerals have different solubilization rates and do not accumulate equally, which influences their concentrations in the water. All involved microorganisms and chemical and physical mechanisms of solubilization are not well understood. Under current practices in

RAS, the solid wastes are only partially solubilized as they are mechanically filtered out on a daily basis (Cripps and Bergheim, 2000). These filtered wastes can be externally fully mineralized and reinserted into the hydroponic beds. Given the objective of obtaining a low environmental footprint, a zero-discharge recirculating system concept should be achievable according to (Neori *et al.*,2007), but more research needs to be carried out on fish waste solubilization with the objective to transform all added nutrients into plant biomass. There are two methods for mineralizing organic material that could be implemented: (1) anoxic digestion in special mineralization or settling units using bioleaching abilities of heterotrophic bacteria (e.g., *Lactobacillus plantarum*) (Jung and Lovit, 2011) and/or (2) using earthworm species such as *Lumbricus rubellus* capable of converting organic wastes to water enriching compounds in wet composting or grow beds (Bajsa *et al.,* 2003). Vermiculture can facilitate a high degree of mineralization as worm casts contain micro- and macronutrients broken down from organic compounds (Torri and Puelles, 2010). Addition of external sources (e.g., food waste) of feed for the worms to provide the aquaponic system with additional organic fertilizers has also been suggested (Jorgensen *et al.,* 2009).

Phosphorous

Among the different minerals, phosphorus (P) deserves a specific attention. It is a macronutrient, which is assimilated by plants in its ionic orthophosphate form ($H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}). It is essential for both vegetative and flowering stages of plant growth. In RAS, 30 %–65 % of the phosphorus added to the system via fish feed is lost in the form of fish solid excretion that is filtered out by either settling tanks or mechanical filters (Schneider *et al.*, 2005). Moreover, organic P solubilized as orthophosphate can precipitate with calcium (e.g., hydroxyapatite–$Ca_5(PO_4)_3(OH)$)

making these elements less available in solution. Consequently, aquaponic experiments report a range of 1–17 mg L−1 PO_4-P (Villarroell *et al.,* 2011*).* However, recommended concentrations in standard hydroponics are generally between 40 and 60 mg L^{-1} PO_4-P (Sikawa and Yakupitiyage, 2010*).* This discrepancy suggests that phosphate should be added to aquaponic systems, especially for fruity vegetables that do not yet show satisfying yields in aquaponic. Phosphorus is a finite and scarce mining resource and subsequently, an expensive component of hydroponic solutions. Sufficient phosphorus production will certainly be a major concern in the near future. Therefore, solutions to reuse the discharge of P-rich effluents must be explored (Shu *et al.,* 2006*).*

2.3.9 Spinach

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family *Amaranthaceae* native to central and western Asia. It is an annual plant (rarely biennial), which grows up to 30 cm tall. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, and ovate to triangular and very variable in size from about 2–30 cm long and 1–15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3–4 mm in diameter, maturing into a small, hard, dry, lumpy fruit cluster 5–10 mm across containing several seeds (Bergquist *et al.,* 2007).

Antioxidant benefits of spinach

Most of the flavonoid and carotenoid nutrients found in spinach that provide anti-inflammatory benefits provide antioxidant benefits as well. Given the fact that spinach is an excellent source of other antioxidant nutrients including vitamin C, vitamin E, vitamin A (in the form of carotenoids), and manganese as well as a very good source

of the antioxidant zinc and a good source of the antioxidant selenium it's no wonder that spinach helps lower risk of numerous health problems related to oxidative stress (Cerullo *et al.,* 2002). Our blood vessels, for example, are especially susceptible to damage from oxidative stress, and intake of spinach has been associated with decreased risk of several blood vessel-related problems, including atherosclerosis and high blood pressure. (Interestingly, the blood pressure benefits of spinach may be related not only to its antioxidants, but also to some of its special peptides (Bergquist *et al*., 2007). Peptides are small pieces of protein, and researchers have discovered several peptides in spinach that can help lower blood pressure by inhibiting an enzyme called angiotensin I-converting enzyme.).Two of the carotenoids that are especially plentiful in spinach lutein and zeaxanthin are primary antioxidants in several regions of the eye, including the retina and the macula (Chen and Gallie, 2004). Although we haven't seen specific studies on spinach intake and prevention of eye-related problems like macular degeneration, we have seen studies showing that human blood levels of lutein can be increased by consumption of spinach in everyday amounts. We've also seen at least one group of researchers suggesting that spinach has a likely role to play in prevention of eye problems, including age-related macular degeneration (Kruk *et al*., 2005).

2.4 Ecological Considerations for Intensive Aquaculture

The sustainability of intensive aquaculture on a large scale requires consideration of the environmental resources on which it depends (inputs) and the ecological systems to which it discharges wastes (outputs) (Chen and Galie, 2004). Net pen production occurs within a host ecosystem (e.g. coastal waters) with wastes from a high concentration of animals freely flowing out of the production area and into the surrounding environment (Bunting, 2013). Flow-through raceway production systems

receive a constant supply of water from a river, spring or well and continuously discharge effluent as water leaves the farm (Lovelace, 2009; Pillay, 2004). But RAS (including aquaponics) resolves the output or pollution problems inherent in other forms of intensive aquaculture (Bunting, 2013). As a form of tank aquaculture RAS is independent of aquatic ecosystems.

This separation of production from the natural environment creates a point of intervention that, if properly managed, virtually precludes the discharge of effluents as pollutants (Bunting, 2013). The question of sustainability of inputs remains relevant for RAS as it does for other forms of intensive aquaculture. Due to filtration water usage is extremely low in RAS and especially in aquaponic (Lovelace, 2009). However, formulated aqua feeds are an input of critical importance in terms of the sustainable growth of the aquaculture sector and the health of the world's fisheries (Tacon *et al*., 2011).

2.5 Fish feed and feeding used

Tilapia fish are largely omnivores and respond well to commercial fish feed. Their diets need to be well balanced in terms of amino acids, proteins, fats, vitamins, minerals and carbohydrates (Riche and Garling, 2003). In natural environments, wild tilapia feed on algae which (low in protein) and small animals such as worms whereas small-scale aquaponic growers may choose to feed their fish with a mixture of these materials, however optimum tilapia growth will be obtained by the use of commercial feed pellets. Fish in culture require less food than wild fish as they need less energy to survive and obtain food, thus the controlled use of fish feed pellets gives the grower complete control of the nutrient inputs into the aquaponic system (Rich and Garling, 2003).

In recirculating aquaculture, feeding rates for tilapia will vary with fish size. Food to be given is measured as a percentage of the average body weight of the fish in the tank. Also, as the average fish weight increases, the percent body weight fed to the fish decreases. The daily feed ratio should therefore be adjusted to account for fish growth.

In aquaponic systems, tilapia fish grow best when fed three times daily ad libitum (the amount of food that they will eat in 30 minutes) (Bailey *et al.,* 2004), where the feed is composed of 32% protein. Determining amounts of fish feed per tank per day over the growing period of the tilapia based on average fish weight is considered an over-complication by aquaponic experts. Instead, empirical values have been established for the amount of daily fish feed per area of hydroponic grow bed. This allows for the calculation of the number of fish the system can grow and consequently the volume of water needed to stock the fish. Overfeeding fish result accumulation of excess feed and waste which compromised with water quality, lower feed efficiency, reduced health of fish and increased costs (Riche and Garling, 2003).

Physiological response

Tilapia culture is widely practiced in many tropical and subtropical regions of the world and constitutes the third largest group of farmed finfish, right after carp and salmonids, with an annual growth rate around 11.5%. Much of the rapid increase in aquaculture production has come from the increasing of existing systems (Bittencourt *et al.,* 2003). Nutrient supplementation in fish diets has been an economically promising method for improving the performance of different intensive fish production systems. Iron amino acid chelate is among the most important a nutrient influencing the fish immune system, and the supply of Iron amino acid chelate can

reduce mortality and improve fish performance, while increasing specific immune responses (Puangkaew *et al.,* 2004).

Hematological parameters are used as an index to detect physiological changes and to assess structural and functional status of health during stress conditions in a number of fish species (Suvetha *et al.,* 2010). Fish blood is sensitive to pollution-induced stress, and changes on the hematological parameters, such as hemoglobin content, hematocrit and number of erythrocytes can be used to monitor stress caused by pollutants such as heavy metals (Barcellos *et al.,* 2004).

Copper, iron and zinc are trace elements essential for all organisms including fishes, and often need to be supplemented to commercial feeds (Watanabe *et al.,* 1997). The amounts of copper, iron and zinc required by fishes were 1–5 g, 30–170 g and 15–40 g kg^{-1} dry diet respectively (Watanabe *et al.,* 1997).

Therefore, supplementary iron in fish feed needs to be well balanced to fulfil iron requirement and to avoid iron toxicity (Mertz, 1993). The nutritional and toxic effects of Cu, Fe and Zn in fishes have been well documented (Lorentzen *et al.,* 1998), but the mechanisms of their actions are not fully understood. For example, the effects of high levels of dietary Cu, Fe and Zn on the activities of digestive enzymes in fishes remain largely unknown, although in vitro studies suggest some metal ions may affect the activities of digestive enzymes (Kirchgessner *et al.,* 1976). Therefore study was conducted to investigate the effect of iron amino acid chelate supplementation in fish diets on the growth performances of Nile tilapia and spinach in aquaponic system.

2.6 Water Quality

Good water quality must be maintained at all times in a recirculating fish tank to maintain optimal growth conditions and health of the fish. Regular water quality testing is essential and can be performed using water quality testing kits obtained from aquaculture supply companies. The most critical water quality parameters to monitor are dissolved oxygen concentrations, temperature, pH, and nitrogen from ammonia, nitrate and nitrite (Devillers *et al.,* 2009). Nitrogen in the form of nitrate and nitrite usually does not present a water quality problem in aquaponic fish tanks as nitrite is quite quickly converted to nitrate and nitrate itself is only seriously toxic to fish at very high levels (300-400 mg L^{-1}). The biofiltration mechanism in aquaponic systems also removes nitrates quite well and can keep their concentration at much lower levels than this (DeLong *et al.,* 2009). Thus the most important water quality parameters to design and make practice recommendations for are temperature, dissolved oxygen and ammonia. Other important parameters include salinity, phosphate, chlorine and carbon dioxide. Other factors that influence the quality of fish tank water include the stocking density of the fish, their growth rate, the rate at which they are fed, the volume of water in the system and environmental conditions (Diver, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted at University of Eldoret hatchery of the Fisheries and Aquatic Sciences Department for a period of 119 days from August to December 2016. The university is situated 9 Km north east of Eldoret Municipality, on the Eldoret - Ziwa road. Global position of 0°35°N and 35 °N-12 °E at an altitude of 2180 m above sea level. Temperatures 8.4 °C - 25 °C, 2 rainy seasons – 900 mm to 1,200 mm per annum, the hatchery is 500 m from 2.6 acre university fish farm. The farm has 42 earthen ponds, and rears Nile tilapia, goldfish and African catfish.

3.2 Source of Fish

Three hundred and sixty experimental fish were obtained from the University of Eldoret fish farm. Ready Nile tilapia brooders were selected and mixed with the ratio of 3 female and 1 male of the same size for two week to spawn then after two weeks eggs was removed from their mother's mouth and incubated using the hatching jar, after eggs hatched fry were then removed using the scoop net and placed on another aquaria for conditioning. The hatched fry were administered with 60 mg kg^{-1} methyltesteosterone sexed reversal diet for 30 days. The selected Nile tilapia fry were graded for uniformity and conditioned for 3 days to empty their gut to avoid any stress during transportation to hatchery. After conditioning they were introduced in one packaging polythene with half full of water, oxygen cylinder was used to induce oxygen then finally tightened with rubber band to avoid oxygen loss. This was done in the morning because of low temperatures which lowers metabolic rate and which

thus reduced fish excretion. Selected fingerlings were then transported to the hatchery and acclimatized with hatchery water for temperature and pH stabilization before stocking according to project experimental research designed.

3.3 Aquaponic system

Three hundred sixty (360) fish was stocked in 12 aquaria of dimensions 45 cm by 35 cm by 35 cm. Each of these tanks was each filled with 50 L de-chlorinated water. The fry of the same initial weight (0.475 ± 0.025 g), length (3.127 ± 0.063 cm) was stocked in each experiment treatment; each of these treatments was replicated thrice. The tanks were placed on raised platform where it was placed a 1.0 m away from the walls and ½ m above the ground.

3.4 Experimental Design

The experiments were conducted in an aquaponic system, which consist of 12 rectangular indoor aquaria fish tank (45 cm by 35 cm by 35 cm each) and 12 rectangular plastic plant tanks (1 m by 0.5 m) with gravel substrate. Water was continuously recirculated from the rearing tanks through sump and pumped through to the filter plants and gravels then back to the fish culture. Fish with the same initial mean weight (0.475 ± 0.025 g) and mean length (3.127 ± 0.063 cm) was selected randomly and stocking densities of thirty (30) fish were stocked in each aquaponic unit; with the same plants density carries 9 spinach in each aquaponic unit. The treatments were replicated three times in a completely randomized design layout. Treatment diets was formulated with the same crude protein levels (30 %) and supplemented with10 g Fe kg^{-1}, 20 g Fe kg^{-1} and 30 g Fe kg^{-1} and control (0 g Fe kg^{-1}) iron amino acid chelate then administered respectively on the set experiment.

3.5 Sampling

3.5.1 Fish sampling

Each week, 30 fish from each aquarium were individually weighed and their total length measured. All the fish were removed from each tank using a minnow seine, and returned to the tank following measurement. Electronic balance (readability 0.01 g) was used to record fish weight and a meter ruler to the nearest 0.1 cm were used to estimate total length. Fish growth performance was assessed using the following equations (Ricker, 1979):

i) $Weight\ gain\ (W) = Final\ weight\ (W_t) - Initial\ weight\ (W_0)$

ii) $Food\ Conversion\ Ratio\ (FCR) = \frac{Total\ feed\ :F_1}{Total\ weight\ gain\ :W_1}\ or\ \frac{Dry\ weight\ of\ feed\ :g_1}{Weight\ gain\ :g_1}$

iii) $Specific\ Growth\ Rate = \frac{100 \times (Ln\ W_t - Ln\ W_0)}{t}$

iv) $Relative\ Weight\ Gain\ (RWG\%) = \frac{(W_t - W_0)}{W_t} \times 100$

v) $Daily\ \ Weight\ Gain\ (DWG) = \frac{(W_t - W_0)}{T}$

vi) $Percentage\ Survival\ (\%) = \frac{No\ of\ fish\ at\ end\ of\ experiment}{No\ of\ fish\ at\ beginning\ of\ experiment} \times 100$

Wt is the weight (g) of fish at the end of the experiment

Wo is the weight (g) of fish at the beginning of the experiment

T is the number of experimental days

Nf is the number of surviving fish in each treatment by the end of the experiment

No is the number of fish in each treatment at the start of the experiment

3.5.2 Water sampling

Water quality parameters which were measured include Dissolved oxygen, temperature, pH, Ammonia, nitrite and nitrate. Where water temperature, dissolved oxygen, conductivity and pH were measured using a thermometer, an Oxymeter (YSI 200 model) and a portable field pH meter, respectively, three times a week in fish and plants components. While ammonia (NH_4^+), nitrate (NO_3^-) and iron were measured weekly using YSI 9500 photometer

3.5.3 Plants sampling

Each week, 9 Spinach from each tank were individually measured only for height and leaf number counts while, wet and dry weights were only measured at initial and harvesting stage. A vernier caliper (westward, 0.01 mm) were used to measure height and a digital scale (Ohaus, 0.01 g) was used to record wet weight and dry weight. Growth biomass of the plants was calculated as the growth rate which is the height of plants (cm) / culture period (days).

3.6 Mineral Analysis

The elements K, Ca, Na, of spinach leaves were determined using flame photometer while Fe, Mn and Zn were determined using an atomic absorption Perkin-Elmer 4000 spectrophotometer. Phosphorous and nitrogen were analyzed by automated colorimetry in a Technicon Acta CIII auto-analyzer. The total nitrogen was measured by automated colorimetry after a Kjeldahl digestion method.

3.7 Determination of Proximate Composition of diets, spinach and fish carcass

The proximate composition of diets ingredients, plants samples, and fish carcass (whole body fish excluding viscera) were determined according to the standard methods of the AOAC (2003) as follows: Dry matter of the collected samples was dried in oven at 105°C for 24 h until constant weight achieved while the Crude protein was obtained (N × 6.25) by the Kjeldahl method after acid digestion, lipids were measured by hexane extraction in a Soxhlet system, ash samples were measured by incineration in a muffle furnace at 550 °C for 24 h, while nitrogen-free extract (NFE) was calculated by differences between Dry mater, Crude protein, Lipids and moisture content.

The gross energy contents of the diets and the fish were calculated on the basis of their crude protein, lipid and carbohydrate contents using the equivalents of 22.2, 38.9 and 17.15 kJ g^{-1} respectively (Luquet and Moreau, 1989). Experimental diets and fish were analyzed for mineral composition (iron), using microwave digestion and atomic absorption spectrophotometer (Varian SAA 110) air acetylene flame (AOAC, 2003). All the samples were analyzed in triplicate and the mean of each value were derived.

3.8 Chlorophyll Determination on spinach leaves

Accurately weighted 2 g of fresh plant leaf sample was taken, and homogenized in tissue homogenizer with 10 ml of 95% ethanol solvent. Homogenized sample mixture were centrifuged for 10,000 rpm for 15minutes at 4°C. The supernatant was separated and 0.5ml of it mixed with 4.5ml of the 95% ethanol solvent. The solution mixture was analyzed for Chlorophyll-a and b content in spectrophotometer (Parkin). The equation used for the quantification of Chlorophyll-a and Chlorophyll-b are given below:

i) Chlorophyll a, Ch-a=13.36A664 – 5.19 A649 (mg Chl-a mL^{-1})

ii) Chlorophyll b, Ch-b=27.43A649 – 8.12 A664 (mg Chl-b mL^{-1})

iii) Total chlorophyll concentration (a+b), Chl (a+b) = Chl-a + Chl-b (mg Chl mL^{-1}).

3.9 Physiological Response of Nile tilapia

Physiological Response Analysis was carried out at Moi Referral and Teaching Hospital Ampath Department. Blood glucose was measured using a TRUE track® glucose meter (Nipro Diagnostics Inc., Osaka, Japan) which was calibrated by inserting a test strip into the test slot. A drop of blood from the syringe was added on the designated area of the test strip to measure the glucose (Mustafa *et al*., 2000). This method has been validated for use in glucose analysis for fish (Iwama *et al.,* 1995).

Packed Cell Volume was measured using a single glass capillary tube which was filled with blood from the syringe and then one end was capped with Critocaps (Gensic *et al*., 2004). The tubes were kept standing for 15 minutes at room temperature. After sampling all the 24 fish, the blood-filled tubes were centrifuged in the micro centrifuge machine for 1,000 rpm for 5 minutes, resulting separation of the plasma from the blood cells. The hematocrit levels were determined using Micro-Hematocrit Capillary Tube Reader (Monoject Scientific, St. Louis, MO) after the centrifugation (Mustafa *et al*., 2000).

3.10 Feeds

3.10.1 Feed Ingredients

Fish ingredients diets were locally purchased and feed formulation was done at the same crude protein (30 % CP) but with different supplement level of iron amino acid chelate (0 g Fe kg^{-1}, 10 g Fe kg^{-1}, 20 g Fe kg^{-1} and 30 g Fe kg^{-1}) respectively which were commercially bought. Example in Diet 1, Diet 2, Diet 3 and Diet 4 (Table 1).

3.10.2 Feed preparation and proximate analysis

Fish feed ingredients were subjected to proximate analysis before diet formulation to determine the crude protein, moisture content, lipid, ash and NFE. Experimental diets were formulated using the Winfeed (Version 2.8) computer program at 30% crude protein content (Table 2).

Table 1: Feed ingredients required for formulation of experimental Diets

Ingredients	Diet 1 (0 g Fe kg^{-1}) (Control)	Diet 2 (10 g Fe kg^{-1})	Diet 3 (20 g Fe kg^{-1})	Diet 4 (30 g Fe kg^{-1})
	CP (30 %)	CP (30 %)	CP (30 %)	CP (30 %)
Yellow corn (g)	48	48	48	48
Soy bean meal (44%) (g)	18.5	18.5	18.5	18.5
Fish meal (72%) (g)	26.0	26.0	26.0	26.0
Corn oil (g)	5	5	5	5
Vitamin premix (g)	1.5	1.5	1.5	1.5
Starch (g)	1	1	1	1
Iron amino acid chelates	0 g kg^{-1}	10 g kg^{-1}	20 g kg^{-1}	10 g kg^{-1}

Table 2: Experimental diet proximate composition after iron amino acid chelate supplementation

Composition	Experimental diets			
	0 g Fe kg^{-1}	**10 g Fe kg^{-1}**	**20 g Fe kg^{-1}**	**30 g Fe kg^{-1}**
Dry matter (%)	91.20 ± 0.01	90.75 ± 0.02	90.55 ± 0.01	91.02 ± 0.241
Ash content (%)	12.93 ± 0.97	12.98 ± 0.04	12.43 ± 0.17	14.14 ± 0.119
Crude lipids (%)	14.18 ± 0.66	13.21 ± 0.38	14.07 ± 0.16	12.49 ± 0.313
Crude protein (%)	30.47 ± 0.39	32.49 ± 0.55	34.45 ± 0.55	34.60 ± 1.33
Moisture content (%)	0.64 ± 0.35	0.49 ± 0.01	0.54 ± 0.28	0.45 ± 0.124
Crude fibre (%)	3.21	2.98	2.95	2.82
NFE	36.22	36.35	35.10	34.98

The ingredients was weighed and ground to small particle size and thoroughly mixed with water to obtain a 30 % moisture level. Corn Oil, Vitamins and minerals mixture were added to the diets. Iron amino acid chelate was included in diet formulations to serve as traces iron and amino acid supplements.

3.11 Data Analysis

All data were subjected to normality test using Kolmogorov–Smirnova. One way analysis of variance (ANOVA) followed by Tukey's multiple-comparison post hoc test were applied to determine differences among all treatment in plants growth, fish growth, dry weights, wet weights, plants heights, plants minerals, proximate composition levels, chlorophyll levels and physiological parameters of fish while Kruskal Wallis were used to test significance difference on the spinach number of leaves. The regression lines comparisons procedure was designed to compare the slope lines relating the growth rate in terms of log number of leaves and plant heights of spinach in relation to the number of days in aquaponic system using single factor classification for multiple slopes implemented using Statgraphics Ver. 16 (StatPoint Technologies, 2010).

CHAPTER FOUR

RESULTS

4.1 Fish Growth

4.1.1 Growth of fish in aquaponic system

The growth parameters of fish at different levels of iron amino acid chelate treatments in terms of mean weight (g), total length (cm), % weight gain, SGR (%), FCR and survival (%) were calculated and as shown in Table 3. The final mean fish weight, in aquaponic system treatments was 4.354 ± 0.295 g, 6.207 ± 0.318 g, 7.406 ± 0.306 g and 11.606 ± 0.55 g for 0 g Fe kg^{-1}, 10 g Fe kg^{-1}, 20 g Fe kg^{-1} and 30 g Fe kg^{-1} treatments respectively. Total length of the fingerling at the end of experiment was significantly different ($F_{0.05,\ 3}$ = 59.74; p-value = 0.001) between treatments. The highest was observed in 30 g Fe kg^{-1} (8.949 ± 0.16 cm) followed by 20 g Fe kg^{-1} (7.4578 ± 0.097 cm), 10 g Fe kg^{-1} (7.102 ± 0.128 cm) and least 0 g Fe kg^{-1} (6.062 ± 0.143 cm). The specific growth rate (SGR) recorded significantly different in all treatment and was 1.744 ± 0.02, 2.023 ± 0.012, 2.162 ± 0.02 and 2.516 ± 0.01 for 0 g, 10 g, 20 g and 30 g Fe kg^{-1} treatments respectively. Daily weight gain was significantly different ($P < 0.05$) for the all treatment where 30 g Fe kg^{-1} had the highest (8.764 ± 0.413 g) and 0 g Fe kg^{-1} had the lowest (3.054 ± 0.212 g). The mean food conversion ratio (FCR) in all treatments was significantly ($p < 0.05$) higher in 0 g Fe kg^{-1} (2.081 ± 0.797) than in the other treatments (Table 3).

Table 3: Fish growth parameters of *O. niloticus* in an aquaponic system at four different of iron amino acid chelate levels

Parameters	Treatments			
	0 g Fe kg^{-1}	**10 g Fe kg^{-1}**	**20 g Fe kg^{-1}**	**30 g Fe kg^{-1}**
Initial length (cm)	3.13 ± 0.063^{a}	3.13 ± 0.063^{a}	3.13 ± 0.063^{a}	3.13 ± 0.063^{a}
Final length (cm)	6.06 ± 0.143^{a}	7.10 ± 0.128^{b}	7.46 ± 0.097^{c}	8.95 ± 0.160^{d}
Initial weight (g)	0.48 ± 0.025^{a}	0.48 ± 0.025^{a}	0.48 ± 0.025^{a}	0.48 ± 0.025^{a}
Final weight (g)	4.35 ± 0.295^{a}	6.21 ± 0.318^{b}	7.41 ± 0.306^{c}	11.61 ± 0.550^{d}
Weight gain (g)	3.88 ± 0.270^{a}	5.73 ± 0.293^{b}	6.93 ± 0.281^{c}	11.13 ± 0.525^{d}
Daily weight gain (g)	0.03 ± 0.002^{a}	0.05 ± 0.002^{b}	0.05 ± 0.002^{c}	0.09 ± 0.004^{d}
% Daily weight gain	3.05 ± 0.212^{a}	4.51 ± 0.235^{b}	5.46 ± 0.221^{c}	8.76 ± 0.413^{d}
SGR	1.74 ± 0.020^{a}	2.02 ± 0.012^{b}	2.16 ± 0.020^{c}	2.52 ± 0.010^{d}
% SGR	174.401	202.322	216.228	251.601
Survival %	98.33 ± 0.293^{a}	98.71 ± 0.127^{a}	99.08 ± 0.103^{c}	98.71 ± 0.112^{a}
FCR	2.08 ± 0.797^{a}	1.18 ± 0.038^{b}	1.17 ± 0.015^{c}	1.10 ± 0.107^{d}

Superscript in the same row sharing a common letter were not statistically different

4.1.2 Fish growth trends in aquaponic system during the experimental period

The treatment at 30 g Fe kg^{-1} had the highest growth throughout the experiment followed by 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and last 0 g Fe kg^{-1} of iron amino acid chelate supplementation. In the first weeks the growth were slower in all treatments but after two weeks fingerlings show significantly ($p = 0.001$) difference on length (Fig. 1) and weight (Fig. 2) up to the entire period of 17 weeks. One-Way ANOVA showed significantly different in weights and length ($F_{0.05, 3} = 59.74$; p-value = 0.001) and ($F_{0.05, 3} = 71.84$; p-value = 0.001) respectively.

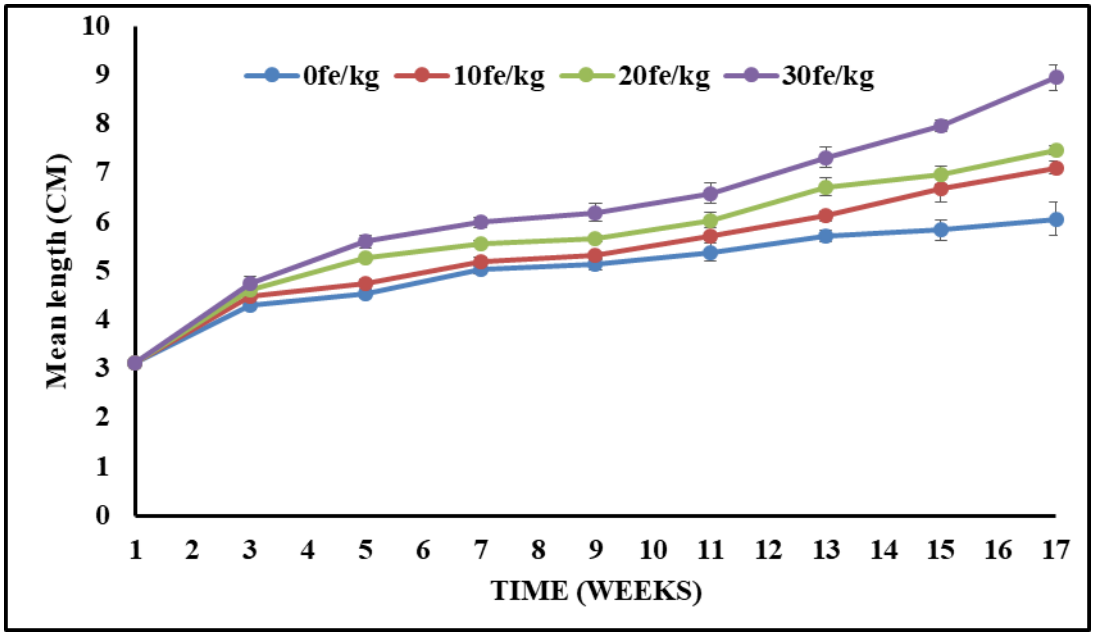

Figure 1: Mean length ± SE of *O. niloticus* fingerlings for 4 treatments over the experimental period of 17 weeks

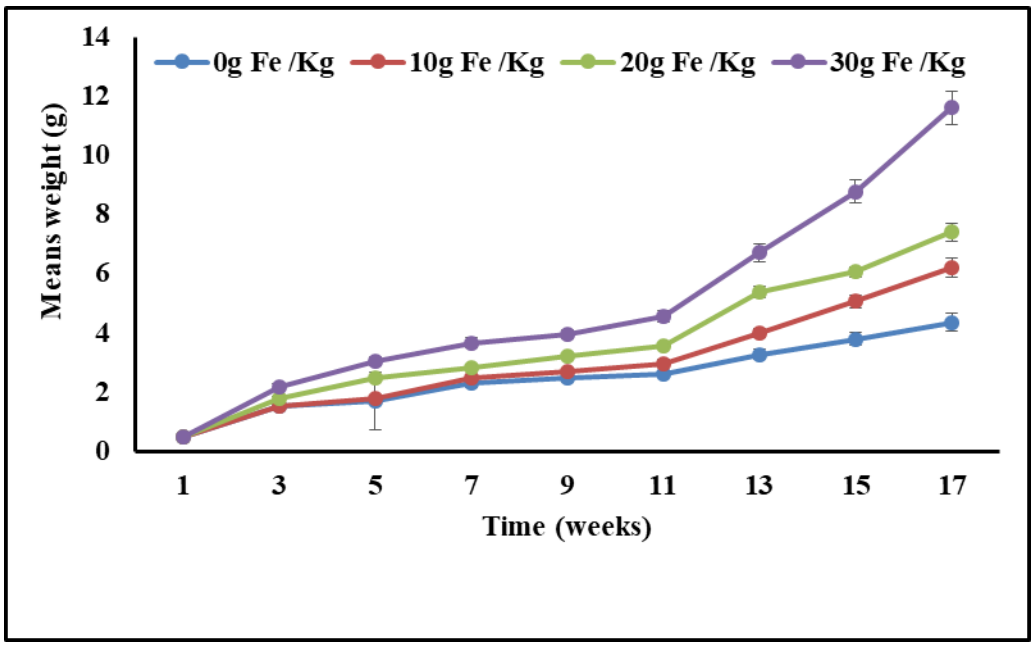

Figure 2: Mean weights ± SE of *O. niloticus* fingerlings for 4 treatments over the experimental period of 17 weeks

4.2 Plants Growth in Aquaponic System

The results of *Spinacia oleracea* growth in the different treatments are represented in Table 4. The mean final spinach wet weight (113.6 ± 9.46 g), number of leaves (19) and plant height (52.44 ± 0.798 cm) was highest in the 30 g Fe kg^{-1} treatment whereas the 0 g Fe kg^{-1} treatment had the lowest mean wet weight (30.65 ± 2.15 g), number of leaves (10 ± 0.225) and plant height (25.36 ± 0.723 cm).

There was a significant difference ($p < 0.05$) in mean *S. oleracea* wet weight. The 30 g Fe kg^{-1} treatment gave the highest mean final wet weight (113.6 ± 9.46 g) and mean final dry weight (32.973 ± 0.253 g) as compared with other treatments which had 107.39 ± 9.48 g, 59.75 ± 2.8 g, 30.65 ± 2.15 g wet weight and 23.796 ± 0.215 g, 6.7422 ± 0.0445 g, 4.1704 ± 0.0816 g mean dry weight for (20 g Fe kg^{-1}, 10 g Fe kg^{-1} and 0 g Fe kg^{-1} respectively.

Table 4: Growth performance of Spinach (*Spinacia oleracea*) in an aquaponic system at different levels of iron amino acid chelate

Parameters	Treatments			
	0 g Fe kg^{-1}	**10 g Fe kg^{-1}**	**20 g Fe kg^{-1}**	**30 g Fe kg^{-1}**
Initial leaves no	2.00 ± 0.131^{a}	2.00 ± 0.131^{a}	2.00 ± 0.131^{a}	2.00 ± 0.131^{a}
Final leaves no	10.00 ± 0.225^{a}	13.00 ± 0.16^{b}	15.70 ± 0.509^{c}	19.33 ± 0.392^{d}
Initial heights (cm)	3.00 ± 0.131^{a}	3.00 ± 0.131^{a}	3.00 ± 0.131^{a}	3.00 ± 0.131^{a}
Final heights (cm)	25.36 ± 0.723^{a}	33.33 ± 1.37^{b}	41.52 ± 0.633^{c}	52.44 ± 0.798^{d}
Initial wet weights (g)	0.59 ± 0.523^{a}	0.59 ± 0.523^{a}	0.59 ± 0.523^{a}	0.59 ± 0.523^{a}
Final wet weights (g)	30.65 ± 2.150^{a}	59.75 ± 2.800^{b}	107.39 ± 9.480^{c}	113.60 ± 9.460^{c}
Initial dry weights (g)	0.20 ± 0.017^{a}	0.20 ± 0.017^{a}	0.20 ± 0.017^{a}	0.20 ± 0.017^{a}
Final dry weights (g)	4.17 ± 0.082^{a}	6.74 ± 0.045^{b}	23.80 ± 0.220^{c}	32.97 ± 0.250^{d}
Wet weight gain (g)	30.06 ± 1.627^{a}	59.16 ± 2.277^{b}	106.80 ± 8.957^{c}	113.01 ± 8.940^{c}

Superscript in the same row sharing a common letter were not statistically different

Mean number of leaves was significantly ($p < 0.05$) among the treatments. The highest mean number of leaves was recorded in 30 g Fe kg^{-1} (19 ± 0.392) and the lowest of the same was recorded in 20 g Fe kg^{-1} (15 ± 0.509) (Fig. 3). Kruskal-Wallis non-parametric showed that the number of leaves (Figure 3) counted in all treatment was significantly varied (H = 37.79, DF = 3, p-value = 0.001). 30g Fe kg^{-1} gave the highest (19.33 ± 0.392) and 0 Fe kg^{-1} gave the lowest (10 ± 0.225).

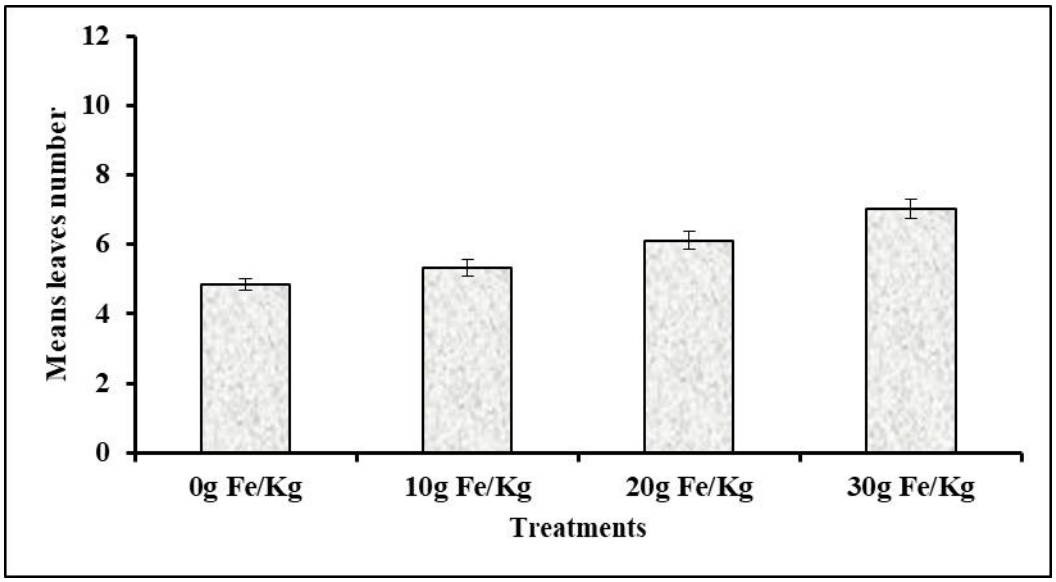

Figure 3: Mean ± SE number of leaves of *Spinacia oleracea* in an aquaponic system from four different iron amino acid chelate supplementation treatments

Regression comparison of spinach heights in aquaponic system are summarized in (Table 5). Higher slope of spinach heights value was recorded for Diet 4 treatment (0.430084) and the lowest for diet 1 treatment (0.233699). In the first to twentieth days the spinach heights were not statistically different in all treatments but after twentieth day spinach heights show significant ($p = 0.0005$) difference up to the entire period of 119 day (Fig 4). The R-Squared statistic indicates that the model as fitted explains 87.1182 % of the variability in Height (cm). The adjusted R-Squared statistic, which is more suitable for comparing models with different numbers of

independent variables, is 87.0648 %. The Durbin-Watson (DW) statistic tests the residuals to determine if there is any significant correlation based on the order in which they occur. Since the P-value is less than 0.05, there is an indication of possible serial correlation at the 95.0 % confidence level. Because the P-value for the slopes is less than 0.01, there are statistically significant differences among the slopes for the various values of Treatment at the 99 % confidence level (Fig 4).

Table 5: Regression model describing the relationship between *Spinacia oleracea* heights, sampled period (days) at four treatments in aquaponic system

	Coefficients	
Treatment	***Intercept***	***Slope***
Diet 1	1.12658	0.233699
Diet 2	1.12658	0.302334
Diet 3	1.12658	0.336801
Diet 4	1.12658	0.430084

Analysis of Variance

Source	***Sum of Squares***	***Df***	***Mean Square***	***F-Ratio***	***P-Value***
Model	160429.	4	40107.3	1633.24	<0.00005
Residual	23722.0	966	24.557		
Total (Corr.)	184151.	970			

Post-hoc ANOVA

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-Ratio**	**P-Value**
Days	135229.	1	135229.	5506.74	0.0001
Slopes	25200.4	3	8400.13	342.07	0.0001
Model	160429.	4			

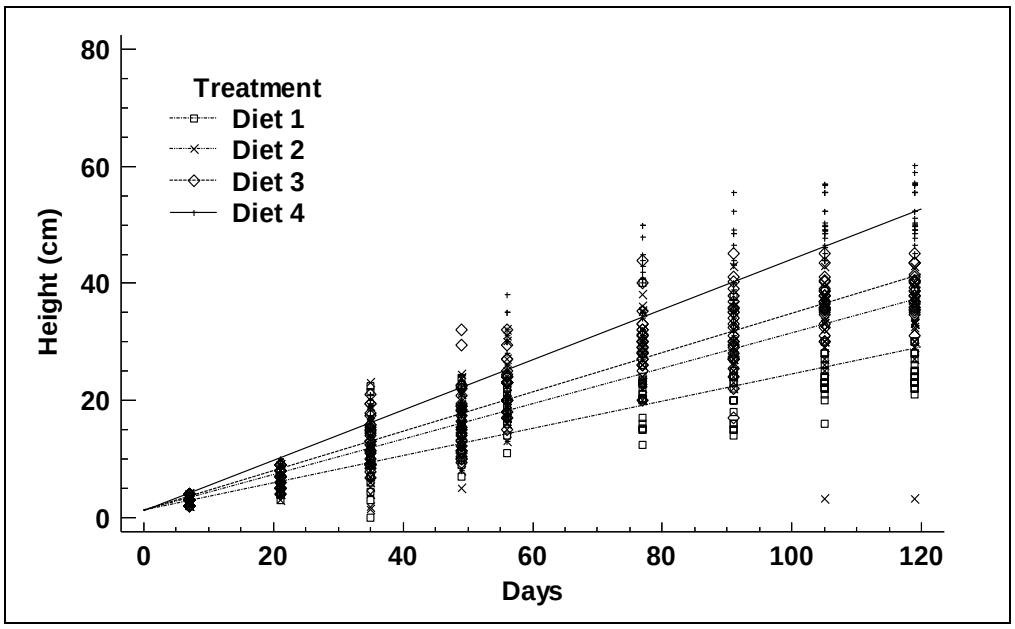

Figure 4: Linear regression model describing the relationship between Height (cm) of *Spinacia oleracea*, sampled period (days) at four treatments in aquaponic system

Regression comparison in terms of spinach number of leaves are summarized in (Table 6). There was a higher slope on spinach number of leaves value in Diet 4 treatment (0.00733283) and the lowest for diet 1 treatment (0.00513697). In the first days the number of leaves were slightly the same in all treatments but after twentieth day spinach leaves show significantly ($p = 0.0001$) difference up to the entire period of 119 day (Fig 5). The differences among the treatments were found to be significant ($p < 0.05$) difference where diets 4 treatments performed better than the other three treatments (Table 6). The highest mean number of leaves was recorded in Diet 4 treatments and the lowest was recorded in Diet 1 treatment (Fig 5). The R-Squared statistic indicates that the model as fitted explains 88.4333 % of the variability in Log number of leaves. The adjusted R-Squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 88.3854 %. The Durbin-Watson (DW) statistic tests the residuals to determine if there is any

significant correlation based on the order in which they occur in data file. Since the P-value is less than 0.05, there was an indication of possible serial correlation at the 95.0 % confidence level.

Table 6: Regression model describing the relationship between *Spinacia oleracea* number of leaves, sampled period (days) at four treatments in aquaponics system

Coefficients

Treatment	Intercept	Slope
Diet 1	0.470001	0.00513697
Diet 2	0.470001	0.00610702
Diet 3	0.470001	0.00670522
Diet 4	0.470001	0.00733283

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	54.225	4	13.5562	1848.30	<0.00005
Residual	7.09241	967	0.00733444		
Total (Corr.)	61.3174	971			

Post-hoc ANOVA

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Days	50.9262	1	50.9262	6943.43	0.0001
Slopes	3.29879	3	1.0996	149.92	0.0001
Model	54.225	4			

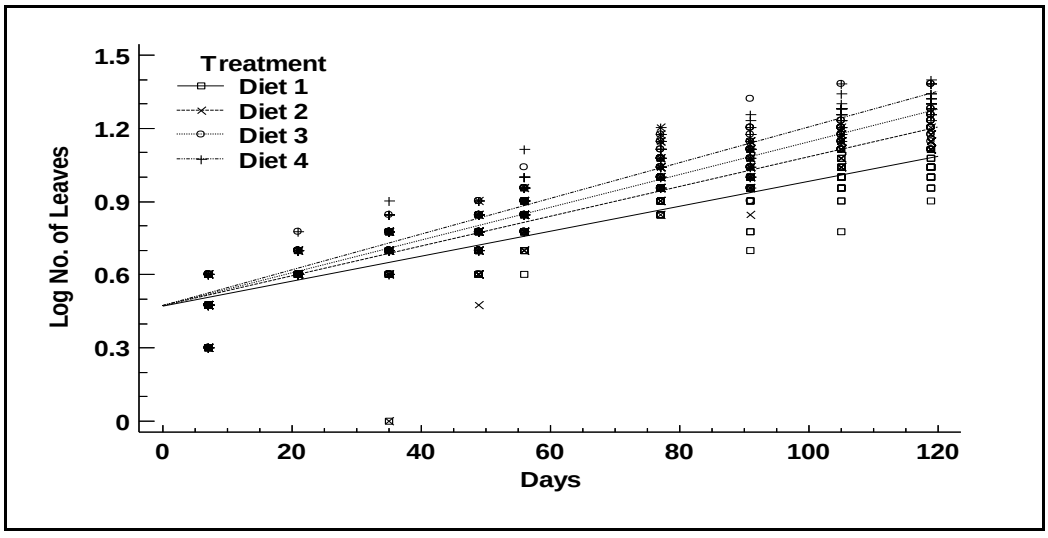

Figure 5: Linear regression model describing the relationship between *Spinacia oleracea* number of leaves, sampled period (days) at four treatments in aquaponic system

The results of *Spinacia oleracea* dry and wet weights in different treatments in the aquaponic system have been represented in (Fig 6 and 7). There was a significant ($p < 0.05$) difference in mean *S. oleracea* wet weights and dry weights. The 30 g Fe kg^{-1} treatment gave the highest mean final wet weight (113.60 ± 9.460 g) and mean final dry weight (32.97 ± 0.253 g) as compared with other treatments which had 107.39 ± 9.480 g, 59.75 ± 2.800 g, 30.65 ± 2.1500 g wet weight and 23.80 ± 0.215 g, 6.74 ± 0.045 g, 4.17 ± 0.0816 g mean dry weight (20 g Fe kg^{-1}, 10 g Fe kg^{-1} and 0 g Fe kg^{-1}) respectively. The parametric One-Way ANOVA in both the dry and wet weight of *S. oleracea* in all treatments differed significantly difference (Dry: F (3,104) = 6432.221, p-value = 0.0001) (Fig 6) and (Wet: F (3,104) = 32.9886, p- value= 0.0001) respectively (Fig. 7).

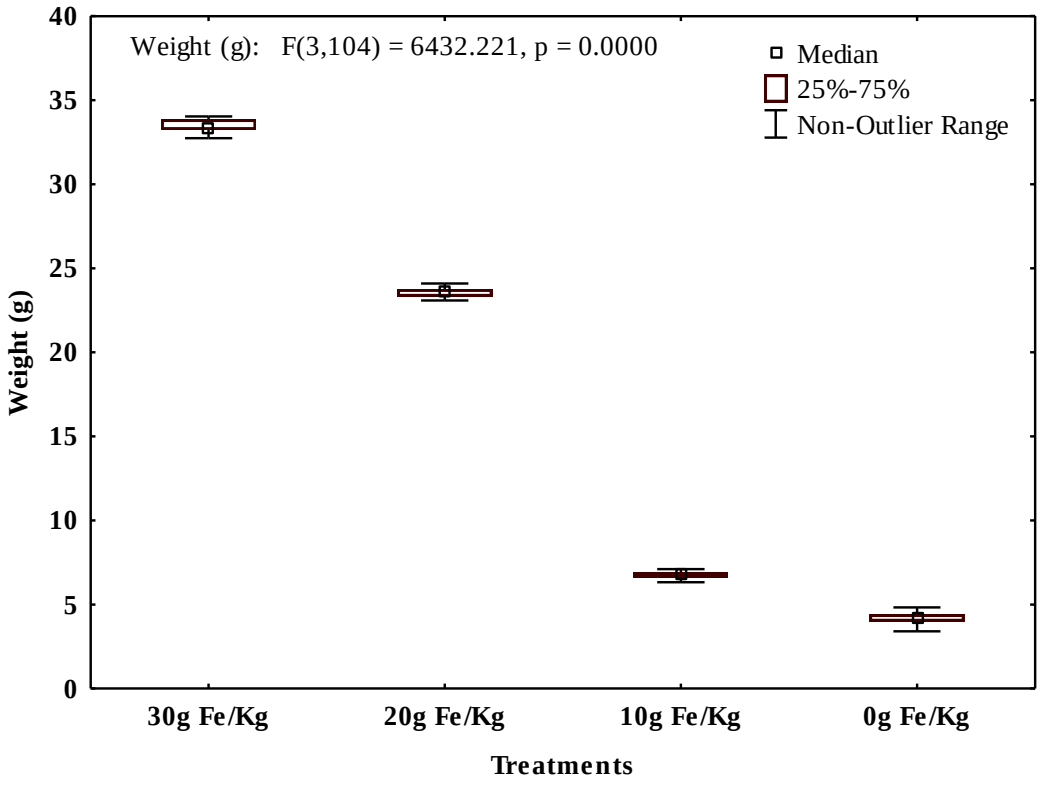

Figure 6: Dry weights of *Spinacia oleracea* in an aquaponic system at four different iron amino acid supplementation treatments

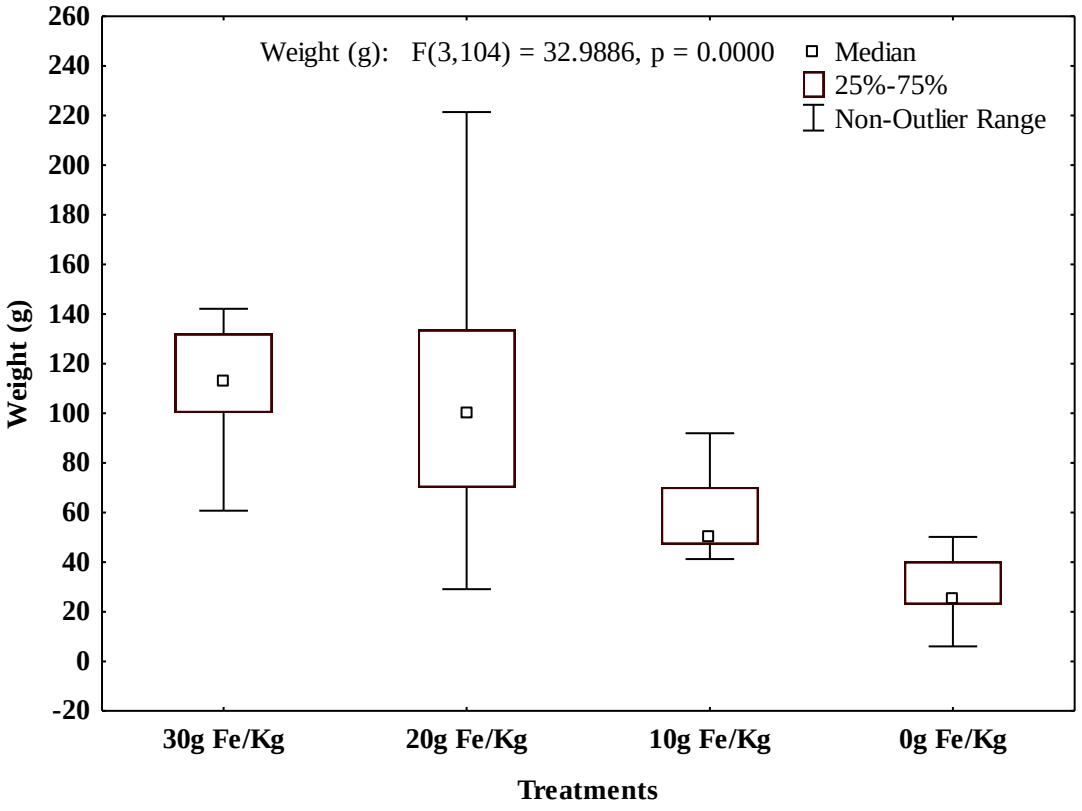

Figure 7: Wet weights of *Spinacia oleracea* in an aquaponic system from four different iron amino acid supplementation treatments

4.2.3 Plants minerals in aquaponic system

The concentrations of phosphorus, zinc, iron, manganese, total nitrogen, and potassium and sodium levels of spinach leaves in different treatments in aquaponic system are shown in Table 7. Final iron concentration was no significant ($p = 0.085$) in the 0 g Fe kg^{-1} (2.62 ± 0.205 mg L^{-1}) and 10 Fe kg^{-1} (0.41 ± 0.024 mg L^{-1}) but there was significantly ($p < 0.05$) differences in the 20 g Fe kg^{-1} and 30 g Fe kg^{-1} treatments. The iron concentration of *S. oleracea* leaves recorded in all treatment was significantly different ($F_{0.05,\ 3} = 6.14$; p-value = 0.001). 30 g Fe kg^{-1} gave the highest (5.20 ± 0.218 mg L^{-1}) and 0 g Fe kg^{-1} gave the lowest (2.62 ± 0.205 mg L^{-1}) of iron concentration.

The 30 g Fe kg^{-1} treatment gave the highest mean final phosphorus levels (67.51 ± 2.420 mg L^{-1}) as compared with other treatments which had 59.42 ± 1.65 mg L^{-1}, 50.35 ± 1.610 mg L^{-1}, and 38.51 ± 3.610 mg L^{-1} mean Phosphorus respectively. Phosphorus (P) showed no significant difference ($F_{0.05, 3}$ = 1.82; p-value 0.152) in all treatments. Mean Zinc concentration of *S. oleracea* was significantly ($F_{0.05, 3}$ = 6.86; p-value = 0.0001) difference among the treatments. The highest mean zinc concentration of *S. oleracea* was recorded in 30 g Fe kg^{-1} (9.07 ± 0.450 mg L^{-1}) and the lowest of the same was recorded in 0g Fe kg^{-1} (3.04 ± 0.320 mg L^{-1}).

The highest mean final total nitrogen concentration (11.25 ± 0.141 mg L^{-1}) was observed in aquaponic system treatment at 30 g Fe kg^{-1} iron amino acid chelate supplement, whereas the lowest which was (9.08 ± 0.711 mg L^{-1}) recorded in 0 g Fe kg^{-1} treatment. The differences among the treatments was not significant ($F_{0.05, 3}$ = 0.65; p-value = 0.586) difference.

Table 7: Minerals concentration for Spinach (*Spinacia oleracea*) leaves in an aquaponic System

Minerals (mg L^{-1})	**0 g Fe kg^{-1}**	**10 g Fe kg^{-1}**	**20 g Fe kg^{-1}**	**30 g Fe kg^{-1}**
Phosphorus	38.51 ± 3.61^{a}	50.35 ± 1.610^{b}	59.42 ± 1.650^{c}	67.51 ± 2.420^{d}
Zinc	3.04 ± 0.320^{a}	4.06 ± 0.121^{b}	5.71 ± 0.569^{c}	9.07 ± 0.450^{d}
Iron	2.62 ± 0.205^{a}	2.94 ± 0.388^{a}	3.99 ± 0.008^{b}	5.20 ± 0.218^{c}
Manganese	3.19 ± 0.142^{a}	4.10 ± 0.378^{b}	5.38 ± 0.205^{c}	7.66 ± 0.344^{d}
Total nitrogen	9.08 ± 0.711^{a}	10.31 ± 0.167^{b}	10.69 ± 0.082^{c}	11.25 ± 0.141^{d}
Potassium	4.04 ± 0.369^{a}	5.16 ± 0.235^{b}	5.96 ± 0.235^{c}	7.33 ± 0.454^{d}
Sodium	3.26 ± 0.056^{a}	5.37 ± 0.056^{b}	5.73 ± 0.097^{c}	7.22 ± 0.028^{d}

Superscript in the same row sharing a common letter were not statistically different

The *S. oleracea* plant growth in the 30 g Fe kg^{-1} treatment had the highest *S. oleracea* Manganese concentration and was significantly (p = 0.0001) different from plants grown in 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and 0 g Fe kg^{-1} treatments. Similarly, the

Manganese concentration were also significant difference ($F_{0.05, 3}$ = 6.00; p-value = 0.001) in all treatment, but no significant (p = 0.684) difference in treatment 20 g Fe kg^{-1} and 30 g Fe kg^{-1} and in treatment 0 g Fe kg^{-1} and 10 g Fe kg^{-1} were recorded.

Mean final Potassium (K) concentration was significantly ($F_{0.05, 3}$ = 7.06; p-value = 0.0001) different among all treatments at 7.33 ± 0.454 mg L^{-1}, 5.96 ± 0.235 mg L^{-1}, 5.16 ± 0.235 mg L^{-1} and 4.04 ± 0.369 mg L^{-1} for 30 g Fe kg^{-1}, 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and 0 g Fe kg^{-1} treatments respectively.

The final mean Sodium (Na) concentration was also significantly ($F_{0.05, 3}$ = 5.08; p-value = 0.003) different of *S. oleracea* in all treatment and was 3.26 ± 0.056 mg L^{-1}, 5.37 ± 0.056 mg L^{-1}, 5.73 ± 0.097 mg L^{-1} and 7.22 ± 0.028 mg L^{-1} for 0 g, 10 g, 20 g and 30 g Fe kg^{-1} treatments respectively (Table 7).

4.3 Carcasses of *O. niloticus* proximate Composition in Aquaponic System

Proximate analysis for *O. niloticus* carcasses are summarized in Table 8**.** The four tested treatment indicated a significance difference in crude protein, crude lipid and ash content, ($F_{0.05, 3}$ = 461.6; p-value = 0.0001), ($F_{0.05, 3}$ = 21.07; p-value = 0.001) and ($F_{0.05, 3}$ = 5.07; p-value = 0.009) respectively. Crude protein indicated significantly varied levels at difference iron amino acid chelate supplementation. Crude lipid content were relatively higher in treatment 0 g Fe kg^{-1} (18.20 ± 0.465 %) as compared with other treatments which had 16.57 ± 0.353 %, 14.49 ± 0.114 % and 12.20 ± 0.256 % (10 g Fe kg^{-1}, 20 g Fe kg^{-1} and 30 g Fe kg^{-1}) respectively. Ash content was higher in treatment 30 g Fe kg^{-1} (16.35 ± 0.030 %) as compared to the others treatments. There were no significant differences (p = 0.087) in moisture content between the treatments (8.25 ± 1.025 %, 8.21 ± 0.984 %, 7.87 ± 0.241 % and 7.68 ± 0.541 %) in 0 g Fe kg^{-1}, 20 g Fe kg^{-1}, 30 g Fe kg^{-1} and 10 g Fe kg^{-1} respectively.

Table 8: Carcass composition of *O. niloticus* fed on different diets in aquaponic system

Composition (%)	Treatments			
	0 g Fe kg^{-1}	**10 g Fe kg^{-1}**	**20 g Fe kg^{-1}**	**30 g Fe kg^{-1}**
Ash content	10.59 ± 2.120^{a}	12.11 ± 1.040^{a}	15.85 ± 0.230^{b}	16.35 ± 0.030^{b}
Crude lipids	18.20 ± 0.470^{a}	16.57 ± 0.350^{b}	14.49 ± 0.114^{c}	12.20 ± 0.256^{d}
Crude protein	59.67 ± 0.680^{a}	63.79 ± 0.21^{b}	64.89 ± 0.080^{c}	65.61 ± 0.74^{c}
Moisture content	8.25 ± 1.025^{a}	7.68 ± 0.541^{a}	8.21 ± 0.984^{a}	7.87 ± 0.241^{a}

Superscript in the same row sharing a common letter was not statistically different

4.4 Water quality in aquaponic system

The physico-chemical parameters of aquaponic water are represented in Table 9. The ammonia value between treatments was significantly different ($F_{0.05, 3}$ = 8.29; p-value = 0.0001).The mean ammonia in fish component was 0.52 ± 0.127 mg L^{-1}, 0.40 ± 0.059 mg L^{-1}, 0.29 ± 0.048 mg L^{-1} and 0.25 ± 0.040 mg L^{-1} (Table 3) for 0 g, 20 g, 10 g and 30 g Fe kg^{-1} treatment respectively.

The highest mean nitrate concentration was recorded in 30 g Fe kg^{-1} (1.57 ± 0.157 mg L^{-1}) followed by 20 g Fe kg^{-1} (1.23 ± 0.145 mg L^{-1}), 10 g Fe kg^{-1} (0.69 ± 0.094 mg L^{-1}) and 0 g Fe kg^{-1} treatment (0.41 ± 0.042 mg L^{-1}), nitrate in all treatments showed significantly ($F_{0.05, 3}$ = 19.54; p-value = 0.0001) different. The mean iron concentration on fish aquaria was significantly ($F_{0.05, 3}$ = 33.92; p-value = 0.0001) different. The highest mean iron concentration was recorded in 30 g Fe kg^{-1} (1.03 ± 0.122 mg L^{-1}) followed by 20 g Fe kg^{-1} (0.58 ± 0.047 mg L^{-1}), 10 g Fe kg^{-1} (0.04 ± 0.006 mg L^{-1}) and 0 g Fe kg^{-1} treatment (0.04 ± 0.006 mg L^{-1}). The pH values ($F_{0.05, 3}$ = 73.27; p-value = 0.0001) in fish aquarium were significant different. The highest was recorded in treatment 0 g Fe kg^{-1} (7.83 ± 0.043) followed by 10 g Fe kg^{-1} (7.76 ± 0.0307), 20 g Fe kg^{-1} (7.28 ± 0.038) and 30 g Fe kg^{-1} (7.12 ± 0.048) treatments.

The results further showed that conductivity was statistically significantly ($F_{0.05,\ 3}$ = 31.2; p-value = 0.0001) different between the treatment but there was no significant ($p > 0.05$) difference in treatment 30 g Fe kg^{-1} (976.10 ± 53.900) and 20 g Fe kg^{-1} (1118.1 ± 71.900). However Dissolved oxygen and temperature indicated no significant difference ($F_{0.05,\ 3}$ = 2.59; p-value = 0.054) and ($F_{0.05,\ 3}$ = 0.07; p-value = 0.976) respectively between the treatment.

Table 9: Mean water chemistry parameters for the four treatments iron amino acid chelate levels in aquaponic system

Parameter	Fish Component			
	0 g Fe kg^{-1}	**10 g Fe kg^{-1}**	**20 g Fe kg^{-1}**	**30 g Fe kg^{-1}**
Ammonia (mg L^{-1})	0.52 ± 0.127^{a}	0.29 ± 0.048^{b}	0.40 ± 0.059^{c}	0.25 ± 0.040^{d}
Nitrate (mg L^{-1})	0.41 ± 0.042^{a}	0.69 ± 0.094^{b}	1.23 ± 0.145^{c}	1.57 ± 0.157^{d}
Iron (mg L^{-1})	0.04 ± 0.006^{a}	0.53 ± 0.045^{c}	0.58 ± 0.047^{c}	1.03 ± 0.122^{d}
pH	7.83 ± 0.043^{a}	7.76 ± 0.031^{b}	7.28 ± 0.038^{b}	7.12 ± 0.048^{d}
DO (mg L^{-1})	3.68 ± 0.096^{a}	3.51 ± 0.088^{a}	3.86 ± 0.086^{a}	3.74 ± 0.093^{a}
Temperature (^{o}C)	22.95 ± 0.217^{a}	23.07 ± 0.194^{a}	23.06 ± 0.201^{a}	23.02 ± 0.194^{a}
Conductivity (μS cm^{-1})	421.70 ± 26.100^{a}	795.60 ± 53.600^{b}	1118.10 ± 71.900^{c}	976.10 ± 53.900^{c}
Parameter	**Plants component**			
	0 g Fe kg^{-1}	**10 g Fe kg^{-1}**	**20 g Fe kg^{-1}**	**30 g Fe kg^{-1}**
Ammonia (mg L^{-1})	0.55 ± 0.089^{a}	0.19 ± 0.034^{b}	0.30 ± 0.051^{c}	0.21 ± 0.036^{d}
Nitrate (mg L^{-1})	1.40 ± 0.154^{a}	1.68 ± 0.182^{b}	2.37 ± 0.220^{c}	2.95 ± 0.259^{d}
Iron (mg L^{-1})	0.04 ± 0.006^{a}	1.08 ± 0.118^{b}	2.24 ± 0.206^{c}	2.89 ± 0.153^{d}
pH	7.86 ± 0.035^{a}	7.75 ± 0.029^{b}	7.29 ± 0.037^{c}	7.14 ± 0.045^{d}
Conductivity (μS cm^{-1})	427.30 ± 25.700^{a}	794.60 ± 53.500^{b}	1108.00 ± 70.400^{c}	972.40 ± 53.200^{c}
Temperature (^{o}C)	22.65 ± 0.240^{a}	23.01 ± 0.198^{a}	22.90 ± 0.213^{a}	22.86 ± 0.204^{a}

Superscript in the same row sharing a common letter were not statistically different

In plants component results of one-way ANOVA test indicated a significant difference in ammonia, nitrate, iron, pH and conductivity ($F_{0.05, 3}$ = 8.63; p-value = 0.0001), ($F_{0.05, 3}$ = 11.34; p-value = 0.0001), ($F_{0.05, 3}$ = 79.88; p-value = 0.0001), ($F_{0.05, 3}$ = 89.95, p-value = 0.0001) and ($F_{0.05, 3}$ = 30.74; p-value = 0.0001) respectively among the treatments. However, there was no significantly different ($F_{0.05, 3}$ = 0.5; p-value = 0.679) in temperature between treatments respectively (Table 9).

4.5 The physiological response of *O. niloticus* in aquaponic system

Summary for hematological (glucose, hemoglobin and hematocrits) levels are presented in Table 10. Significant variation in concentration of glucose in blood was observed among all four treatments (p = 0.00001), the 0g Fe kg^{-1} (26.80 ± 0.029 mg dL^{-1}) treatment having the highest glucose level and 30 g Fe kg^{-1} iron amino acid chelate supplementation (13.43 ± 0.169 mg dL^{-1}) had the lowest. On the blood hemoglobin Hb (g dL^{-1}) the values were significantly increase with the increase concentrations of iron amino acid chelate in the fish diets. The values (means ± SE) of Hb (g dL^{-1}) in the treatments 30 g Fe kg^{-1}, 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and 0 g Fe kg^{-1} were 16.63 ± 0.235, 14.72 ± 0.058, 10.83 ± 0.087 and 9.92 ± 0.071, respectively. Also Among the different treatment groups, there was a significant difference ($p < 0.05$) in the percentage of blood hematocrit level. The values (means ± ES) (%) of hematocrits were highest 30 g Fe kg^{-1} (49.89 ± 0.704) and lowest in 0 g Fe kg^{-1} (29.77 ± 0.213) treatment (Table 10).

4.6 Spinach leaves chlorophyll content in aquaponic system

The effect of iron amino acid chelate at difference treatments on chlorophyll a and b content are summarized in (Table 11). Spinach leaves chlorophyll content both a, and b, was significantly increased by iron amino acid chelate supplementation. In all treatments there were significantly different ($F_{0.05, 3}$ = 152.42; p-value = 0.0001) and ($F_{0.05, 3}$ = 98.42; p-value = 0.0001) in chlorophyll a and b respectively where 30 g Fe kg^{-1} (10.28 ± 0.220 mg mL^{-1}) had the highest chlorophyll a followed by 20 g Fe kg^{-1} (9.76 ± 0.074 mg mL^{-1}), 10 g Fe kg^{-1} (7.24 ± 0.326 mg mL^{-1}) and last 0 g Fe kg^{-1} (3.76 ± 0.270 mg mL^{-1}). Furthermore 30 g Fe kg^{-1} (11.67 ± 0.250 mg mL^{-1}) recorded highest chlorophyll b and 0 Fe kg^{-1} (5.25 ± 0.280 mg mL^{-1}) recorded the lowest.

Table 10: Mean haematological level of *O. niloticus* from difference treatments in aquaponic system over a period of 119 days

Haematological parameter	0 g Fe kg^{-1}	10 g Fe kg^{-1}	20 g Fe kg^{-1}	30 g Fe kg^{-1}
Glucose ($mgdL^{-1}$)	26.80 ± 0.029^{a}	23.77 ± 0.017^{b}	21.80 ± 0.029^{c}	13.43 ± 0.169^{d}
Haemoglobin ($mg\ dL^{-1}$)	9.92 ± 0.071^{a}	10.83 ± 0.087^{b}	14.72 ± 0.058^{d}	15.63 ± 0.935^{d}
Haematocrits (%)	29.77 ± 0.213^{a}	32.49 ± 0.261^{b}	44.15 ± 0.475^{c}	45.09 ± 0.704^{d}

Superscript in the same row sharing a common letter were not statistically different

Table 11: Mean spinach chlorophyll a, and b, for the four treatment iron amino acid chelate supplement in aquaponic system over a period of 119 days

Chlorophyll levels	Treatments			
	0 g Fe kg^{-1}	10 g Fe kg^{-1}	20 g Fe kg^{-1}	30 g Fe kg^{-1}
Chlorophyll a ($mg\ Chl\text{-}a\ mL^{-1}$)	3.76 ± 0.270^{a}	7.24 ± 0.326^{b}	9.76 ± 0.074^{c}	10.28 ± 0.220^{d}
Chlorophyll b ($mg\ Chl\text{-}b\ mL^{-1}$)	5.25 ± 0.280^{a}	7.10 ± 0.270^{b}	8.61 ± 0.297^{c}	11.67 ± 0.250^{d}

Superscript in the same row sharing a common letter were not statistically different

CHAPTER FIVE

DISCUSION

5.1 Growth of Nile tilapia in aquaponic system

Iron amino acid chelate has been proven to be one of the element for animals to absorb Fe compound supported by studies on Fe absorption mechanism (Saltman, 1965). Many studies have showed that Fe-Gly has a high bioavailability in the bodies of rats, human beings, aquatic animals and other animals (Ashmead, 2001; Allen, 2002). The current study demonstrates the benefit of dietary supplementation with iron amino acid-chelate on the growth of *O. niloticus*. The growth rate was significantly different between all the treatments where there was an increased growth rate with an increase supplementation of iron amino acid chelate. The results of this experiment showed that supplementation of 30 g Fe kg^{-1} iron amino acid chelate had the best mean final weight, total length, weight gain SGR followed by that of 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and lastly 0 g Fe kg^{-1}. These findings concurs with the findings of (Apines-Amar *et al*., 2004; Sharif *et al*., 2012) who indicated that micronutrients particularly iron, zinc are known to be essential for growth both in fish, humans and other animals. These results also agrees with work done by Anant *et al*. (2014) reporting high growth at high supplementation of chelated amino acid. Reduced growth at a low 0 g Fe kg^{-1} supplementation of iron amino acid chelate is possibly due low iron concentration which improved growth and the immune system of the fish and also high concentration of ammonia and high FCR which negatively affect the growth of the fish. El-Shafai *et al.* (2004) and El-Sherif and El-Feky (2008) had shown that chronic exposure to concentration levels higher than 0.1 mg L^{-1} of NH_3 can significantly inhibit growth in tilapia fingerlings, and that growth performance

decreases and FCR increases as the concentration of NH_3 increases. Satisfactory performance of *O. niloticus* was recorded in high supplementation of iron amino acid chelate which might be due to presence of lysine amino acid which also improved the protein contents of used treatment diet. Marcouli *et al.* (2006) reported that dietary lysine supplementation is reported to advantages on weight gain, feed conversion, nitrogen retention and reduction in body lipid. This is also concurrent with the results of the present study indicating better Feed Conversion Ratio, Specific Growth Rate and Daily Weight Gain when iron amino acid chelate is supplemented to the fish diet. Salama *et al*. (2013) found out that increasing levels of lysine and methionine + cystine in the diet improved the protein efficiency ratio, and the feed conversion ratio (FCR).

Feed conversion ratio (FCR) involve finding out how much feed given actually goes into building fish body biomass. The lower the FCR the better the quality of the diet. This is because a smaller quantity of feed is needed to convert fish body mass to flesh. Food conversion ratio (FCR) value of 30 g Fe kg^{-1} was significantly low followed by 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and 0 g Fe kg^{-1} respectively. This might be due to the higher digestibility and proper utilization of feed, it is a phenomenon that largely concurs with work done by Rahman and Shirajum (2013) who observed decrease trends of FCR values with decrease digestibility and utilization of the fish diets.

5.2 Survival of Nile tilapia in aquaponic system

The survival rate of Nile tilapia in the experimental study was generally high and was not affected by dietary iron level. The lowest survival observed in fish fed the 0 g Fe kg^{-1} and highest in 20 g Fe kg^{-1} diet and could not be attributed to the dietary iron level or source since there was no discernable trend among the values obtained from treatments. Likewise, (Chhorn and Klesiu, 1996) and (Gatlin and Wilson, 1986) indicated that dietary iron level had no effect on the mortality of channel catfish. Also similar finding was observed by Kohinoor *et al*. (2009) from their fry experiments with various carp, barb, Nile tilapia and catfish species.

5.3 Proximate analysis (Carcass composition) of *O. niloticus* in aquaponic system

The obtained results of fish carcass composition showed that dietary supplementation of iron amino acids chelate at 30 g Fe kg^{-1} and 20 g Fe kg^{-1} had increased significantly in crude protein percentage compared with the 10 g Fe kg^{-1} and 0 g Fe kg^{-1} control treatment; but in the same treatment, carcass lipid content was lowest although its highest fish weights which may be due to the highest crude protein content in fish carcass since there is a negative relationship between protein and lipid content, as well as due to iron amino acids chelate which is necessary for metabolism of carbohydrates, lipids and many aspects of fish metabolism (Pan *et al*., 2003). The obtained results also are supported by that of El-Sayed *et al*. (2010) who reported that dietary supplementation of iron amino acid chelate (up to 120 mg kg^{-1}) to Nile tilapia (*O. niloticus*) diets has significantly increased crude protein content and decreased lipids concentration. Moreover, Liu *et al*. (2010) found that dietary iron amino acid chelated significantly lowered carcass lipid content in grass carp (*Ctenopharyngodon idellus*). Further, more the ash content of fish carcasses vary with the experimental

diets treatments. The fish had a significant increase in ash content in carcasses with increased level of iron amino acid chelated which might be due to some mineral content in supplemented treatment. Costanzo *et al*. (2011) reported that the ash content of good quality fish average between 17 and 25 %, also more ash indicates a higher mineral content, especially calcium, phosphorus and magnesium which could be traced in iron amino acid chelated and less in the other treatment especially in 0 g Fe kg^{-1} treatment. However, the moisture content of fish carcasses did not vary with experimental treatments.

5.4 The physiological response of *O. niloticus* in aquaponic system

Fish fed the diet without supplemental iron developed hypochromic microcytic anemia characterized by decreased hemoglobin, hematocrit, MCV and MCH (Chhorn and Klesius, 1996). Physiological response varied among the treatment. High supplementation of iron amino acid chelate increases hemoglobin, hematocrit and reduce glucose level of the fish. The present research 30 g Fe kg^{-1} showed improved hematological values and the differences were significantly, but all the glucose, hemoglobin and hematocrit was within the standard range of *Oreochromis niloticus*, indicating less severe iron deficiency (Suvetha *et al.*, 2010). Increases of hemoglobin and hematocrit with reduction of blood glucose indicated that iron amino acid chelate does not induce stress to *O.niloticus* fingerlings. Zubair (2012) reported that the elevated increase amount of glucose may be due to the high requirement of energy, due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their new energy demands. Similar results were reported in *Clarias gariepinus* (Abalaka *et al*., 2011) and common carp (Hossain *et al*., 2015). Alterations in blood glucose levels have been reported in *Heteropneustes fossilis* exposed to sub-lethal concentration of testosterone (Chowdhury, 2000). Hematological values did not differ

among the groups of fish fed diets supplemented with 20 g Fe kg^{-1} and 30 g Fe kg^{-1} treatments. This indicates that both sources of iron were equally available for fingerling *O. niloticus* which indicated that it was sufficient to maintain normal hematological values. This is in agreement with Gatlin and Wilson (1986) who determined that the dietary iron requirement of catfish was 30 mg kg^{-1} diet. Ahmed *et al*. (2016) stated that decrease in hemoglobin levels might be due to the disruptive action on the erythropoietic tissue as a result of which the viability of the cells might be affected.

5.5 Growth of Spinach (*Spinacia oleracea*) in aquaponic system

Iron is one of the most important micronutrients for plant growth and productivity and its availability affects many plant morphological, physiological and biochemical parameters (Steer and Hocking 1984). Iron traces element is the essential for the plants growth but limiting micronutrient in aquaponic system. From the results it is concluded that iron amino chelate and some nitrogen deficiency significantly decreased growth parameters of spinach cultivated in aquaponic system, such as biomass accumulation, plants heights and the number of leaves, in agreement with earlier findings with plants like salvia (*Salvia splendens* L.), dill (*Anethum graveolens* L.), thyme (*Thymus vulgaris* L.) and lettuce (*Lactuca sativa* L.) (Kang and Van Iersel, 2004). According to Golcz *et al*. (2006) and Olfati *et al*. (2010), nitrogen fertilization has been also shown to directly correlate with the growth, yield, and essential oil content of basil plants, in contrast with Jacimovic *et al.* (2010).

Both plant species of the all treatment showed poor growth at first week, probably due to the sensitivity of the root system on nutrients characteristics present in the system (Licamele, 2009). Growth in terms of plants, slopes, means final height, number of

leaves, wet weight, weight gain and dry weight of *S. oleracea* was significantly different in all treatments, higher in 30 g Fe kg^{-1} treatment where iron amino acid chelate supplementation was high compared to other treatments although the same plants was applied at an equal ratio in all the plants treatments. The causes might include high nutrients due to higher iron amino acid supplementation which stabilizes pH and allows the present of nitrate and iron bioavailability. Similar with findings of Danaher (2013) who reported better growth in terms of heights, number of leaves, diameter, dry and wet weight in supplementation of iron amino acid chelate in aquaponic system also further indicated that supplementation of iron amino acid chelate stabilized pH to around neutral level and provide adequate nutrients for the plants growth. The number of leaves was significantly difference where 30 g Fe kg^{-1} had the highest and 0 g Fe kg^{-1} had the lowest this might be probably because of nutrient reflection with the increment of iron supplementation (Hamid and Simin, 2012) reported that when the amount of nutrients does not suffice, the growth of leaves and then, leaf areas index can be limited due to either the low level of photosynthesis or insufficient cell elongation. The cells of leaves are smaller in plants suffering from N deficiency (Hamid and Simin, 2012). These effects arise from the decrease in water conductance which results in water deficiency in the sheaths of growing leaves (Hamid and Simin, 2012). Yield on the dry and wet weights was statistically significant difference among all the treatment 0 g Fe kg^{-1} treatment had the lowest as compared with 10 g Fe kg^{-1}, 20 g Fe kg^{-1} and 30 g Fe kg^{-1}. these give an indication that supplementation of iron amino acid chelated promote the accumulation of K and N which decreases longitudinal growth and increases the formation of auxiliary roots (Hamid and Simin, 2012) reported that K and N deficiencies increase longitudinal growth and reduce the formation of auxiliary roots.

5.6 The minerals composition spinach (*Spinacia oleracea*) in aquaponic system

Nutrient content of leaves in aquaponic treatments is represented in Table 7. There was a significant difference ($P < 0.05$) in all the treatments in terms of nutrient contents of plant organs. Regardless of the treatments, the concentrations all of the studied elements were lowest in 0 g Fe kg^{-1} treatment compared to other treatments. Kaya and Higgs (2002) found similar result for Zn, but they reported that K, Mg and Fe content of the leaves and the fruits of 'Blizzard' cultivar of tomato were in the same levels. The results showed that supplementation of iron amino acid chelate in aquaponic statistically difference affecting N, P, Na, K, Zn, Mn and iron absorption of spinach leaves, 0 g Fe kg^{-1} treatment had lower N, P, Na, K, Zn and Iron as compared to other treatments. The result indicated that supplementation of iron amino acid chelate affect the macro and micronutrient of the plants in aquaponic system. P concentration was higher in treatment 30 g Fe kg^{-1} followed by 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and lower in 0 g Fe kg^{-1} which might be due not suffice released of phosphate in the other treatments and also probably because of lower availability and release of nutrients from un-supplemented iron amino acid chelate and in fish excretion.

The concentrations of Mg, Na, Fe and Zn were higher in the leaves of aquaponic-grown plants at 30 g Fe kg^{-1} followed by 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and lower at 0 g Fe kg^{-1} treatment. This is may be due to various fractions of dissolved organic matter and traces of mineral in the diets (as a result of microbial decomposition of fish food and feces), which form organometallic complexes with Fe and Zn, thereby increasing the availability of these micronutrients to plants. Hamid and Mohsen (2013) report similar result in concentration of Mn, Na, Fe, and Zn in high supplementation of foliar fertilizers with high supplementation of iron. 0 g Fe kg^{-1} had the lowest Mn, Na, Fe,

and Zn this is partly due to low or lack concentrations of iron amino acid chelate in aquaponic system as well as the low mobility of them in plant. Thus, the translocation of them is limited from old shoot tissues to young tissues, and heir xylem transport into organs that do not have a high transpiration rate are low. On the other hand, in contrast of the results related to the leaves, chelated application of iron amino acid supplementation caused a significant increment of macro and micronutrients element concentrations in the spinach leaves in aquaponic system. This is due to high concentrations of the nutrients used during iron amino acid chelate in fish diet supplementation. Kaya and Higgs (2002) found similar results for Zn chelate, in which the concentration of Zn in the leaves and fruits of hydroponic-grown tomatoes increased linearly with the increasing Zn chelate levels as a foliar spray.

5.7 Leaves chlorophyll content of spinach in aquaponic system

Iron is important in chlorophyll formation, photosynthesis, enzyme systems, chloroplast development and respiration of plants (Miller *et al*., 1995; Halvin *et al*., 1999). The effect of iron amino acid chelate on chlorophyll content in the leaves of spinach plants at 0 g Fe kg^{-1}, 10 g Fe kg^{-1}, 20 g Fe kg^{-1} and 30 g Fe kg^{-1} was significantly increased by iron amino acid chelate supplementation as compared with the control treatment (0 g Fe kg^{-1}). The highest value of leaf chlorophyll content was recorded from plants with 30 g Fe kg^{-1} treatment and followed by 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and 0 g Fe kg^{-1}. The lowest values of chlorophyll content was recorded in 0 g Fe kg^{-1} where spinach plant received only fish diet without iron amino acid chelate supplementation. The increment of chlorophyll a and b could be due to iron traces element present in the supplemented diets compared to the control (0 g Fe kg^{-1}) non supplemented diet although Fe is not a constituent of chlorophyll, but the essential for

chlorophyll biosynthesis (conversion of Mg proporphyrin to protochlorophyllide) (Marschner, 1995). This results concurs with Hamzeh and Florin (2014) who reported increased chlorophyll content of wheat as iron chelate (Fe-DTPA (6% Fe)) is supplemented. This is concurrent with the findings of (Ai - Qing *et al*., 2011; Mohsen, 2013 and Kobraee *et al*., 2011) who demonstrated that adding Fe alone or in combination with other micronutrients increased chlorophyll content of plants. De la Guardia and Alcantara (2002) stated that if Fe in plant tissue is not available or inadequate, the synthesis of chlorophyll is impaired.

5.8 Water quality in aquaponic system

Physico- chemical parameters played a significant role in the maintenance of a healthy aquatic environment and production of natural food organism. Aquaculture waste nutrients should ideally meet the requirements of plants co-cultured in aquaponic systems (Trang *et al*., 2010).In fish and plants component temperature and dissolved oxygen showed no significant ($p > 0.05$) difference among the treatments. Water temperature for all treatment varied with the average value of (23^{o}c) was within the normal range for the survival of Nile tilapia, but according to Colt (2006) optimal temperature for the life of tilapia is 28^{o}C.

The concentration of dissolved oxygen indicated no significant difference among the treatments ($p > 0.05$). Dissolved oxygen is an important parameter, in the process of oxidation of ammonia and the major limiting factor for the survival of fish. Dissolved oxygen concentration was higher in treatment 20 g Fe kg^{-1} followed by 30 g Fe kg^{-1}, 10 g Fe kg^{-1} and least in treatment 0 g Fe kg^{-1} but was within the optimal levels, low DO in treatment 0 g Fe kg^{-1} might be probably due to high concentration of ammonia recorded in the treatment. The optimum DO concentration for optimal fish growth

should be maintained above 5 mg L^{-1} (Colt, 2006), and the DO concentration below 2 mg L^{-1}, ammonia and nitrite oxidation by nitrifying bacteria becomes inefficient anymore (Hargreaves, 2006).

The observed and recorded pH values were within the accepted levels for Nile tilapia and Spinach plants significant difference ($p < 0.005$) was also recorded among all the treatments. High pH was recorded in 0 g Fe kg^{-1} followed by 10 g Fe kg^{-1}, 20 g Fe kg^{-1} and low in 30 g Fe kg^{-1} where supplementation of iron amino acid chelate was high. Supplementation of iron amino acid reflects reduction in pH that is likely caused by the respiration of fish and bacteria that produce carbon dioxide. The presence of CO_2 will shift the equilibrium carbonate reaction, produces H^+ ions, and lowers the pH. Decrease in the pH was presumably associated with the oxidation process undertaken by bacteria in the system. According to Princic *et al.* (1998), in environments with high inputs such as ammonia from aquaculture wastewater, oxidation of this compound produces CO_2 and lowers the pH. The present result agrees with the findings of Hefni *et al*. (2016) who reported that treatments with supplementation of micronutrients result to decrements of pH in relation to increment of the micronutrient in the diets and vice versa in aquaponic system.

In fish component ammonia and nitrate in fish culture units were minimal to none detectable during the experiments for all treatments. Ammonia levels were undetectable after two weeks in all the experiment treatments. Nitrite range from 0.1 mg L^{-1} to 0.19 mg L^{-1} throughout the entire experiment for all treatments. Significant differences were recorded in all treatments. However, high ammonia content was recorded in aquaponic system with treatments of 0 g Fe kg^{-1} iron amino acid supplement which might be due to poor utilization of the diets by the fish resulting to

high accumulation of waste in the system also might be because of high pH more than 7.5 which cannot support survival of bacteria which can easier nitrification process to occur these findings are in conformity with the findings of Kohinoor *et al*., (2009) and Simeonidou *et al.* (2012). Nitrate concentration also increased during the experiment, and the concentration at the end is greater than the beginning of the experiment. Ammonia (NH_4) assimilation occurs relatively rapidly by plants and metabolic reactions are more efficient than NO_3. The low NO_3 removal by lettuce has been documented in other aquaponic systems (Buzby and Lin, 2014). During the experiment, the concentration of NO_3 was still supportive for the life of Nile tilapia and spinach in aquaponic system. According to Watson and Hill (2006), NO_3 should be maintained below 100 mg L^{-1}. Nitrate concentration was highest in treatment 30 g Fe kg^{-1} and lowest in 0 g Fe $kg^{-1.}$ The possible cause was the amount of oxygen supply. At 30 g Fe kg^{-1} treatments oxygen supply was adequate for NO_3 oxidation process and offered favorable condition for bacteria to convert ammonia level to nitrate. However, in plants component ammonia and nitrate varied among all the treatments, ammonia was high in 0 g Fe kg^{-1} treatment as compared with other treatments probably due to poor nitrification of ammonia by bacteria to nitrate which was also reflected to be low nitrate at 0 g Fe kg^{-1} treatment. Lower nitrate removal rate and higher ammonia concentration rate which were accounted for in 0 g Fe kg^{-1} treatments were in accordance with the findings of Endut *et al*. (2009) in a study of aquaculture effluent treatments under different hydraulic loading rates using *Ipomoea aquatica*. Supplementation of iron amino acid chelated indicated that it affect ammonia and nitrate concentration in aquaponic system, this might be also due to the influence of iron amino acid chelate on lowing the pH and thus influence the nitrification process by bacteria. In plants and fish components iron concentration

varied among the treatments 30 g Fe kg^{-1} had the highest and 0 g Fe kg^{-1} had the lowest. There was an increase of iron concentration in aquaponic system in relation with the increment supplementation of iron amino acid chelated. The increase of iron concentration might be due to the present of iron traces in the diet.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Based on the objectives and results in this study, the following conclusions can be drawn:

i) Iron chelate amino acid supplemented at 30 g Fe kg^{-1} exhibited the highest fish growth performance and survival as compared to 20 g, 10 g and 0 g Fe kg^{-1} treatments respectively. Fish proximate composition with supplementation of iron amino acids chelate at 30 g Fe kg^{-1} indicated highest crude protein and ash content in the fish flesh as compared to 20 g Fe kg^{-1}, 10 g Fe kg^{-1} and 0 g Fe kg^{-1}. However, 30 g Fe kg^{-1} supplementation exhibited the lower levels of crude lipids while 0 g Fe kg^{-1} supplementation demonstrated highest crude lipid content. There was no significant difference in moisture content of Nile tilapia in all the treatments. This is an indicator that iron amino acids chelate affect growth and nutrient composition of Nile tilapia positively.

ii) Iron amino acids chelate are important in improving the physiological response of fish. Despite the limited literature on the use of iron amino acid chelate on physiological factors, the present study at 30 g Fe kg^{-1} iron amino acids chelate supplementation improved glucose level, hematocrits and hemoglobin. Therefore the physiological response tends to improve with increase in supplementation of iron amino acids chelates.

iii) The present study on Spinach (*Spinacia oleracea*) growth has confirmed that iron chelate amino acids supplementation have positive effect on the growth index such as wet weight, dry weight, heights of the plants and the number of

leaves of treated compared to the non- supplemented diets. According to the obtained results supplementation of iron amino acids chelate at 30 g Fe kg^{-1} exhibited best spinach (*Spinacia oleracea*) growth in terms of wet weights, dry weights, height of the plant and the number of leaves than the non-supplemented control diet with lower spinach growth parameters.

iv) Furthermore the supplementation of iron amino acids chelate at 30 g Fe kg^{-1} resulted in high concentrations of Mn, Na, Fe, K, N, P and Zn macro and micro- nutrients in the leaves of spinach while the non-supplemented control diets consisted of lower macro and micro- nutrients composition. Chlorophyll results also showed that iron amino acid chelate supplementation of 30 g Fe kg^{-1} significantly increased leaf chlorophyll a and b content while the control (0 g Fe kg^{-1}) treatments had the lower values of chlorophyll a and b levels. The study reports that an increase in macro and micro nutrients composition corresponds with an increase in the supplementation of iron amino acids chelates in the fish diet. Therefore the findings indicated that iron amino acids chelate supplementation can effectively alleviate macro - nutrients and micro - nutrient deficiencies in the leaves of spinach grown on aquaponic.

v) Water quality parameters in iron amino acids chelate supplementation treatments in fish component indicated significance differences in both plants and fish component where 30 g Fe kg^{-1} treatment resulted in high nitrate, conductivity, iron concentration, oxygen with low ammonia and pH levels compared to the other treatments in both plant and fish components. The present results reported temperature to be at optimal ranges for survival of fish and spinach in the aquaponic system.

vi) The experiment therefore demonstrated the ability of aquaponic systems to produce spinach (*Spinacia oleracea*) and Nile tilapia (*Oreochromis niloticus*) using iron amino acid chelate supplementation in fish diets as the nutrient sources. Generally our study reports that fish growth performance, carcass composition and physiological response improved with increased supplementation of iron amino acid chelates in fish diet. Similarly, the growth of spinach in regard to its minerals and chlorophyll content will greatly depend on the amount of iron amino acid chelates supplemented in the fish diet.

6.2 Recommendation

On the basis of results and conclusions drawn from this study, the following recommendations are proposed:

i) Thus study recommends the incorporation of 30 g Fe kg^{-1} iron amino acid chelate due to the better growth parameters of *O. niloticus*

ii) Present result recommends 30 g Fe kg^{-1} iron amino acid supplementation in fish diet due to the improved carcases composition of *O. niloticus.*

iii) The present finding recommends 30 g Fe kg^{-1} iron amino acid chelate supplementation because improved physiological response parameters was record in 30 g Fe kg^{-1} treatments than the other treatments.

iv) The study recommends 30 g Fe kg^{-1} iron amino acid supplementation for both *O. niloticus* and spinach growth pertaining to the improved results in growth parameters and water quality obtained in both plant and fish components.

v) The study recommends the incorporation of 30 g Fe kg^{-1} iron amino acid chelate in on-farm formulated diets where complete diets are not easily accessible for small scale farmers.

vi) Further studies should consider the use of solar panel, mineralization of fish, water evaporation and organic source of iron amino acid chelate effect on the growth of fish and spinach in aquaponic system.

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