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ADDIS ABABA UNIVERSITY

ENQUIRY. MASTERY. SERVICE.

Effects of Niger cake inclusion in fish feed as a major protein source on anion dynamics in Nile tilapia-lettuce Aquaponics System

By

Liyuwork Amare

A Thesis submitted to

The Department of Zoological Sciences, College of Natural & Computational Sciences

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Aquatic Sciences, Fisheries and Aquaculture)

ADDIS ABABA UNIVERSITY

Advisors: Prof. Abebe Getahun

Dr. Abebe Tadesse

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List of acronyms

ANOVA	Analysis of variance
ATP	Adenosine tri phosphate
DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen
EC	Electrical conductivity
EIAR	Ethiopian Institute of Agricultural Research
H	Hydroponic
LSD	Least significant difference
LNSV	Lettuce Necrotic Stunt Virus
M	Molar
NFARC	National Fisheries and Aquaculture Research Center
NFT	Nutrient Film Technique
RAS	Recirculating aquaculture System
RNA	Ribonucleic Acid
SD	Standard deviation
T	Treatment
TBSV	Tomato Bushy Stunt Virus
µS/cm	Micro Siemens per centimeter

Abstract

The aquaponics systems culture different organisms that are dependent on nutrients generated from feed. Most of the time the major protein source of this system is fish meal. However, the availability of fish meal is very scarce and the cost is not affordable for developing countries. So, this study was carried out to examine Niger seed cake as replacement of fish meal and also to compare the anion dynamics of Niger seed cake with fish meal. The experiment was conducted in five treatments, each treatment in duplicate, with the proportion of Niger seed cake; 0% (Control), 12.5% (TA), 25% (TB), 37.5% (TC), 42.5% (TD). Also a hydroponic treatment (H) was included to examine performance of each treatment on anion dynamics. Samples of fish, lettuce and water were collected every week for about five weeks. Both phosphorus and nitrogen groups (ammonia, nitrite and nitrate) were analyzed by spectrophotometer. The result of the experiment had shown that as the Niger seed cake inclusion increased in the fish feed (43%), TD, the increment of anion in the system was observed, which was comparable with the control diet. However, it had low anion loading than the hydroponic solution. In the lettuce, as percentage of the alternative feed increased, the concentration of anion became higher and comparable with the control fish feed. In general, the result of the experiment had shown little difference with the fish meal. Therefore, higher percentage of alternative feed from plant sources such as Niger seed cake could be taken as supplementary source of feed.

Key words Anion dynamics, Aquaculture, Aquaponics, Fish meal, Hydroponics, Lettuce, Niger seed cake, Nile tilapia

1. Introduction

The world human population is growing very fast. According to latest estimate provided by the United Nations, world population reached 7.4 Billion in June, 2016 (United Nations, 2016). This increment can lead to rapid depletion of natural resources especially water and land. The depletion of earth's natural resources is the major problem that the world is facing at this time. Especially, developing countries like Ethiopia, which are mostly dependent on subsistence farming by waiting for rainfall, are facing huge problems. The other problem is the spread of urbanization that decreased the land to be farmed. These problems brought new ideas and technologies that could develop suitable agricultural production system for developing countries (Connolly *et al.*, 2010).

The word aquaponics is derived from two different words, aquaculture and hydroponics. Aquaculture refers to fish farming while hydroponics refers to cultivation of plants in soilless media. Aquaponics is the production of food that combines recirculating fish production systems (aquaculture) with plant production system (hydroponics) to use the nutrients efficiently (Lennard, 2009). Aquaponic systems recirculate water and nutrients, which are used efficiently thus producing food in a sustainable manner with little environmental impact. In this system vegetables and fruits can be grown with fish waste. This system has mutualistic relationship, the fish with the plant and nitrifying bacteria.

In aquaponic system, the fish in the water tank secrete wastes through urine and their gills. Those waste compounds (ammonia) are toxic to the fish which can lead to fatality. But the waste of the fish is important for the plant because it has high nutritional value that can be used as an organic fertilizer (Nelson, 2008). This nutrient-rich effluent is used to supply a connected hydroponic bed while delivering nutrients for the plants at the same time. The nutrients are gained from the fish feed. As the fish eats the feed, it excretes out toxic nutrient in the form of ammonia to the environment (water). The excreted ammonia is converted by nitrifying bacteria into forms that can be readily taken up by plants for energy and growth (Marschner, 1995). Basically, the hydroponic system and its crops serve as a biofilter for the fish because waste water will be cleaned by the plant before it is returned to the fish tank. Thus, the waste of one biological system becomes nutrients for another biological system (Diver, 2006). As mentioned above, aquaponics depends on a balanced relationship between fish, bacteria and plants. Certain nitrifying bacteria that are found in biofilter tank (*Nitrosomonas*) can change ammonia into nitrite and other bacteria (*Nitrobacter*) can change nitrite to nitrate which is non toxic and important nutrient for plant growth (Britto *et al.*, 2002).

Elements are usually divided into macronutrients and micronutrients. The macronutrients are Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Sulphur (S). These six elements are most needed by plants. In addition, plants need small amounts of Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Boron (B) and Molybdenum (Mo). These are called micronutrients (Arnon *et al.*, 1939).

The anion groups are the nitrogen and phosphorus family, while the cation groups are calcium, potassium, magnesium, iron, zinc, manganese, copper, boron and molybdenum.

Nitrogen (N) is the basis of all proteins. It is important for building structures, photosynthesis, cell growth, metabolic processes and the production of chlorophyll. Nitrogen is the most common element in plants after oxygen and carbon, in which both are obtained from the air while nitrogen is obtained from different sources through the root (FAO, 2003). Therefore, nitrogen is the key element in the aquaponic nutrient solution and serves as an easy-to-measure proxy indicator for other nutrients. Usually, plants can utilize the dissolved nitrogen in the form of nitrate. Nitrogen deficiencies are obvious, and include yellowing of older leaves, thin stems, and poor vigor (FAO, 2003). Excess nitrogen can cause vegetative growth, resulting in lush, soft plants susceptible to disease and insect damage, as well as causing difficulties in flowering and fruiting (FAO, 2003).

Phosphorus (P) is used by plants as the backbone of DNA, structural component of phospholipid membranes, and ATP (the component to store energy in the cells). It is important for photosynthesis as well as the formation of oils and sugars. It encourages germination and root development in seedlings. Phosphorus can be reallocated within plant tissues and therefore is mobilized from older leaves and delivered to new growth, which is why deficiencies are seen in older growth. Phosphorous deficiencies commonly occur in plants because of poor root development or energy cannot be properly transported through the plant; older leaves appear dull green or even purplish brown, and leaf tips appear burnt (FAO, 2003).

The anions are the most important nutrients for the growth of plants and animals. However, excess amount of nitrogen and phosphorus have negative impact on aquatic ecosystems and their deficiencies are also causing serious problems in the growth and development of organisms (FAO, 2003).

Therefore, the goal of this study was to investigate the anion dynamics in an aquaponics system in which the protein source is Niger seed cake used in the fish diet. The effects of Niger seed cake on the physicochemical parameters were also examined.

1.1 Statement of the problem

The water quality in the aquaponics system is fundamental for optimal growth and health of fish and plants. Treatment of dissolved minerals, organic matter, dissolved gases, pH, temperature, salinity, etc. contribute to ensure good water quality (Cripps *et al.*, 2000). While use of recirculating aquaculture system (RAS) has reduced the discharge of waste water to the environmnet, the accumulation of potentially harmful substances increase within the system (Wood *et al.*, 2012). Treatment of recirculating water is one of the limiting factors for production capacity of an aquaponics system. Plants need some nutrients in large amounts for growth and health. Carbon, Hydrogen and Oxygen are macronutrients but taken up through water and air. Other minerals such as Nitrogen and Phosphorus serve as nutrients for plants. However, they are also of concern due to the discharge of aquaculture effluent and can cause eutrophication (Lin *et al.*, 2002). All the nutrients that are important for health and growth of both fish and plants are obtained from fish feed. Fish feed is composed of protein, fat, carbohydrate, minerals and vitamins. Ingredients used to make the fish feed are either natural products or industrially made. But, the feed might not contain all the elements in good quantity. The hydroponic nutrient solution contains the acceptable concentration of most nutrients which are added as fertilizer (Goddek *et al*., 2015). Whereas, most nutrients in aquaponics system that are needed for the proper growth of plants, are obtained from the fish wastes. But fish wastes do not give all important nutrients in the required quantity. Therefore, the fish feed used in aquaculture is very important to improve the concentration of the nutrients in aquaponics system. Fish meal is used as a major protein source in fish feed for the culture of Nile tilapia, *Oreochromis niloticus*. However, its affordability due to its expensive price made it difficult for poor farmers to use. So, it is necessary to conduct a research on nutrient dynamics (anion dynamics specifically) in Nile tilapia–lettuce aquaponics system by using fish feed that is made from locally available products (Niger seed cake) as a major protein source.

1.2 Objectives

1.2.1 General Objective

To evaluate effects of Niger seed cake inclusion in fish diet on anion dynamics for improved productivity in tilapia.

1.2.2 Specific Objectives

- To determine the amount of nitrogen family within the aquaponics system
- To determine the amount of phosphorus family within the aquaponics system

1.3 Literature Review

1.3.1 Aquaculture

Aquaculture, also called aqua farming, is rearing of aquatic organisms such as fish, crustaceans, mollusks, zooplanktons and phytoplankton with human interventions. It includes growing freshwater and saltwater fish species under controlled or semi controlled culture conditions. Aquaculture, at this time, is the fastest growing sector of animal food production. Fish farming is the most common form of aquaculture that involves raising fish commercially in tanks, ponds, or enclosures, usually for food. Aquaculture practices must, therefore, work with environmental and fisheries policies to supply a productive and sustainable management system for the future.

Cultivations of aquatic organisms have been practiced long time ago in China. Aquaculture rose from ancient water-oriented civilizations of the East, where fish was the main part of people's diet. The Chinese people have been practicing integrated aquaculture (fish and plant culturing) systems for centuries. During Tang Dynasty, carp farming flourished in China and, hence, China is the leader in aquaculture production but also one of the major importers of frozen and fillet tilapia (Rakocy *et al.*, 2006).

Africa is home to at least 3000 species of freshwater fish, representing 483 genera and 76 families (Le ´veque, 1997; Pullin *et al.*, 2001). Many of these species are endemic and at least 100 are important in local food security (Pullin *et al.*, 2001). African aquaculture production remains very small but is increasing significantly.

Fish farming in Ethiopia started in 1975 by the Sebeta Fish Breeding and Research Centre (Rothuis *et al.*, 2012). This center is now called the National Fisheries and other Aquatic Life Research Center (NFARC) and is mandated to conduct and coordinate fisheries, limnology and aquaculture research under the Ethiopian Institute of Agricultural Research (EIAR) (Rothuis *et al.*, 2012). The first program consisted of the stimulation of extensive aquaculture by stocking and enhancing artificial lakes, reservoirs and small water bodies (culture-based fisheries). Aquaculture operations other than culture-based fisheries are scarce in Ethiopia. More controlled forms of aquaculture practices are still in an infant stage of development. However, FAO (2011) estimated that in recent years, production from aquaculture sub-sector increased from 15 to 25 tons annually.

Nile tilapia, *Oreochromis niloticus*, is a widely cultured species (Rakocy *et al*., 2006; Le ´veque 1997; Pullin *et al*. 2001). This is because it has many suitable characteristics including fast growth rate, lives in wide range of temperature and salinity, resistance to disease and stress, can breed easily, feed on variety of food items and easily adapt to artificial feeds after yolk-sack absorption (El-Sayed, 2013). In Ethiopia, Nile tilapia culture in cages is well documented (Abebe Tadesse, 2007; Ashagrie Gibtan *et al.*, 2008; Belsti Fetene, 2008; Solomon Hailu, 2008; Asfaw Alemayehu, 2011) and pond culture of Nile tilapia is also considerably assessed (Kassaye Balkew and Gjoen, 2012; Zenebe Tadesse *et al.*, 2012; Adamneh Dagne *et al.*, 2013; Kassaye Balkew *et al.*, 2014).

Based on water temperature preference of freshwater culture species, aquaculture can be broadly categorized as warm water aquaculture and cold water aquaculture.

> Warm water aquaculture: refers to raising plants and animals that have the characteristics to grow fast in warm and fresh water. Examples are catfish, crayfish and Nile tilapia.
>
> Coldwater aquaculture: this type of farming is raising plants and animals that can grow fast in cool and fresh water. Examples are salmon and trout.

There are also different types of culture systems.

Pond Culture usually involves inland artificial ponds. It is common to see aeration systems connected to the pond. This increases the supply of oxygen in the pond.

Recirculating System involves a closed set of units, for example tank, while fish is placed in one tanker and water is treated in another. It is extremely dependent on power supply, so that water has to be pumped constantly into the fish tank. As water flows through the treatment tank, suspended solids and other wastes are cleared out and air added. This system controls the salinity, temperature, oxygen and anything that can cause harm to the fish. It is considered as an environmentally friendly approach system because little water is added to replace evaporated water and nutrient loads are treated.

Open-net pen and cage systems are often found in offshore fresh water lakes. These systems use public water, therefore environmental regulation and some authorization protocols must be respected.

1.3.2 Hydroponics

Hydroponics is the culture of plants in a soil-less media using mineral nutrient solutions in a water solvent (Santos, 2013). Terrestrial plants may grow in water that has important mineral solution. The root is supported by an inert medium, such as perlite or gravel. The nutrients in hydroponics might be either from different animals' wastes like fish, duck, or nutrient solutions produced in experimental laboratories. The ability to produce vegetables all year round enables growers to obtain high prices for vegetables out of season when supply is decreased. In hydroponic systems the plant roots reside in a hydroponic solution formulated for optimal plant growth (Hanan, 1998). The solution can be tailored to a specific species (Resh, 2001). Another advantage of hydroponic production methods is that the risk of soil borne viruses is reduced because the plants reside in an aquatic medium. There are many soil borne viruses that usually affect crops of economic viability. Lettuce dieback is a disease caused by at least two soil borne viruses in the family Tombus, Viridae: lettuce necrotic stunt virus (LNSV) and tomato bushy stunt virus (TBSV) (Resh, 2001). Symptoms include chlorosis and necrosis in older leaves and eventually causing plant stunting and death (Resh, 2001).

There are different methods of hydroponic systems such as Water culture method, Aeroponics, Aggregate Culture and Continuous Flow Systems.

Water culture method- This method is simple to set up on small scale. In this system, the roots of plants are totally immersed in a nutrient solution. The main weaknesses in this system are the amount of water required per plant is massive and there is a need to aerate the solution continuously. So, the system should supply mechanisms to support the plant, aerate the solution, and prevent light from reaching the tanker that contains the solution (avoiding the growth of algae). The solution is oxygen saturated by an air pump combined with porous stones. With this method, the plants grow much faster because of the high amount of oxygen that the roots receive. Some plants that can grow this system are lettuce, spinach, cucumber, tomato, paper and many others.

Aggregate Culture**-** Growing plants in aggregates such as sand or gravel is often preferred to the water culture method since the aggregate helps to support the roots. The aggregate is held in the same type of tank as is used for a water culture system. The nutrient solution is held in a separate tank and pumped into the aggregate tank to moisten the roots as needed. After the aggregate has been flooded it is drained to provide aeration. Enough water and nutrients cling to the aggregate and roots to supply the plant until the next flooding.

Aeroponics**-** In the aeroponic system the roots of the plant grow in a closed container. A misting system bathes the roots in a film of nutrient solution and keeps them near 100 percent relative humidity to prevent drying. The container may be of almost any design as long as it is moisture proof and dark. Tomatoes may be grown in tall, narrow containers lined with plastic.

Continuous Flow Systems**-** Most commercial hydroponic systems direct a continuous flow of nutrient solution over the plant roots. One continuous flow system uses polyvinyl chloride (PVC) pipe of the type commonly used for household waste plumbing. A 2-inch pipe for lettuce or a 4- to 6-inch pipe for tomatoes may be set up with a slight gradient to allow for flow of the solution. Lettuce plants will support themselves if they have been started in growing cubes. Tomato plants must be supported with wire or string.

1.3.3 Aquaponics

Aquaponics, is defined as the integration of two separate, established farming technologies - recirculating fish farming and hydroponic plant farming (Lennard, 2009).

This means, it is a manmade environment that is used to grow both plants and fish that depend on different symbiotic relationships between the fish, plant and bacteria. The integration of these two systems, aquaculture and hydroponics, manage the dismissal of nutrients (primarily nitrates and phosphates), those are dangerous wastes for the fish, from the system decreasing the need for water changes and thus conserving water. However, water is necessary to be filled at the beginning of the system. Dissolved nutrients from fish culture system should be the same as nutrients needed by plants in the hydroponic system (Rakocy *et al*., 2006). When nutrient rich waste water is flown to the plants, it can be environmentally sound optional treatment of minerals in the water and has economic value (Rakocy *et al.,* 1987). It is thus a sustainable technology that will have more production per unit area as resources become limited.

Removal of nutrients from fish effluent via plant nutrient uptake is an efficient and productive method of filtration (McMurtry *et al*., 1990). The nutrients in the system can achieve a steady state with given inputs (feed) and outputs (fish, plants, particulate matter). The concept of aquaponics system is to balance the nutrients within a given system so that there is no excess accumulation of nutrients, nitrogen in particular. Nutrients are delivered to the system via feed which is consumed, assimilated and excreted by the fish. Balancing the amount of nutrients produced from the fish system with the nutrient requirements of the plants can lead to optimized resource utilization and system productivity. Excess nutrients can decrease water clarity thus initiating algal, fungal or bacterial growth (Rakocy *et al*., 2006).

As a sustainable food production technology, aquaponics can play a big role in raising the availability of nutritious food in current and future food systems. Small to medium-scale aquaponics systems need very little space which can be utilized in homes, backyards, basements, balconies and rooftops to raise personal and community food security (Fitzsimmons, 1992). Consumers are becoming more conscious about the significance of their food choices for both their own health and the environment, and aquaponics system can comply with the demand of this growing market. Increasing consumer consciousness and demand of food choices, combined with the manageability of aquaponics technology, puts the aquaponics industry in a beneficial place for future growth of food production.

Aquaponics allows for the growth of a full nutritional meal (protein from fish and fiber, nutrients and minerals from vegetable, fruit, or herb production) in one closed-loop system, where the cultivation of two types of crops (fish and plants) are accomplished using only one body of water and one infrastructure. Crops are grown in an intensive manner without suspecting the health of the system while greatly reducing the required input of water resources (Nelson, 2008) and increasing the value received from the continuously cleaned and recycled water (Considine, 2007).

Aquaponics is the most effective food production system regarding the amount of yield produced per volume of water. It is an emerging industry with huge potential uses 75% less energy than mechanized agriculture, and consumes 80-90% less water (Rakocy *et al*., 1992). Moreover, the fish provides a protein source which many smallholder farmers lack. Aquaponics can grow coupling fish aquaculture with hydroponic plant culture, than conventional agriculture systems (Rakocy and Nair, 1987; Rakocy and Hargreaves, 1993). Intensive RAS can produce more fish per liter of water than other types of aquaculture systems (Timmons *et al*., 2002). It takes approximately 100 liters of water to raise a fish in a recirculating aquaculture system (Timmons *et al*., 2002). Water loss in fish systems occurs mainly from water changes or discharge and evaporation. The integration of fish and plant systems reduce the amount of water used per kilogram of food produced. Water from RAS systems can be used in greenhouse hydroponics to intensify production by utilizing resources more efficiently potentially reducing water usage by 20-27% compared to conventional agriculture (Chavez *et al*., 2000). Aquaponic systems can yield similar crop production to hydroponic systems (Sabidov, 2004).

Protein content in the feed dictates the amount of nitrogen that is available to the plants after the fish assimilate and process the nutrients (Timmons, 1996). The density of fish, protein content in the feed, and the feeding rate drive the nutrient loading of the system. Nitrogen is used for protein and amino acid synthesis. Proteins form structural tissues, catalyze biochemical reactions as enzymes, regulate reactions as hormones, and transport oxygen or hemoglobin (Parker, 2002). Protein is the most costly ingredient in fish diets. Protein is the primary nutrient for fish growth and can account for more than 60 percent of fish feed cost (Hatch and Kinnucan, 1993). High body fat reduces the shelf life of the frozen fish, reduces dressing percentage possibly decreasing market value. Increasing the protein content in the feed will improve the quality of the fish (Hatch and Kinnucan, 1993). Protein has an effect on the growth of fish, quality of fish, cost of feed, and the water chemistry of the system. Aquatic animals convert feed at better rates than other terrestrial food animals (Parker, 2002). Fish require less energy for body support and are cold blooded therefore expending less metabolic energy for body temperature regulation. This will yield low feed conversion ratios (FCR) thus yielding more product per feed unit. The conversion of feed to usable energy and somatic growth in fish is 25-30% (Rakocy and Hargreaves, 1993).

The technique makes it possible to maintain complete control over the growing conditions (i.e. light, nutrients, pH, temperature, etc.), resulting in larger and more predictable yield of plants. The reason why it is possible to grow plants both with and without soil are the mineral elements (Diver, 2006). The mineral elements serve as nutrition for the plant and are absorbed from soil, sand, gravel or water. The physicochemical parameters of the water and soil may limit the availability of elements. Light and temperature affect photosynthesis, translocation and respiration.

1.3.3.1 Main Aquaponics designs

Raft system- This system is also called deep water culture of aquaponics and used in small to large scale productions. It is designed where the plants are placed in floating rafts, and the roots dangle in the nutrient-rich water. The nutrient rich water comes from the fish tank through biofilter (Thorarinsdottir *et al.*, 2015). Plants use the nutrients then the water becomes clean and go back to the fish tank (Fig 2).

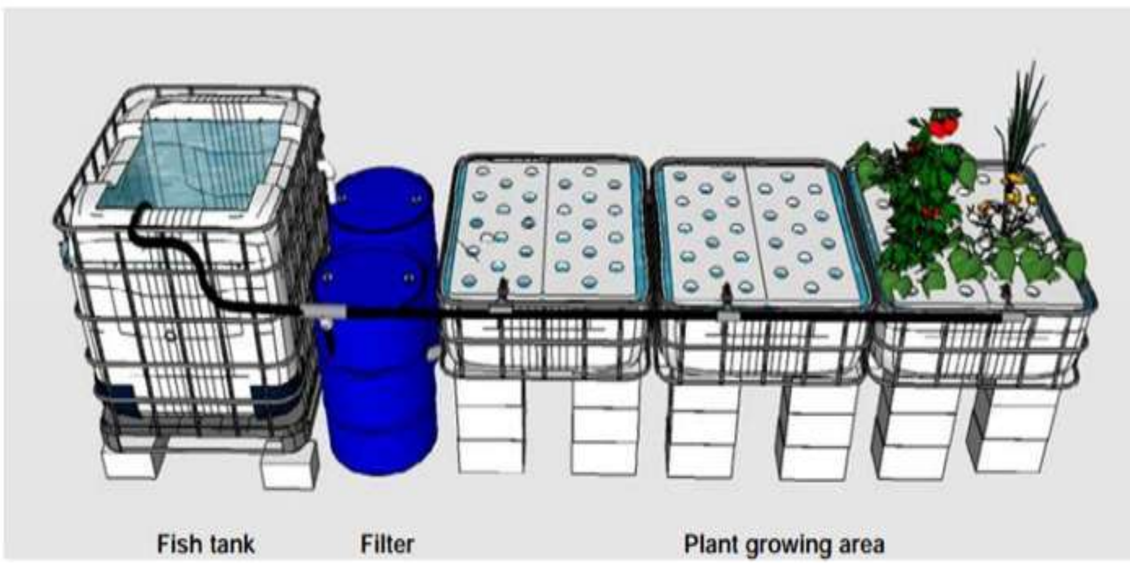

Figure 1. Raft system of aquaponics (Source: Somerville *et al.*, 2014).

Raft system has its own advantages and disadvantages. Some of the advantages are:-

- All type of plants can be grown on this system including tall and fruiting plants.
- Requires relatively low electrical energy than other systems.
- The medium captures and mineralizes solids easily.

The disadvantages are:-

- Media can be expensive.
- Media might be unavailable.
- Higher evaporation than the two systems.
- Requires careful calculation of the water volume.
- Takes space

Nutrient Film Technique- It is a system where plants are placed in long, narrow channels, and a thin stream of continuously flowing water passes through (Fig 3). The system can also be oriented vertically to reduce the amount of required space. Plants absorb nutrients in this system while the water flows into the PVC pipe. This configuration works well with indoors with artificial lighting, but it is not as effective outdoors because not all the plants receive full sunlight.

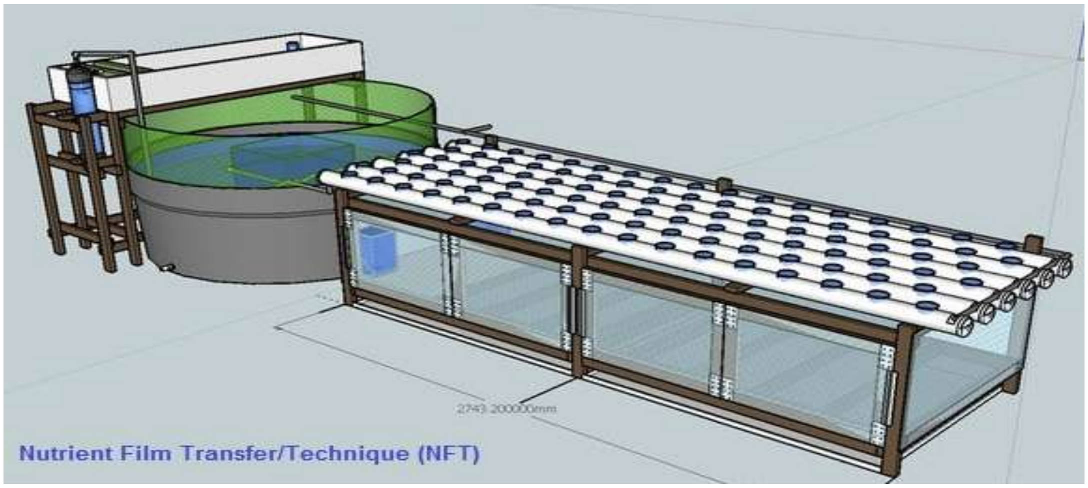

Figure 2. Nutrient Film Technique (NFT); (Source: Somerville *et al*., 2014)

Nutrient film technique has its own advantages and disadvantages. Some of the advantages are:-

- Best method to use on rooftops.
- More cost effective.
- Comfortable for herbs and leafy green vegetables.
- Water loss by evaporation is almost negligible.
- Small water volume required for the plants.
- Plants can be harvested simply.
- Takes less space

The disadvantages are:-

- Filtration techniques are more complex.
- Water pump and air pump are mandatory.
- It has high variation of temperature which could stress fish.
- Sensitive to power failure.

Media-filled bed system- It is a system where the container is filled with gravel, perlite, or another medium to support the plant (Fig 4). While water flows continuously through the other two systems, in the media-filled design, water is periodically dumped onto the bed and allowed to drain into the fish tank. The media-filled bed is simpler to operate because it does not require a filter, but its production is much lower than the other two methods.

Figure 3. Media bed unit. (Source: Somerville *et al*., 2014)

Media-filled bed system has its own advantages and disadvantages. Some of the advantages are:-

- Large water volume keeps water quality good.
- Water loss by evaporation is almost negligible.
- Can be used at high stocking density.
- Can tolerate short interruptions of electricity.

The disadvantages are:-

- Filtration methods are more complex.
- Very heavy unit to carry and move.
- It requires high amount of dissolved oxygen.
- Only short plants are easy to be supported by the system.
- Large water volume increases humidity and result in fungal disease.

1.3.4 Fish and Plant Species

1.3.4.1 Fish

Common fish species used in worldwide fish farming include carp, salmon, tilapia, and catfish. Tilapia is the most consumed aquaculture product in the world (FAO, 2003). Next come catfish and salmons. Tilapia mostly belongs to Africa and the Middle East (Fitzsimmons and Posadas, 1997). It is tight, deep bodied and have broken lateral line. The most famous tilapia species are the Nile Tilapia (*Oreochromis niloticus*). Nile tilapias generally have noticeable vertical strip while mature and male Nile tilapia acquires gray or pink color on the throat area (Fitzsimmons and Posadas, 1997). Tilapia has many suitable features for aquaculture production. It can live with poor water quality, wide salinity and water temperature ranges, low dissolved oxygen levels, and is less affected by ammonia concentrations than other fish species (Fitzsimmons and Posadas, 1997). It is fast growing fish with a low protein need and is omnivorous. It is easy to raise, absorbs seasonings well, mild in flavor, consumed globally and has a relatively fast growing time in comparison with other species. This makes them a primary target for cultivating in aquaculture (Rakocy *et al*., 2006). They consume phytoplankton, zooplankton, aquatic macrophytes, algae, benthic invertebrates, larval fish, detritus and decomposing materials (Timmons *et al.*, 2002). Tilapia can tolerate a pH from acidic to alkaline (pH 5-10) (Chervinski, 1982) and a wide range of salinity concentrations. This is because it is very easy to breed, tolerates low Dissolved Oxygen (DO) levels (200mg/l); high Total Nitrate levels (>400 mg/l); high Total Ammonia Nitrogen levels and low pH levels (< 5.0) (Watanabe *et al*., 2002).

1.3.4.2 Plants

Lettuce is the first salad crop to be cultivated and commercialized internationally. It has the ability to accumulate nitrogen and phosphate (Seawright, 1998). It also has a quick growth cycle and can be harvested within four to five weeks allowing for quick realized profit and turnover of nutrients in the system (Rakocy *et al*., 2006). This makes lettuce a good target crop for hydroponic systems with a heavy bio-load and thus high nitrogen accumulation. But, other plants like spinach, paper, tomato, even potato etc. can grow in hydroponics system. Biotic (genetics, growth and disease) and abiotic (temperature, light, water potential and nutrient availability) factors influence growth and development of plants. These factors can also influence pigment concentrations in plants (Both *et al.*, 1994). Plants grow best and uptake nutrients at a lower pH (5.5-6.5) (Resh, 2001).

Specifically, lettuce will grow well in a pH range of 5.5-6.5 (Resh, 2001, Islam *et al*., 1980). Lettuce exhibits normal growth at oxygen levels of the solution greater than 0.10 mM (McMurtry *et al*., 1997). Lettuce is tolerant of lower oxygen levels in comparison to other plants around 2 mg/l (Watanabe *et al*., 2002). Electrical conductivity levels range between 200-2000 µS/cm for hydroponic lettuce production (Resh, 2001).

1.3.5 Feed

1.3.5.1 Fish Meal

Fishmeal is a commercial product mostly made from bones and other leftover parts of fish after consumptions by humans *(*Jacobs, 2017). It is a good protein source that could help the good performance of fish because it has high protein source and edible by the fish (Akewake Geremew *et al*, 2015). It has high nutritional value since it is found from animal. But it is very expensive to afford for developing countries like Ethiopia (Akewake Geremew *et al.*, 2015). So, replacement of fish meal with cheaper ingredients of plant origin in fish feed is necessary.

1.3.5.2 Niger Seed Cake

Niger (*Guizotia abyssinica*) belongs to the family *Compositae* and the genus *Guizotia*, which has only six species, of which five are native to Ethiopia (Baagoe, 1974). It is one of the sources of oil that is used for making food. The meal remaining after oil extraction is free from any toxic substance and contains approximately 30% protein and 23% crude fibre (Getinet and Sharma 1996). It is used as feed for animals, fertilizer or fuel.

According to Rumsey (1993), increased use of plant protein supplements in fish feed can reduce the cost of fish meal. According to Abebe Tadesse (2017), the proportion of Niger seed cake should not more than 45%.

1.3.6 Water Quality Parameters of Aquaponic System

In aquaponics system, the growth and health of both fish and plants depend on water quality. Because these parameters can affect the physiology of the organisms within the system they should be monitored and managed for optimal system performance. Some of these parameters are mentioned below.

1.3.6.1 pH

pH is one of the most important water quality parameters in aquaponics systems. It is considered a "master variable" because the change of pH influences many other parameters. It is important to adjust pH at acceptable level to fish, nitrifying bacteria and plants. For example, tilapia requires pH to be in the range of 5.0 to 10.0. Plants, on the other hand, grow best when pH levels are below 6.5. Nitrifying bacteria perform optimally at pH levels greater than 7.5 and basically stop working when pH levels fall below 6 (Antoniou *et al*., 1990). The compromise that is optimal to all three components of an aquaponics system fish, plants, and nitrifying bacteria is a pH of 6.8 to 7.0. However, maintaining pH within such a narrow window can be difficult and may lead to unnecessary adjusting and tweaking. As long as the pH is maintained between 6.4 and 7.4 it will be tolerable to all three components of the system (Watanabe *et al.*, 2002).

1.3.6.2 Temperature

Water temperature affects all aspects of aquaponic systems. It influences not only what type of fish can be reared but also plant growth and the performance of the biofilter. Temperature has an effect on DO as well as on the toxicity (ionization) of ammonia; high temperatures have less DO and more unionized (toxic) ammonia (FAO, 2003). Fish species are temperature-dependent. Warm-water species choose temperatures ranging from 18^{0}C to 29°C, while cold-water species such as trout thrive at temperatures in the range of 13^{0}C to 18°C (Resh, 2001). Tilapia is a warm water fish so that it prefers temperatures of 27^{0}C–29°C for maximum growth. But it can resist some low level temperature range. When water temperature drops below 21°C, growth slows dramatically, reproduction stops, and the incidence of disease increases (Tyson *et al.*, 2007). The optimum temperature for the proper growth of lettuce is $16\text{-}25^{0}$C (Licamele, 2009). The temperature for optimum growth of nitrifying bacteria is 25-30° C. And growth rate of bacteria is decreased when the temperature is less than 18° C (Licamele, 2009). So, the general temperature range for the three aspects is 18–30 °C.

1.3.6.3 Dissolved Oxygen

Dissolved oxygen (DO) is the other most important parameter for the growth of fish, plants and is also very important to nitrifying bacteria (Lennard, 2009). The DO level describes the amount of molecular oxygen within the water, and it is measured in milligrams per litre. It has the most immediate and drastic effect on aquaponics. Indeed, fish may die within hours when exposed to low DO within the fish tanks. Thus, ensuring adequate DO levels is crucial to aquaponics (FAO, 2003). Oxygen dissolves directly into the water surface from the atmosphere. In natural conditions, fish can survive in such water, but in intensive production systems with higher fish densities, this amount of DO diffusion is insufficient to meet the demands of fish, plants and bacteria. Water movement and aeration are critical aspects of every aquaponic unit, and their importance cannot be overstressed. The optimum DO levels for each organism, fish, lettuce and nitrifying bacteria, to thrive are 5–8 mg/litre (Lennard, 2009). The concentration of dissolved oxygen should not be below 2mg/l to culture fish for their performance and nitrification will not occur if concentrations drop to 2.0 mg/l (ppm) or less (Licamele, 2009). Tilapia is oxygen tolerant and it can live in lower levels of DO, but its growth rate will be affected. If the level of DO decreases in the tank, it will come to the surface for oxygen-rich water. DO levels are related to temperature and this means warm water holds less oxygen (Yoshida *et al.,* 1997).

1.3.6.4 Conductivity

Conductivity is the other parameter that is used to indicate the strength of the nutrients which are found in aquaponics system. It does not show concentration of each element that are found in the system but indicates the total concentration of nutrients in the system. Electrical conductivity levels range between 200-2500 µS/cm for hydroponic lettuce production (Resh, 2001). Both the higher and lower concentrations of electrical conductivity affect plant growth and yield. So, it is very important to maintain conductivity level. This means, it is needed to add water when the conductivity is higher and needed to add nutrient when the conductivity is lower.

1.3.7 Nutrients in aquaponics system

Nutritional requirements of plants change at different stages of development. Nitrogen is the most important nutrient that drives plant growth and elongation. It is part of a large number of essential organic compounds such as amino acids, proteins, coenzymes, nucleic acids, and chlorophyll (Resh, 2001). Phosphorous plays a vital role in the synthesis of organic compounds such as sugar phosphates, ATP, nucleic acids, phospholipids and coenzymes (Resh, 2001).

Calcium is an important component of plant cell wall. It is also used as an enzyme cofactor and assists in protein synthesis and carbohydrate transfer (Tiruken Aziz, 2016). Iron is used as an oxygen carrier and an enzyme catalyst which make it useful in the process of chlorophyll production, protein synthesis and respiration (Tiruken Aziz, 2016). Potassium is used as an enzyme activator in reactions occurring in plants. Since potassium controls the closing and opening of the stomata, it helps the plant to regulate their water utilization efficiency (Tiruken Aziz, 2016). During photosynthesis it helps to balance the electrical charges at the site of ATP production and is also involve in protein synthesis. Potassium helps in translocation of sugars which could be used for the growth of plants or stored in fruits or roots (Tiruken Aziz, 2016).

Deficiency of nutrients affect the growth of plants (Resh, 2001). Some of the deficiencies are listed below in Table 1.

Table 1 Symptoms of Nutrient Deficiencies (James *et al*., 1914)

Nutrients	Symptoms of Deficiencies
Nitrogen	Small and light green leaves, weak and lighter lower leaves than upper ones, not much leaf drop.
Phosphorus	Lower leaves sometimes turn yellow between veins, dark green foliage, purplish color might appear on leaves or petioles.
Potassium	Yellowing at leaf margins continuing toward the center, lower leaves may be spotted, dead areas may be seen near tips and margins.
Calcium	Tips of leaves are hooked-shaped, tips of young leaves and tips of shoot die.
Magnesium	Leaf margins may curl up or down, in other words leaves may contrast, lower leaves become yellow between veins (veins remain green), leaves die in latter stages.
Sulfur	Tips of the shoot stays alive, leaf veins become lighter than the surrounding areas, upper leaves become light green.
Iron	New upper leaves turn yellow between veins, large veins remain green, tips of the shoot stays but edge and tips of leaves may die.
Manganese	Tips of the shoot stay alive, leaves may appear trapped because of small veins remain green, new upper leaves have dead spots over surface.
Boron	Tips of the shoot dies, stems and petioles are fragile (breakable).

1.3.7.1 Nutrient Dynamics of Anions

Nitrogen is essential nutrient for all living organisms. It is the building block of amino acids. In this study the three nitrogen groups are well studied. Ammonia is a toxic substance that is the waste product of the fish. The concentration of ammonia increases due to many circumstances. According to Tyson *et al.* (2008) the aquaponics system is balanced with ammonia levels between 0 to 1.0 ppm (1 ppm = 1 mg/L) with pH range between 6.4 and 7.4. Ammonia comes from the fish waste and removed by consumer bacteria (*Nitrosomonas*). Nitrite is another toxic substance for the fish. Like ammonia Tyson *et al.* (2008) stated that nitrite levels should be between 0 to 1.0 ppm (1 ppm = 1 mg/L) with pH range between 6.4 and 7.4. Nitrite is an oxidized form of ammonia in the process of nitrification. Nitrite comes from the bacteria (*Nitrosomonas*) and removed from the system by its consumer bacteria (*Nitrobacter*). Nitrates are not toxic for the fish rather it is important nutrient for the plant. Nitrate is the primary source of nitrogen for plants in hydroponic nutrient solutions at concentrations from 20 to 280 mg/l NO3-N (Resh, 2004).

Phosphorus is an important mineral in nucleic acids and cellular membranes, the main representative of the structural components of the skeletal tissues, and it is directly involved in energy processes (NRC, 1993). Fish can absorb this mineral from the water, but due to the low waterborne Phosphorus concentration, dietary supplementation is necessary. Maximum absorption of phosphorus is 0.52 g/ 100g (which is 52%) of dry matter, and when the dietary level is higher, the amount of excreted phosphorus also increases (Rodehutscord *et al*., 2000).

2. Materials and Methods

2.1 System description

This research was carried out in the experimental aquaponics set up that was built within the compound of the College of Natural and Computational Sciences, Addis Ababa University. The experimental setup constituted 10 fish tanks (circular, plastic) with 250 L water holding capacity. Each tank was attached to 80 L biofilter tank, which contains 0.25 Kg bioballs and attached to 20 L clarifier. The Nutrient Film Technique (NFT) was the system that was used in this experiment with 44 planting pots attached to each fish tank. The fish tanks had equal biomass of juvenile fish (*Oreochromis niloticus*), and there was one hydroponic garden that uses hydroponic solution and was used as a control. The hydroponic solutions was made up from inorganic salts with a property of high solubility in water, and that are important for the growth of lettuce (Abebe Tadesse, 2017). Fertilizers can be grouped based on the nutrient of interest as macronutrient fertilizers and micronutrient fertilizers (Rush, 2012). Macronutrients include Magnesium sulfate, Calcium chloride, Calcium sulfate, Phosphoric acid, Triple supper phosphate, Monocalcium phosphate, Potassium sulfate, Potassium chloride, Monopotassium phosphate, Amonium monohydrogen phosphate, Ammonium nitrate, Ammonium dihydrogen phosphate, Calcium nitrate, and Potassium nitrate (Trejo-Téllez and Gómez-Merino, 2012). Micronutrient fertilizers include Ferrous sulfate, Ferric chloride, Iron Chelate, Boric acid, Disodium octaborate, Sodium tetraborate, Copper sulfate, Manganese sulfate, Manganese chloride, Zinc sulfate, Zinc chloride, Ammonium molybdate, Sodium molybdate, Zinc chelate and Manganese chelate (Rush, 2012).

2.2 Collection and stocking of Nile tilapia juveniles

Juveniles of Nile tilapia (*Oreochromis niloticus*) were collected from Ziway (Batu) Fisheries Research Center and the National Fisheries and other Aquatic Life Research Center (NFARC) at Sebeta by using beach seine net with a size of 50m×2.5m (20 mm mesh size). After juveniles were captured, Nile tilapia was identified from other fish species with their external morphology and their weight were measured. The selected as experimental fish of juveniles measured 30-50gm. They were kept in Polyethylene plastic bags that were filled with water and oxygen as stated by Ashagre Gibtan *et al.* (2008). The fish then were transported to the experimental site (Addis Ababa University aquaponics center). When they reached the study site the polyethylene bags that contain juveniles were immersed for about 40 minutes to the acclimatized tanks to balance water condition (Kassaye Balkew and Gjoen, 2012). After 3 days of acclimatization period, the fish were stocked in each experimental tank at stocking density of 20kg/m^3 (Somerville et al., 2014).

2.3 Fish Feed

Feed were prepared from locally available feed ingredients (Table 2). The experimental feed was fed twice a day that is in the morning and afternoon at feeding rate of 3% of their body weight (Abdel-Tawwab *et al*., 2010). Five experimental diets were formulated containing five different Niger seed cake inclusion (percentages) with 0 % (control), 12.5% (TA), 25% (TB), 37.5% (TC), and 42.5% (TD) which was guided by duplicating the system per treatment (Table 2). Ingredients were homogenized for 40 minutes in juice mixer to get all ingredients mixed equally then the experimental diets were prepared by passing the hot paste of ingredients through 2 mm diameter size meat mincer to get pelletized (Abdel-Tawwab *et al*., 2010). Pellets were dried in shadow and preserved in deep freeze at -18 0C till consumption (Al-Souti *et al*., 2012).

Table 2 Proportion of ingredients in the formulated feed (Abebe Tadesse, 2017)

Ingredients (gKg-1)	Treatment				
	Control	Treatment A	Treatment B	Treatment C	Treatment D
Niger seed cake	0	125	250	375	425
Fishmeal	550	425	300	175	125
Meat bone meal	150	150	150	150	150
Wheat grain	150	150	150	150	150
Wheat bran	100	100	100	100	100
Limestone	7	7	7	7	7
DiCalcium phosphate	10	10	10	10	10
Vitamin-mineral premix1	3	3	3	3	3
Fish oil (mlkg-1)	2	2	2	2	2
Soyabean oil (mlkg-1)	20	20	20	20	20
Percentage composition (%)[2]					
Crude protein	32.2	30.6	29.2	28.9	28.8
Dry matter	93.8	92.9	92.2	92.17	92.16
Fat	5	5.3	5.9	6.7	7.2
Ash	15.96	13.57	11.32	10.61	9.97
K	0.297	0.388	0.389	0.391	0.483
Ca	6.47	6.95	5.6	4.4	3.2
Fe	0.0019	0.0016	0.0014	0.0013	0.0018
NC:FM ratios	-	0.29	0.83	2.14	3.4

2.4 Lettuce transplantation

Lettuce (*Lactuca sativa longfolia)* was planted on the ground and after 3 weeks the lettuce seedlings were transplanted into the aquaponics system. During this time the roots of the seedlings were washed with water to avoid mud which may affect the filtration process.

2.5 Data collection

Total wet weight measurements of fish were taken weekly from each treatment. Six fish from each treatment were sacrificed and preserved at -18 ^{0}C analysis of feed and fish were done according to standard procedure (AOAC, 2000). Water samples were collected weekly by Polyethylene plastic bottles for the experimental data analysis of elements especially the anions. Those samples were collected from fish tank outlet, bio filter outlet and hydroponic outlet trough for each treatment. After collecting the samples, they were filtered by sterile filter paper and kept in deep freeze for analysis.

2.5.1 Physicochemical parameters

The physicochemical parameters were measured by portable digital meters (Jenway pH and EC meter). The measurement was taken every week for the entire experimental period. Physicochemical parameters include Dissolved oxygen, Temperature, Electrical conductivity, and pH.

2.5.2 Analyzed Nutrients (Anions)

Nutrients (anions) of fish were analyzed according to standard procedures of AOAC (2000) using spectrophotometer. Proximate composition (moisture content, crude protein, crude fat and ash content) analysis of feed and fish were done according to standard procedure (AOAC, 2000); Moisture content by drying at 80 ^{0}C for 48 h, crude protein using Kjeldahl nitrogen and total lipid by di-ethyl ether extraction with Soxlet and ash by incineration in muffle furnace at 550 ^{0}C for 6 h. The phosphorus in feed and fish were analyzed photometric method using spectrophotometer absorbance at 400 nm (AOAC, 2000). The analytical experiment of water sample was conducted for each nutrient (anion) by using different standard procedures described by AOAC (2000). The Phenate method, using spectrophotometer with absorbance at 640 nm was applied for Ammonia (Ammonium) analysis (AOAC, 2000). Nitrite was measured using spectrophotometer with absorbance at 543 nm (AOAC, 2000). Nitrate was measured with spectrophotometer with absorbance at 420 nm (AOAC, 2000). The total nitrogen in water was analyzed using Kjeldahl method with spectrophotometer absorbance at 425 nm (AOAC, 2000). Phosphate was measured with spectrophotometer with absorbance at 885nm (AOAC, 2000).

2.6 Data Analysis

Statistical analysis was done using the IBM SPSS version 20. Data were analyzed using one way ANOVA (Al-Souti *et al*., 2012). Treatments with significant difference were further tested using post-hoc LSD test. Treatment effects were considered significant at $p < 0.05$ for mean difference analysis (Koch *et al*., 2016).

3. Result and discussions

3.1 Water quality parameters

The average water quality parameters recorded during the experiment are listed in Table 3. The pH level observed during the experiment was at optimal level for tilapia and lettuce growth. As Watanabe *et al.*(2002) mentioned, the pH level would be tolerable to all three components of the system (fish, plants and bacteria) in a range between 6.4 and 7.4. So, the experiment had better pH level except the control and hydroponics solution. The control treatment of the aquaponics water had higher pH level (7.51) while hydroponics solution had lower pH level (6.11). The pH of the hydroponics solution is favorable for the plants (Rakocy *et al*., 2006).

The average temperature of the aquaponics water was between 23 and 27 ^{0}C, which was favorable condition for growth of both Nile tilapia and lettuce. Resh (2001) reported that warm-water fish species choose temperatures ranging from 18^{0}C to 29°C. Therefore, the experiment had optimum water temperature for the fish and also for lettuce since Licamele, (2009) stated that the optimum temperature for the proper growth of lettuce is 16-25^{0}C. The nitrifying bacteria better grow in temperature range of 18-30°. Therefore, all the three organisms had favorable temperature for their growth performance.

The mean DO concentration observed during the experiment was very low as compared to Lennard (2009) in which the range of DO concenteration should be 5–8 mg/liter for all organisms. Most of the time low DO level below 2 mg/ liter has no effect on survival of Nile tilapia, nitrifying bacteria and plant but, it has a great effect on fish growth. The control had lowest oxygen concentration (1.09mg/l) than experimental treatments (A to D) and the hydroponic solution. The DO concentration in this experiment was very low as compared to Al-Hafedh *et al*. (2008) observations in aquaponics system and Al-Hafedh *et al*. (2003) in recirculating aquaculture system in which the range of DO concentrations were 4 to 6.7 mg/l, averaged 5.5mg/l and 4.8 to 6.9 mg/l averaged, 6.12mg/l, respectively. However, an experiment conducted by Bishop *et al*. (2009) in Tilapia-Basil and Tilapia-Okra aquaponics system, had low dissolved oxygen, which were between 2.31 to 2.94 mg/l and averaged 2.72 mg/l. Low oxygen concentration in the control could be due to the presence of higher organic compounds in fish meal which might have consumed higher amount of oxygen for decomposition. The concentration of DO should be 2mg/l and above for better performance of warm water fishes (Licamele, 2009). But Nile tilapia can withstand a very low concentration of DO even less than 0.3 mg/l, which is not the case in other fish species (Popma and Massar, 1999). So, the treatments were in a safe range as compared to the control. The hydroponic water had significantly higher (6.95mg/l) DO concentration than the aquaponics water. This might be because the hydroponic tank did not have competition with fish, plant and bacteria to share the DO.

The electrical conductivity of treatment C had shown very low value compared to others. Treatment A and control had significantly higher ($P<0.05$) electrical conductivity than other aquaponic treatments. It is evident that EC doesn't show the concentrations of each ion. This result also indicates that the effects of the combinations of both anions and cations. The hydroponic solution had shown higher amount of EC value than the aquaponic treatments.

Table 3 The Concentration of Water Quality Parameters (Mean ±SD)

TREATMENT	**Physicochemical Parameters**			
	PH	**Temp**	**DO mg/l**	**EC µs/cm**
Control	7.5 ± 0.08	24.3 ± 2.16	1.09 ± 0.82	351.53 ± 56.52
HYDRO	6.11 ± 0.39	23.52 ± 3.82	6.947 ± 0.48	1510.80 ± 32.16
TA	6.98 ± 0.36	24.91 ± 4.18	3.558 ± 1.38	316.80 ± 42.69
TB	7.01 ± 0.16	25.37 ± 2.21	3.572 ± 1.24	246.40 ± 60.54
TC	7.21 ± 0.13	23.15 ± 1.94	2.739 ± 1.29	204.36 ± 23.61
TD	7.17 ± 0.30	27.069 ± 1.58	2.55 ± 1.40	276.29± 51.94

3.2 Nitrogen dynamics among system components in different treatments

3.2.1 Total Nitrogen of Feed

The total nitrogen content of the feed had decreased with increasing amount of the Niger seed cake (Fig 4). The control feed (fish feed without the Niger seed cake) had significantly highest ($P<0.05$) concentration of total nitrogen than other experimental feeds (those that contain Niger seed cake). Treatment A had significantly higher total nitrogen than other treatments. However, the other treatments, (treatments B, C and D) had almost the same concentration of total nitrogen. The concentration of total nitrogen had shown decreasing pattern starting from the control through all the treatments. This is because the fish feed made from fish meal contains higher concentration of nitrogen than that of feed made up of plant source, Niger seed cake. The content of fish meal is highly enriched with total nitrogen (Jacobs, 2017).

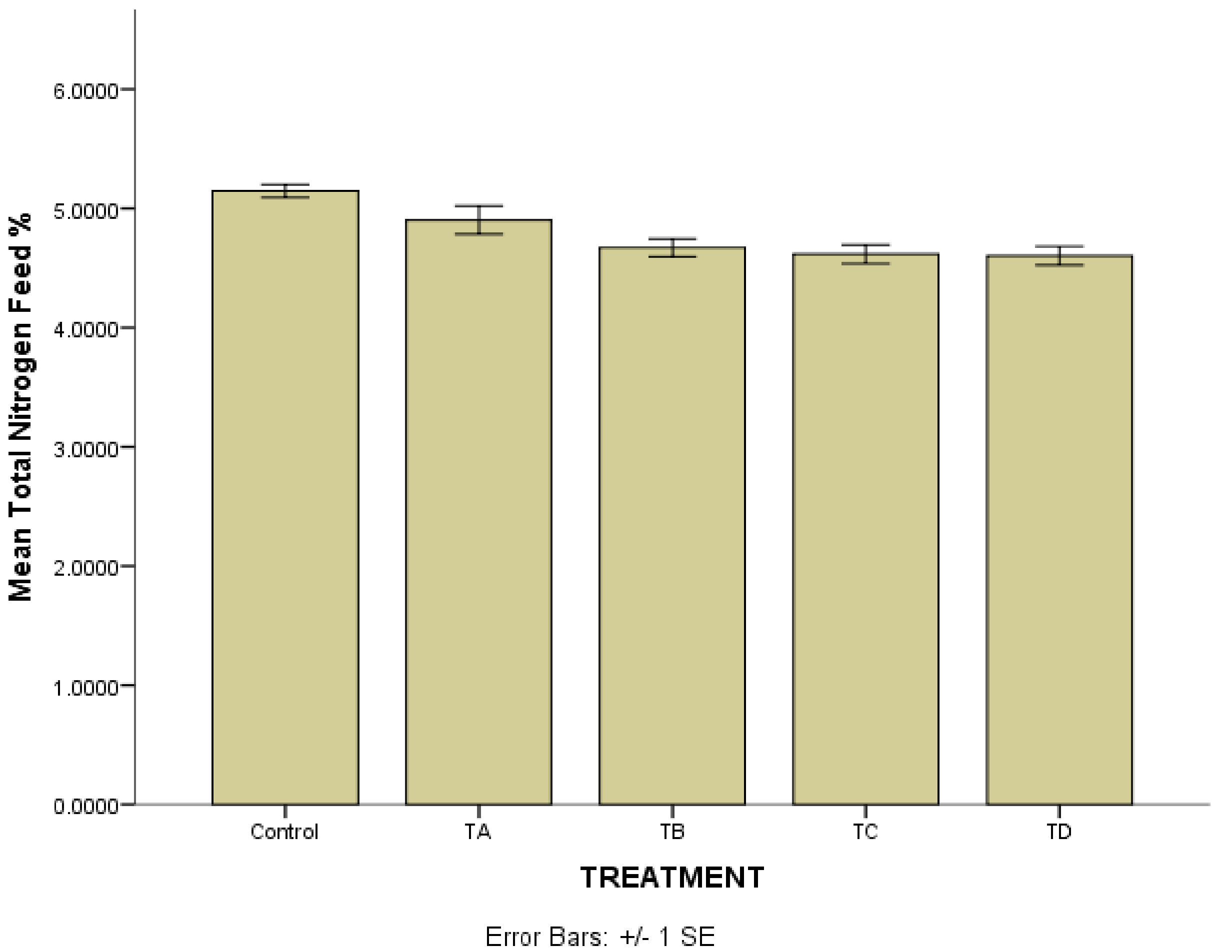

Figure 4. Total Nitrogen of Feed

3.2.2 Total Nitrogen of Fish

As shown in fig. 5, the fish in the control had significantly higher ($P<0.05$) concentration. Whereas, the difference of the control and treatment A was very low. Treatment C had better concentration of total nitrogen than treatment B and treatment D. Treatment D had ($p<0.05$) lowest concentration of total nitrogen campared to the others.

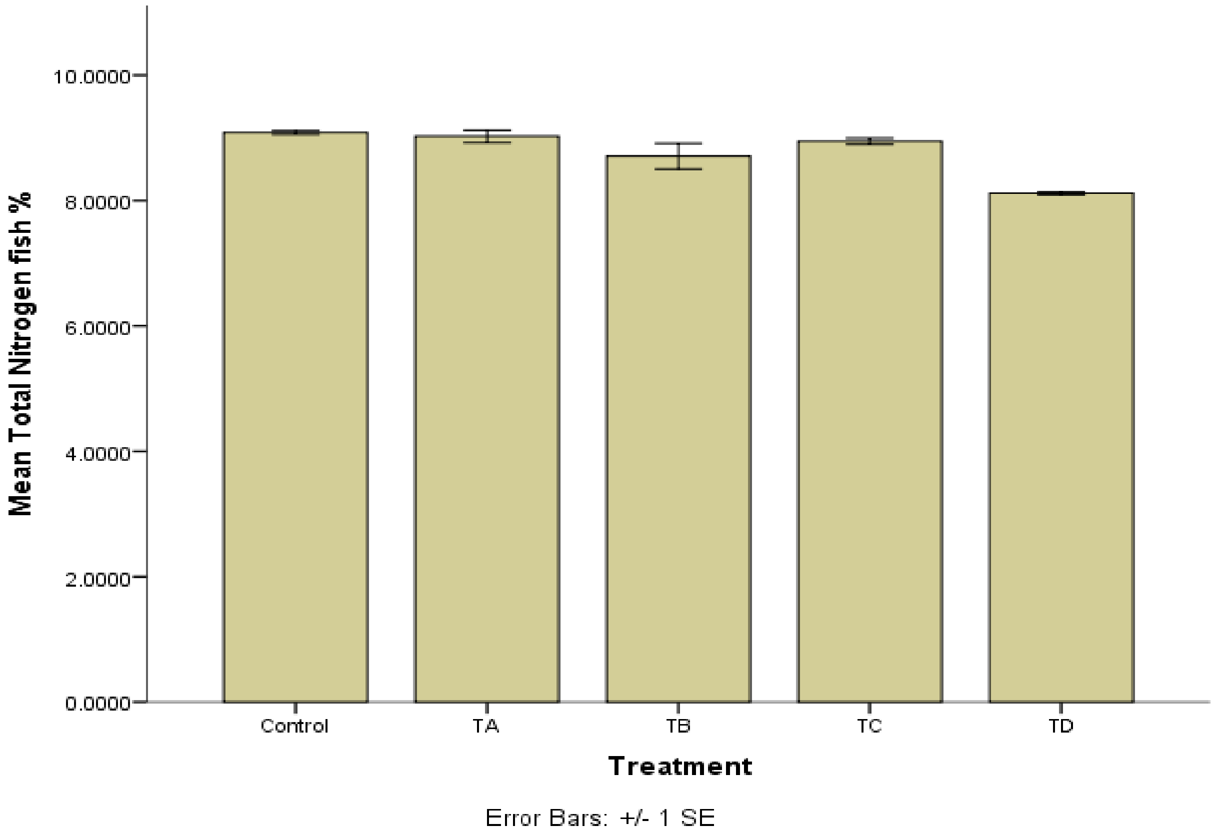

Figure 5. The Total Nitrogen of Fish

It is known that fish meal has high nitrogen content (Jacobs, 2017*)* and hence the fish in the control also had high total nitrogen content compared, at least, to treatment D. In the feed, treatment D had almost equal concentration of total nitrogen compared to other treatments (Fig. 4) whereas the fish in treatment D had the least concentration of total nitrogen in fish (Fig. 5). It was also observed that, lettuce of treatment D had high concentration of total nitrogen (Fig. 6). This might be because the feed in treatment D is less digestible due to high plant material in it. Generally, in this study, the range of total nitrogen in fish is around 8.2- 9.6%.

3.2.3 Total Nitrogen in Lettuce

As shown in fig. 7, the lettuce in the control had higher total nitrogen concentration. Treatment D had the highest total nitrogen concentrationfrom the treatments. Treatment A had significant difference with treatment C. While treatment B had the lowest concentration as campared to the other treatments.

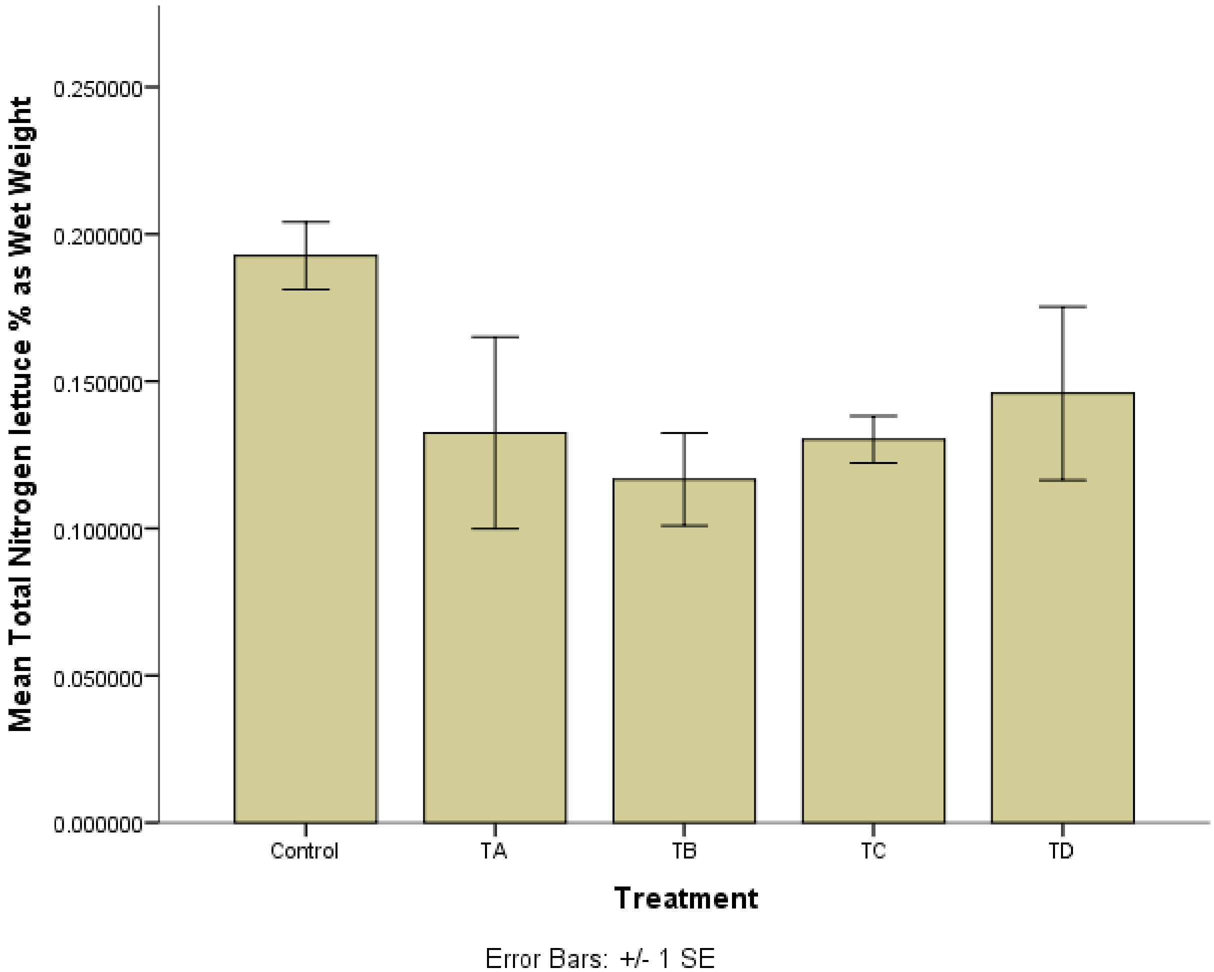

Figure 6. Total Nitrogen in Lettuce

It is obvious that the fish meal has higher total nitrogen concentration (Jacobs, 2017) and hence the lettuce in the control group had also shown highest concentration of total nitrogen. The concentration of total nitrogen had decreased until treatment B but it had again increased as the amount of Niger seed cake increased. Lettuce of treatment D had high concentration of total nitrogen (Fig. 6). This is because, the fish in treatment D had excreted more nitrogen and the lettuce might use it as stated by NRC (1993). Plant leaves contain between 0.14% and 5.0% total nitrogen on weight basis (Jacobs, 2017). The control group had almost fit the range while treatment B had below the range.

3.2.4 The Nitrogen group in the water sample

3.2.4.1 Ammonia

There was significant difference in the concentration of ammonia among the treatments as shown in Fig 7. Treatment D had the highest concentration of NH_3 compared to the others. This might be due to the fact that the fish in Treatment D had released relatively higher concentration of NH_3 which could, in turn, might have affected the concentration in the water. The second highest is Treatment B. Treatment A was very low compared to the others.

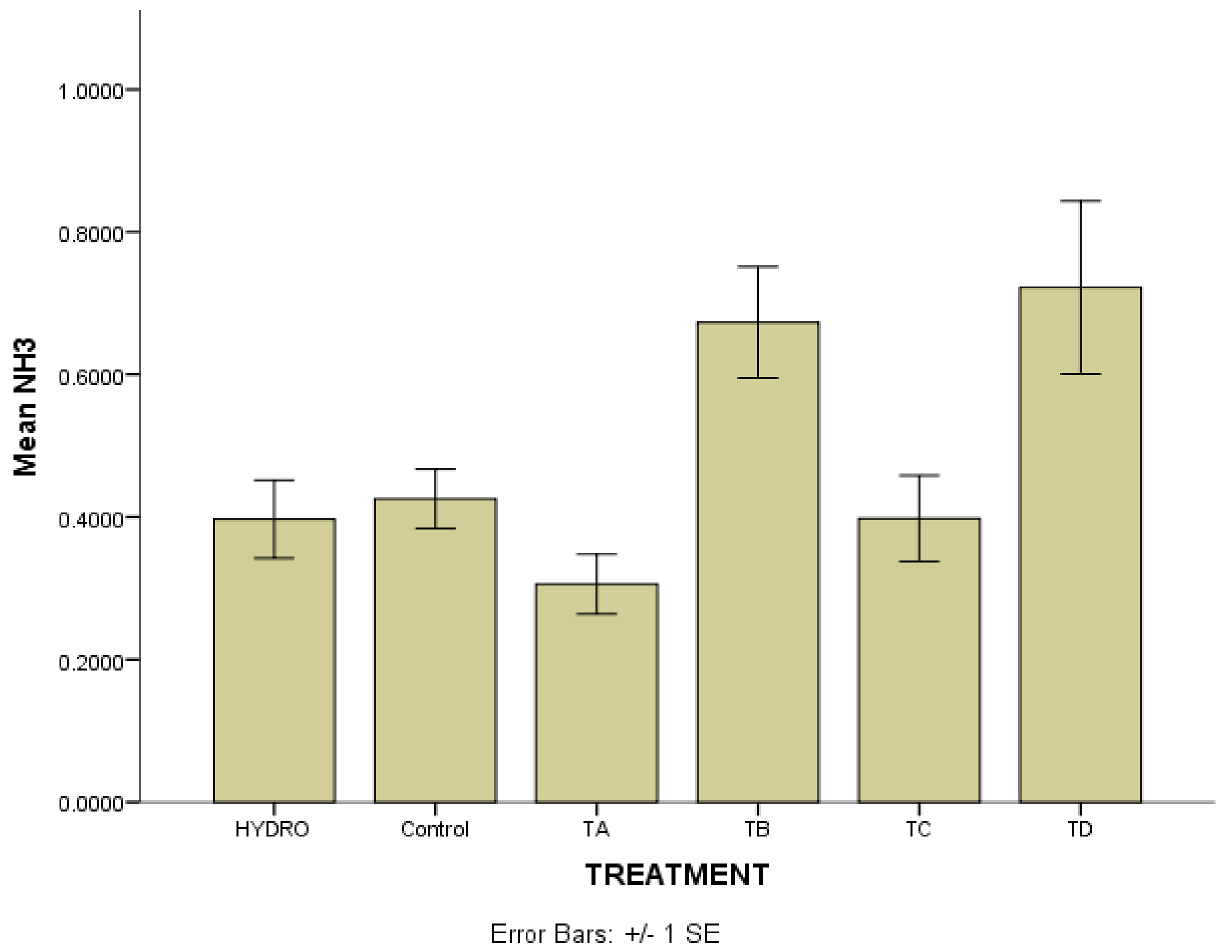

Figure 7. The mean Ammonia concentration of the water sample

The concentration of ammonia is very important because when the concentration of ammonia increases the survival of the organisms will be at risk. According to Tyson *et al.* (2008), the aquaponic system is balanced with ammonia levels between 0 to 1.0 ppm mg/L with pH range between 6.4 and 7.4. When aquaponic tanks are consistently experiencing high concentration of ammonia levels >2.0 mg/l, it could put the fish into stress. In this study the mean concentration of ammonia in the aquaponics water ranged from 0.39 to 0.72 mg/l with the range of pH 6.11-7.4.

3.2.4.2 Nitrite

There was significant difference in nitrite concentration among the treatments as shown in Fig 8. Treatment D had the lowest ($P<0.05$) concentration of NO_2 compared to the others. This might have happened due to the lower number of nitrifying bacteria in treatment D as compared to the others. It was also noted that treatment D had the highest ammonia concentration. Whereas, the hydroponic tank had highest concentration of NO_2. The second highest is Treatment A. Treatment C had the third concentration of NO_2.

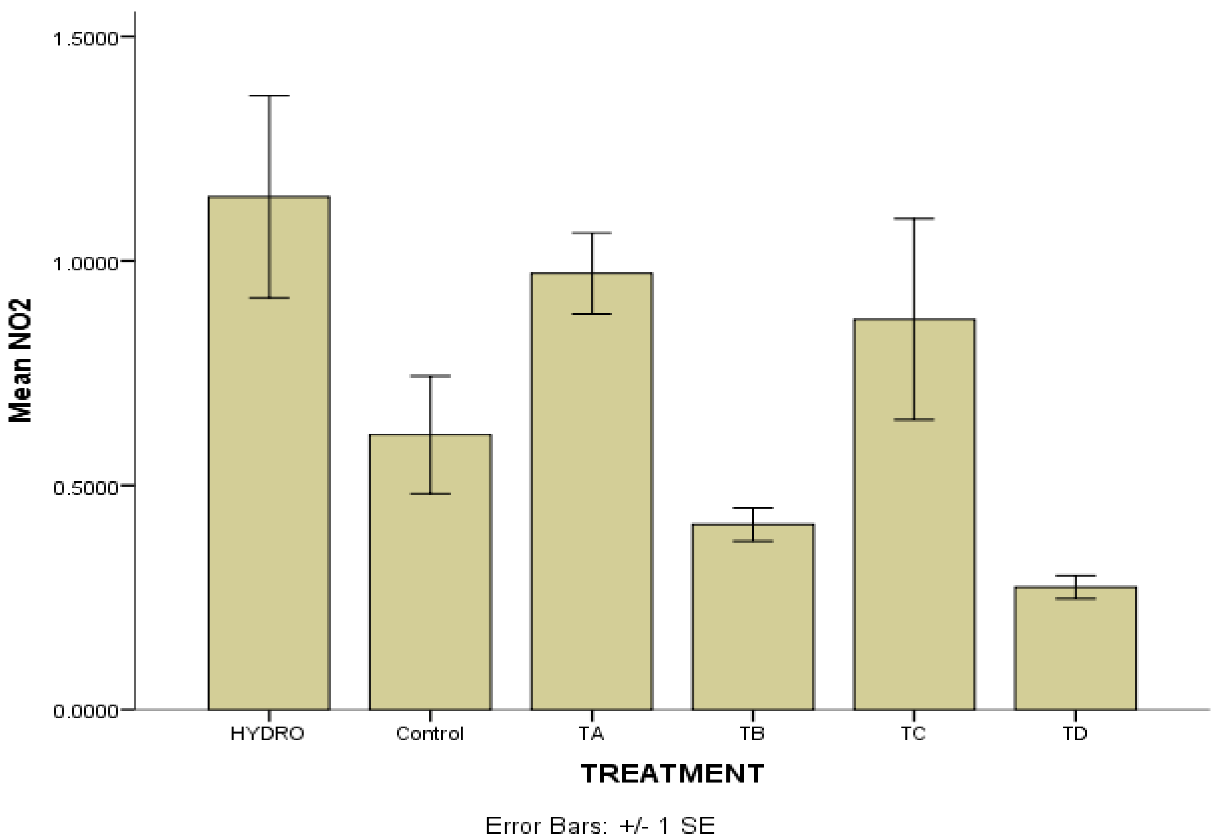

Figure 8. The mean Nitrite concentration of the water sample

The concentration of nitrite is very important because when the concentration of nitrite increases, it could cause death to the fish. The increasing nitrite concentration sometimes could be the sign of less number of bacteria. According to Tyson *et al.* (2008), the aquaponic system is balanced with nitrite levels between 0 to 1.0 ppm mg/L with pH range between 6.4 and 7.4. In this study the mean concentration of NO_2 in the aquaponics water ranged from 0.27- 0.97 mg/l with pH range of 6.11-7.4. The hydroponic tank had high concentration level of nitrite but because there was no fish in it, its effect was not significant.

3.2.4.3 Nitrate

There was significant difference ($P<0.05$) between the treatments as shown in Fig 9. The hydroponics treatment had the highest concentration of NO_3 which was around 227.17mg/l. From the aquaponics treatments, Treatment D had highest concentration of NO_3 next to the control. The third is Treatment B and Treatment C was the lowest compared with the others.

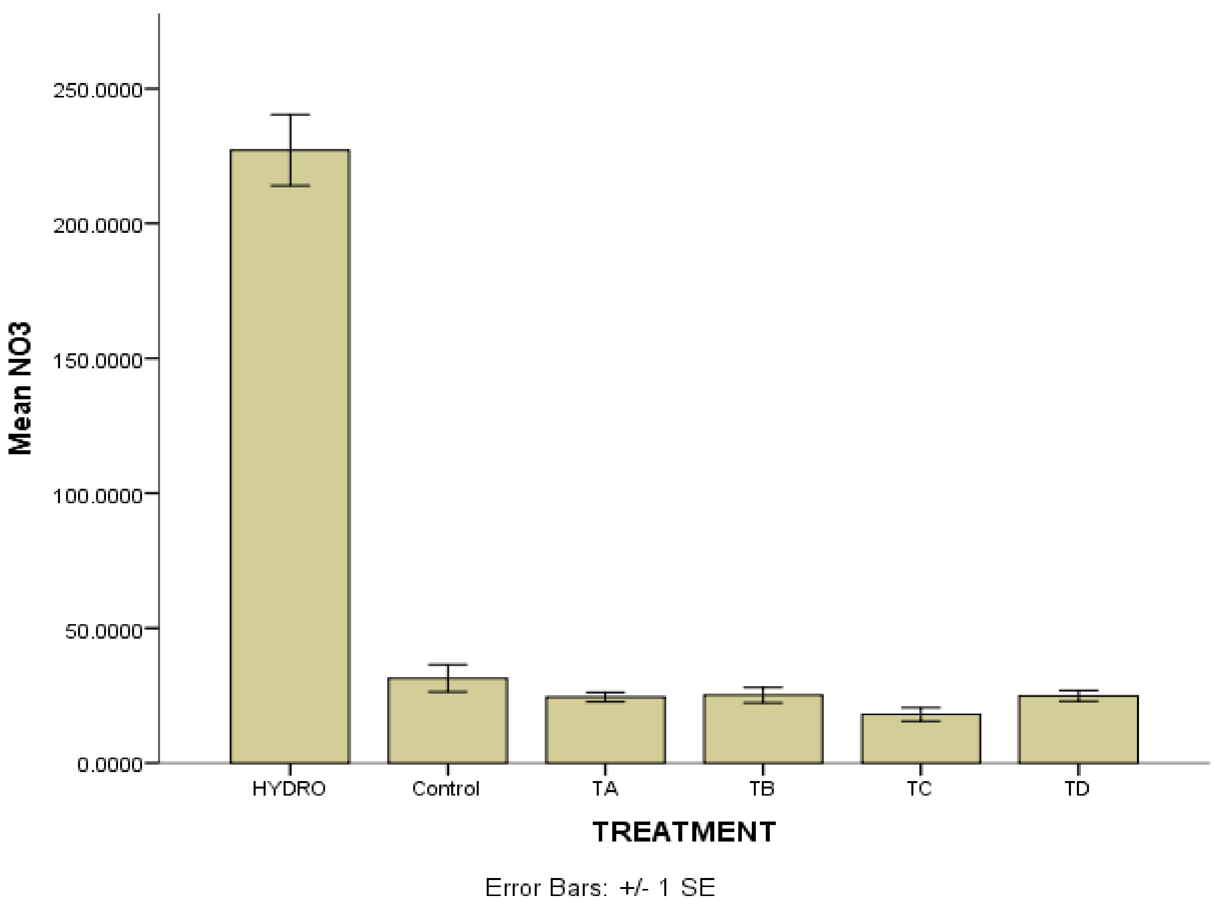

Figure 9. The mean Nitrate of Water Sample

Nitrate is the primary source of nitrogen for plants in hydroponic nutrient solutions at concentration ranging from 20 to 280 mg/l NO_3-N (Resh, 2004). It is less toxic for the fish than other nitrogen groups within this range. But when it is excess it could be dangerous for the fish. In this study the mean concentration of NO_3 in the aquaponics water ranged from 17.97 to 31.40 mg/l with a pH range of 6.11-7.4 which had high difference between the treatments and the hydroponic tank, which had 227.17 concentration of NO_3. But still all the treatments are within the range provided by Resh (2004).

3.2.4.4 Total Nitrogen

There was significant difference between the treatments as shown in Fig 10. The hydroponics had the highest concentration of total nitrogen which was around 228.71mg/l. From the aquaponics treatments, Treatment D had high ($P<0.05$) concentration of total nitrogen next to the control. The third is Treatment B and Treatment C was the lowest compared with the others.

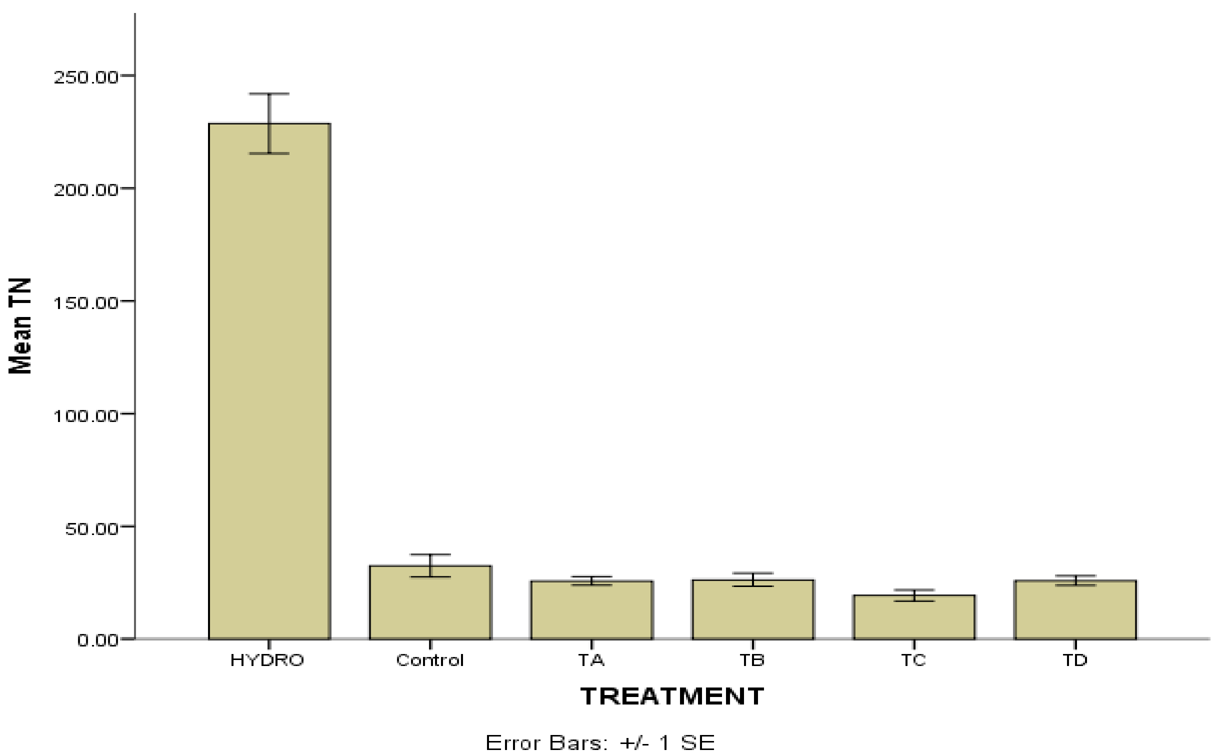

Figure 10. Total Nitrogen of the water sample

Nitrogen is essential nutrient for all living organisms. It is the building block of amino acids. Total nitrogen is the combination of ammonia, nitrite and nitrate. As known, the first source of nitrogen is feed. In this study the total nitrogen concentration of water samples was taken from the combination of the three nitrogen groups (ammonia, nitrite and nitrate). The concentration of nitrate in the hydroponic tank was higher so that the total nitrogen concentration of hydroponic tank became higher. As shown in fig. 10 the total nitrogen in the control was higher than other aquaponics treatments. The reason was Fig.8 and 9 showed that the control group had much higher result than that of treatment D which had high ammonia shown in fig 7. The control group had no Niger seed cake instead it had only important nutrients that are added as solutions.

3.3 Phosphorus dynamics among system components in different treatments

3.3.1 Total Phosphorus in Feed

There was significant difference between the treatments as shown in Fig 10. The feed in treatment C had higher concentration of total phosphorus than treatment D which was not significant. The feed in the control had the lowest ($P<0.05$) concentration of total phosphorus compared to the others. The feed in the treatment A had highest concentration of total phosphorus, while the second highest is the feed in treatment B.

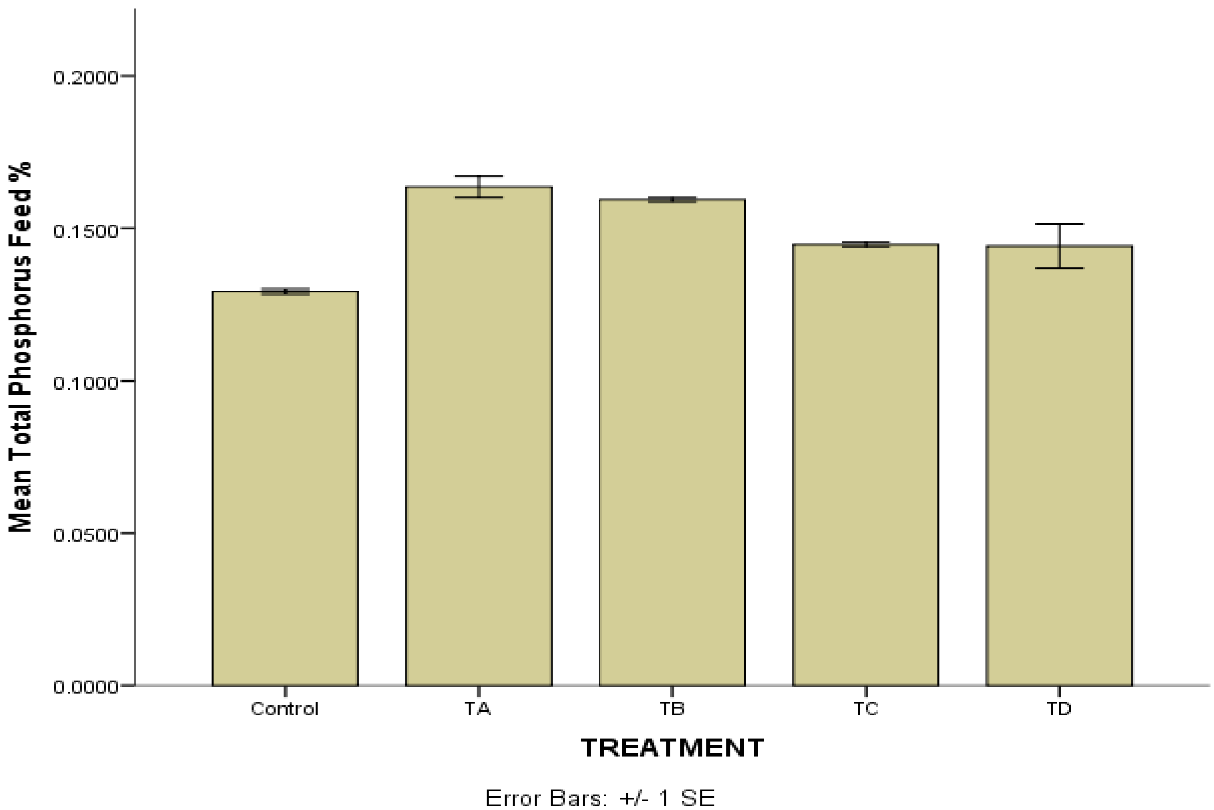

Figure 11 Total Phosphorus of Feed

Unlike the concentration of total nitrogen, the phosphorus content of the control feed (fish meal) had been observed lower than treatment D feed (Niger seed cake) (Fig 11). This might be because the Niger seed cake had high supplementation of phosphate than the fish meal.

3.3.2 Total Phosphorus in Fish

There was significant difference between the treatments as shown in Fig 12. The fish in treatment C and the control had relatively lowest concentration ($P<0.05$) of total phosphorus compared to the others. The fish in treatment D had significantly highest ($p<0.05$) concentration of total phosphorus. The second highest is the fish in treatment B. The fish in treatment A had the third concentration of total phosphorus.

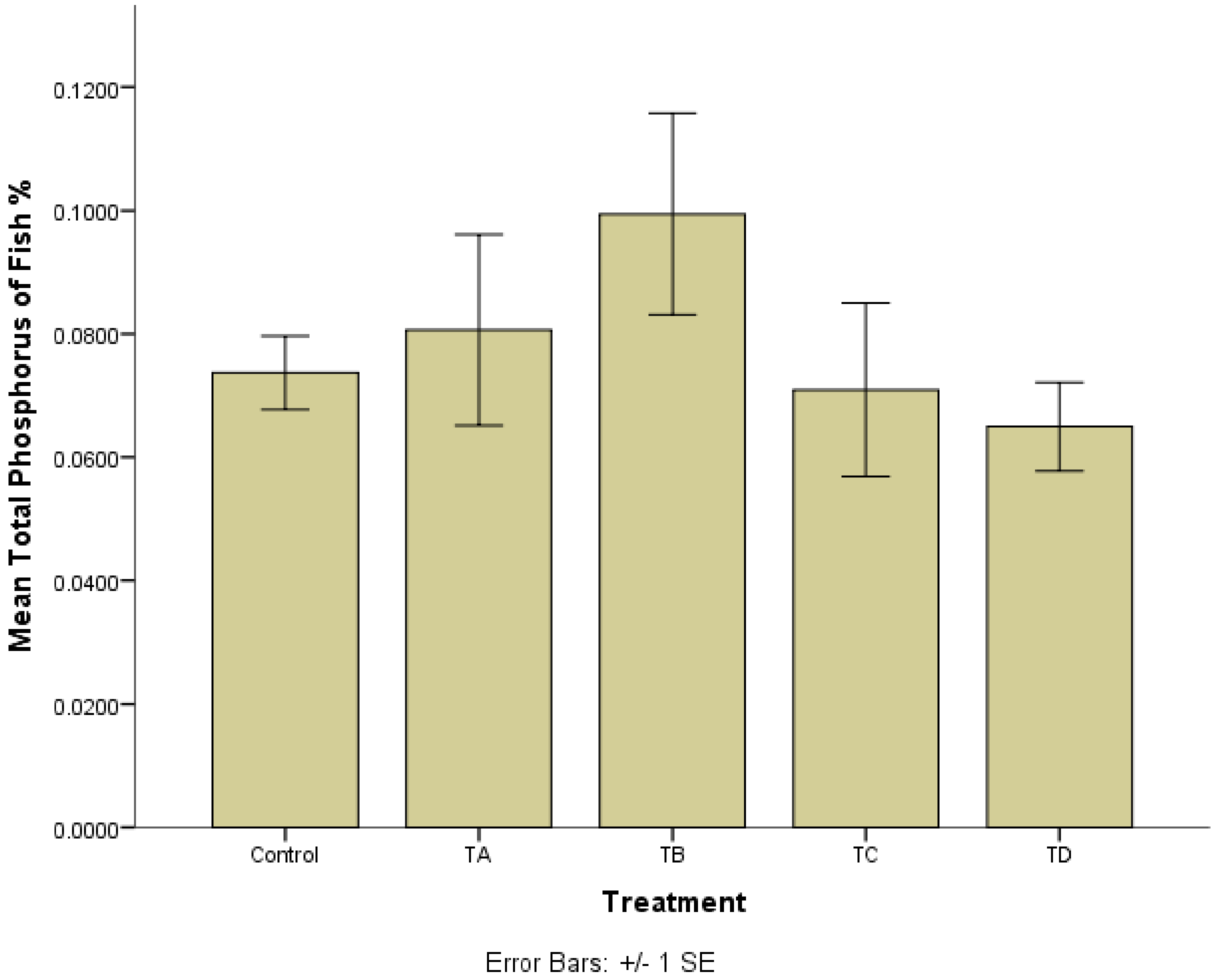

Figure 12. Total Phosphorus in Fish

As mentioned by Rodehutscord *et al.*, 2000, maximum of total phosphorus absorption is 0.52 g/100g of dry matter. This study had a range of total phosphorus from 0.07- 0.48 mg/ 100 g. This result was lower than the result of Abelti (2017) who reported the concentration of phosphorus in Nile tilapia to be between 16.4 mg/100 g to 17.1 mg/100 g. It, therefore, appears that the rest of total phosphorus was excreted into the environment.

3.3.3 Total Phosphorus in Lettuce

There was significant difference between the treatments as shown in Fig 13. The lettuce in treatment C had the lowest ($P<0.05$) concentration of total phosphorus compared to the others. The lettuce in treatment A had highest concentration of total phosphorus. The second highest is the lettuce in the control. The lettuce in treatment D had significantly higher concentration of total phosphorus than treatment B.

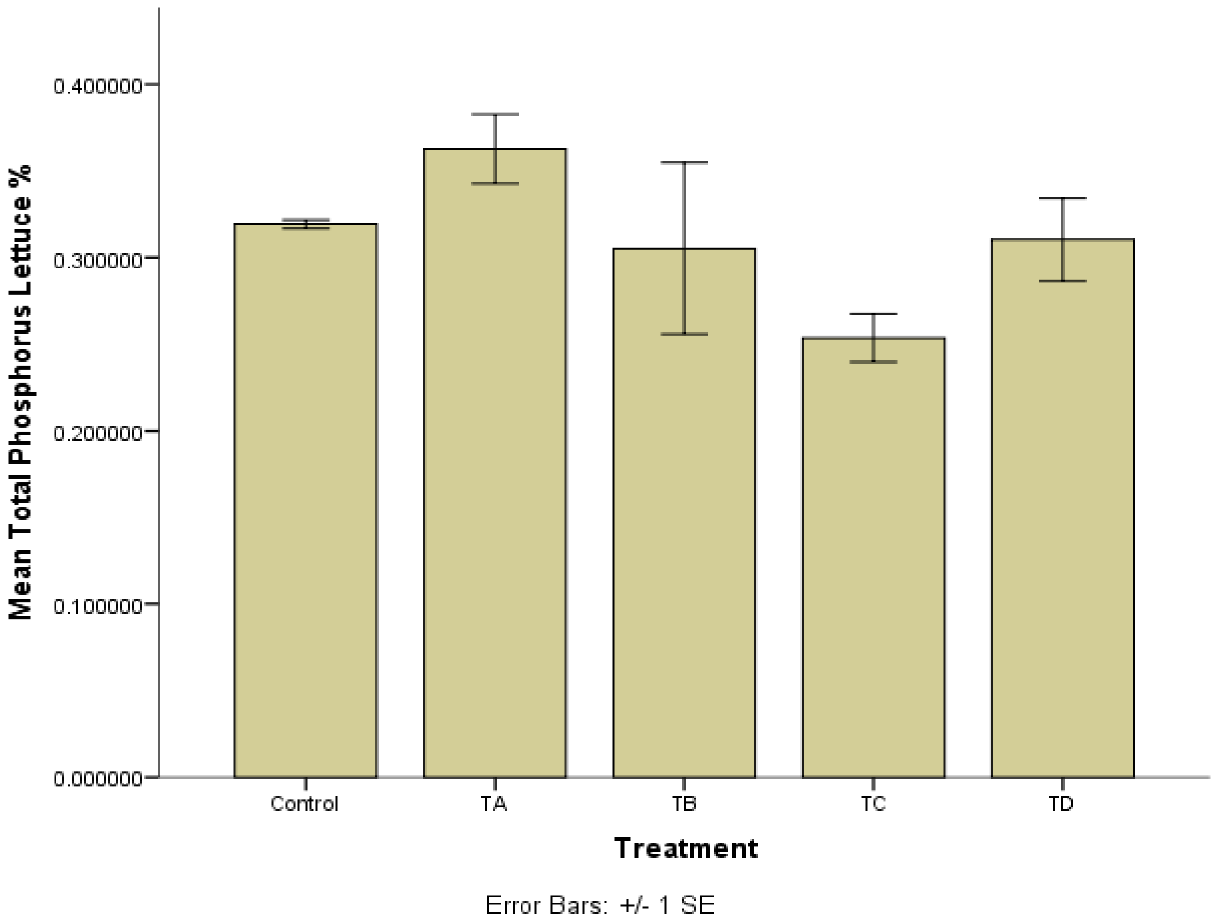

Figure 13. Total Phosphorus in Lettuce

Phosphorus is very mobile in the plant. When leaves become older, they show up deficiencies. This is because phosphorus leaves the older leaves to satisfy the needs of new growth. The range of total phosphorus in lettuce is 0.25 to 0.6% (Rodehutscord *et al*., 2000). When leaves have only about 0.1% phosphorus, deficiency will be shown. In this study, the total phosphorus was in the range of 0.27-0.48%. So, it was within the range provided by Rodehutscord *et al*. (2000).

3.3.4 The Mean Phosphate in water sample

There was significant difference between the treatments as shown in Fig 14. Treatment C had the lowest ($P<0.05$) concentration of PO_4 compared to others. The hydroponic tank had highest concentration of PO_4. The second highest is treatment A that is equal to treatment B. Treatment D had the third concentration of PO_4.

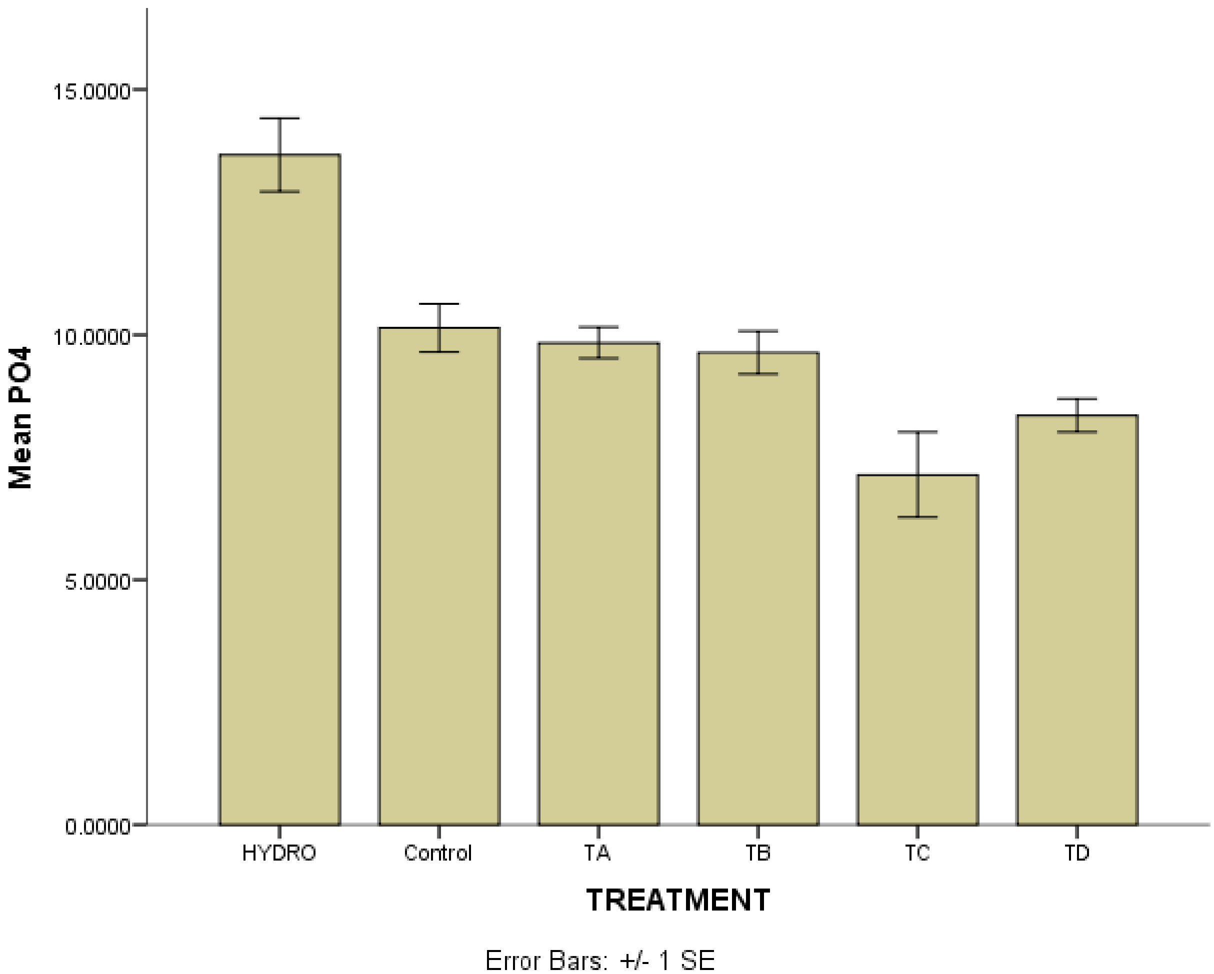

Figure 14 The Mean Phosphate in water sample

The concentration of phosphate is very important because when there is excess concentration of phosphate, it could bring algal blooms in unshaded tanks. A good level of phosphate in an aquaponics garden is between 10 and 20 mg/l for vegetable crops (Rodehutscord *et al*., 2000). In this study, the mean concentration of phosphate in the aquaponics water ranged from 8.00 to 14.5 mg/l with a pH range of 6.11-7.4. So the phosphate concentration is almost acceptable according to Rodehutscord *et al*. (2000), but treatment C and treatment D were below the expected range.

4. Conclusion

- In this study, the experimental feeds (with Niger Cake) had lower concentration of total nitrogen than the control feed (with fish meal).
- The fish in the control group had higher concentration of total nitrogen than the fish in the experimental groups.
- The total nitrogen content of the lettuce showed that higher proportion of fish meal in fish diet provided higher total nitrogen concentration.
- Even though the levels of these anions were lower than standard hydroponic solution, there was no nutrient deficiency symptom on leaves of lettuce throughout the experimental period.
- The feed in the control had the lowest concentration of total phosphorus compared to the experimental feeds.
- The fish in the experimental groups had relatively higher concentration of total phosphorus than the fish in the control group.
- The phosphorus content of the lettuce showed little fluctuations from fish meal in fish diet to the treatments. But with the increase in Niger Seed Cake inclusion phosphorus concentration also increased.
- In the present study all the anions generated by Nile tilapia were not specified in amounts that need to be found in a standard hydroponic system for the production of lettuce.
- This study showed that as the percentage of Niger seed cake increased, it could provide comparable total nitrogen and phosphorus concentration for lettuce production with the expensive fish meal.

5. Recommendations

- From this research, it is recommended to use Niger seed cake inclusion in feed instead of utilizing unavailable and too expensive fish meal as a major protein source.
- It is also recommended to use supplementation of nutrients to generate comparable anion concentration with that of standard hydroponics for better quality production.
- In general from this research we recommend that higher percentage of Niger seed cake (42.5%) inclusion in fish diet with supplementation could provide better minerals for the fish and lettuce.

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