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# EVALUATING DIFFERENT DENSITIES OF LETTUCE (Lactuca sativa L) WITH VARYING LEVELS OF FEEDING REGIMES ON TILAPIA FISH (Oreochromis niloticus L) IN AQUAPONICS SYSTEM

Ahmed Salem Mohammed Suhail Alkaabi

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## United Arab Emirates University

College of Food and Agriculture

Department of Aridland Agriculture

## EVALUATING DIFFERENT DENSITIES OF LETTUCE (*Lactuca* sativa L) WITH VARYING LEVELS OF FEEDING REGIMES ON TILAPIA FISH (*Oreochromis niloticus* L) IN AQUAPONICS SYSTEM

Ahmed Salem Mohammed Suhail Alkaabi

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Horticulture

Under the Supervision of Dr. Shyam S.Kurup

November 2018

## **Declaration of Original Work**

1, Ahmed Salem Mohammed Suhail Alkaabi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled *"Evaluating Different Densities of Lettuce (Lactuca sativa L) with Varying Levels of Feeding Regimes on Tilapia Fish (Oreochromis niloticus) in Aquaponics System"*, hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Shyam S. Kurup, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

7

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### Abstract

Aquaponics uses waste generated by fish as plant nutrients within a re-circulating system that returns clean water back to the fish. The purpose of this study was to cultivate high quality of lettuce (Lactuca sativa L.) and Tilapia (Oreochromis niloticus L.) production in an integrated aquaponic system with recirculating aquaculture system in the UAE climatic condition on three different densities and feeding regimes. An experiment was conducted under greenhouse condition in the United Arab Emirates in 2016 (from April to August). The evaluation of production was based on three parameters viz., head of lettuce production, total weight and leaf number under three different densities of lettuce (12, 18 and 28 in foam) and three different feeding regimes (1, 2 and 3 per day). Based on the results, the total fresh weight and head weight showed a significant increase. The finding of leaf number proved that different densities do not impact the number of leaves. Control densities (18 plants) showed the best results on total fresh weight and head weight, compared to other densities. However, the feeding frequency regime (3 times a day) had no significant effect on plant production. The results also showed both Ca and Na had no significant differences under different plant densities. The outcomes of Fe and Mo elements showed no significant differences among all treatment densities although, feeding regime has been changed among them. The level of pH showed a marginal decrease during the period of experiment. The current aquaponic system has been established for the lettuce/tilapia fish integration and concluded that the low feeding frequency (one time a day) is optimum for the aquaponics system in the UAE climatic conditions for better productivity. However, future studies on other crop and/or fish system combinations in aquaponics to determine how crop yields are affected by operating at specific pH levels of water for long term sustainability of production.

Keywords: Lettuce, density, growth, different feeding regime, aquaponics.

## **Title and Abstract (in Arabic)**

## تقييم كثافات مختلفة لمحصول الخس في مستويات مختلفة من التغذية السمكية لأسماك البلطي في نظام الأكوابونيك

الملخص

يقوم نظام Aquaponics باستخدام الفضلات الناتجة من الأسماك كمغذيات نباتية ضمن نظام يعيد المياه نظيفة الأسماك. تهدف هذه الدراسة إلى إنتاج نوعية عالية من محصول الخس (Lactuca sativa L) وإنتاج البلطى في نظام Aquaponic متكامل مع نظام الاستزراع المائي المعاد تدوير ه في دولة الإمار ات العربية المتحدة على ثلاثة أنواع من الكميات المختلفة ونظام التغذية المختلفة. أجريت هذه التجربة في مساطب مبردة في ظروف دولة الإمارات العربية المتحدة في 2016 (من أبريل إلى أغسطس). استند تقييم الإنتاج على (وزن رأس الخس، الوزن الكلى و عدد الأوراق) تحت ثلاث كثافات مختلفة من الخس (12 ، 18 و 28 في الرغوة) وثلاثة أنظمة تغذية مختلفة (1 ، 2 و 3 في اليوم) تمت على النظام. استنادا إلى النتائج، أظهر الوزن الإجمالي ووزن الرأس زيادة كبيرة في بعض المعاملات. أما عدد الأوراق لم يتأثر بتغيير الكثافات من معاملة إلى أخرى حيث أثبت هذه الدراسة أن الكثافات المختلفة لا تؤثر على عدد الأوراق. أظهرت الكثافة (18) أفضل النتائج مقارنة بالكثافات الأخرى. ومع ذلك، فإن نظام التغذية المختلف (3 مرات في اليوم) لم يكن له تأثير كبير على زيادة نوعية الخس من ناحية الوزن. أظهرت البيانات أيضًا أن كلا من عنصر الكالسيوم والصوديوم لم يحصلا على اختلافات كبيرة تحت الكثافة النباتية المختلفة. وبطريقة مشابهة، لم تظهر نتائج عناصر الحديد و الموليبديوم \ فروقا إحصائية بين جميع معاملات الكثافة رغم أن نظام التغذية تغير فيما بينها. أظهر مستوى الأس الهيدر وجيني انخفاضًا طفيفًا في فترات التجربة. تم إنشاء نظام aquaponic الحالي لنظام أسماك الخس / البلطي و الخلاصة أن التغذية (بمعدل مرة واحدة يوميا) ملائمة لنظام aquaponics في حالة المناخ في دولة الإمارات العربية المتحدة. ومع ذلك، ينبغي إجراء المزيد من الدراسات المستقبلية على مجموعات أخرى من المحاصيل المائية / أسماك البلطي في نظام aquaponic لتحديد كيفية تأثر إنتاج المحاصيل بالعمل في مستويات الأس الهيدر وجيني لزيادة الاستدامة على المدي الطوبل.

**مفاهيم البحث الرئيسية**: الخس، الكثافة، النمو، نظام التغذية المختلفة، الأكوابونيك، الماء.

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Specially thank my parents, wife, son, brothers, and sisters who helped me all the way during the research. Dedication

To my beloved parents and family

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## List of Abbreviations

HLR	Hydraulic loading rate
RAS	Recirculating aquaculture system
TAN	Total ammonia nitrogen
TDS	Total dissolved nitrogen

### **Chapter 1: Introduction**

Aquaponics is an exclusive system that produce more food per unit space by recycling the waste material which can be integrated with fish and crop. In this way, smaller amount of resources are used and restricted area to grow beds are mandatory (Endut *et al.*, 2011).Therefore, aquaponics is increasing productivity with limited impact to the environment. This production technology focuses more on sustainable fish production, vegetables and above all conservation natural resources.

Recirculating aquaponics system (RAS), is aimed to harvest two products simultaneously is both fish and plants. This method ensures controlled culture condition for the fish and it offers enriched waste management, decreased use of water, enhanced water quality and recycling of nutrients (Hamlin et al., 2008; Endut et al., 2009; Martins et al., 2010; Lam et al., 2014). In RAS, the plants absorbs the wastes excreted by fish (e.g. ammonia) and nutrients (nitrites, nitrates) from the microbial breakdown of fish feed. These nutrients enhances growth, there by promotes the elimination of unwanted materials from the water by plants and the purified water is then recycled for fish culture. The faster growth and advanced production of fish and plants can be achieved with the help of these biological activities. RAS provides a symbiotic atmosphere for production of fish and plants by utilizing the generated fish waste in the form of nutrients for the plants and thus creates a symbiotic environment in a closed system (Martins et al., 2010). The pH of the system influences the availability of nutrients for RAS. In plants, the availability of copper, zinc, iron, manganese, and boron is restricted by a pH higher than 7.0. The solubility of calcium, magnesium, phosphorus and molybdenum into the system get regulated by a pH lower than 6.0 (Rakocy *et al.*, 2006).

The advantages of integrated agriculture production systems are reducing the cost of water and the quantity of chemical fertilizer preferred for crops, regulating quality of water required for fish pond, decrease the environmental effect of releasing nutrient rich water (Ghate and Burtle, 1993, Billard and Servrin-Reyssac, 1992, Brune, 1994; Azevedo, 1998). Higher productivity is a major benefit by incorporating agriculture with aquaculture as it can generate two crops by using same quantity of water and it maximizes yield per unit area by using two or more production technologies (Dhawan and Sehdev, 1994).

Leafy vegetables such as lettuce are better adapted in aquaponics system, as these vegetables are harvested in a minimum duration with relatively less problems of pests and diseases compared to fruit vegetables. (Diver 2006; Rakocy et al., 2006). Dunn (2012) stated that modern aquaponics is a viable resource to maintain sustainability in production. The system relies on fish waste to provide nutrients to help the plants grown in turn, the water will be recycled back to the fish creating a symbiotic relationship. The system was designed for lettuce (Parker et al., 1990; Seawright, 1993), tomatoes (McMurtry et al., 1993) and other crops (Racocy et al., 1993). In the case of field crops, few studies were conducted for integrating aquaculture and agriculture (Al-Jaloud et al., 1993; Olsen et al., 1993; Khan, 1996; Palada *et al.*, 1999).Water spinach, lettuce, tomato, cucumber and pepper are commonly used in aquaponics (Endut et al., 2010, 2011; Effendi et al., 2015a, Simeonidou et al., 2012; Effendi et al., 2015b; Wahyuningsih et al., 2015, Roosta and Hamidpour 2011, Tyson et al., 2008; Graber and Junge 2009, Roosta and Mohsenian 2012).

Fish, beneficial bacteria, and plants are the three kinds of living organisms widely used in aquaponic system, as the correlation between them are extremely multifarious and symbiotic (Tyson *et al.*, 2011). Ammonia excreted by the fish in the system is considered as a toxic constituent, (Bittsánszky *et al.*, 2015) which has to be deactivated. Food production using aquaponic method is extremely competent, as the nutrients confined in fish feed and fish waste can be used again to cultivate the crop plants in an environmental condition (Love *et al.*, 2015).

In order to accomplish food security in the twenty-first century, increased food production using agro-ecological methods is required. Dietary importance of lettuce is considerable as it contains numerous health-promoting bioactive compounds and dietary minerals such as iron (Fe), zinc (Zn), calcium (Ca), phosphorus (P), magnesium (Mg), manganese (Mn), and potassium (K) as these micro nutrients are essential for human health (Kim et al., 2016). Colo-rectal cancer and lettuce consumption is associated inversely in a reported case study (Fernandez et al., 1997). Carotenoids and phenolic compounds contribute to the beneficial health properties of lettuce (López et al., 2014). Crisphead, butterhead, romaine, green and red leaf lettuces contains large quantities of carotenoids like  $\beta$ -carotene, lutein, phenolic acids and anthocyanins (Mou, 2005; Nicolle et al., 2004). Fatty acid composition in different lettuce types has not been reported and the major fatty acid in lettuce are  $\alpha$ -linolenic acid, (Le Guedard et al., 2008; Pereira et al., 2001).

Therefore, the main aim of this study to determine the ideal plant density of lettuce (*Lactuca sativa* L) in an aquaponics production system with different feeding frequency regime of tilapia (Oreochromis aureus) under UAE conditions.

## **Chapter 2: Literature Review**

## 2.1 Aquaponics production system

Recirculating aquaculture systems (RAS) have been expansively renovated to achieve viable production of agriculture productsv. In order to enhance filtration RAS exploits specified equipment and mechanically remove waste (Timmons and Ebeling, 2002). Recirculation of water by filtration is a major achievements in RAS that enhances fish production also promote a better way to save water resource. 5 to 10% daily water exchange is needed for most of RAS (Popma and Masser *et al.*, 1999). When compared to the 0.005 to 0.007 lbs, densities of 0.5 pounds per gallon or greater is essential for RAS to be cost effective. (Popma and Masser *et al.*, 1999)

As mentioned earlier, aquaponics is an integrated system with fish and crop plants, which are living symbiotically in closed recirculating systems allowing fish, and plants to grow harmoniously. (Medina *et al.*, 2015). Initially, fish consumes food in the tank and released it as a fertilizer, which obviously is ammonia. Ammonia is converted to nitrite and nitrate by bacteria, which is available as plant nutrition and returned to the tank (Khater and Ali, 2015). Other benefits of aquaponics include production of organic produce, which is healthier to human beings. In arid regions like Middle- East where precipitation is very low and freshwater resources are limited, aquaponics will constitute the best choice of production (Chalmers *et al.*, 2004).

In Aquaponic systems fish waste offers a nutrient basis for nitrifying bacteria, which convert toxic waste of the fish to useful nutrients for plants (AL-Hafedh *et al.* 2008). The integrated type of bio-filter and the ratios between plants, fish, daily feed input were normalized in aquaponic systems (McMurtry *et al.*, 1993).

#### 2.2 Tilapia (Oreochromis niloticus) in aquaponics

An increase in demand for fish and seafood throughout the world, aquaculture is considered as a fast-growing industry and it is developing at a prompt pace than other areas concerning animal culture. (Qin *et al.*, 2005).

Tilapia (Oreochromis niloticus) is one of the foremost fish species to be cultured extensively and has been cultured for more than 3,000 years. Tilapia is native to Africa and the Middle East and it is a successful type of fish used in the aquaponics system (Delis et al., 2015; Liang and Chien 2013; Love et al., 2015; Wang et al., 2016). Nile tilapia is well-grown in aquaponic system using vegetables, and has a high economic output and has good tolerance to various environmental conditions (Diver, 2006). Tilapia is sold in international markets for consumption and provides welfare for the marginal farmers by paving the way to be reared in all any levels of production systems. The amount of nitrate produced in a fish culture system is directly proportional the amount or density of fish in the system and the amount and protein content of the food (Endut et al. 2010 and Timmons (1996 & 2002), Nile tilapia has the capacity to tolerate different environmental conditions, like variable water temperatures, disease tolerance and high tolerance to pH levels ranging from 5 to 11 (Liang and Chien, 2013; Effendi et al., 2016). In commercial farming operations, elevated ammonia concentrations, wide salinity ranges, water temperature ranges and low dissolved oxygen levels have less impact on tilapia than other fish species grown (Popma and Masser 1999).



Figure 1: Fish cultivation in aquaponics

Lettuce can grow well in a pH range of 5.5-6.5 as it can up-take nutrients at a lower pH (Resh, 2001). Tilapia can tolerate a wide range of salinity concentrations and pH from acidic to alkaline (pH 5 - 11) (Watanabe *et al.*, 2002). Lettuce deposit a large amount of nitrogen to its leaves and the nitrogen deposition can be manipulated by plant density and nitrogen availability (Seawright, 1998). In hydroponic and aquaponic systems, lettuce (*Lactuca sativa cv.*) is regularly cultured because it can tolerate lower oxygen levels when compared to other plants. For hydroponic lettuce production, electrical conductivity levels range between 1 to 12 mS/cm and below the levels (2000 mS/cm) is toxic to tilapia (Resh 2001, Timmons 2002). The optimal growth levels of Tilapia is greater than 2 mg/L and the fish can survive low dissolved oxygen levels (Watanabe *et al.*, 2002). Lettuce grows best at water temperatures between 21- 25 °C and the optimal water temperature for Nile tilapia (*Oreochromis niloticus*) ranges between 28 - 35 °C (Resh, 2001).

Jamu and Piedrahita (2002) conducted studies in organic matter and nitrogen dynamics model for the ecological analysis of integrated aquaculture/agriculture systems: and reported the results as under. As a means of supporting system efficiency and justifying the negative ecological effects of aquaculture effluents, the incorporation of aquaculture and agriculture accomplishments is of principal awareness for aqua-culturists and agricultural ecologists. The role of incorporation in supporting system productivity and dropping the undesirable conservational influences of aquaculture can be upgraded if more data about the combined system is added. The component interactions, processes and mechanisms regulating the operation of the integrated system is known as Integration of Aquaculture and Agriculture Activities (IAAS). The IAAS denotes a significant main stage in expressing a model that consist of the important constituents of an incorporated aquaculture agriculture arrangement, and that measures the complex relations between the different constituents of the system.

Rakocy *et al.* (2004) established a `profitable scale aquaponics system in which no main modifications in the system had been employed since 2000, 2002 and 2003, where trials were conducted to evaluate the construction of basil and okra. Batch and staggered production of basil in the aquaponics system was associated to field construction of basil using staggered production method. Savidov *et al.* (2005) stated that numerous ranges of plant species can be developed in aquaponics system. These collapse into three main groups based on the solution conductivity factor (CF) in which the plants accomplish best. Group- 1 involves plants with high CF and contains tomato and eggplant. Group- 2 plants include lettuce, basil, and cucumber and have medium CF. Group -3 consists of plants with low CF and includes water cress.

Salah (2006) studied aquaponics production of bell pepper (*capsicum annuum*L) in re-circulating water system using tilapia (*Oreochromis niloticus*). Kuhn and Gregory (2007) evaluated the effect of Tilapia effluent for marine shrimp

production in a recirculating aquaculture system with ion supplementation and found that effluent of fish is the main factor for money loss for farmers but application of shrimp in the effluent as an alternative crop is a possible solution that can offer a sustainable and money gaining operation.

Jchappell *et al.* (2008) found that using tilapia and tomato culture together provides an effective incorporated system approach. Jason (2009) found that Nile tilapia (*O. niloticus*) fed 2% of their body weight daily yields on average 4.7 kg m-2 of lettuce (L. sativa cv. Rex) in 35 days in aquaponics system under the specified environmental conditions of 5 kg m-3. Results showed that there was no significant difference (P $\leq$ 0.05) in chlorophyll concentration index in lettuce grown with aquaponics water.

Endut *et al.* (2010) reported that using African catfish (*Clarias gariepinus*) and water spinach (*Ipomoea aquatica*) in recirculation aquaponics system developed optimal hydraulic loading rate and plant ratios. This study established that the variations in amounts of nutrients in aquaponics system vary due to difference between the relative quantities of accessible nutrients produced by fish and nutrients by plants.

Roosta and Hamidpour (2011) reported that foliar use of some macro-and micro-nutrients affects the growth of tomato plants. When compared to aquaponics the biomass gains of tomatoes were higher in hydroponics. Dediu *et al.*, (2012) studied the effect of wastewater effluents evolving from sturgeon aquaculture were considered as potential nutrient source for production of hydroponic lettuce. Ingrid (2013) studied the effect of small-scale re-circulating system using wastewater from smolt manufacturing unit for growing lettuce in commercial scale.

Blidariu *et al.* (2013a &b) concluded that nitrate levels of lettuce produced in aquaponics (mean=810.69) are higher that the nitrate levels (R1=708.80 R2=686.65) from their roots by conducting a study in green lettuce by assessing the nitrate and phosphorous levels under natural conditions and aquaponics system.

Petrea *et al.* (2014) studied phosphorus and calcium dynamics in aquaponic system with different crop densities (BH1–59crops/m<sup>2</sup>, BH2–48crops/m<sup>2</sup> and BH3– 39crops/m<sup>2</sup>) using combined rainbow trout and spinach (Nores variety). Results showed that among the three tested densities in terms of water chemical treatment plant density applied, BH1 showed highest values of phosphorus (P<sub>2</sub>O<sub>5</sub>) and calcium (Ca<sup>2+</sup>) removal rates. Khater and Ali (2015) reported that nutrients uptakes were diminished with enhanced flow rate and the length of gully by studying the role of nutrient, flow rate and length of gully. The total nutrient uptake values were higher in nutrient solution than those in effluent fish water.

Delaide *et al.*, (2016) conducted a study to decide changes in development rates while revealing lettuce plants to normal (AP), CAP, and HP solutions and results showed that there was a significantly increased growth rate in the CAP treatment on Lettuce (*Lactuca sativa L.* var. Sucrine).

## **Chapter 3: Materials and Methods**

## 3.1 System description

A greenhouse experiment was conducted during 2016 to study and evaluate the response of changed density of lettuce per growing foam and fish feed frequency. The experiment was carried out in Falaj Hazza campus unit of of the College of Food and Agriculture, UAEU in Al Ain city, 160 km East of Abu Dhabi the capital city of the United Arab Emirates. The greenhouse environment was simulated for temperature and relative humidity. Accordingly, during the experimental period, the temperature of the greenhouse was maintained at 24±2°C. The greenhouse had the source of the natural light (80%) and hence artificial light was not applied. The methodologies adopted are described below. Three aquaponics units, each one inside a 400  $m^2$ greenhouse with a  $120 \text{ m}^2$  growing area in four fiber glass turfs (each 24.4\*1.23\*0.42 m<sup>2</sup> L W H covered with 2-inch-thick perforated Styrofoam sheets), two circular (3 m diameter and 1.2 m high) fish tanks each with 7.7  $m^2$ . The fish tanks connected to water treatment units include circular with cone shaped bottom (2 m<sup>2</sup> diameter with water volume of 4.5 m<sup>3</sup>) swirl separator for mechanical filtration connected to U-tube to remove sludge by siphoning followed by two connected biological filters for nitrification, (1.8\*80\*0.6 m<sup>3</sup> each) tanks one third filled (35 kg) with plastic media (HDPE polymer with very high surface area; 899  $m^2/m^3$ ) from Pentair's Sweetwater USA. Then water from the biological filters move to a CO2 stripping tank (1\*0.6\*0.6 m<sup>3</sup>) before moving to the four plantation raceways. Water moves in the system at a rate of a 10 m<sup>3</sup>/hour from fish tanks to the water treatment system and plantation raceways by gravity and return to fish tanks using a 3 Hp water tanks. Total water volume 58 m<sup>3</sup>. The system was aerated by air blower (S53-AQ Sweetwater Regenerative Blower 2.5 HP. (MFD BY; Aquatic Eco Systems, INC Apopka, Florida USA) through one inch PVC pipe and a rubber hoses. Each fish tank has 20 silicon air stones (each 20 cm length) and each water trough has 10 air stones (each 10 cm in length). Water consumption from evaporation and evapotranspiration and cooling system were measured using two water meters (KENT PSM 15 mm water meter PN 16, GRUNDFOS, England. Electricity consumption was measured using one electrical meter (Elster A1100 polyphase meter by: Elster metering Ltd. Stafford). One air cooler fan: Euroemme® EM50n, Exhaust fan with 1.5 HP motor, Propeller diameter 1,270 mm, 6 Kista, blade, Sweden. One water pump for cooling pad: GRUNDFOS DK-8850, 1 HP single phase motor Capacity of water pulling a 5 m<sup>3</sup>/h.



Figure 2: Weighing the head of lettuce

## **3.1.1 Culture conditions**

Fish tanks were stocked with 100 fish m<sup>-3</sup> of *Oreochromis niloticus* fingerlings with an average weight of 5 g. Nile tilapia were fed with 36% protein commercial tilapia diet from Arabian Agricultural Services Company ARASCO, Saudi Arabia. Fish were fed to satiation three times a day.

Raceways were planted in Styrofoam at a rate of 24 lettuces *Lactuca Sativa* seeds per square meter. Lettuce seeds were inserted in a piece one-inch Rockwool cube 2-inch length inside a perforated bottom plastic cub. Lettuce was harvested every 30 days and a new seed was planted to start new crop. Lettuce characteristics of each harvest was evaluated by measuring length (green to root), green length, root length, total weight, green weight (head), leaf weight, leaf length, leaf width, and average no of leafs.

## **3.1.2 Planting details**

One race way surface area is 30 m<sup>2</sup>

There were four raceway plant cultivation area is which is 120 m<sup>2</sup>

Total no of plants in a greenhouse = 2808 Nos

Therefore, per m<sup>2</sup> surface area contained plants (2808 plants/120 m<sup>2</sup>) = 24 plants per m<sup>2</sup>

Sludge was collected daily by siphoning from the swirl separator in a plastic bucket the left to settle the solids for one hour then transferred to  $2 \text{ m}^2$  tray for air drying. Floating sludge was collected using fine net three times a day and added to the above try to dry. Sludge from each aquaponics unit was collected separately.

## **3.2 Measurement and analysis**

2.4.1 Light intensity was measured by the LUX meter (Make: Tekemura; Model: DM - 28) weekly.

2.4.2 Water quality from tanks were analyzed once every week for pH, Temperature and Electrical conductivity was measured using HACH HQd portable meter (Make: HACH; Model: HQ 40d), TDS (HACH TDS meter Pocket pro<sup>™</sup> (HACH; Model: DR 900)

## 3.2.1 Chemical analysis

TAN (Total Ammonia Nitrogen) (Salicylate method) Nitrite (USEPA Diazotization Method), Nitrate (Cadmium Reduction Method) and Fe (FerroVer® Method) using HACH portable calorimeter (HACH; Model: DR 900). DO, Orion star<sup>TM</sup> and Star plus meter (Make Thermo Scientific; Model: Orion 4 star), Total Alkalinity and acidity to be measured by the Titration method of APHA standard methods 2003, Minerals Analysis was done using ICP-OES (Inductively Coupled Plasma Optic Emission Spectroscopy (ICP\_OES) Model 710- ES, Varian, United States).

## 3.2.2 Experimental diet, fish, lettuce and sludge sample analysis

These samples were analyzed in triplicate for moisture using a forced air oven, crude protein by macro-Kjeldahl, crude fat by ether extraction method total ash by muffle furnace (550 °C) for 24 h, and CF (for feed samples only) using Lab. Conco (Lab. Conco Corporation, Kansas City, MO, USA). Growth energy was calculated based on standard energetic values for protein (23.67 MJ kg<sup>-1</sup>), carbohydrate (17.17 MJ kg<sup>-1</sup>) and lipids (39.79 MJ kg<sup>-1</sup>) (NRC 1993).

## 3.2.3 Bacterial analysis

Enumeration of ammonia-oxidizing autotrophic bacteria, nitrite oxidizing autotrophic bacteria, total heterotrophic bacteria and total coliforms from water of growth troughs of every aquaponics unit was carried out. The isolation of ammoniaoxidizing autotrophic bacteria (Nitrosomonas spp.). The isolation of heterotrophic bacteria and total coliforms from the water samples was carried out using the membrane filter technique on M- heterotrophic plat count agar (HPC), and m Endo total coliform broth (BD Difco, Becton, Dickinson, Franklin Lakes NJ, USA), respectively.

## **3.3 Statistical analyses**

Data were subjected to one-way ANOVA to determine significant (P>0.05) differences among the treatment means. Student–Neuman–Keuls multiple range test All statistical analyses were conducted using a system for Windows (version 8.0, SAS Institute, Cary, NC, USA, 1995).

## **3.3.1** Calculated parameters

Several parameters were calculated like head weight (Figure 2), total fresh weight, leafs number root length and etc.

## **Chapter 4: Results**

## 4.1 Plant length

The results in the Table 1 show that under feeding regime-1 (F1), with planting time (M1) under different planting densities there is a significant difference in length of the control plant (77.0 cm) with that of D2 with 28 plants (66.0 cm). There is no significant difference between D2 and D1. In May (M2) planting, it was observed that control with 18 plants recorded significantly tall plants (85.3 cm) compared to a plant density of 28 plants, also found to be significantly different to 12 plants. During June (M3) planting it was observed as that of M1 planting. In feeding regime-2 (F2), control showed no significant difference comparing with 12 plants (D1) in May, however there is significant difference compared with 28 plants with 60.6 cm height. During the next month with the same feeding regime (F2), control plants, showed significant increase in plant height compared to D2 plant densities but on par with D1 (45.5 cm). In July, all treatments show significant difference between each other. In (F3), planting in June, under different densities there was no significant difference in length. However, in July, control treatment recorded significantly, higher plant length (43.1 cm) compared to a density of 28 plants (D2), but showed to be on par with D1-plant density. Similarly, in Aug planting, control treatment compared with D2-density showed significant difference, but found to be on the par with D1 plant density.

	F1			F2			F3			
Plant	(Feed	ing one time/	day)	(Fee	(Feeding two time/day)			(Feeding three time/day)		
density	M1	M2	M3	M1	M2	M3	M1	M2	M3	
	April	May	June	May	June	July	June	July	Aug	
12 plants	74.1 <sup>ab</sup>	39.4 <sup>b</sup>	39.4 <sup>ab</sup>	69.0 <sup>a</sup>	45.5 <sup>a</sup>	46.8 <sup>b</sup>	66.7 <sup>a</sup>	44.8 <sup>a</sup>	38.5 <sup>a</sup>	
(D1) (cm)										
28 plants	66.0 <sup>b</sup>	40.2 <sup>b</sup>	36.7 <sup>b</sup>	60.6 <sup>b</sup>	40.1 <sup>b</sup>	41.7 <sup>c</sup>	69.8 <sup>a</sup>	39.2 <sup>b</sup>	33.0 <sup>b</sup>	
(D2) (cm)										
Control	77.0 <sup>a</sup>	58.3 <sup>a</sup>	40.2 <sup>a</sup>	69.8 <sup>a</sup>	45.6 <sup>a</sup>	49.3 <sup>a</sup>	69.1 <sup>a</sup>	43.1 <sup>a</sup>	37.1 <sup>a</sup>	
(18) (cm)										
Mean±SE	72.3±2.81	45.3±2.0	38.7±0.82	66.4±1.63	43.7±1.14	45.9±0.68	68.5±2.0	42.3±0.82	36.2±0.80	

Table 1: Plant length under different plant density (D), feeding frequency regime (F) and staggered sowing date (M)

Each value is the mean  $\pm$  SE

Mean values in each column have different subscript (a, b, c) are significantly different a P<0.05

## 4.2 Leaf number

Table 2 explains the response of plants to leaf number under different plant densities. The lettuce leaf number has not shown any significant difference on F1 in April. However, in May, with the same feeding regime (F1) control recorded the highest mean number of leaves with a significant difference (19.1) when compared to other treatments (D1 and D2). In June, the whole treatments were found to be on the par with no significant difference. In the second greenhouse during May, the control showed a significant difference with D2 plant density, but it was on the same level with D1 plant densities. In June, for the same feeding regime, D1 plant densities recorded the highest number of leaves with 14.9 and it showed a significant difference. However, other treatments recorded 12.4 for D2 density and 13.4 for control plants with no significant difference in comparison to D1 plant densities. In July, control showed a significant difference with 12.1 cm compared to other treatments, but D1 plants and D2 plant densities were on the same level. In feeding regime 3 (F3), the first month (June) showed no significant difference between D1 density and control 14.7, 17.5 respectively, but they showed a significant difference compared to D2 plants. Similarly, in July, D1 densities and control showed a significant difference between each other however, D2 plants got the lowest number of leaf in this month. In the month of August, the control got the highest score compared to other treatments with a significant difference, but D1 and D2 densities showed no significant difference between each other.

	F1			F2			F3		
Plant density	(Feeding one time/day)			(Feeding two time/day)			(Feeding three time/day)		
	M1	M2	M3	M1	M2	M3	M1	M2	M3
	April	May	June	May	June	July	June	July	Aug
12 plants (D1) leaf number	17.5 <sup>a</sup>	10.2 <sup>b</sup>	10.7ª	14.3 <sup>ab</sup>	14.9 <sup>a</sup>	10.9 <sup>b</sup>	14.7 <sup>a</sup>	13.2ª	11.8 <sup>b</sup>
28 plants (D2) leaf number	15.8ª	10.9 <sup>b</sup>	10.8 <sup>a</sup>	13.0 <sup>b</sup>	12.4 <sup>b</sup>	11.3 <sup>ab</sup>	16.2 <sup>ab</sup>	11.5 <sup>b</sup>	11.9 <sup>b</sup>
Control (18) leaf number	17.4 <sup>a</sup>	19.1 <sup>a</sup>	10.9 <sup>a</sup>	16.1ª	13.4 <sup>b</sup>	12.1 <sup>a</sup>	17.5 <sup>a</sup>	12.9 <sup>a</sup>	13.4 <sup>a</sup>
Mean± SE	16.9±0.50	13.4±0.54	10.8±0.41	14.4±0.61	13.5±0.39	11.4±0.26	16.1±0.51	12.5±0.32	12.7±0.37

Table 2: Leaf number under different plant density (D), feeding frequency regime (F) and staggered sowing date (M)

Each value is the mean  $\pm$  SD

Mean values in each column have different subscript (a, b, c) are significant different a P<0.05

#### 4.3 Total fresh weight

Table 3 elaborates the response of plants to total weight (biomass) under different plant densities. In April, with F1 the control recorded the highest total weight with 634.62 g and was found significantly higher compared to other treatments (D1 and D2), which were on the par between them. In May and June, a similar trend was noticed as in M1. In the F2, the control was significantly showed higher biomass compared to D2, but found to be same as D1. In June, all the treatments of plants densities showed no significant difference. However, In July, all the 3 different densities (control, D1, and D2 plants) showed a significant difference between each other. In F3, during the month of June, a significant difference was found between control and D2, 459.04, 358.83 g respectively. During July (M2), the control with 253.31 g was the highest biomass whereas in August (M3) all the plant densities showed a significant difference between each other.

	F1			F2			F3		
Plant	(Feeding one time/day)			(Feeding two time/day)			(Feeding three time/day)		
density	M1	M2	M3	M1	M2	M3	M1	M2	M3
	April	May	June	May	June	July	June	July	Aug
12 plants	489.04 <sup>b</sup>	234.6 <sup>b</sup>	224.6 <sup>b</sup>	286.1 <sup>ab</sup>	249.06 <sup>a</sup>	247.28 <sup>b</sup>	390.97 <sup>ab</sup>	225.17 <sup>b</sup>	243.28 <sup>b</sup>
(D1) (g)									
28 plants (D2) (g)	428.93 <sup>b</sup>	246.3 <sup>b</sup>	211.97 <sup>b</sup>	241.97 <sup>b</sup>	228.23ª	212.97°	358.83 <sup>b</sup>	212.97 <sup>b</sup>	205.57°
Control (18) (g)	634.62ª	483.97 <sup>a</sup>	246.3ª	318.83 <sup>a</sup>	265.46 <sup>a</sup>	292.55 <sup>a</sup>	459.04 <sup>a</sup>	253.31ª	259.7ª
Mean±SE	517.53±35.6	321.6±9.55	227.6±4.58	282.3±13.3	247.5±10.6	250.8±4.86	402.9±27.8	230.4±7.4	236.1±4.2

Table 3: Total fresh weight of the plant with lettuce head under different plant density (D), feeding frequency regime (F) and staggered sowing date (M)

Each value is the mean  $\pm$  SD

Mean values in each column have different subscript (a, b, c) are significant different a P<0.05

## 4.4 Head weight of lettuce

The results on the response of response of plants to head weight of lettuce (Table 4) under different plant densities, during April month, the data showed that control significantly increased the fresh weight than 28 plants (D2) and 12 plants (D1), and showed the same trend during May planting (M1) also. However, planting in June (M2), the control plants, D2 and D1 showed a significant difference among all of them. For the F2, in May, the control plants and D1 (12 plants) showed no significant difference, but there was a significant difference compared to D2 densities (28 plants). In the following month (June), the control with 221.34 g showed significantly higher weight of head compared to D2 but equal to D1. In July (M1), the data showed a significant difference among all the treatments. In June (M2), for F3, the treatments were on par, but in the month of July again the control showed the highest head weight compared to D2, but similar to D1. During the planting in August (M3), all the three densities got a significant difference.

	F1			F2			F3		
	(Feeding or	eding one time/day)			time/day)		(Feeding three time/day)		
Plant	M1	M2	M3	M1	M2	M3	M1	M2	M3
density	April	May	June	May	June	July	June	July	Aug
12 plants	433.01 <sup>b</sup>	202.93 <sup>b</sup>	202.93 <sup>b</sup>	243.41 <sup>a</sup>	210.66 <sup>ab</sup>	224.93 <sup>b</sup>	342.27 <sup>a</sup>	184.0 <sup>ab</sup>	228.93 <sup>b</sup>
(D1) (g)									
28 plants	358.37 <sup>b</sup>	222.1 <sup>b</sup>	184.23 <sup>c</sup>	185.93 <sup>b</sup>	178.43 <sup>b</sup>	187.33 <sup>c</sup>	312.52 <sup>a</sup>	170.22 <sup>b</sup>	188.93 <sup>c</sup>
(D2) (g)									
Control	567.7 <sup>a</sup>	429.46 <sup>a</sup>	222.1 <sup>a</sup>	272.52 <sup>a</sup>	221.34 <sup>a</sup>	260.1 <sup>a</sup>	403.01 <sup>a</sup>	213.94 <sup>a</sup>	247.2 <sup>a</sup>
(18) (g)									
Mean±SE	453±33.3	284.8±9.16	203.0±4.72	233.9±11.6	203.4±10.4	224.1±5.78	352.5±26.0	189.3±9.49	221.6±4.51

Table 4: Head weight of lettuce under different plant density (D), feeding frequency regime (F) and staggered sowing date (M)

Each value is the mean  $\pm$  SD

Mean values in each column have different subscript (a, b, c) are significant different a P<0.05

## 4.5 Root length

Table 5 shows the response of plants to root length under different plant densities. During April, the results in F1 showed no significant differences among the three treatments D1, D2 and control. However, in May, the control showed higher root length than D1 and D2 with a significant difference, but D1 and D2 were observed to be on the same level. In June, D1 was significantly higher than D2 and control, but D2 showed no significant difference compared to control. Under F2, D1 recorded higher length than the control, which was in the month of May, but there was no significant difference. However, in June, the treatments showed no significant differences at all. D1 and the control were observed to be on par between each other, but significantly differed to D2. In F3, the first month (M1) was found to be on the par with all treatments. In July (M2), D1 got higher root length than other treatments, but it showed to be on the par with the control. In the last month August (M3), D1, D2 and control showed significant differences with 15.5 cm for the D1 and 12.0 cm (D2).

	F1			F2			F3		
Plant	(Feeding one	e time/day)		(Feeding two time/day)			(Feeding three time/day)		
density	M1	M2	M3	M1	M2	M3	M1	M2	M3
	April	May	June	May	June	July	June	July	Aug
12 plants	46.7 <sup>a</sup>	16.1 <sup>b</sup>	16.1 <sup>a</sup>	44.1 <sup>a</sup>	19.8 <sup>a</sup>	20.6 <sup>a</sup>	40.7 <sup>a</sup>	20.1 <sup>a</sup>	15.5 <sup>a</sup>
(D1) (cm)									
28 plants	40.0 <sup>a</sup>	15.8 <sup>b</sup>	14.0 <sup>b</sup>	33.9 <sup>b</sup>	17.2 <sup>a</sup>	17.4 <sup>b</sup>	43.2 <sup>a</sup>	15.2 <sup>b</sup>	12.0 <sup>b</sup>
(D2) (cm)									
Control	45.8 <sup>a</sup>	53.5 <sup>a</sup>	15.8 <sup>ab</sup>	43.2 <sup>a</sup>	19.4 <sup>a</sup>	21.8 <sup>a</sup>	42.6 <sup>a</sup>	18.7 <sup>a</sup>	14.4 <sup>a</sup>
(18) (cm)									
Mean±SE	44.1±2.36	28.4±1.68	15.3±0.55	40.4±1.50	18.8±1.2	19.9±0.87	42.1±1.90	18±0.72	13.9±0.58

Table 5: Plant root length under different plant density (D), feeding frequency regime (F) and staggered sowing date (M)

Each value is the mean  $\pm$  SD

Mean values in each column have different subscript (a, b, c) are significant different a  $P \le 0.05$ 

## 4.6 Head height

Data on Table 6 shows the response of height of lettuce head under different plant density. In feeding regime one, during April, control significantly increased than D1 and D2 with 31.2 cm. Similarly, in May, the control got a greater length of green leaves compared to the D1 and D2. However, D1 and control were on the par in June, but they were significantly higher than D2. In F2 feeding frequency, D2 increased more than other treatments with 26.7 cm, but it was on the par with the control. In June (M2), D1 and control showed no significant differences. Treatments on the following month (July) showed no significant differences except control, which was significantly higher, compared to D2. On the Feeding regime 3 (F3), the first two months (June and July) showed no significant differences at all. However, in August (M3), D1 and control were on the par, but showed a significant difference compared to D2.

	F1			F2			F3			
Plant donsity	(Feeding one time/day)			(Feeding two	(Feeding two time/day)			(Feeding three time/day)		
T fullt defisitly	M1	M2	M3	M1	M2	M3	M1	M2	M3	
	April	May	June	May	June	July	June	July	Aug	
12 plants	27.4 <sup>b</sup>	23.3 <sup>b</sup>	23.3 <sup>ab</sup>	24.9 <sup>b</sup>	25.7 <sup>a</sup>	26.2 <sup>ab</sup>	26.0 <sup>a</sup>	24.7 <sup>a</sup>	23.0 <sup>a</sup>	
(D1) (cm)										
28 plants	26.0 <sup>b</sup>	24.4 <sup>b</sup>	22.7 <sup>b</sup>	26.7 <sup>a</sup>	22.9 <sup>b</sup>	24.3 <sup>b</sup>	26.6 <sup>a</sup>	24.0 <sup>a</sup>	21.0 <sup>b</sup>	
(D2) (cm)										
Control (18)	31.2 <sup>a</sup>	31.8 <sup>a</sup>	24.4 <sup>a</sup>	26.6 <sup>ab</sup>	26.2 <sup>a</sup>	27.5 <sup>a</sup>	26.5 <sup>a</sup>	24.4 <sup>a</sup>	22.7 <sup>a</sup>	
(cm)										
Mean±SE	28.2±0.61	26.5±0.48	23.4±0.44	26.0±0.49	24.9±0.31	26±0.63	26.3±0.39	24.3±0.55	22.2±0.42	

Table 6: Head height under different plant density (D) feeding frequency regime (F) and staggered sowing date (M)

Each value is the mean  $\pm$  SD

Mean values in each column have different subscript (a, b, c) are significant different a P<0.05

## 4.7 Calcium and Sodium

Table 7 shows the response of plants to Ca and Na under different plant densities. Based on lab analysis, F1M1, F2M1 and F3M3, the data showed no significant differences at all on the Ca content. Na also showed no significant differences in the lab analysis. However, apparently differences were noticed among all the treatments.

Table 7: Concentration of Ca and Na under different plant density (D) and feeding frequency regime (F)

Plants		Ca (mg/l)		Na (mg/l)			
density	F1M1	F2M1	F3M1	F1M1	F2M1	F3M1	
12 plants	1.65 <sup>a</sup>	1.78ª	1.97ª	2.98ª	3.14 <sup>a</sup>	2.71 <sup>a</sup>	
28 plants	1.54 <sup>a</sup>	1.82ª	1.80 <sup>a</sup>	3.15 <sup>a</sup>	3.21 <sup>a</sup>	2.74 <sup>a</sup>	
Control	1.48 <sup>a</sup>	1.88ª	1.77ª	2.21ª	3.11 <sup>a</sup>	2.76 <sup>a</sup>	

Each value is the mean  $\pm$  SD

Mean values in each column have different subscript (a, b, c) are significant different a P<0.05

## 4.8 Molybdenum and Iron

Table 8 shows the response of plants to Fe and Mo under different plant densities. The data showed the percentage of Fe and Mo on the plants and showed no significant differences in the lab analysis. However, apparently differences were noticed among all the treatments.

Table 8: Concentration of Fe and Mo under different plant density (D) and feeding frequency regime (F)

Plants	Fe (mg/l)			Mo (mg/l)			
density	F1M1	F2M1	F3M1	F1M1	F2M1	F3M1	
12 plants	331.35 <sup>a</sup>	487.30 <sup>a</sup>	456.0 <sup>a</sup>	3.98 <sup>a</sup>	3.34 <sup>a</sup>	$2.22^{a}$	
28 plants	295.09 <sup>a</sup>	492.20 <sup>a</sup>	368.90 <sup>a</sup>	5.00 <sup>a</sup>	3.05 <sup>a</sup>	5.07 <sup>a</sup>	
Control	287.13 <sup>a</sup>	523.60 <sup>a</sup>	386.90 <sup>a</sup>	4.54 <sup>a</sup>	4.00 <sup>a</sup>	2.66 <sup>a</sup>	

Each value is the mean  $\pm$  SD

Mean values in each column have different subscript (a, b, c) are significant different a P<0.05

## 4.9 Ammonia, Nitrate, Nitrite and pH

Table 9 shows the status of ammonia, nitrate, nitrite and pH under different feeding frequency. The ammonia rate increased when the feeding ratio was increased. The nitrate and nitrite levels increased when ammonia increased. The pH level also showed marginal decrease during the period of experiment

	F1			F2			F3		
	(Feeding one time/day)			(Feeding two time/day)			(Feeding three time/day)		
Nutrients	M1	M2	M3	M1	M2	M3	M1	M2	M3
Average	April	May	June	May	June	July	June	July	Aug
pН	7.25	6.29	6.46	6.21	6.52	6.67	7.17	6.92	6.23
Ammonia	1.860	1.756	0.856	2.450	2.380	1.850	1.180	0.890	0.768
(mg/l)									
Nitrate	13.95	15.216	16.124	16.350	15.080	15.500	17.260	16.180	14.650
(mg/l)	0								
Nitrite	0.183	0.278	0.301	0.226	0.650	0.265	0.173	0.270	0.293
$NO_2$									
(mg/l)									

Table 9: Ammonia, Nitrate, Nitrite and pH under different feeding frequency regime (F) and sowing date (M)

## **Chapter 5: Discussion**

During our experiment, no plant diseases had occurred expect mosquitoes breeding was noticed on the raceway, which did not influence the experiment. Lettuce plants is a short day crop, which prefers winter or spring season than summer months for producing optimum growth.

## 5.1 Plant length

The plant height was the highest during April month, which was the best month for growth and development. The most important observation was that the feeding regimes as expected did not affect the plant height even though nutrient addition was more with higher feeding regimes. This finding was in conformity with the observation of (Yina Zon, 2015) who reported that low feeding of fish resulted in high extract of nutrients, which led to having higher yield of plants. Another critical finding was that the plant densities (D1)12 and control 18 produced similar plant heights which were significantly higher under different feeding regimes and at different growing months, indicating that the optimum growing space was available in the above densities at 28 plants per growing board where the plants were crowded and might be giving high competition for nutrients thus producing less plant height. This observation was in agreement with the findings of Takahashi (2014) who claimed that less competition for low densities of different lettuce cultivars.

## 5.2 Leaf number

Based on the observation, there was almost no interaction between different sowing dates, feeding regime frequency and the number of leaves per plant. Our experiment showed that when plants were planted in different densities they usually did not influence on the number of leaves. This observation was in an agreement with the findings of Calori *et al.* (2014) reported that the number of leaves and length were not influenced by different spacing between the plants (densities). However, in a few months after growth, our results showed significant differences on the number of leaves, which agreed with Maboko (2013) who observed that the big space between plants would give more leaves, and large ones. Our study also focused on the different feeding regimes during the entire period of experiment. However, the numbers have fluctuated between the treatments and the different feeding regimes but overall, F1 gave the best results compared to others. This finding was in an agreement with the observation of Licamele (2009) who found that feeding of fish could give different yield of lettuce. In addition, feed requirements should consider properly through feeding regime or fish density because high level of these two might have impacted the availability of some nutrients for aquaponics (Villarroel et al., 2016).

## 5.3 Head weight and total fresh weight

Both the total fresh weight and fresh weight of head showed significant differences between the treatments. The total fresh weight for control densities were significantly high when compared to D1 and D2 densities. The spacing between plants could have initiated a competition for nutrient resources, like light, temperature, humidity etc, (Calori, 2014). This could be due to the spacing between the plants, which could play a main role on the height, leaf number, and leaf area (Maboko, 2013). The most important observation was that the increased head weight of lettuce was as a result of longer leaf. This finding correlated with the study of Gonnella and F. Serio (2003). Further, low densities resulted in wider spacing between the plants, which ended up with a lesser competition within plants. These outcomes agreed with the findings of Takahashi (2014) who reported that there is a relationship between spacing (plant densities) and the weight of plants. Another finding was that during April month, control plants were on the optimal conditions and thus resulted in highest biomass compared to all other treatments. Turbin *et al.* (2014). Reported that the decrease in space led to having a low mass of lettuce head. The FAO Fishers and Aquaculture technical paper reported that there were guidelines for 12 different vegetables and the ideal densities for lettuce ranged between 20 to 25 m<sup>2</sup>.

## 5.4 Root length and head length

Our experiment showed fluctuated results on the shoot length among the treatments in all months. Mostly, shoot length for control was more than D1 and D2. The densities of D2 were higher compared to control and D1, which should promote increased length of the leaf. This finding was in disagreement with the reports of Gonnella and Serio (2003) with different varieties. Decreasing plant space could promote the leaves to competition for the light source, which led to higher leaf area and leaf number (Maboko, 2013). This observation was validating the reason for control getting higher leaf length than D1. However, Turbin *et al.* (2013) and Takahashi (2014). Reported that increasing the distance between plants could promote increased of leaves, which was in an agreement with our study. Another finding showed Nitrate and Nitrite which gradually increased during the experiment. This point to losing of nitrification bacteria due to the harvesting of lettuce. Removal of lettuce is removing of roots, which is holding most of nitrification bacteria.

## 5.5 Calcium, Sodium, Molybdenum and Iron

In the present study, the different feeding regime did not affect the Ca, and Na amount in different densities of lettuce planting in the aquaponics system. The findings of Ca, and Na showed no significant differences among treatments (Table 6) simultaneously the same trend was observed in both Fe and Mo levels on the leaves of lettuce (Table 7). The present results agreed with the studies of Tucker (2014) and Kim *et al.* (2016) who reported that there is a correlation between fresh vegetable consumption and reduced risk of chronic diseases.

Lettuce contains numerous nutritional constituents such as magnesium (Mg), manganese (Mn), potassium (K), iron (Fe), zinc (Zn), calcium (Ca), phosphorus (P) and other health-promoting bioactive compounds that are essential for human health (Kim *et al.*, 2016).Recently scientists are investigating the role of lettuce consumption in disease prevention by conducting few human clinical studies. Fernandez *et al.*, 1997 conducted a case-control study and reported that a counter relationship between and lettuce consumption and colorectal cancer. Due to low calcium in vegetarian diets vegetarians have a higher risk of bone fracture and low bone density (Tucker, 2014). In our study, the findings of sodium (Na) content in lettuces agreed with the statement of Kim *et al.* (2016) who reported that the risk of hypertension can be decreased by lowering the intake of Na and increasing K intake. In our study the mineral content was generally higher than the results found by Baslam, et al. 2013. The lettuce leaf nutrient levels also agreed with the findings of Hartz & Johnstone, (2007).

## 5.6 Ammonia, Nitrate, Nitrite and pH

In the current study, the pH level showed marginal decrease in the period of experiment. This might be due to the increment of fish metabolic waste dissolved in water and dissolved oxygen reduction by plants and fishes. The ammonia rate was increased when the feeding ratio was increased. When the ammonia was increased, simultaneously the nitrate and nitrite levels increased. This indicated that the biological system was working well in the aquaponics system. The present results came in line with the finding of Kuhn et al. (2007) who reported, throughout the experiment period, the water quality maintained more optimal level than the findings of previous researchers (Wortman & Dawson, 2015).

The stabilization of pH plays a major role in aquaponic system and in all living organisms effective within a cycling system which controls metabolism of fish bacterial activities and affecting the nitrogen availability in plants. In each living organism the optimal pH is different. In order to enhance the uptake of nutrients. Most plants need a pH value between 6 and 6.5. In order to achieve sustainability of all the biological interactions occurring in an aquaponics system, it is essential to distinguish the optimal pH range for complete growth rate of plant, bacteria and fish. Even at higher pH levels, the plant roots, bacteria, and fish absorb nutrients thereby providing the optimal pH for every part in a challenging system. The most significant parameter in the aquaponics systems is the pH solution because it controls the metabolism of fish, activities of microorganisms and also influences the accessibility of nitrogen to plants.

## **Chapter 6: Summary**

This study was carried out in the greenhouse on the area reserved for experiments in the College of Food and Agriculture at Falaj Hazza campus ALA in, UAE. In this study a small-scale aquaponics system with a grow bed form producing tilapia (*Oreochromis aureus*) lettuce (*Lactuca Sativa*) were used as the fish and the plant materials, respectively.

Treatments were arranged in complete randomize block design with 3 replicates as follows. Tilapia (*Oreochromis niloticus*) were stocked at three different regimes: feeding one time per day (F1), Feeding two times per day kg/m3 (F2) and feeding three times per day (F3). Lettuce plants were sown in vegetation foam plates each with 12, 18 and 28 plants.

Water quality parameters including temperature, pH, TDS, EC, ammonium, nitrite, nitrate, iron, alkalinity, acidity and light intensity except water temperature showed significant differences (p<0.05) with times and the experimental groups.

The ammonia rate decreased in F1 comparing between M1 and M3 which was 1.86 and 0.856 mg/L respectively. Similarly, F2 and F3 ammonia rates were decreasing. The nitrate and nitrite levels increased when ammonia increased. The pH level also showed marginal decrease during the period of experiment.

It was observed that Feeding frequency regime (F) has no impact to the production of lettuce as increasing the feeding frequency. F1 shows significant value of some parameters like total weight and head weight comparing to other feeding frequency (F2 and F3). The most important observation was that the feeding regimes as expected did not impact some parameters even though nutrient addition was more

with higher feeding regimes. This finding was in conformity with the observation of who reported that low feeding of fish resulted in high extract of nutrients, which led to having higher yield of plants. The highest mean values of plant total weight and head Wight of lettuce were observed in April then the plant appears lighter with time. Feeding frequency one a day highest mean plant total weight and head weight of lettuce.

Ca, Fe, Mo and Na has been tested one time only during the experiments due to limited of labor and lab equipment. The data show no significant different between all treatments. The good head weight, green leafs matter and good texture were higher in F1. The highest mean values were obtained with using 18 plants in foam.

## Conclusion

The aquaponic food production is derived from fish feed through bio filter nitrification and excretory waste of fishes there by incorporating nutrient circulation which is highly effective. This system reprocesses the nutrients enclosed in fish feed and fish feces to propagate the crop plants in an environmental cycle. Plants can function as bio filters and reuses the system effluent which are eliminated to the atmosphere. The effort in developing an average environmental condition among plants, fish, and culture in aquaponics has lead to reduced integration of the systems than would be ideal for increasing the space and arrangement, thus decreases the overall flexibility of aquaponics.

## Recommendation

It is evident that the aquaponic systems management has been built for the lettuce/tilapia fish system, but more long term research/demonstrations should be conducted on other aquaponic crops/tilapia fish system combinations. It appears to be

an ideal solution for ecological aquaculture and hydroponic practices by supporting the information that aquaponics follows water and nutrient recycling. The effectiveness of the production technique depends on scientific improvements, climatic and geographic conditions that is essential to be evaluated. Aquaponics as a sustainable food production technique will be validated using these factors. Based on this experiment, it is suggested that under aquaponic conditions another hydroponic crop species should be tested to study how crop yields are regulated to maximize longterm sustainability by operating at pH levels more appropriate for bio-filter nitrification. Balancing the environment for aquaponic system which provides optimum growth of organisms will be a significant subject for future studies.

### References

- Al-Hafedh, Y.S., Alam, A., and Beltagi, M.S. (2008). Food production and water conservation in a recirculating aquaponic system in Saudi Arabia at different ratios of fish feed to plants. J. the world aquaculture society, 39(4), 510-520.
- Al-Jaloud, A.A., Hussain, G., Alsadon, A.A., Siddiqui, A.Q., and Al-Najada, A. (1993). Use of aquaculture effluent as a supplemental source of nitrogen fertilizer to wheat crop. Arid Soil Res. Rehabil, 7, 233-241.
- Azevedo, C.M.S.B. (1998). Nitrogen transfer using <sup>15</sup>N as a tracer in an integrated aquaculture and agriculture system. Ph.D. Dissertation, University of Arizona, Tucson, Arizona
- Billard, R. and Servrin-Reyssac, J. (1992). Les impacts negatives delapisci cultured'e ' tangsurl' environment. In: Barnabe ', G., Kestemont, P. (Eds.), Production, Environment and Quality. European Aquaculture Society (special publication), 64, 17-29.
- Bittsánszky, A.A., Uzinger, N., Gyulai, K., Mathis, A., Junge, R., Villarroel, M., Kotzen, B. and Kornives, T, (2016). Nutrient supply of plants in aquaponic systems. Ecocycles, 2(2), 17-20.
- Baslam, M., Morale, F., Garmendia, I., and Goicoechea, N. (2013). Nutritional quality of outer and inner leaves of green and red pigmented lettuces (Lactuca sativa L.) consumed as salads. Scientia Horticulturae, 151, 103-111. http://dx.doi.org/10.1016/j.scienta.2012.12.023
- Blidariu, F., Alexandru, D., and Adrian G. (2013b). Evaluation of Phosphorus Level in Green Lettuce Conventional Grown under Natural Conditions and Aquaponic System. Bulletin UASVM Animal Science and Biotechnologies, 70(1), 128-135.
- Blidariu, F., Isidora, R., Dacian, L., Alexandru, D., and Adrian, G. (2013a) Evaluation of Nitrate Level in Green Lettuce Conventional Grown under Natural Conditions and Aquaponic System. Animal Science and Biotechnologies, 2013, 46(1), 244-250.

- Brune, D.E. (1994). Sustainable aquaculture systems. Report prepared for the Office of Technology Assessment. U.S. Congress, Food and Renewable Resources Program, Washington, DC
- Calori, A.H., Faztor, T., Junior S., Moraes, L., Barbosa, P., Tivelli, S., and Purqueria L. (2014). Elecrical conducitity and plant spacing on baby leaf table beet and lettuce production. Horticultura Brasileira, 32, 426-432.
- Chalmers, G.A., (2004). Aquaponics and food safety, Lethbrige, Alberta.
- Dediu, L., Cristea, V. and Xiaoshuan, Z. (2012). Waste production and valorization in an integrated aquaponic system with bester and lettuce. African Journal of Biotechnology, Nairobi 11, 2349-2358.
- Delaide, B., Simon, G., James, G., Helene, S., and Haissam Jijakli, M. (2016). Lettuce (Lactuca sativa L.var. Sucrine) Growth Performancein Complemented Aquaponic Solution Outperforms Hydroponics Water, 8, 467-474. doi:10.3390/w8100467.
- Delis, P.C., Effendi, H., Krisanti, M. and Hariyadi, S. (2015). Treatment of aquaculture waste water using Vetiveria zizanioides (Liliopsida, Poaceae). AACLBioflux, 8, 616-625.
- Dhawan, A. and Sehdev, R.S. (1994). Present status and scope of integrated fish farming in the north-west plains of India. In: Mathias, J.A., Charles, A.T., Baotong, H. (Eds.), Integrated Fish Farming. CRC press, Boca Raton, New York, 295-306.
- Diver, S. (2006) Aquaponics-integration of hydroponics with aquaculture. National Center for Appropriate Technology (NCAT), Butte, pp 1-28.
- Dunn, P. (2012). An Aquaponics Experiment Where Tilapia and Tomatoes can be best Buddies. Newspaper Source: Times-News, The (Twin Falls, ID). Web 24-Jan, 2013.
- Effendi, H., Utomo, B.A., and Darmawangsa, G.M. (2015a) Phytoremediation of freshwater crayfish (Cherax quadricarinatus) culture waste water with spinach (Ipomoea aquatica) in aquaponic system. AACL Bioflux, 8, 421-430

- Effendi, H., Utomo, B.A., Darmawangsa, G.M., and Hanafiah, D.A. (2015b) Waste water treatment of freshwater crayfish (Cherax quadricarinatus) culture with lettuce (Lactuca sativa). Int J Appl Environ Sci, 10(1), 409-420.
- Effendi, H., Wahyuningsih, S., and Yusli, W. (2016). The use of nile tilapia (Oreochromis niloticus) cultivation waste water for the production of romaine lettuce (Lactuca sativa) in water recirculation system. Appl Water Sci, 54, 102-109. DOI10.1007/s13201-016-0418-z.
- Endut, A., A. Jusoh, N. Aói, W.B. Wan Nik and A. Hassan, (2010). A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. Bioresour Technology, 101, 1511-1517.
- Endut, A., Jusoh, A., Ali, N., and Nik, W.B.W. (2011) Nutrient removal from aquaculture waste water by vegetable production in aquaponic recirculation system. Desalin Water Treat, 32, 422-430. doi:10.5004/dwt.2011.2761.
- Endut, A., Jusoh, A., Ali, N., WanNik, W.N.S. and Hassan, A. (2009). Effect of flow rate on water quality parameters and plant growth of water spinach (Ipomoea aquatica) in an aquaponic recirculating system. Desalin Water Treat, 5, 19-28.
- Fernandez, E., LaVecchia, C., Davanzo, B., Negri, E., and Franceschi, S. (1997). Risk factors for colorectal cancer in subjects with family history of the disease. British Journal of Cancer, 75(9), 1381-1384. <u>http://dx.doi.org/10.1038/bjc.1997.234</u>
- Ghate, S.R. and Burtle, G.J. (1993). Water quality in channel catfish ponds intermittently drained for irrigation. In: Wang, J.K. (Ed.), Techniques for Modern Aquaculture. ASAE,93, 177-186.
- Gonnella, M., Serio, F., Conversa G and Santamaria P. (2014). yield and quality of lettuce grown in floating system using different sowing density and plant spatial arrangements. International symposium on protected cultivation in mild winter climates.
- Graber, A. and Junge, R. 2009. Aquaponic Systems: Nutrient recycling from fish waste water by vegetable production. Desalination, 246, 147-156.
- Hamlin, H.J., Michaels, J.T., Beaulaton, C.M., Graham, W.F., Dutt, W., Steinbach, P., Losordo, T.M., Schrader, K.K., and Main, K.L. (2008). Comparing denitrification rates and carbon sources in commercial scale up flow denitrification biological filters in aquaculture. Aquac. Eng., 38, 79-92.

- Hartz, T., Johnstone, P., Smith, R and Cahn, M. (2007). Soil Calcium Status Unrelated to Tipburn of Romaine Lettuce. HORT SCIENCE, 42(7), 1681-1684.
- Hussain, M.G. (2004). Farming of Tilapia-Breeding Plans, Mass Seed Production and Aquaculture Techniques. Bangladesh Fisheries Research Institute, Mymensingh 2201, Bangladesh, p. 149.
- Ingrid, G. (2013). Study of Water Quality of Recirculated Water in Aquaponic Systems (Study of speciation of selected metals and characterization of the properties of natural organic matter). Thesis submitted to Norwegian University of Science and Technology.
- Jamu, D.M., Piedrahita, R.H. (2002). An organic matter and nitrogen dynamics model for the ecological analysis of integrated aquaculture/agriculture systems: I. model development and calibration, Environmental Modelling & Software, 17, 571-582.
- Jason, L. (2009). Biomass production and nutrient dynamics in an aquaponics system. A Dissertation Submitted to the Faculty of the department of agriculture and biosystems engineering In Partial Fulfillment of the Requirements for the Degree of doctor of philosophy In the Graduate College. The University of Arizona.
- Jchappell, J. A., Brown, T.A., and Purcell, T. (2008). A demonstration of tilapia and tomato culture utilizing an energy efficient integrated system approach. 8<sup>th</sup> International Symposium on Tilapia in Aquaculture 2008.
- Khan, M.A. (1996). Utilization of aquaculture effluent to supplement water and nutrient use of turf grass and native plants. Ph.D. Dissertation. New Mexico State University, Las Cruces, New Mexico, pp. 218.
- Khater, E.S. and Ali, S.A. (2015) Effect of Flow Rate and Length of Gully on Lettuce Plants in Aquaponic and Hydroponic Systems. J Aquac Res Development, 6, 318-325. <u>http://dx.doi.org/10.4172/2155-9546.1000318</u>.
- Kim, M. J., Moon, Y, Tou, J. C., Mou, B., and Waterland, N. L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). Journal of Food Composition and Analysis, 49, 19-34. <u>http://dx.doi.org/10.1016/j.jfca.2016.03.004</u>
- Kuhn, D.D., and Gregory, D.B. (2007). Evaluation of Tilapia Effluent with Ion Supplementation for Marine Shrimp Production in a Recirculating Aquaculture System. Journal of World Aquaculture Society, 38, 74-84.

- Lam, S.S., Ma, N.L., Jusoh, A., and Ambak, M.A. (2014). A study on the optimal tank design and feed type to the growth of marble goby (Oxyeleotris marmorata Bleeker) and reduction of waste in a recirculating aquaponic system. Desalin. Water Treat, 52, 1044-1053.
- Le Guedard, M., Schraauwers, B., Larrieu, I., and Bessoule, J.J. (2008). Development of a biomarker for metal bioavailability: The lettuce fatty acid composition. Environmental Toxicology and Chemistry, 27(5), 1147-1151. <u>http://dx.doi.org/10.1897/07-277.1</u>
- Liang, J.Y. and Chien, Y.H. (2013). Effects of feeding frequency and photoperiod on water quality and crop production in a tilapia water spinach raft aquaponics system. Int Biodet Biodeg, 85, 693-700. doi:10.1016/j.ibiod.2013.03.029.
- Licamele, J. (2009). Biomass production and nutrient dynamics in a aquaponics system. (PhD dissertation), The University of Arizona. Tucson.
- López, A., Javier, G. A., Fenoll, J., Hellin, P., and Flores, P. (2014). Chemical composition and antioxidant capacity of lettuce: Comparative study of regularsized (Romaine) and baby-sized (Little Gem and Mini Romaine) types. Journal of Food Composition and Analysis, 33(1), 39-48. http://dx.doi.org/10.1016/ j.jfca.2013.10.001
- Love, D.C., Uhl, M.S., and Genello, L. (2015) Energy and water use of a small scale raft aquaponics system in Baltimore, Maryland, United States. Aquacult Eng, 68, 19-27. doi:10.1016/j.aquaeng.2015.07.003.
- Maboko, M and Du Plooy, C. (2013). Effect of plant spacing on growth and yield of lettuce (Lactuca sativa L.) in a soilless production system. South African Journal of Plant and Soil, 52, 195- 198.
- Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., d'Orbcastel, E.R., and Verreth, J.A.J. (2010). New developments in recirculating aquaculture systems in Europe: a perspective on environmental sustainability. Aquac. Eng., 43, 83-93.
- McMurtry, M.R., Sanders, D.C., Nelson, P.V., and Nash, A. (1993). Mineral nutrient concentration and uptake by tomato irrigated with recirculating aquaculture water as influenced by quantity of fish waste products supplied. J. Plant Nutr., 16(3), 407-419.

- Medina, M.D., (2014). Effect of Aquafeed on Productivity of Red Amaranth and on Water Quality under Aquaponic. Florida International university.
- Mou, B. (2005). Genetic variation of beta-carotene and lutein contents in lettuce. Journal of the American Society for Horticultural Science, 130(6), 870-876.
- Nicolle, C., Carnat, A., Fraisse, D., Lamaison, J.L., Rock, E., Michel, H. and Remesy, C. (2004). Characterisation and variation of antioxidant micronutrients in lettuce (Lactuca sativa folium). Journal of the Science of Food and Agriculture, 84(15), 2061-2069. <u>http://dx.doi.org/10.1002/jsfa.1916</u>
- NPC (National Research Council), (1993). Nutrient requirements of fish, Washington D.C., National Academy Press.
- Olsen, M.W., Fitzsimmons, K.M. and Moore, D.H. (1993). Surface irrigation of cotton using aquaculture effluent. In: Wang, J.K. (Ed.), Techniques for Modern Aquaculture. ASAE pub. 02-93, St. Joseph, MI, pp. 159-165.
- Palada, M.C., Cole, W.M. and Crossman, S.M.A. (1999). Influence of effluents from intensive aquaculture and sludge on growth and yield of bell peppers. J. Sustain. Agric., 14, 85-103.
- Parker, D., Anouti, A. and Dickenson, G. (1990). Integrated fish/plant production system: experimental results. ERL report 90-34. University of Arizona, Tucson, Arizona. pp. 12.
- Pereira, C., Li, D., and Sinclair, A. J. (2001). The alpha-linolenic acid content of green vegetables commonly available in Australia. International Journal for Vitamin and Nutrition Research, 71(4), 223-228. <u>http://dx.doi.org/10.1024/0300-9831.71.4.223</u>
- Petrea, S.M., Victor, C., Lorena, D., Maria, C., Mirela, C., Alina, A., Marian, T. C. and Alexandru-C.B. (2014). A Study of Phosphorus and Calcium Dynamics in an Integrated Rainbow Trout and Spinach (Nores variety) Aquaponic System with Different Crop Densities. Animal Science and Biotechnologies, 47(2) 196-206.
- Popma, T. and Masser, M. (1999). Tilapia Life History and Biology. Southern Regional Aquaculture Center. Publication No. 283.
- Qin, G., Liu, C.C.K., Richman, N.H., and Moncur, J.E.T. (2005) Aquaculture waste water treatment and reuse by wind-driven reverse osmosis membrane technology: a pilot study on Coconut Island, Hawaii. Aquacult Eng, 32, 365-378. doi:10.1016/j.aquaeng.2004.09.002.

- Racocy, J.E., Hargreaves, J.A., and Bailey, D.S. (1993). Nutrient accumulation in a recirculating aquaculture system integrated with vegetable hydroponics. In: Wang, J.K. (Ed.), Techniques for Modern Aquaculture. ASAE pub. 02-93, St.Joseph, MI, pp. 148-158.
- Rakocy, J.E., Bailey, D.S., Shultz, C., and Thoman, E.S. (2004). Update on tilapia and vegetable production in the UVI aquaponic system. Proceedings from the 6<sup>th</sup> International Symposium on Tilapia in Aquaculture, 2, 676-690.
- Rakocy, J.E., Masser, M.P., Losordo, T.M., (2006). Recirculating Aquaculture Tank Production Systems: Aquaponicse Integrating Fish and Plant Culture. Southern Regional Aquaculture Center Publication, Stoneville, 45, 16-25.
- Resh, H. M. (2001). Hydroponic Food Production. Woodbridge Press Publishing Company. Santa Barbra, CA 93012.
- Roosta, H.R. and Hamidpour, M. (2011) Effects of foliar application of some macro and micronutrients on tomato plants in aquaponic and hydropinic systems. Sci Hortic, 129, 396-402. doi:10.1016/j.scienta.2011.04.006
- Roosta, H.R. and Mohsenian, Y. (2012) Effects of foliar spray of different Fe sources on pepper (Capsicum annum L.) plants in aquaponic system. Sci Hortic, 146, 182-191.
- Salah, M.K. (2006). Aquaponic production of nile tilapia (oreochromis niloticus) and bell pepper (Capsicum annuum l.) in recirculating water system. Egypt. J. AquaL Bioi & Fish, 10, 85-97.
- Savidov, N. (2005). Evaluation of aquaponic technology in Alberta, Canada. Aquaponics Journal, 37, 20-25.
- Seawright, D.E. (1993). A method for investigating nutrient dynamics in integrated aquaculture hydroponic system. In: Wang, J.K. (Ed.), Techniques for Modern Aquaculture. ASAE, 93, 137-147.
- Seawright, D.E., Stickney, R.R., Walker, R.B., (1998). Nutrient dynamics in integrated aquaculture-hydroponics systems. Aquaculture, 160, 215-237.
- Simeonidou, M., Paschos, I., Gouva, E., Kolygas, M., and Perdikaris, C. (2012). Performance of a small-scale modular aquaponic system. Aquaclture, Aquarium, Conservation & Legislation. International Journal of the bioflux Society, 5(4) ,182-188.
- Takahashi K and Cardoso A., (2014). Plant density in production of mini lettuce cultivars in organic system management. Horticultura Brasileira bras., 32, 342-346.

- Timmons, M.B. (1996). Successes and failures in commercial recirculating aquaculture. Aquaculture Engineering Society Proceedings II. NRAES 98(1), 141-150.
- Timmons, M.B., J.M. Ebling, F.W. Wheaton, S.T. Summerfelt and B.J. Vinci (2002). Recirculating Aquaculture Systems 2<sup>nd</sup> Edition. Northern Regional Aquaculture Center Publication No. 011002. Cayuga Aqua Ventures Ithaca, New York.
- Tisdell, C. (1999). Overview of Environmental and Sustainability Issues in Aquaculture. Aquaculture Economics and Management, 3, 1-5. http://dx.doi.org/10.1080/13657309909380228
- Turbin, V., Sokolov, A., Kosterna, E. and Rosa, R. 2014. effect of plant density on the growth, development and yield of brussels sprouts (Brassica oleracea L. var. gemmifera L.). Acta Agrobotanica, 67(4), 51-58.
- Tucker, K.L. (2014). Vegetarian diets and bone status. The American Journal of Clinical Nutrition, 100, 329-335. http://dx.doi.org/10.3945/ajcn.113.071621.
- Tyson, R.V., Danielle, D.T., and Eric, H.S. (2011). Opportunities and Challenges to Sustainability in Aquaponic Systems. Hort Technology, 21(1), 6-13.
- Tyson, R.V., Simonne, E.H., Treadwell, D.D. (2008) Reconciling pH for ammonia biofiltration and cucumber yield in are circulating aquaponic system with perlite biofilters. Hort Sci, 43, 719-724.
- Wahyuningsih, S., Effendi, H. and Wardiatno, Y. (2015). Nitrogen removal of aquaculture waste water in aquaponic recirculation system. AACL Bioflux, 8, 491-499.
- Wang, C.Y., Chang, C.Y., Chien, Y.H. and Lai, H.T. (2016). The performance of coupling membrane filtrationin recirculating aquaponic system for tilapia culture. Int Biodet Biodeg, 107, 21-30. doi:10.1016/j.ibiod.2015.10.016.
- Watanabe, W. O., T. M. Losordo, K. Fitzsimmons and F. Hanley (2002). Tilapia production systems in the americas: technical advances, trends and challenges. Reviews in Fisheries Science, 10(34), 465-498.
- Wortman, S.E. and Dawson, J.O. 2015. Nitrogenase Activity and Nodule Biomass of Cowpea (Vigna unguiculata L. Walp.) Decrease in Cover Crop Mixtures. Communications in Soil Science and Plant Analysis, 46(11), 1443-1457. https://doi.org/10.1080/00103624.2015.1043457

Yina, Z., Hu, Z., Jian Z.H., Xie, S., Liang, J., and Yan, R.W. 2015. Attempts to improve nitrogen utilization efficiency of aquaponics through nitrifies addition and filler gradation. Environmental Science and pollution Research, 23(7), 6671-6679.