Effect of Different Fish Diets on Mustard Production in Aquaponic System Associated With Tilapia Culture

By

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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Date: 14 December 2017

I certify that the report of this final year project entitled "Effect of Different Fish Diets on Mustard Production in Aquaponic System Associated with Tilapia Culture" by Noorazizah Binti Tahir, matric number F14A0188 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honors, Faculty of Agro-based Industry, Universiti Malaysia Kelantan.

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ABSTRACT

The nutrition of fish or fish feed requires an expensive cost in the farming production. Expensive and limited source of the main ingredient in fish feed, such as fish meal leads to the requirement of alternative protein source in fish diet. In addition, waste pollution from aquaculture activity is one of the biggest contributions to environmental pollution. Integrated farming such as aquaponic could be the best solution to overcome these problems. Hence, this research was conducted to investigate the effect of two types of fish diet towards both tilapia and mustard growth performance within the recirculation nutrients in designed aquaponic system. Sixty tilapias were divided into two diet groups with three replicates namely, Treatment 1 which represented diet of solely commercial pellet and Treatment 2 which represented diet of commercial pellet associated with *Azolla pinnata*. Approximately 72 mustard seeds were germinated using peat moss as soilless medium. Aquaponic aquarium design was installed manually using pipes, water pump and aerators. Tilapia culture and mustard culture with hydroponic floating raft were prepared within two separate aquariums which connected vertically from each other based on aquaponic aquarium design. Growth performance of mustard were observed based on height, number of leaf and weight after harvest while tilapia weight as well as water parameter were monitored once a week throughout 15 weeks of culture. The findings showed Treatment 1 gave better mustard growth performance compared to Treatment 2 based on mustard weight after harvest, where Treatment 1 had average weight of 7.13 ± 0.22 g while Treatment 2 produced smaller size with average weight of 1.68 ± 0.04 g. Statistically, the findings showed significance difference ($p<0.05$) between both groups, with Treatment 1 showed better performance than Treatment 2. The best diet for tilapia culture in aquaponic system was diet with solely commercial pellet. Hence, type of fish diet in aquaponic had really influenced the performance of both plant and fish.

Keywords: Aquaponic system, commercial pellet, *Azolla pinnata,* performance, integrated farming

Kesan Terhadap Penghasilan Sawi Dalam Sistem Akuaponik Bersekutu Dengan Pembiakan Tilapia Menggunakan Dua Jenis Makanan Ikan yang Berbeza

ABSTRAK

Nutrisi ikan atau makanan ikan memerlukan kos yang mahal dalam pengeluaran ternakan. Sumber utama untuk bahan makanan ikan yang mahal dan terhad seperti tepung ikan menyumbang kepada keperluan untuk mencari sumber protein yang lain dalam diet pemakanan ikan. Tambahan pula, sisa pencemaran daripada kegiatan pertanian, terutamanya kegiatan akuakultur, dimana ia merupakan salah satu penyumbang terbesar kepada pencemaran alam sekitar. Pertanian bersepadu seperti akuaponik adalah cara terbaik untuk menyelesaikan masalah ini. Justeru, kajian ini telah dijalankan untuk menguji keberkesanan dua jenis diet pemakanan ikan terhadap prestasi pertumbuhan ikan tilapia dan sawi melalui edaran semula nutrisi dalam sistem akuaponik yang telah dirancang. Sebanyak 60 ekor ikan tilapia dibahagikan kepada dua kumpulan diet dengan tiga replikasi iaitu Rawatan 1 mewakili diet yang menggunakan pelet komersial manakala Rawatan 2 mewakili diet yang menggunakan pelet komersial bersama-sama *Azolla pinnata*. Kira-kira 72 biji benih sawi telah disemai menggunakan mos gambut sebagai media tanpa tanah. Pemasangan sistem akuaponik telah dilakukan secara manual menggunakan paip, pam air akuarium dan alat pengudaran. Kultur tilapia dan kultur sawi menggunakan rakit hidroponik terapung telah disediakan dalam dua akuarium yang berasingan, dimana ianya disambungkan secara menegak berdasarkan reka bentuk akuarium akuaponik. Prestasi pertumbuhan sawi telah diperhatikan berdasarkan tinggi, jumlah helaian daun dan berat selepas tuai, manakala berat ikan tilapia serta parameter air turut diawasi seminggu sekali sepanjang 15 minggu tempoh kultur. Hasil penilaian menunjukkan Rawatan 1 memberikan prestasi pertumbuhan yang lebih baik berbanding Rawatan 2 berdasarkan berat sawi selepas tuai, dimana Rawatan 1 mempunyai purata berat sebanyak 7.13 ± 0.22 g, manakala Rawatan 2 menghasilkan saiz yang lebih kecil dengan berat purata 1.68 ± 0.04 g. Secara statistik, hasil kajian menunjukkan perbezaan bererti ($p<0.05$) antara kedua-dua kumpulan. Diet yang terbaik untuk tilapia kultur dalam sistem akuaponik adalah diet yang menggunakan komersial pelet. Oleh itu, jenis diet ikan dalam akuaponik telah mempengaruhi prestasi untuk kedua-dua pokok dan ikan.

Kata kunci: Sistem akuaponik, pellet komersial, *Azolla pinnata,* pertumbuhan, edaran semula nutrisi

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

NH_4^+	Ammonium
NO_3^-	Nitrate
NO_2^-	Nitrite
EFA	Essential Fatty Acid
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
DPN	Diphosphopyridine
TPN	Triphosphopyridine
N	Nitrogen
P	Phosphorus
K	Potassium
ANOVA	Analysis of Variance
Sig.	Significant
d.f	Degree of freedom
ppt	part per thousand
XRF	X-ray fluorescence

CHAPTER 1

INTRODUCTION

1.1 Research Background of Aquaponic

Sustainable farming is the latest farming method of the new millennium. Aquaponic, is one of the farming methods that fits into this sustainable philosophy very well, which fish waste is not been categorized as “waste,” but as a “commodity (Lennard, 2004).

Aquaponic is an integrated aquaculture farming that has an ancient history since 1970. Started with the raised of plant on raft on the surface of lakes in approximately 1000 A.D at Maxico, it is also known as chinampas. The plants were grown at the shallow lake and waste water was dredged from the chinampa canals to irrigate the plants. The cultivation of rice in paddy fields, combine with fish farming is one of the examples of ancient aquaponic system that being practiced in South China, Thailand and Indonesia, until the present modern integrated aquaponic systems were used (Bradley & Kirsten, 2014). In Bangladesh which known as world's most densely populated country, a team from Department of Aquaculture at Bangladesh Agricultural University, has innovated low-cost aquaponics system plan in order to encourage chemical-free usage in fish production for people living in a disadvantageous areas where at the southern area are salinity-prone and the eastern area are flood-prone (Rahman & Amin, 2016).

One of the earliest hydroponic system is floating systems on polycultural fish ponds that were installed on a huge scale area of growing paddy, wheat, canna lily and other crops (Rahman & Amin, 2016). To date, there are vary types of system that being used in aquaponics depends on the farmers or researcher's preference such as different fish feed, crop types, fish species, light sources and the system design.

Generally, aquaponic, is related to the management of nutrient manure of the fish and the return of nutrient from plant which give massive effect to both of the products in aquaponic system. Previous research were done by Love et al. (2014), to investigate on the effect of different light sources, such as artificial fluorescence light and original sunlight, on the growth of crop in aquaponic system, as crops need light for photosynthesis. In aquaponic, the main concern input is the feed that will serves as the major nutrients source for both animals and plants (Medina, Jayachandran, Bhat & Deoraj, 2016). In an earlier research done by Al-Shamsi, Hamza and El-Sayed (2006), they studied the effect of different food sources which are live feed, artificial feed and combination of both of them, towards the growth rates and survival of Nile tilapia fry in a normal culture. In another previous research by Medina et al. (2016), they determine the plant growth, water quality and economic effects from the used of plant-based protein aqua feed in the aquaponic, which is very related to this present study.

1.2 Problem Statement

Aquaculture activities give profitable yield to the farmers and consumers, but at the same time gives side effect in terms of waste, if inappropriate managed. Fish farming is one of the main farming activities that lead to this problem. Since the fish waste could beneficial to the crops as organic fertilizer, hence integrated farming system of fish farming with crops plantation or aquaponic could be practiced in order to manage the waste from the fish culture and supply the nutrient to the crops in circulating the crops nutrients to the fish. Excess nutrients that available in fish culture water or manure are depending on the feed source; hence the type of feed may plays a crucial role in providing better nutrient to fish in order to produce better quality of manure. There are various types of feed being used for fish in aquaponic system. According Love et al., (2014), in general, most societies use feed pellets (94%), aquatic plants (33%), live feed (30%) and human food scraps (13%). But there is no documentation yet in determining the specific types of feed that can devote the best nutrient performance to the fish culture and nutrient biomass of the manure. Hence, this study was done using two types of feed as in the research, which were commercial pellet and one of the aquatic plants, *Azolla pinnata* as supplemented feed.

Nutrition cost is the most highly expenses in intensive aquaculture industry. Meanwhile, protein ingredients in feed express about 50% of feed cost in intensive culture. The best dietary protein is crucial for successful tilapia culture practices, especially in aquaponics (El-Sayed, 2004). There are different protein and nutrient level in each types of feed or diet. There are varying types of alternatives diet for the fish practiced by farmers such as artificially diets, either solely using complete commercial pellet or addition with supplemented feed (Craig & Helfrich, 2002). There are also fish diets by using natural or living organisms such as plankton in the pond, or live feed such as *Artemia nauplii*, worms and others.

Farmers will use commercial pallet that contain animal-based protein from fish meal and fish oil as main ingredient and other plant-based ingredients such as soybean meal. The use of fishmeal in feed is to produce higher valued fish. It's increasing usage considered as unacceptable and lead to the limited nature of the resource (Brinker & Reiter, 2011). Vast demand and cost of protein source in feed from traditional resources gives constraint for developing countries farmers to merge with cheap but easy to access ingredients for fish feed. Due to that, there are many strategies of the fish diet in order to boost growth of the fish and profit but at the same time could reduce or avoid high spent in feeding activities. One of them is supplement feed or additional feed. Nowadays, the usage of aquatic plants as supplement feed contains high food value. It had been highly produced and developed at the level of cost effective for animals feed (Sithara & Kamalaveni, 2008).

In aquaponics, good feed strategies lead to good nutrients supplied for both growth of plants and fish since feed is the main nutrient source in the aquaponic cycle. Thus, the aim of this study was to determine the best types of diet strategy between diets of commercial pellet and commercial pellet associated with *A. pinnata* as supplement in the aquaponics system towards growth of both mustard and tilapia.

1.3 Hypothesis

H_0: There is a significant between diets contain commercial pellet and commercial pellet with *A. pinnata* towards growth performance of mustard and tilapia.

H_a: There is no significant between diets contain commercial pellet and commercial pellet with *A. pinnata* towards growth performance of mustard and tilapia.

If $p < 0.05$, H_0 was accepted.

If $p > 0.05$, H_0 was rejected.

1.4 Objectives

1. To determine the effect of two different fish diets; commercial pellet and commercial pellet associated with *A. pinnata* on mustard growth performance in aquaponic system associated with tilapia culture.

1.5 Scope of Study

The scope of study was related to aquaponic system which also known as integrated system of fish culture and hydroponic. The present study was focused on the management of waste from fish culture as beneficial nutrients uptake and benefits to the crops in aquaponic system.

1.6 Significance of Study

This study indicated the importance of waste management that can be practiced in an integrated farming system. This research would determine the efficiency of the diets contained solely commercial pellet and commercial pellet with *A. pinnata* as supplement in the aquaponic system. Different diets or feed types contain different nutrients levels content in each feeds as well as their effect on crops and fish growth performance. The nutrient levels could be investigated by observation on growth performance of the mustard and tilapia. From this study, the findings could assist farmers and societies to choose appropriate types of feed or diets for the profitable output of aquaponic system based on their nutrients content.

CHAPTER 2

LITERATURE REVIEW

2.1 Growth of Tilapia and Mustard

2.1.1 Tilapia

Nile tilapia is one of the extremely popular freshwater fish for commercial aquaculture production due to their high nutritional qualities, rapid growth and resistance to illnesses. Nile tilapia is a mouth-brooder. According to Rakocy, Shultz, Bailey, and Thoman (2004), the female will spawned few hundred offspring for each spawning which is every four to six weeks. For young tilapia, they will spawn every less than six months. Female tilapia will lay the eggs which then will be fertilized by male tilapia. Tilapia eggs will hatch in five to seven days and the fry will be kept in the female mouth for four to seven days after hatched. Tilapia growth is relying on their stocking density, feed and quality of water. Males Tilapia grows 10% to 20 % faster than females. Newly hatched fry depend on their yolk sacs for nutrients and later they will eat the smallest phytoplankton present in the pond. As the fry grow, they eat bigger organisms and supplemental feeds such as rice bran, fishmeal and others. Tilapia culturing period is usually within 24 weeks or six months (Rakocy et al, 2004).Tilapia will usually eat variety of phytoplankton as their primary feed items. Commercial pellet will then be given as the size of tilapia increases. According to Riche and Garling (2003), tilapia feeding rate depends on their size and weight. Tilapia fingerlings sized around 0.5 inch to 2.5 inch, weighed around 1 g to 10 g. The feeding rate of tilapia fingerlings is approximately 10% from their average body weight (Riche & Garling, 2003). Tilapia is the most common species for commercial culturing and integrated system due to the demand worldwide.

2.1.2 Mustard Growth

Mustard is one of the of the cabbage family members. There are varying types of mustards such as green mustard and white mustard. Mustard will be grow excellently either in cold, hot or even rainy season (Nurshanti, 2010). According to Rakhaman, Lanya, Bustomi Rosadi, and Kadir (2015), the height of the matured or harvested size of Mustard is around 20 cm to 25 cm with yellow flowers, which is around 30 days to 40 days from seed germination. The seed will sprout as early as three days and as late as ten days after seed being germinated. The germination period is usually around one week to two weeks. Since mustard is in the same family as lettuce, the harvested time is quite similar, which is around five weeks to six weeks after seedlings being transferred into hydroponics system (Ako & Baker, 2009). Mustard plant had been researched for their growth in both aquaponics and hydroponic system which resulted better performance in hydroponic system (Rakhman et al., 2015).

2.2 Aquaponic System

Aquaponics systems are the combination of two important production techniques, which are hydroponics and aquaculture (Bakhsh & Khoda, 2008). The systems are basically related to recycling or reuse waste nutrient from the fish tank and supply them to the plant in an integrated cultured together. Due to some problems and issues appear when there are plenty of waste residues from aquaculture farming, hence the idea of integrated farming between the aquaculture and other types of industry are evolved. There are many types of integrated farming of aquaculture such cattle-fish, pig-fish, rabbit-fish, goat-fish, poultry-fish, duck-fish, paddy-cum fish and the most commonly being practiced is plant-fish integration, known as aquaponics. The traditional integrated fish farming practiced in China had been introduced to Asian by the Chinese immigrant (Ahilan, Avaneshwaran, & Kumaravel, 2011). In a survey done by Love et al. (2014), the most common methods of crops culturing used in aquaponic were media beds, followed by floating rafts and nutrient film technique. For water usage in aquaponics system, majority use traditionally drinking waters sources meanwhile for feed most of the communities use feed pallet followed by aquatic plants and live feed.

Aquaponics related to waste water treatment process in conjunction with crops plantation focus on maximizing the recycling rates of phosphorus, nitrogen and potassium as well as fulfill the quality demands of plants products. Approximately 60% of removed nitrogen from the fish farming will be recycled back to the crops (Graber & Junge, 2009). The nutrients that available for vegetable consumption such as nitrogen, potassium, phosphorus, and other elements in water, such as ammonia, nitrate and nitrite will dissolve in the media. The elements and nutrients then will be absorbed by the plant roots to optimize the usage of nutrients and water. This will reduce the fish manure that related to better environmental impact (Munguia-Fragozo et al., 2015). The ratio of 57 g to 60 g of feed per m^2 of plant growing area per day reduce the rate of nutrient accumulation and sufficient nitrification were able to present in the hydroponic tanks (Rakocy, 2007).

The benefits from aquaponics system are basically could reduce overall cost of farming, increase the production yield and reduce the environmental pollute by the farming activities. Aquaponics could be done indoor and outdoor, in small and large scale, depending on the types of design that the farmers wanted to apply.

2.2.1 Nitrification Process

The process of nitrification in aquaponics was carried out with oxidizing of ammonia and nitrate. This nitrification enhance the growth of microbial community such Nitrosomonas and Nitrobacter, known as nitrifying bacteria. These microorganisms contribute to the processing of particulate and dissolved manure such as ammonia from feces, carbon and nitrogen accumulation from feed waste and feces in aquaculture farming (Munguia-Fragozo et al., 2015). In nitrification, nitrifying bacteria will convert NH_4^+ (ammonium) to NO_3^- (nitrate). This nitrification bacteria play a crucial role in efficient aquaponics system (Hu et al., 2015). Transformation of nitrogen in aquaponics can be directly influenced by plant species. Nitrification is facilitated by the continuous aeration of the system. The best pH range for conversion of NH_4^+ to nitrite (NO_2^-) is between pH 5.8 and pH 8.5 (Enduta, Jusoh, Ali, & Wan Nik, 2011).

2.2.2 Aquaponic Tilapia Culture

In aquaponics, the fish that is most commonly used is Nile tilapia (*Orechromis niloticus*). This is due to tilapia is known as the second most farmed fish as it is highly demanded protein sources in the world. In fact, in 2009, the tilapia production were over three million metric ton per year (Ng & Romano, 2013). Some of the characteristics of tilapia that makes them as the farmers choices in the aquaponics and highly demand in the aquaculture industry are their resistance to crowding, high market price, easy to spawn and breed within the year, high resistance towards disease, success in other polyculture and their ability to tolerate and consumed with low cost diet (Ng & Romano, 2013).

In recirculation aquatic system or aquaponics, tilapia was commonly being cultured with lettuce and aubergine plant. However, the most common plant being cultured together in aquponic are vegetable or green leafy plants depending on the cost and design used. The stages of tilapia that being used in aquaponics are varying from fry until juvenile. The most common stages of tilapia growth being used are fry and fingerlings due to its performance and growth until marketable size.

2.2.3 Aquaponics Mustard Culture

According to survey done by Love et al. (2014), the most plant being used in aquaponics are basil, tomatoes and salad greens. Meanwhile according to Effendi, Wahyuningsih and Wardiatno (2016), water spinach, spinach lettuce tomato, cucumber and pepper are been researched as the most common plant being cultured in the aquaponics system. Mustard plants are basically less famous in the aquaponic culture. In a study conducted by Rakhman et al. (2015), mustard plant have better performance in hydroponic itself rather than integrated with aquaculture farming. The study was tested on mustard with hydroponic independently and with the culture with tilapia. The results obtained stated that Mustard gave positive performance when culture in hydroponics rather than aquaponics. Hence, this study were done in order to test the growth performance of mustard effected from different fish diets of commercial pellet and commercial pellet with *A. pinnata* in aquaponic system. Mustard is one of the vegetable that are mostly consumed by consumer in Malaysia due to its cheap price, high acceptance from multi races and suitable to be cooked in vary of dishes (Tan, Yen, Hasan, & Muhamed, 2014).

The recent studies by Rakhaman et al. (2015), showed mustard being cultured together with tilapia in aquaponics, rather than other common types of fishes. The favorable performance of both mustard and tilapia in the aquaponic system had led to this present study.

2.2.4 Medium for Hydroponics

Hydroponics is related to the method of growing plants using a nutrient solution rather than soil. It can be divided into methods of using non-soil growing medium or no growing medium at all. Growing medium should fully inert and could support the plants and their root developments, where nutrient solution could move and absorbed freely by plants. In 1981, Rockwool was first used in Australia in hydroponics culture of cut flowers (Wahome, Oseni, Masarirambi & Shongwe, 2011). The hydroponic media that commonly being used are in the form of solid, inert materials such as peat, vermiculite, and solid plus inert materials, coconut coir, sawdust, sand, gravel, Rockwool, expanded clay, perlite, brick shards and polystyrene or marbles to supports the plant roots (Wahome et al., 2011).

2.3 Effects of Aquaponic Culture

2.3.1 Effects on Tilapia (*Oreochromis nilloticus*)

In farming, including aquaculture, the cost of land, constructions and fish feed are the highest in the farm investment (Ahilan et al., 2011). Hence, these problems could be avoided within the system called integrated farming. The most common integrated system being commercialized by the farmers and communities is aquaponics. Since aquaculture farming also contributed to the environmental pollution from the waste and manure of the fishes, hence this aquaponics could reduce the pollution by nutrient recycling to the crops cultured. Most of the system in recent study were using tilapia as the fish species in the aquaponics, hence this could increase the production thus yield hygiene and better quality of tilapia (Effendi et al., 2016; Graber & Junge, 2009; Love et al., 2014; J. Rakocy et al., 2004).

From the aquaponics system itself, the nitrification process gives plentiful advantages to the cultured tilapia. The ammonia from the fish manure will be transformed by nitrification bacteria through nitrification process to the more safe substances or gaseous form. The substance then being absorbed by the aquaponic plants roots and act as biological fertilizer (Effendi et al., 2016). In return, the plant will gives back its oxygen and nutrients as they will removed those substance in the water to the condition that valuable to the tilapia (Graber & Junge, 2009). Hence, there will be less pollution or accumulated suspended substance in the tilapia tank.

2.3.2 Effects on Mustard plant

The integration of agriculture with aquaculture is the finest method to save water usage in farming activities, appropriate disposing aquaculture wastewater with providing fertilizer to the crops. Manure discharges from fish have nutrients, organic and inorganic compounds such as ammonium and phosphorus. As nitrification process occurs, the manure from the fish tanks will be biodegraded or transformed to suitable nutrient for plant that acts as fertilizer. Plant demanded the main nutrients such as nitrogen, phosphorus and potassium. Those contents are not being fully absorbed by fish during feeding. Hence, it present in the fish manure which considered as competent amount for plant as fertilizer when being transformed through nitrification process. Mustard green is able to downturn the wastewater pollution rate of aquaculture. The diversity in the structure and formation of roots interpret crucial consequences for the deprivation of wastewater components and uptake of nutrients (Enduta et al., 2011). For sure, this will reduce the cost for crops culture in terms of fertilizer cost because the organic nutrients obtained through wastewater treatment.

2.4 Nutrient requirements

2.4.1 Nutrient Requirements of Tilapia

Protein requirement for larval stages of tilapia is the highest, which around 35% to 50% and it will decreases when the size of the fish increases. Protein is the biggest needs of nutrient in fish feed. According to El-Sayed (2004), for juveniles, they need 30% to 40% of protein, while adult tilapia requires 20% to 30% of dietary protein. Tilapia brood stock demanded 35% to 45% of dietary protein in order to get the maximum reproduction, spawning efficiency, larval growth and survival. There are ten essential amino acids required for the fish such as arginine, lysine, histidine, valine, leucine, methionine, phenylalanine, and tryptophan. However, none of the specific requirements of essential amino acid had been determined by research for tilapia (El-Sayed, 2004). For fingerlings, the amount of nutrients required is similar as other stages of tilapia, but the percentage must be slightly higher especially protein for growth performance.

Approximately 15.4 g/kg of digestible lysine and 25% to 30% dietary protein is demanded for Nile tilapia fingerlings (Furuya et al., 2012; Millamena, 1994). Vitamin, lipid, minerals, carbohydrates and energy are also needed in certain quantities for all growth stages. Lipids should be available as 15% in fish feed, to supply essential fatty acids (EFA) and act as transporters for fat-soluble vitamins. Carbohydrates are kept as glycogen and transported to supply energy for fish. Carbohydrate is known as major energy source for mammals, but not used efficiently by fish. Hence the amount may lesser.

Micro-minerals which are the trace minerals that are less needed and act as components in enzyme and hormone systems. Common trace minerals are copper, chromium, iodine, zinc and selenium. Fish can obtain varies amount of minerals from water with the aid of their gills and skin. This is in order to allow them to recoup to some extent for lack of minerals in their diet (Steven, 2009). The amount of energy obtained from fat is only 4% to 8% of the diet. Higher fat content is fed to smaller size of fish and will gradually decreased when fish size increases.

Majority of fish species utilize only 20% to 30% of nitrogen supplied by the feed given. Approximately 70% to 80% of the nitrogen from feed when it is being released in water as waste. Phosphorous retention by fish are within 15% to 40% and released as waste into the water by 60% to 85% (Lazzari & Baldisserotto, 2008).

2.4.2 Nutrient Requirements of Mustard

There are many nutrients needed by the plant for growth. The most nutrients needed are nitrogen, phosphorus and potassium. Nitrogen is highly demanded but it is mostly limited nutrient for mustard culture. In a study done at western Canada, mustard showed positive responds when nitrogen being added in the fertilizer (Silva & Uchida, 2000). The primary nitrogen that will be takes up by the roots is known as nitrate (NO_3-). In photosynthesis and respiration, phosphorus acts as main role in energy storage which it being transferred as adenosine diphosphate (ADP), adenosine triphosphate (ATP), diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN). Phosphorus deficiency caused poor maturity. Phosphorus also leads to poor development of seed and fruit. Presence of potassium is crucial for plant growth because potassium is known as an activation agent of enzyme that promotes metabolism. Potassium assist in regulating the usage of water by plants by controlling the opening and closing of leaf stomata (Silva & Uchida, 2000).

2.5 Diet Types in Aquaponics

2.5.1 Azolla pinnata

According to Alalade & Iyayi (2006), green plants is the most economical and plenty potential of protein source. This is due to its ability to synthesizing amino acids from an unlimited sources and readily available primary materials. *Azolla pinnata* is one of tiny aquatic plants or freshwater ferns which have the symbiotic relationship with nitrogen fixing cyanobacteria, *Anabaena azollae*. It has six species distributed widely throughout warm temperate and tropical zones.

Azolla plant is a fern frond which the main stem growing at the surface of the water, with alternate leaves and adventitious roots at regular intervals along the stem. Secondary stems develop at the axil of certain leaves. Plant diameter is from 1 cm to 2.5 cm for small species, such as *A. pinnata*, and more than 15 cm for *A. nilotica*. A shallow fresh water pond which identical to the environment found in a taro is the best environment for Azolla. When taro reaches their matured age, it shades out the Azolla below the canopy and kill them. Hence there will be nutrient release into the soil-water system, available for taro plants.

Azolla pinnata act as free food source that rapidly self-propagating that can fixed about 450 kg of N/ha annually and substitute 50% urea (Naz, Mushfaqua, Afrin & Azam, 2014). It had been used for century as fertilizer for rice fields and supplement for pigs and poultry. The use of Azolla as fish, swine and poultry feed resource had been tested with favorable results from previous study (Alalade & Iyayi, 2006). In conjunction, according to Naz et al. (2014), *A. pinnata* had been used as fish supplement for long period of time. The production of *A. pinnata* is big around 1000 kg to 2000 kg/ha/day and it is identical to the production of 10 kg to 30 kg protein (Sudaryono, 2006).

According to Almazan & Pullin (1983), if fish could utilized *A. pinnata* efficiently and economically, it will gives massive impact to aquaculture industries. The previous study was done to analyze the aspects regulating the growth of *Azolla filiculoides* from Japan in eutrophic ponds, its nutrient composition, and the possibility of its usage as fish feed (Shiomi & Kitoh, 2001). Fish need protein-rich diet than those of commercially cultured animals. As protein represents the most costly component in formulated diet, it is important to determine the best level that will support maximum growth performance and survival (Sithara & Kamalaveni, 2008).

Azolla are being used in the feed by dried form and mixed with the commercial feed ingredients, giving dried azolla as supplement or freshly fed to the fish. According to El-Sayed (1999), Azolla could replaced fish meal for tilapia fingerlings and adults respectively, at 0% to 100% of substitution levels. Azolla showed extremely bad performance even at the lowest inclusion level of 25% with *O. niloticus* and *Tilapia rendalli* but produced better growth rates of Nile tilapia fry with diet containing up to 42% of *A. pinnata*. Sudaryono (2006) also had test the replacement of soybean meal with Azolla meal in the commercial pelet ingredients feed for black tiger shrimp juvenile. The result shows that Azolla meal can replace up to 100% of soybean meal as a plant protein source in juvenile *Penaeus monodon* diets without adverse effects on growth, survival, feed intake and feed conversion ratio, which lead to downturn of feed cost. According to Almazan & Pullin (1983), an experiment of giving tilapia fingerlings with solely fresh Azolla at different levels which are 5%, 10% and 20% from body weight which resulted only slightly inrease after 28 days. In an experiment conducted by Shiomi & Kitoh (2001), the diet which contained 20.7% Azolla, shown the same outcome as control group fed with commercial feed, which is highly significant.

In a previous research, Azolla can be effectively substituted with the commercial feed with half of the amount of feed for tilapia production. A 50-50 combination of fresh Azolla and commercial feeds had been resulted a higher tilapia growth rate than solely commercial feeds (Kathirvelan, Banupriya, & Purushothaman, 2015; Sudaryono, 2006).

2.5.1.1 Nutrients Composition of *A. pinata*

Azolla is a good source of protein. Apart from appreciable quantities of vitamin A and vitamin B12 it contains almost all essential amino acids, minerals such as iron, calcium, magnesium, potassium, phosphorus and manganese (Cherryl et al., 2014). It is a potential source of fish fodder due to its high yield, enriched nutrients, good edibility, and lower feed coefficient (Naz et al ., 2014).

The nutrients composition of *A. pinnata* are crude protein with 27% ash, 10.5% and 9.1% crude on a dry weight basis (Almazan & Pullin, 1983). Meanwhile, according to Shiomi & Kitoh (2001), Azolla contain high crude protein (20.3% to 31.2%), but low crude fiber (9.2% to 11.3%) and ash (9.0% to 9.3%) expressed on a dry weight basis. Crude lipid content in Azolla ranged from 6.0% to 6.7% with high nitrogen-free extract content (35.1% to 46.2%). A necessary amino acid, lysine was comparatively more sufficient than in the reported amino acid composition of other aquatic plants though the proline, methionine, and histidine levels were lower than those in commercial feed (Almazan & Pullin, 1983) . Table 1 shows the nutrients composition of *A. pinnata.*

Table 2.1: Nutrients Presents in *Azolla pinnata*

Constituent	Percentage Dry Weight
Nitrogen	4.5
Phosphorus	0.7
Potassium	3.3
Crude protein	27.0
Crude fat	3.2
Crude fiber	9.1

Source: (Almazan & Pullin, 1983)

Higher protein content of feed was considered to be a suitable feed for tilapia. Analysis of ascorbic acid and fatty acid levels of fresh Azolla had larger amount of dehydro-ascorbic acid than L-ascorbic acid. According to Sithara & Kamalaveni (2008), the protein content in fish liver that fed on dried Azolla diet showed an increased rate from 1.45 mg/g to 2.65 mg/g per wet tissue and in muscle, 1.41 mg/g to 2.38 mg/g per wet tissue. This show significant increased and high of protein conversion ratio for fish that fed on diet contained dried Azolla. Carbohydrate content in liver and muscle of fish fed with Azolla diet showed an increment rate from 0.90 mg/g to 2.30 mg/g and 0.65 mg/g to 1.76 mg/g wet tissue respectively. The increase in carbohydrate content in muscle and liver of fish fed with azolla indicated that the mobilization and utilization of glycogenic amino acids for the formation of carbohydrate in the liver and muscle. Lipid content in liver and muscle of fish fed with Azolla diet change from 0.212 mg/g to 0.81 mg/g and 0.292 mg/g to 2.00 mg/g wet tissue respectively. The increase of lipid content suggests the fewer uptakes of lipid components by tissues for utilization(Sithara & Kamalaveni, 2008) .

2.5.2 Commercial Pellet

According to Love et al. (2014), a common type of feed used in aquaponics is feed pellet. Artificial feed or also known as commercial pellet are formulated feed. It must be processes to contain nutrient requirement demanded by fish such as protein, carbohydrates, lipids, minerals and vitamins (Al-Shamsi et al., 2006).

The commercial aquatic feeds production has been traditionally used fishmeal as the major protein source due to its high protein content and balanced essential amino acid profile. Fishmeal is a nutrient-rich feed ingredient which commonly used for poultry and other domestic animals diet. It can be made from almost any type of seafood from wild-caught of small marine fish. Small marine fish had high percentage of bones and oil which are not convenient for direct human consumptions. The nutritive value of fish meal varies depends on their input sources, harvest area and addition of salt for preservation. Incorporation of fish meal to artificial diets boost feed efficiency and growth of fish, through higher feed palatability and boost nutrient uptake, digestion, and absorption (Khan et al., 2012).

Fishmeal is a great source of essential fatty acids, digestible energy, minerals, and vitamins. Fishmeal contribute a balanced amount of all essential nutrients including amino acids, phospholipids, fatty acids and mineral content for ideal development, growth, and reproduction for fish larvae and brood stock (El-Sayed, 1999). The range value of gross energy, fat, dry matter contents, protein, fiber content, ash, and phosphorous diversified from 9.90% to 29.52%, 88.43% to 93.29%, 37.49% to 66.57%, 2.23% to 12.67%, 12.74% to 28.22% and 0.10% to 1.0%, respectively (Khan et al., 2012). Some of the farmers in intensive farm will formulated the pellet feed by themselves according to the nutrient requirements of their cultured fish. Due to primary usage of animal-based protein from fish meal, it leads to elevation supply of fishmeal. This caused the ascending prices and development of alternative protein sources.

In a research conducted by Medina et al. (2016), the result of feeding the plant-based protein and animal-based protein to tilapia showed greater fish mean weight in control group compared to group fed by plant-based protein feed.

CHAPTER 3

METHODOLOGY

3.1 Materials and Equipment

The equipment and apparatus that had been used in this study were twelve aquariums sized 45cm x 30cm x 30. Six aquariums were act as hydroponic tanks while another six aquariums were act as fish tank. Six plant trays sized 43cmx 28cmx 28cm as hydroponics floating raft, polystyrene board that had been cut to hold the tray to float, six water pumps, clay pebbles, peat moss, aerators, multi parameter device, analytical balance, ruler, seed germinating tray, pipes, basins, fish net and strainer. Two kilogram of commercial, 60 tilapia fingerlings sized 2 inches in length; mustard seeds, *A. pinnata* and anti-chlorine crystal were used.

3.2 Methods

Mustard seed were purchased at Mr. Azroyfiq Fertigation Resources farm at Machang. Mustard seed germination was done two weeks prior to aquaponic culturing period. Two batch of mustard germination were done. For first batch, a seed germination tray was prepared. Mustard seed were soaked in water for few minutes. Sponges were cut into cubes to fit in the hole of hydroponic tray. The sponges were cut a little bit at the center to place the seed inside, and were soaked in the water. Seeds were placed in the middle of each of the sponges, and then the sponges were placed in each hole of the germination trays. The germination trays were placed in the container filled with water to maintain the moisture to the sponges and seeds. Due to poor result and no significance different between two treatments of the first batch, it was discarded from the experiment. For second batch, same method was applied, but mustard seed were planted in different medium, replaced the sponges to peat moss in a same seed germination tray.

3.2.1 Installation of Aquaponic Design

The aquaponic systems were divided into two treatments groups, Treatment 1 and Treatment 2 with three replicates. Treatment 1 were using commercial pellet while Treatment 2 were using commercial pellets and *A. pinnata* as diets for tilapia. The aquariums that had been set up with connected pipes were altered a little bit to suit the design and needs in the experiment. Six water pumps were installed for both groups to pump up to the hydroponic tank.

3.2.2 Preparation of Tilapia Culture

The aquariums were filled with water for approximately 25.5 liters respectively with tap water. Anti-chlorine were added and left in the water or aerated overnight. Ten tilapia fingerlings were added in each aquarium for both groups. Aerators were switched on for aeration in the aquariums.

3.2.3 Preparation of Mustard Culture

Each of the hydroponic aquariums was filled up approximately 25.5 liters tap water. Polystyrene boards were cut into few pieces and pasted around the plant trays to float the tray as floating raft. Sponges with mustard seedling of two weeks old were placed in each hole of plant tray for respective hydroponic tank. The mustard plants were cultured within float rafts design of hydroponics, with the roots will be grow vertically downwards in contact with water that will be enriched with the useable nutrients obtained from tilapia manure.

3.2.4 Preparation and Culturation of *A. pinnata*

One kilogram of *A. pinnata* was purchased from a farmer in Bachok, through postage. The Azolla were then washed thoroughly before being transferred into six basins. Six containers were filled with tap water and approximately one table spoon of organic fertilizer were put before dissolved it in the water. Few *A. pinnata* were placed in the basins as long as it is not too packed on the surface of water. The basins were put at the areas that have sunlight but not too direct sunlight.

3.2.5 Calculation Amount of Feed

The feeding rate for tilapia fingerlings that was used in this study was 8% from the fish body weight. The average of initial body weight of tilapia for both treatments was

2 g. The feeding rate was calculated based on average weight of individual replicates for each treatment. However, the feeding rate was changed to 6% started on week eight until the end, due to the increased of the fish weight. The example of calculation formula amount of feed given based on the fish feeding rate and weight was as below;

Amount of feed (g) = Feeding rate (in percent) x Weight of Fish (g)

$$= \frac{8}{100} \times 2g$$

$$= 0.16g$$

3.2.6 Feeding of Tilapia

The fingerlings were fed with two diets, commercial pellet and commercial pellet with fresh *A. pinnata* respectively. The feeding rate of tilapia fingerlings was eight percent of body weight. Tilapia being fed thrice per day which were in the morning, afternoon and evening. Unfed feed were collected and removed from aquariums after one hour of feeding.

3.2.7 Maintaning the Aquaponic System

The water condition were observed and controlled every week. The loss of water in the tanks due to evaporation was replaced with approximately two liter of dechlorinated water respectively. Any suspended manures and algae in each of hydroponic tanks and fish tanks were removed.

3.2.8 Analysis of NPK Elements in Aquaponic

The sample from each treatment were collected and sent for X-ray Fluorescence (XRF) analysis, to determine the nitrogen, phosphorus and potassium concentration for each treatment.

3.2.9 Data Collection

The data collections were performed for 15 weeks for tilapia and five weeks for second batch mustard. The culture of mustard plant and tilapia in aquaponic design were observed daily. The water quality of the tilapia culture such as pH, temperature and dissolve oxygen level were observed and recorded. The parameters of growth performance for both cultures were the weight of the fingerlings and mustard plant and they were recorded weekly. The initial fingerling weights of the fish were recorded as ten samples were taken from each of the treatment. Ten samples from each treatment were recorded for average weight of the fish in every week. The final weight of the fish was weighed from the sample of each treatment.

3.2.10 Data Analysis

The results were tabulated and calculated with statistical analysis in order to get the overall results. One way ANOVA test were used to analyze the result. The differences between the efficiency of diets in Treatment 1 and Treatment 2 could be seen based on the test analysis. The analysis was done by using SPSS software version 10.0.

CHAPTER 4

RESULT

This current study was to investigate the effect on mustard production in aquaponic system associated with tilapia culture using two different types of fish diets which are commercial pellet and commercial pellet associated with *A. pinnata.* There were two major groups based on fish diets where Treatment 1 used commercial pellet, while Treatment 2 used commercial pellet associated with fresh *A. pinnata*. This study had been done for 15 weeks where there were two batches of mustard cultured been done. The first batch was cultured from the first week until Week 10 while the second batch of mustard cultured was continued from Week 8 until Week 15 including germination. Meanwhile, the tilapia cultured was done for the whole 15 weeks starting from the week two until week 15. Water quality parameters in the aquaponic system were recorded twice per week.

4.1 Mustard Growth Performance

The data for the first batch of mustard culture was recorded for ten weeks included two weeks of seed germination. Due to the stunted growth of the mustard this culture had been discarded. However, this study was continued by culturing the second batch mustard.

The second batch of mustard cultured was carried out for seven weeks, including two weeks of seed germination. The second batch data performances showed better results than the first batch. According to Table A.1, Treatment 1 showed higher final number of leaves in Week 5, with average mean of 8 ± 0.35, while Treatment 2 with 6 ± 0.52. The height of mustard in final week in Table A.3 also showed higher in Treatment 1 with 7.56 ± 0.36 cm, while Treatment 2 with 4.22 ± 0.20 cm.

Figure 4.1 which showed the average number of mustard leaves over weeks, Treatment 1 and Treatment 2 started with similar initial numbers of leaves (n=3). During the culturing period, Treatment 1 showed better and had higher amount of leaves than Treatment 2, which gradually increased by weeks. The average number of mustard leaves at final week also showed Treatment 1 had better performance with more than eight leaves, compared to Treatment 2, with average of six leaves (Figure 4.1).

Figure 4.2 showed the average height of mustard over weeks where both treatments started with similar initial height of mustard, approximately 2.30 cm respectively. The height continued arises steady until Week 2 with average of 2.58 cm for both treatments. Then, the height started to rose dramatically for Treatment 1 from Week 2 until final week with 6.92 cm of height, while Treatment 2 rose slowly from week two until final week with only reached 3.90 cm.

The final weight of mustard had been recorded at the last week of the study. The final average weight of mustard for Treatment 1 was 7.13 ± 0.22 g while Treatment 2 of 1.68 ± 0.04 g (Table A.6). In addition, there was a statistically significant difference in mean which p value less than 0.05, for mustard height, number of leaves and final weight of mustard for Treatment 1 and Treatment 2 in the aquaponic system (Table A.2, Table A.4 and Table A.6)

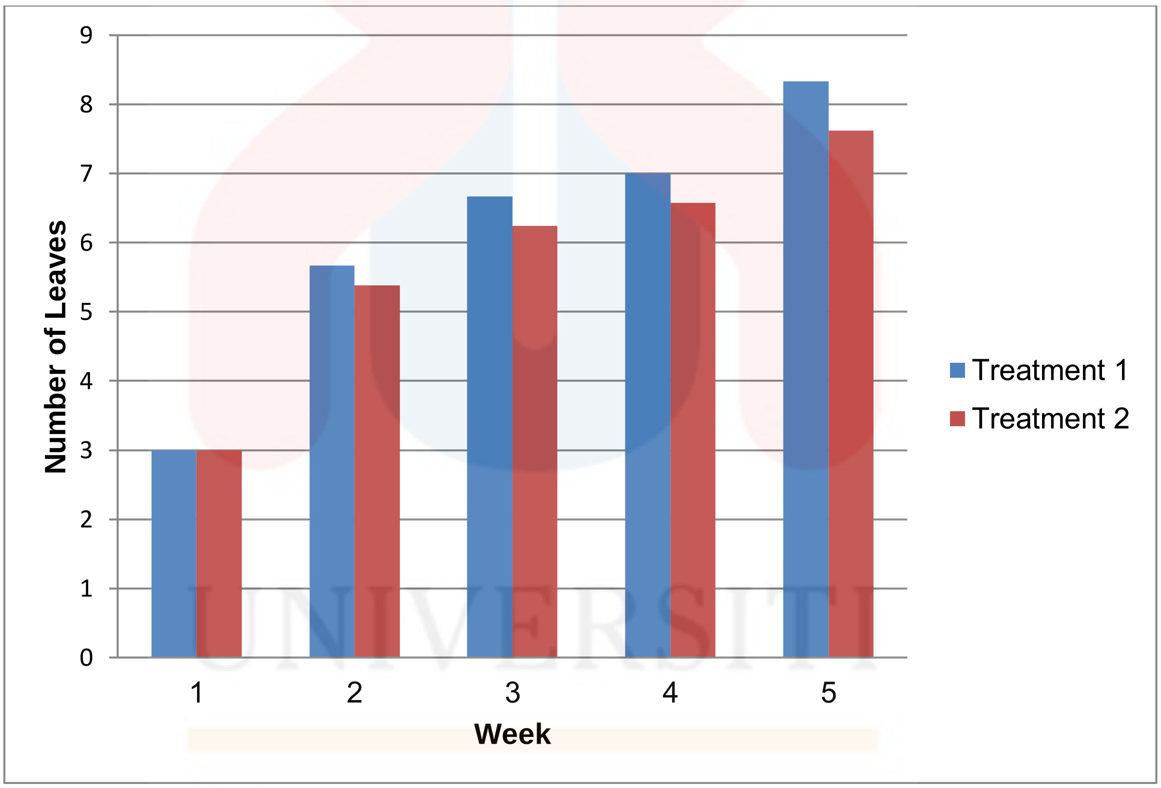

Figure 4.1: Number of leaves of mustard variation between different types of fish diets contain with and without *A. pinnata* in aquaponic system.

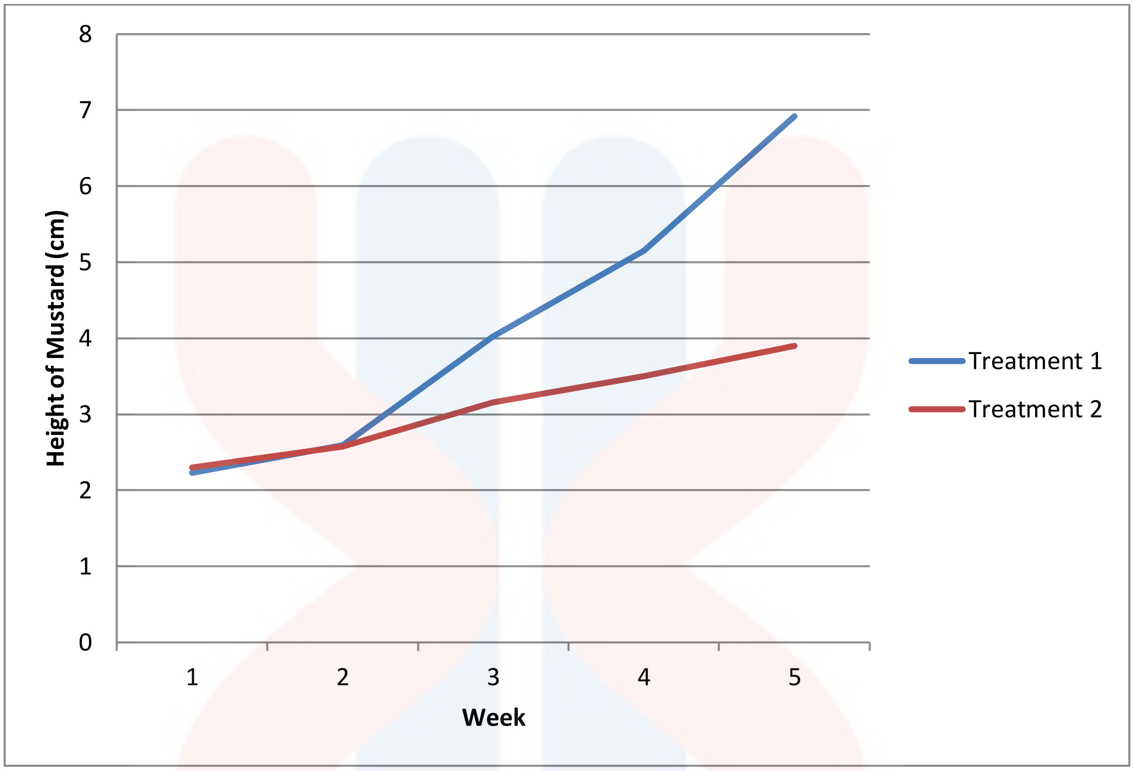

Figure 4.2: Height of mustard over weeks between different types of fish diets treatment with and without *A. pinnata* in aquaponic system.

4.2 Tilapia Growth Performance

From the Figure 4.3, it showed the average weight of tilapia over five weeks. During the initial weight, both treatments had started with almost similar weight of 1.38 g and 1.63 g respectively, and continued increased steadily until Week 4. From Week 5, Treatment 1 showed better performance with rapid growth and the value were steadily increased until the final week with average mean weight of 51.31 ± 3.68 g. Meanwhile Treatment 2 showed slow increased from Week 4 until final week with final weight of 23.16 ± 2.04 g.

The average length of tilapia was shown in Figure 4.4. It showed that Treatment 1 and Treatment 2 also started with almost similar lengths, which were 4.42 cm and 4.45 cm respectively, and maintained stable increased until week four. Started from week four, Treatment 1 showed better growth performance until the final week with length of 13.99 ± 0.37 cm. Meanwhile Treatment 2 only demonstrated poor growth performance compared to Treatment 1 with record of 11.22 ± 0.33 cm in tilapia body length. Treatment 1 and Treatment 2 had significance different with p value less than 0.05 for both final weight and length of tilapia as shown in Table B.2 and Table B.4.

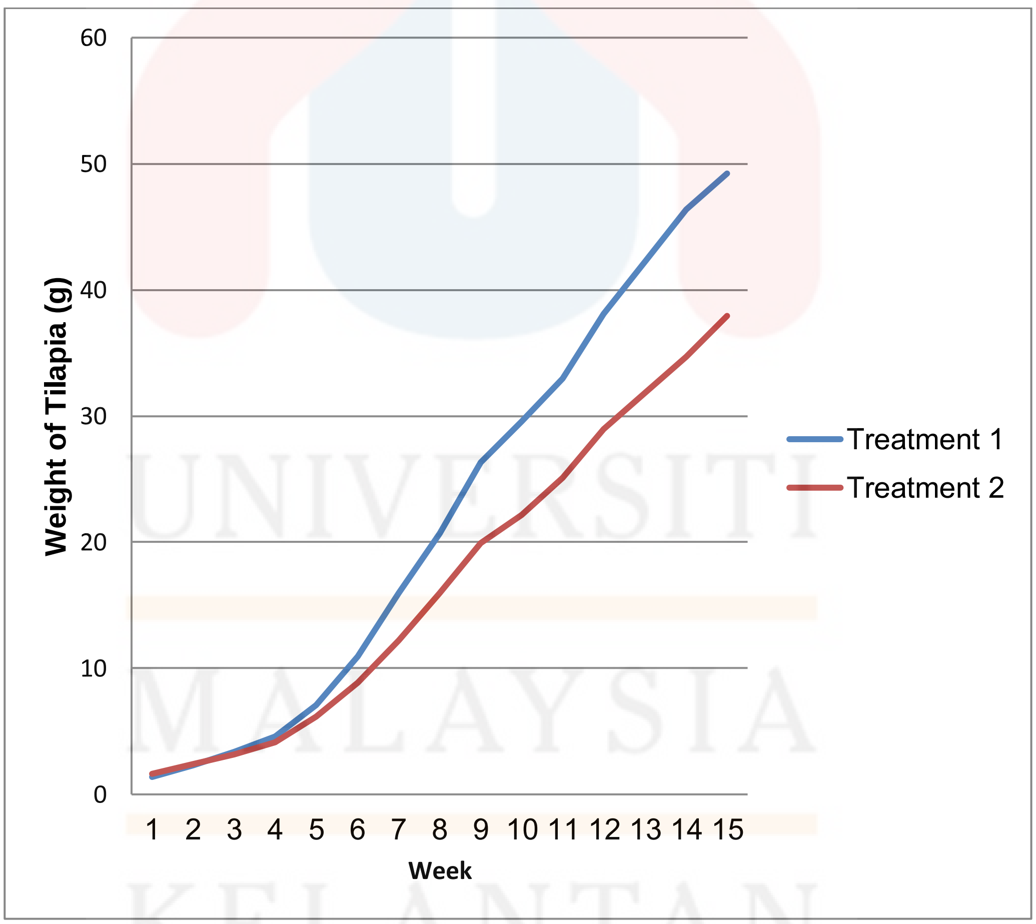

Figure 4.3: Weight of tilapia variation between different treatments of types of fish diets contain with and without *A. pinnata* in aquaponic system.

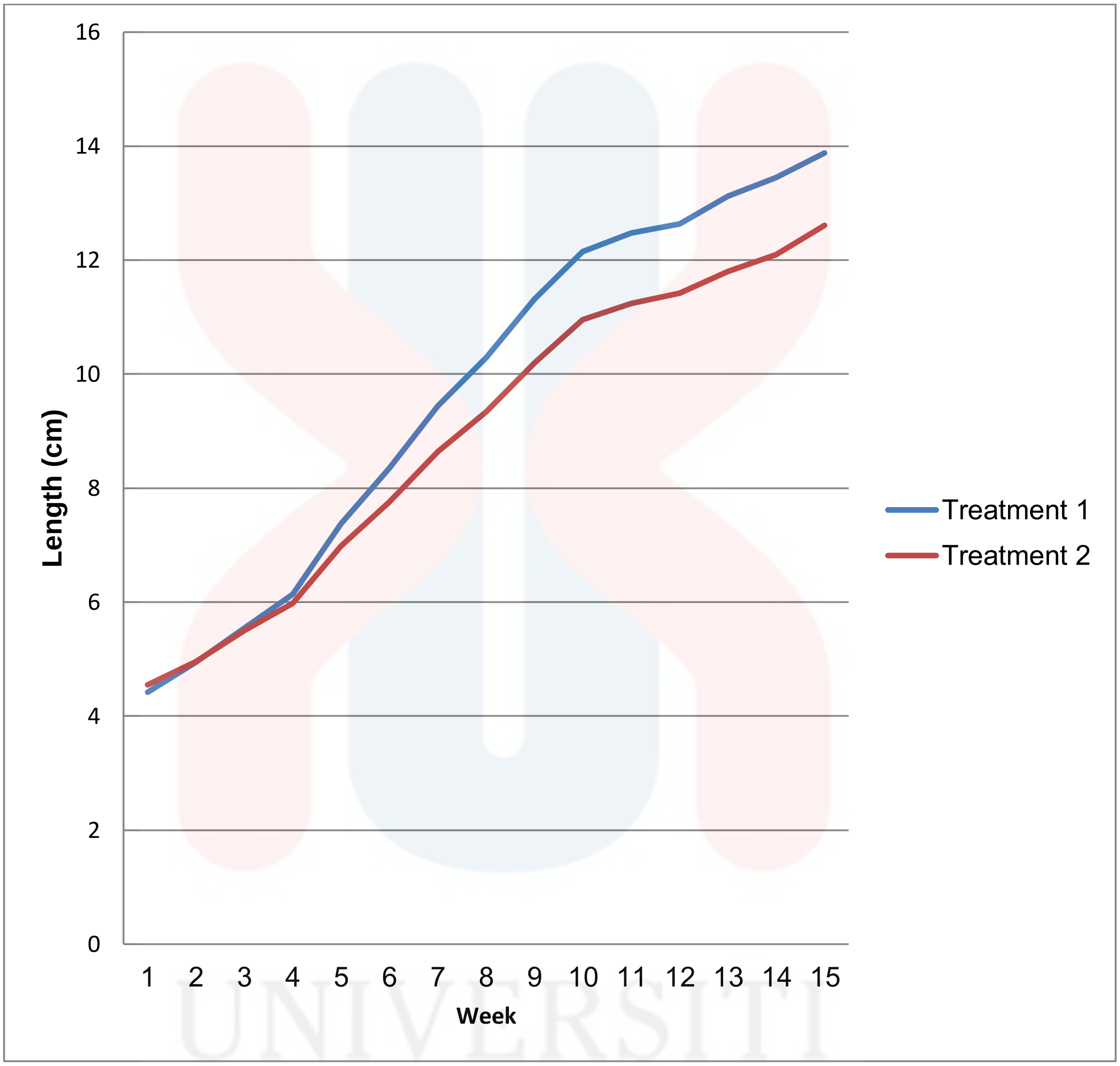

Figure 4.4: Length of tilapia variation between different treatments of types of fish diets contain with and without *A. pinnata* in aquaponic system.

4.3 Water Quality of Aquaponic

Fish and plants are depending on the equilibrium of dissolved nutrients and water quality. Balanced dissolved nutrients and water quality were essential for production of plants and healthy fish (Filep, Dianconescu, Marin, Badulescu and Nicolae, 2016). The water quality parameters in this study were recorded twice a week for monitoring purpose but the initial reading when the system running without the fish and plants were not recorded.

4.3.1 Temperature

Based on the finding in Figure 4.5, the temperature readings showed fluctuation but in similar pattern for Treatment 1 and Treatment 2. The reading of both treatments started with almost similar reading, and stable. During Week 3, Week 6 and Week 9, it had started to fluctuate increased. The increased fluctuation reached the peak on the Week 4, Week 7 and Week 10 with the reading of 2.7^{0}C, 28.7°C and 28.3°C for Treatment 1. Meanwhile for Treatment 2, the peak of point of increased fluctuations were at 27.7°C, 28.2°C and 27.8°C, before started to decreased again. On the Week 11, the temperature were increased steadily until final week with temperature of 28.2°C for Treatment 1 and 27.7°C for Treatment 2, with final reading of 28.5°C and 28.5°C for Treatment 1 and Treatment 2 respectively.

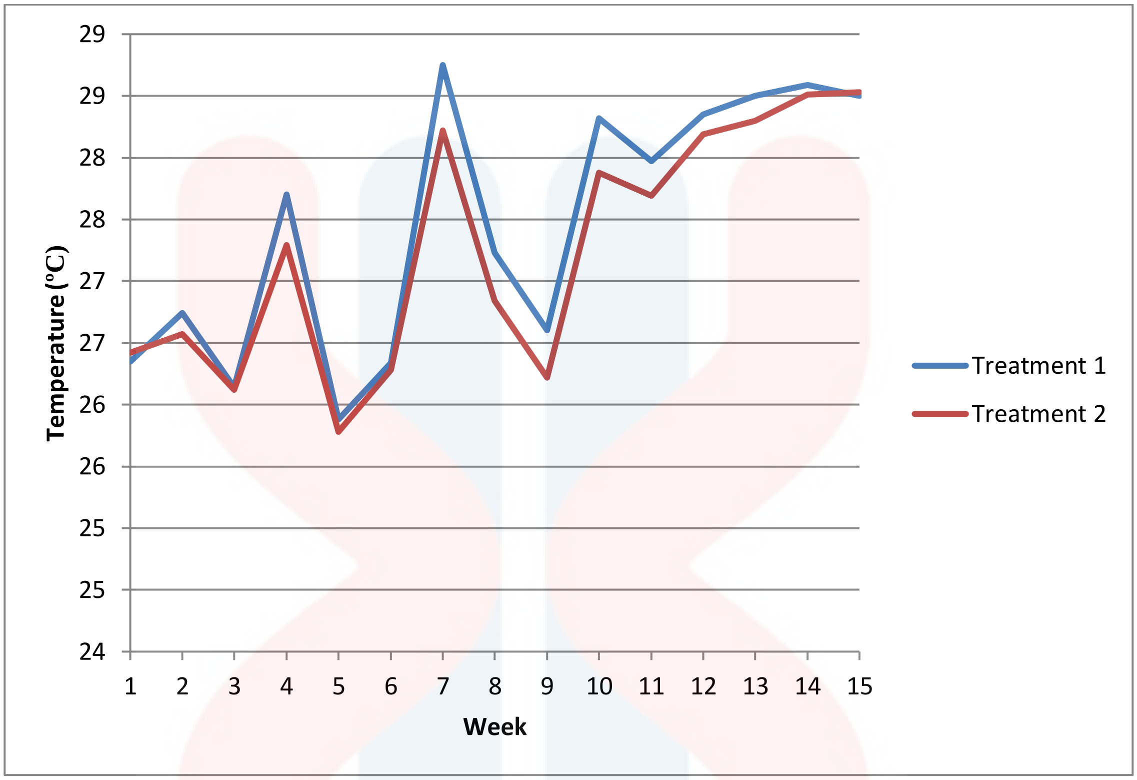

Figure 4.5: Temperature of aquaponic system of Treatment 1 and Treatment 2.

4.3.2 D.O

According to Figure 4.3.2, the dissolve oxygen (D.O) reading in Treatment 1 and Treatment 2 showed fluctuation in most of the weeks and the D.O level continued dropped from initial until final week of experiment. The initial D.O reading was recorded almost similar for Treatment 1 and Treatment 2 which were 6.67 mg/L and 6.64 mg/L respectively. However, during Week 9, the D.O reading showed extreme fluctuation until Week 11 for both treatments. Treatment 1 showed fluctuated from 6.23 mg/L (Week 9) to 4.03 mg/L (Week 11), while Treatment 2 showed fluctuation from 6.09 mg/L (Week 9) to

4.12 mg/L (Week 11). Final D.O reading showed Treatment 1 and Treatment 2 also recorded the similar readings, which were 4.83 mg/L and 4.86 mg/L respectively.

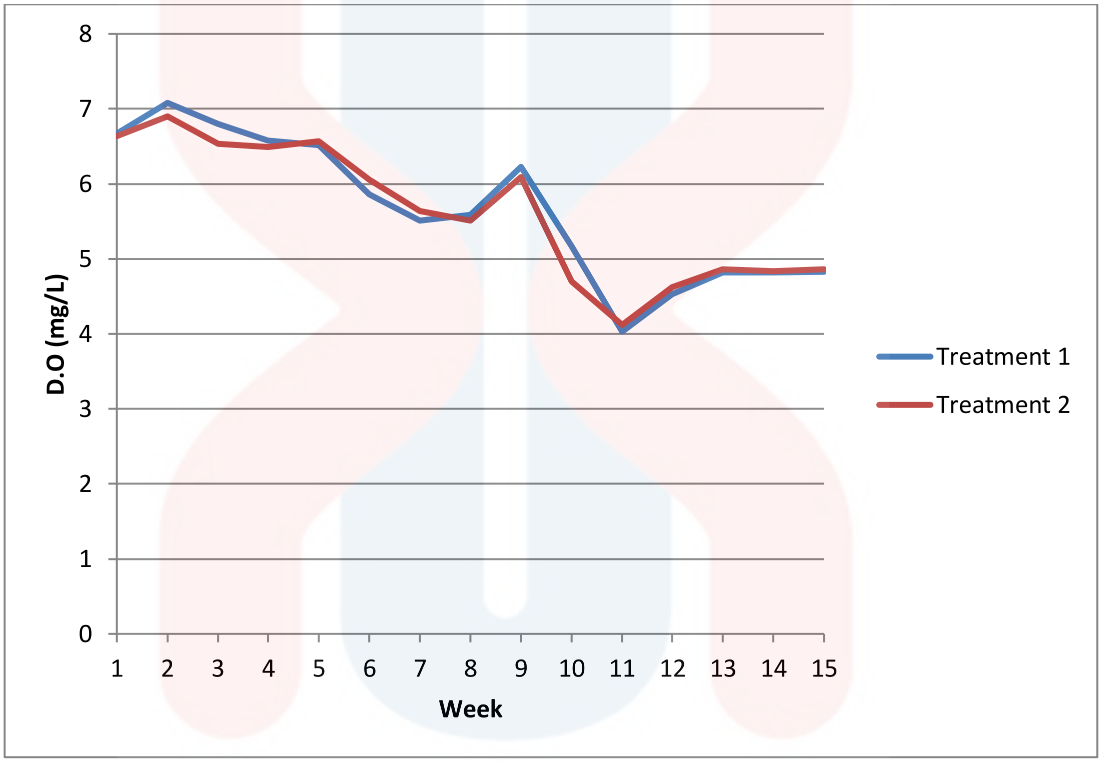

Figure 4.6: Dissolve oxygen reading of aquaponic system of Treatment 1 and Treatment. 2.

4.3.3 Salinity

Figure 4.7 showed the salinity of the two treatments for the 15 weeks. Treatment 1 and Treatment 2 showed similar initial salinity reading, which was 0.04 ppt respectively. The readings were increased steadily for both treatments until Week 4. Started from Week 4, Treatment 1 showed higher salinity and continued steadily increased until Week 10 with reading of 0.48 ppt. Meanwhile Treatment 2 increased slower than Treatment 1 until Week 11 with reading of 0.46 ppt. Treatment 1 showed steadily decreased from Week 10 to final week with final reading of 0.41 ppt, while Treatment 2 also showed steadily decreased from week eleven until final week, with final reading of 0.41 ppt. Both treatments had similar pattern of salinity reading, but Treatment 2 shows slightly lower salinity level than Treatment 1.

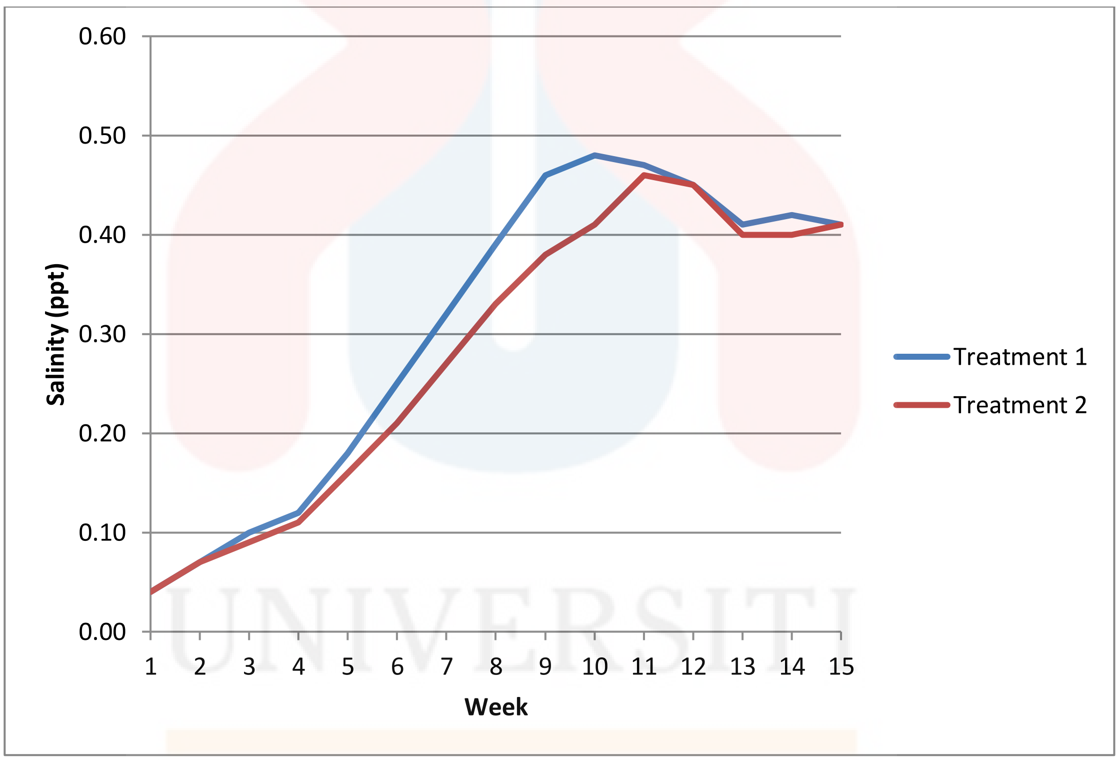

Figure 4.7: Salinity reading of aquaponic system of Treatment 1 and Treatment 2.

4.3.4 pH

Based on Figure 4.8, it showed the pH reading for both treatments over weeks. The initial pH reading of Treatment 1 and Treatment 2 was almost similar which was pH 7.17 and pH 7.01 respectively. It continued steady between pH 7 and pH 8 for initial until Week 3, for both replicates. On Week 3, pH for Treatment 1 and Treatment 2 started to decline dramatically until Week 4 with reading of pH 6.6 and pH 6.35. From Week 4 until Week 10, Treatment 1 and Treatment 2 showed the pH continued steady between pH 6 and pH 7. Started on Week 10, the pH for both treatments rose dramatically until the peak with pH 8.92 for Treatment 1 and 8.86 for Treatment 2 at Week 11. Then it fall steadily from Week 11 towards the final reading, with final pH 7.56 and pH 7.54 respectively.

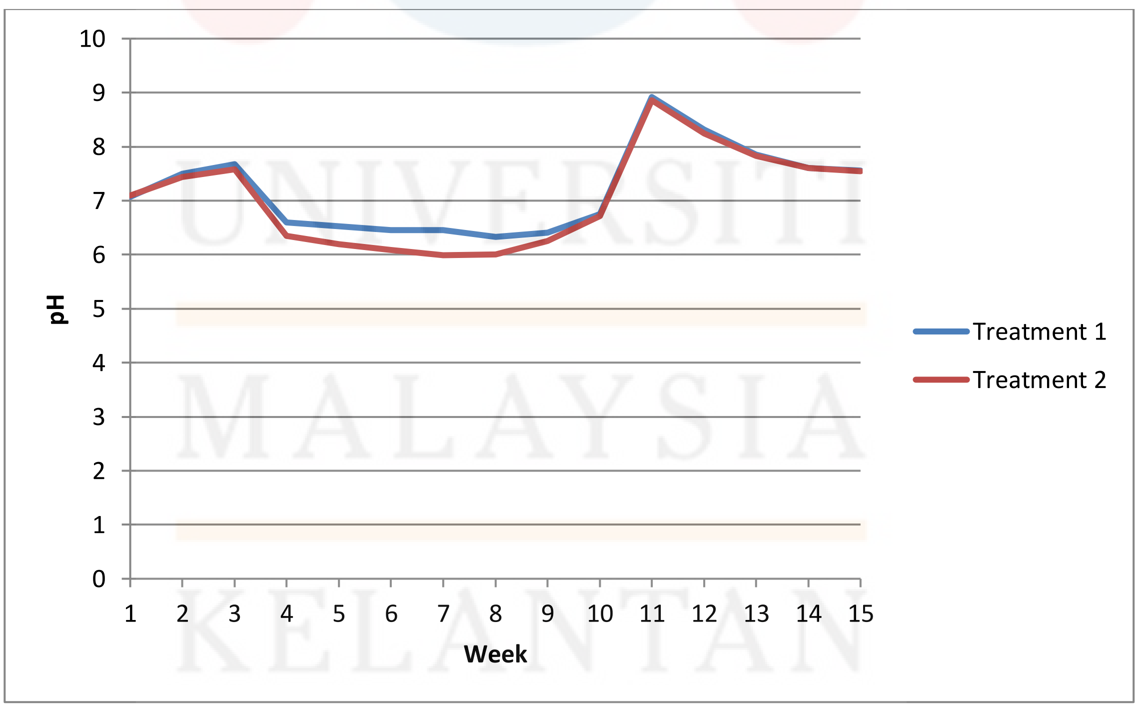

Figure 4.8: pH reading of aquaponic system of Treatment 1 and Treatment 2.

4.4 Analysis of NPK Elements

The nitrogen, phosphorus and potassium (NPK) elements in the aquaponic system were analyzed using X-ray Fluorescence (XRF) machine. The results showed there were vary concentration of those elements in Treatment 1 with diet contain commercial pellet and Treatment 2 with diet contain commercial pellet and fresh Azolla. According Table 4.1, nitrogen concentration in the both treatment were at the same percentage while phosphorus concentration showed slightly higher in concentration in treatment with commercial pellet as fish diets. Meanwhile, there was no detection of potassium in both treatments, which might due to low concentration of this element. From the results, it indicated that Treatment 1 had better elements contents needed by plants compared than Treatment 2.

Table 4.1: The analysis of NPK elements of water sample in both treatments of aquaponic.

	Elements Concentration (%)		
Treatment / Code	N	P	K
Treatment 1 / AQCC	0.21	0.0972	-
Treatment 2 / AQCT	0.21	0.0819	-

CHAPTER 5

DISCUSSION

This study was conducted to identify the effect on mustard production in aquaponic system associated with tilapia culture using two different types of fish diets which are commercial pellet and commercial pellet associated with *A. pinnata.* Treatment 1 used commercial pellet as feed for tilapia diet, while Treatment 2 used commercial pellet associated with fresh *A. pinnata* as feed for tilapia diet.

5.1 Mustard Growth Performance

According to Burris (2001), plant growth is directly depending on enough supply of nitrogen that had been fixed. Fixed nitrogen and water are the most common limiting factors for plant growth. Nitrogen that present in atmosphere is in form of inert gas, N_2, and must be converted chemically or biologically into a more active and safer compound before plants can utilize it. Nitrogen fixation is a process by which free nitrogen from the atmosphere is combined with other element to form inorganic compounds. This conversion is known as nitrification which turned the compound into useful form to be absorbed by plants and other organisms. Symbiotic biological nitrogen fixation occurs in leguminous and non-leguminous plants, and nitrogen is also fixed biologically by free-living and associative nitrogen fixers (Burris, 2001).

In aquaponic, there is also nitrification process which ammonia is converted to nitrites (NO_2-) and then nitrates (NO_3) where it is carried out by specialized bacteria. *A. pinnata* is known for their nitrogen fixation characteristics, which could fix the free nitrogen in the air into useful substance to the plant. Hence, it indicated that the treatment that consists of *A. pinnata* will gives better result to the plant growth in aquaponic system.

Unfortunately, throughout this present study, it gave opposite performance from the previous research. Based on findings, it showed that Treatment 1, which used commercial pellet as fish diets without *A. pinnata,* gave better performance of mustard growth in aquaponic.

Table 4.1 showed the overall concentration of NPK in Treatment 2 which lower concentration than Treatment 1. Even though nitrogen percentages available for mustard in both treatments were similar, Treatment 2 seems lack of phosphorus. According to previous research of Singh and Thenua (2016), the height of mustard was significantly increased with the application of 60 kg phosphorus per hectare. This indicated that the increased in height was due to the increased of phosphorus content to mustard. Hence, Treatment 2 seems to have lower phosphorus level that lead to slow growth and shorter plants.

In addition, there was very low percentage of potassium in Treatment 2 which influenced the growth of the mustard during the cultured period. Potassium has significant roles in producing better crop quality. High content of potassium lead to better physical quality, disease resistance, and shelf-life of fruits and vegetables (Prajapati & Modi, 2012). Potassium deficiency can cause decrease in plant yield and quality (Prajapati & Modi, 2012). Hence to be related with findings, it showed that Treatment 1 with higher potassium percentage had higher average growth performance which indicated better performance than Treatment 2.

5.2 The Failure of First Batch of Mustard Culture

For the present study, there were two batch of mustard that had been cultured in this aquaponic system. The first batch was not successful and had to be discarded because there was no different of growth performance between two treatments. The main reason was due to sponges was not a suitable medium for planting in aquaponics. Meanwhile the medium for the second batch was clay pebbles that commonly used in hydroponic. Based on the observation, it had been justified that mustard roots were not being able to develop well, and then lead to retardation of the plants. According to Wahome et al. (2011) clay pebbles are organic substance and offer excellent drainage to maintain the good condition of the root systems and away from root blight. Clay pebbles could increase aeration to the plants root. It also a good medium to ensure proper support to plants and have a neutral pH level. Expended properties make clay pebbles retain moisture to the roots. Hence, the sponges may retain the moisture but not well in support the roots and its development.

5.3 Tilapia Growth Performance

Usage of aquatic plants and weeds as ingredients in feed lower the cost of feed making. Thus, the sources are ecofriendly and sustainable. Previous studies had reported on improved feed utilization in tilapia and increased growth in Rohu, common carp and silver carp with inclusion of *A. pinnata* in their feeds (Panigrahi, Choudhury, Sahoo, Das, & Rath, 2014; Sithara & Kamalaveni, 2008; Tuladhar, 2006).

Meanwhile in a studies done by Gangadhar et al. (2015), the incorporation of *A. pinnata* in the diet did not affect or does not have significant differences towards the growth, survival and feed conversion ratio in *Labeo fimbriatus* in any levels of substitution. Decline in growth performance and feed conversion in Rohu fry was also recorded when *A. pinnata* were given at higher amount.

In this research, the result of final tilapia weight and length as stated in Table B.2 and Table B.4 showed there was statistically significant difference between both treatments with $p < 0.05$, where Treatment 1 had fish diet with commercial pellet showed better growth performance than Treatment 2. The slow growth of tilapia was observed in treatments group with fresh *A. pinnata* substitution in the diets. Hence, the findings was opposite to the previous aquaponic research using tilapia, rohu and carp with Azolla as inclusion in diets (Panigrahi et al., 2014; Sithara & Kamalaveni, 2008; Tuladhar, 2006). However, the findings was similar to another previous study that used *Labeo fimbriatus* which is one of the carp fish which resulted no significance different when different level of Azolla substituted in the diets (Gangadhar et al., 2015). This was caused by imbalance of amino acid in *A. pinnata*. Protein in *A. pinnata* has deficient in tryptophan and threonine. Hence this cause the higher level of amino acids deficiency when concentration of *A. pinnata* is higher in the diet (Kathirvelan et al., 2015). This indicates the nutrition content of *A. pinnata* may be not sufficiently for the growth of fish in Treatment 2 as the average growth performance were obviously showed the dramatically rose up pattern in the graph for Treatment 1 which indicated better performance than treatment with *A. pinnata*.

According to Table B.1, the final average mean weight of tilapia in Treatment 1 and Treatment 2 were 51.31 ± 3.68 gram and 23.16 ± 2.04 gram respectively. This data showed there was a big difference between the weight of the smallest and the biggest tilapia for both treatments. This was due to the ability of the fish to compete to eat the feed, resulted varying in size of fish in the same treatment groups.

5.4 Water Parameter

5.4.1 Temperature and Dissolved Oxygen (D.O)

The temperature for both treatments had fluctuation pattern during the period of study from initial reading until the final reading being taken in the final week (Figure 4.5). Dissolved oxygen (D.O) also showed fluctuations and decreased from initial to final readings (Figure 4.6). Temperature and D.O had related conditions in this aquaponic system. The findings showed that when the initial temperature for both treatments were steady, the D.O level also steady but when the temperature start to rose dramatically in both treatments, the D.O also decreased steadily (Figure 4.5 and Figure 4.6).

According to Oscar, Fernando, Enrique, & Genaro (2011), temperature is most important for the aquatic animals, because they has relationship and velocity for biochemical processes. Temperature influenced growth performance, rates of development and reproduction in aquatic animals. Higher temperature leads to higher oxygen consumption by bacteria, algae and fish, and fluctuation of D.O level.

According to the findings, temperature were fluctuated together with the increased in fish growth (Figure 4.4 and Figure 4.5). According to Oscar et al. (2011), higher temperature leads to higher fish metabolism that enhances the protein breakdown, lead to higher release of NH_3 by fish. Hence, this indicated that higher temperature lead to increase of tilapia metabolism that produced higher metabolic products such as NH_3, as they were growing up. The resulting combination of high temperatures with high concentration of NH_3 is a toxic environment. This also indicates that in this study, when temperature increased, D.O level also increase, due to high toxic substance in the system. Although aquaponic system abled to change the compound into safer substance for the plant consumption, but small percentage of toxic compounds were still available in the tanks. This also the reason why there was some mortality was recorded in the both of treatments tanks. The fluctuation of temperature also influenced by the unpredictable surroundings temperature due to changes of weather such as raining and sunny day.

In addition, there was also presence of green algae in the aquaponic system. The decreased in oxygen availability in both treatments also may lead to the presence of green algae in the mustard tanks (Figure 4.6). The D.O started to fluctuate after few weeks of cultured period the amount of green algae were increased in the aquaponic system. Algae can consume oxygen and carbon dioxide produced from the organisms in the aquaponic system, depends on the presence of light (Oscar et al., 2011). Hence, the availability of algae in the mustard tanks for both treatments also contributes to the fluctuation and decreasing of D.O level.

5.4.2 Salinity

Salinity was originated from the concentrations of salts in soils or water due to the availability of dissolved salts in water (Bœuf & Payan, 2001). Figure 4.7 showed the salinity for both treatments were similar and increased slowly for the first four weeks. The salinity started to show higher readings and rose steadily in Treatment 1 while Treatment 2 also rose steadily but at lower level than Treatment 1. According to Bœuf & Payan (2001), less amounts of salts that dissolved in natural waters are vital for aquatic animals. Salinity is contributed to fertilization and incubation of egg, resorption of yolk sac, early embryogenesis, inflation of swim bladder and larval growth. Hence, this indicates that the amount of salinity available in the system, contribute to the vital needs of the tilapia and assist their growth performance.

The salinity reading in both treatments was initially originated from the elements in the water source. Meanwhile the salinity increased within weeks, may due to the inclusion of anti-chlorine crystal, when water loss being replaced due to evaporation. Inclusion of anti-chlorine is important for de-chlorinate water for the fish safety. Hence, it may also lead to increase value of the salinity level. The salinity in both treatments had risen steadily, but still in the small level, which is still far from the harmful level.

5.4.3 pH

pH is the acidity and basicity measurement in an aqueous solution which by determining the concentration of hydrogen ions in water. When pH is less than pH 7 the solution is said to be acidic, and when it is more than pH 7 it is named as basic or alkaline. Fish blood has a pH value approximately to pH 7.4. The interaction between water and fish blood is only separated by one or two cells of their gills.

Based on findings in Figure 4.8, the pH showed initial records of neutral pH for both treatments until Week 3. Started from Week 3, the pH declined dramatically until Week 4. pH were maintained stable from Week 4 to Week 10 with acidic pH. This was the point of the second batch of Mustard being cultured in the system until final reading. From acidic, the pH rose to alkaline for both treatments at Week 10 until Week 11. From Week 11, the pH was decreased steadily towards final week. According to Filep, Diaconescu, Marin, Badulescu and Nicolae (2016), if the pH gets too near or above pH 7.2 in an aquaponic system the plants cannot absorb the nutrients in the system and creates a nutrient shutdown. Plants will begin to wither, curly and yellowish leaf, have stunted growth and imperfect growth or blossoms. In effect, the plants are starving to death. Meanwhile Tilapia needs pH 7, which makes the suitable pH for the aquaponic around pH 6 to pH 7. The findings of mustard performance of this study were from second batch cultured during the last five weeks of the cultured period in the system. The pH was rose above neutral during the last five weeks which this affect the growth of the second batch mustard for both treatments. As referred to Figure 4.2 and Table A.3, although Treatment 1 had better performance, but the height of the final week were not in the state of fully matured height which highest in Treatment 1 was 7.56 ± 1.54 cm. This may due to pH fluctuation. Meanwhile for tilapia growth, the average growth performances (Figure 4.1 and Figure 4.2) were increased although the pH fluctuated to undesired condition but mortality recorded total up to 11 fish mortality may due to this fluctuation of pH. The lethal limits are below pH 5 and above pH 10, for most of the fish species. In gills, gaseous interchange of oxygen instead carbon dioxide occurs. This interchange can be hard if pH value is not optimum (Filep et al., 2016).

5.5 NPK Analysis in Aquaponic System

Based on NPK analysis in Table 4.1, it showed that nitrogen percentage for both treatments were similar, with 0.21% although the average growth performance showed better in Treatment 1. However, the growth of the mustard for both treatments still not fully matured and slower compared to the conventional method. Hence, the amount of nitrogen presence in both treatments may not enough to contribute to the growth of mustard. Optimum rate of nitrogen could boost photosynthetic processes, leaf area production, contribute to the dark-green color in plants, and promotes the vegetative part's growth as well as its development and stimulates growth of root (Dongarkar, Pawar, Khawale, Khutate, & Gudadhe, 2005; Leghari et al., 2016). Nitrogen contributed to rapid early growth and enhanced the growth of leafy vegetables (Leghari et al., 2016). It encourages the uptake and utilization of other nutrients including potassium, phosphorous and controls overall growth of plant. Nitrogen deficient may causes reduced growth, changing of the green color into yellow color of leaves, presence of red and purple spots on the leaves and stunt the lateral bud growth (Dongarkar et al., 2005; Leghari et al., 2016).

Phosphorus content in both treatments showed higher concentration in Treatment 1 with 0.0972%, but less in Treatment 2 with 0.0819%. This related to the reason of the mustard growth performance which was better in Treatment 1. Mustard in Treatment 1 seems to have better height and number of leaves, as well as better final weight. According to Singh and Thenua (2016), phosphorus is crucial in the seed germination and growth, flowers, fruit, and roots. When phosphorus is deficient, growth is reduced and leaves fall off prematurely and delay crop maturity. Plants that are lacking in phosphorus will produce dull and purplish or bronze color with brown edges (Singh & Thenua, 2016).

According to Table 4.1, the analysis of NPK showed that there was absent or very small percentage of potassium available in both treatments. Lacking of this element leads to late maturity of both treatments, especially for Treatment 2, based on their growth performance. Potassium is the most commonly deficient nutrient by far and has a complicated relationship with other nutrients in the aquaponic system. For fish, potassium is one of top ten most important elements for body weight. For this reason, fish feed usually contains quite a bit of potassium. Unfortunately, the potassium in fish feed still not enough for plants. Feed is formulated to be efficient for fish feeding only (Singh & Thenua, 2016). Potassium aids in water movement, nutrients, and carbohydrates in plant tissue. Potassium is involved in enzyme activation in plant which affects production of protein, starch and adenosine triphosphate (ATP). The production of (ATP) can regulate the rate of photosynthesis. Potassium also helps to regulate the opening and closing of the stomata which regulates the exchange of water vapor, oxygen, and carbon dioxide. If potassium is less or not supplied in adequate amounts, growth is stunted and yield is decreased (Kaiser et.al, 2016). Hence, the reason of slow maturity and less final weight of mustard in both treatments, especially Treatment 2 may also originate from very small percentage of potassium presence in each treatment.

CHAPTER 6

CONCLUSION

6.1 Conclusion

Based on findings, performance of mustard and tilapia were shown better in Treatment 1, which consists of commercial pellet diet than Treatment 2, which consists of commercial pellet with fresh Azolla in the fish diet. Although the size of harvested mustard were not in fully matured size, but the height of mustard in Treatment 1 was better with average height of 7.56 ± 0.36 cm compared to Treatment 2 with average height of 4.22 ± 0.20 cm. In addition, the growth performance of tilapia in Treatment 1 showed better result with average final weight of 51.31 ± 3.68 g compared to Treatment 2 with average final weight of 23.15 ± 2.04 g. Based on the findings on ANOVA analysis, it showed that p value less than 0.05 for both mustard and tilapia growth performance in both treatments which indicated there was a significant different between Treatment 1 and Treatment 2. Hence, H_0 was accepted. There is a significant between diets contain commercial pellet and commercial pellet associated with *A. pinnata* towards the growth performance of mustard and tilapia. Hence, different types of fish diet in aquaponic system had really influenced the performance of both plant and fish.

6.2 Recommendation

This present study may not have a very satisfied result for some parties due to the final growth of mustard were still not in the matured state, although it had significance different between the two treatment groups. There are some recommendations that could be implemented for future study related to this topic. First is aquaponic design. The aquaponic design used in this study was a very simple yet still systematic to produce the end products of aquaponics. However, in order to get more desirable result for future study, upgraded and more systematic aquaponic design could be used. This is due to the design used in this study was not encourage the rapid change of tilapia metabolic products into safer compound for plants. The fish water in the fish tank pumped up into mustard tank, and only 30% to 40% of the fish manure being pumped into mustard tank, make the low compound content used as fertilizer for mustard. This could be the reason why the plant performance was not that excellent. The left over fish manure in the fish tank only contributes to the water contamination. This contamination may also being the reason of poor water quality in the system. The aquaponic design does not required replacement of water for fish tank. The water in the tank should be clear-colored after being recycled to plant tanks. However, it was still a little cloudy after being recycled. This also indicate that the upgraded system should be used in future study to ensure the water will become clean or clear for the fish after being cycled to the plant tanks.

Next is, the amount of sunlight or light used. This present study was carried out outdoor, but the amount of sunlight achieved was not enough. This was due to the set up area of the experiment was covered with roof. It was favorable for the fish protection from rain drops or any other foreign materials. However, the plants may get insufficient amount of the sunlight on the day time. This may be the reason of late maturity of plant from the lack of photosynthesis process due to lack amount of sunlight. This design or aquaponic design could be install either indoor or outdoor, according to the preference. However, the right amount of sunlight or any artificial light are needed for the plant to undergo photosynthesis to ensure proper growth.

Last but not least, lack of budget is one of the limitations in this current study. Due to the low budget available for the experiment set up, some of the materials that should be used in the study had replaced by the one that was cheaper. It is good to have cheap materials for the experiment set up so that it could be followed and act as reference for future farming ideas. However, it had to be complementary with the reaction of the fish and plant. For instance, the failure of the first batch mustard culturing was due to the unsuitable types of medium being used. As was mentioned in the result, during first batch of mustard culture, the medium being used was cubic sponges, which expected to give good moisture retain to the mustard roots. Purchasing cost of sponge was very cheap. However, from observation, the mustard growth was retarded due to the roots were not being able to grow properly in the sponges. Meanwhile, when the hydroponic medium was changed with clay pebbles which the cost a little higher than sponges, the result were better. Hence, for future study, suitable and appropriate materials should be considered before install the system, not only the low cost as the main concern.

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APPENDIX A

Mustard Growth Performance (Batch 2)

Table A.1: The descriptive analysis of number of mustard leaves.

	N	Mean	Std. Error
1	36	8.89	0.35
2	36	6.69	0.52
Total	72	7.79	0.31

Table A.2: The ANOVA analysis of number of mustard leaves.

ANOVA				
Final Number of Mustard Leaves at Week 5				
	Sum of Squares	d.f	Mean Square	Sig.
Between Groups	86.681	1	86.681	.000
Within Groups	147.194	70	2.103	
Total	233.875	71		

Table A.3: The descriptive analysis of mustard height.

	N	Mean	Std. Error
1	36	7.5611	0.36
2	36	4.2167	0.20
Total	72	5.8889	0.27

Table A.4: The ANOVA analysis of mustard height.

ANOVA				
Height of Mustard on Week 5				
	Sum of Squares	df	Mean Square	Sig.
Between Groups	201.336	1	201.336	.000
Within Groups	126.976	70	1.814	
Total	328.311	71		

Table A.5: The descriptive analysis of mustard weight.

	N	Mean	Std. Error
1	33	7.1261	0.22
2	32	1.6784	0.04
Total	65	4.4442	0.36

Table A.6: The ANOVA analysis of mustard weight.

ANOVA				
Final Weight of Mustard				
	Sum of Squares	df	Mean Square	Sig.
Between Groups	482.131	1	482.131	.000
Within Groups	50.963	63	.809	
Total	533.094	64		

APPENDIX B

Tilapia Growth Performance

Table B.1: The descriptive analysis of tilapia weight.

	N	Mean	Std. Error
1	20	51.3065	3.68
2	29	23.1552	2.04
Total	49	34.6455	2.76

Table B.2: The ANOVA analysis of tilapia weight.

ANOVA				
	Sum of Squares	d.f	Mean Square	Sig.
Between Groups	9380.580	1	9380.580	.000
Within Groups	8537.555	47	181.650	
Total	17918.134	48		

Table B.3: The descriptive analysis of tilapia length.

	N	Mean	Std. Error
1	20	13.9850	0.37
2	29	11.2207	0.33
Total	49	12.3490	0.31

Table B.4: The ANOVA analysis of tilapia length.

ANOVA				
Length of Tilapia Week 15				
	Sum of Squares	d.f	Mean Square	Sig.
Between Groups	90.449	1	90.449	.000
Within Groups	137.913	47	2.934	
Total	228.362	48		

APPENDIX C

Water Quality Result

Table C.1: The average temperature readings for both treatments.

Week	Average Temperature (ºc)	
	Treatment 1	Treatment 2
1	26.35	26.42
2	26.74	26.57
3	26.14	26.12
4	27.7	27.29
5	25.88	25.78
6	26.34	26.28
7	28.75	28.22
8	27.23	26.84
9	26.6	26.22
10	28.32	27.88
11	27.97	27.69
12	28.35	28.19
13	28.71	28.5
14	28.73	28.51
15	28.75	28.53

Table C.2: The average D.O readings for both treatments.

Week	Average D.O (mg/L)	
	Treatment 1	Treatment 2
1	6.67	6.64
2	7.08	6.9
3	6.8	6.53
4	6.58	6.49
5	6.52	6.57
6	5.86	6.06
7	5.51	5.64
8	5.59	5.51
9	6.23	6.09
10	5.17	4.7
11	4.03	4.12
12	4.53	4.62
13	4.82	4.86
14	4.82	4.84
15	4.83	4.86

Table C.3: The average salinity readings for both treatments.

Week	Average Salinity (ppt)	
	Treatment 1	Treatment 2
1	0.04	0.04
2	0.07	0.07
3	0.1	0.09
4	0.12	0.11
5	0.18	0.16
6	0.25	0.21
7	0.32	0.27
8	0.39	0.33
9	0.46	0.38
10	0.48	0.41
11	0.47	0.87
12	0.43	0.4
13	0.41	0.39
14	0.42	0.4
15	0.41	0.41

Table C.4: The average pH readings for both treatments.

Week	Average pH	
	Treatment 1	Treatment 2
1	7.07	7.1
2	7.5	7.44
3	7.67	7.58
4	6.6	6.35
5	6.53	6.19
6	6.45	6.09
7	6.46	5.96
8	6.33	5.85
9	6.41	6.25
10	6.75	6.72
11	8.92	8.83
12	7.87	7.86
13	7.62	7.61
14	7.6	7.6
15	7.56	7.54

APPENDIX D

NPK Analysis Result

Table D.1: The elements analysis result using XRF machine.

Element	Formula	Concentration	
		Treatment 1	Treatment 2
Nitrogen	N	0.21	0.21
Phosphorus	P	0.0819	0.0972
Potassium	K	-	-
Barium	Ba	0.004	0.0025
Calcium	Ca	0.0289	0.0661
Chlorine	Cl	0.18	0.179
Water	H2O	98.6	99.6
Sulphur	S	0.106	0.0973
Tin	Sn	0.0019	0.0018

APPENDIX E

Related Images During Culturing Period

(a) (b)

Figure E.1: Mustard seed germination. (a) Mustard seed had been germinated using peat moss as medium. (b) Mustard seed for germination.

Figure E.2: The prepared setup of the system.

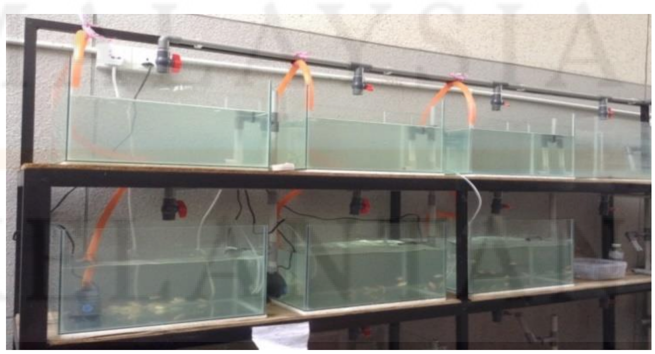
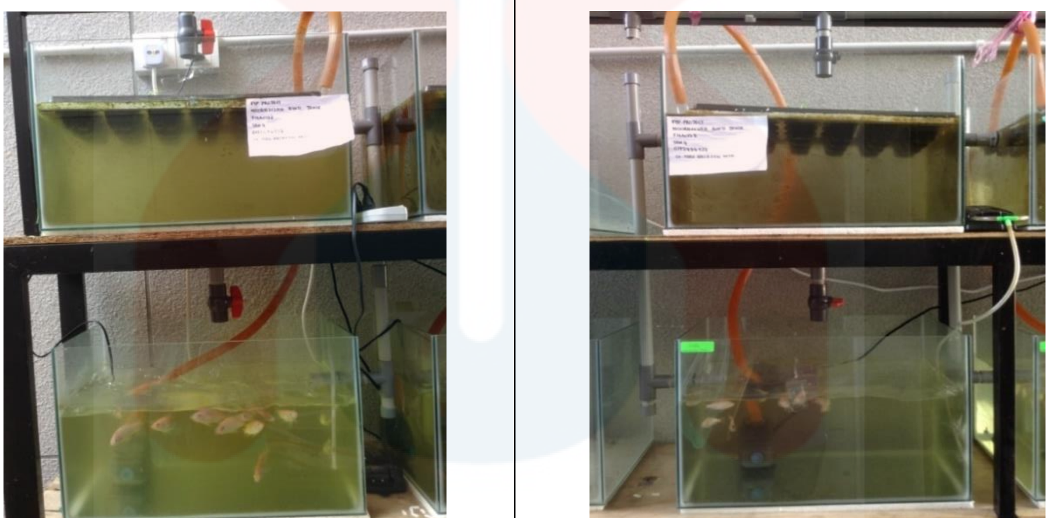


Figure E.3: The first week of tilapia culture in the system, without mustard.

Figure E.4: *Azolla pinnata* that had been prepared in a place that available for sunlight.



(a) (b)

Figure E.5: The aquaponic system after one week mustard cultured. (a) Treatment 1 system after one week mustard being included. (b) Treatment 2 system after one week mustard being included.

Figure E.6: The system after the 5 weeks of mustard in the system.

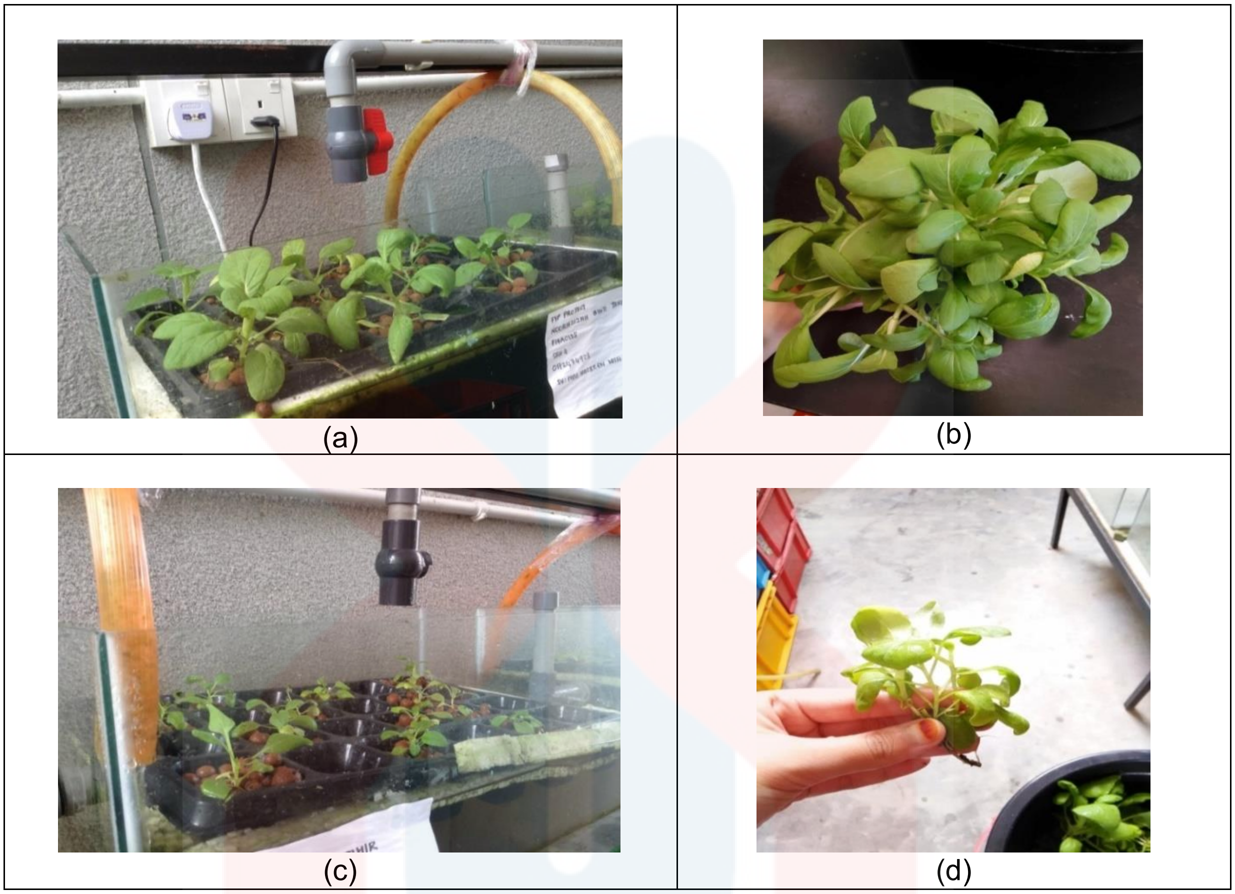


Figure E.7: Final mustard growth on the fifth week. (a, b) Final mustard growth in Treatment 1. (c, d) Final mustard growth in Treatment 2.

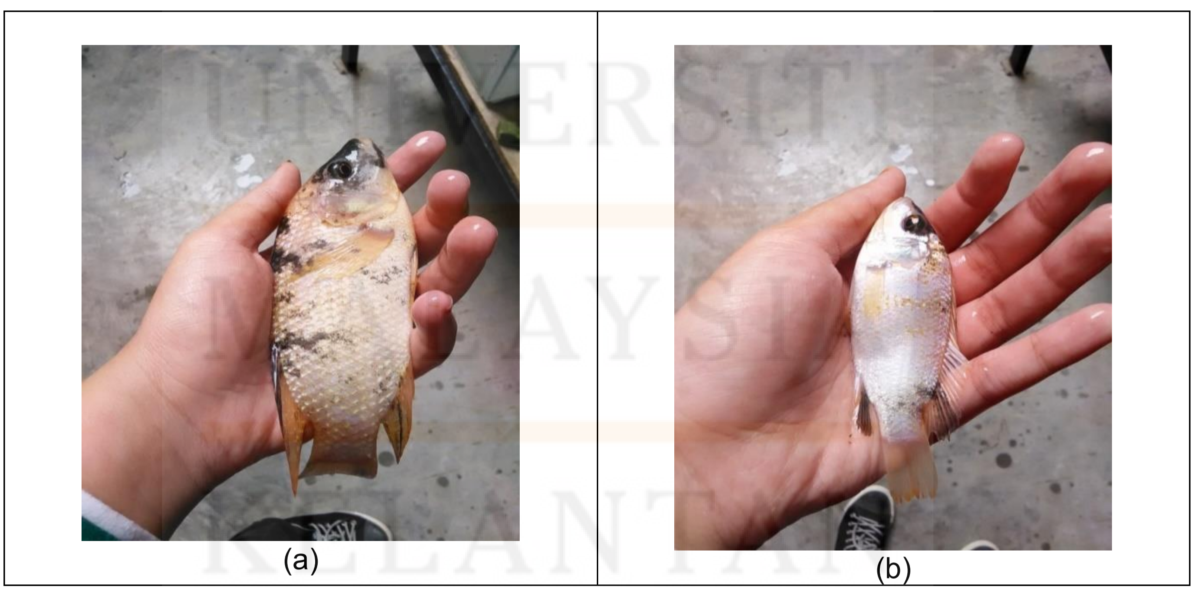
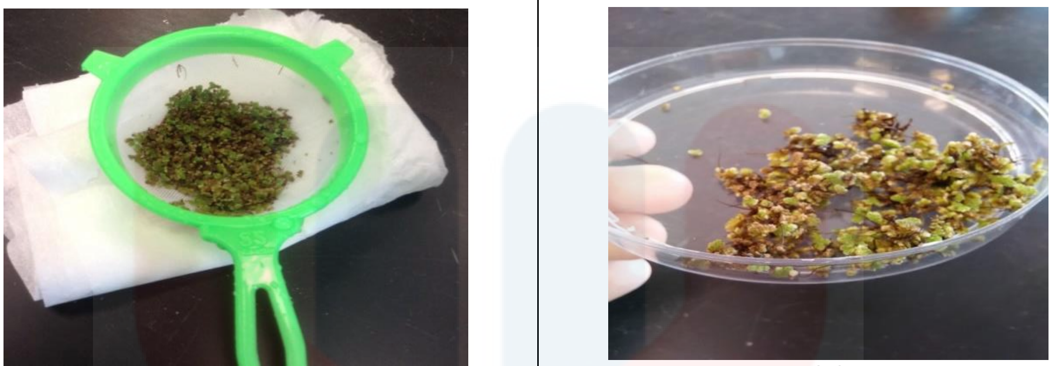


Figure E.8: Final growth of tilapia in the final week. (a) Final growth of tilapia in Treatment 1. (b) Final growth of tilapia in Treatment 2.



(a) (b)

Figure E.9: Fresh *A. pinnata* for weighing process. (a) Azolla being drain off before being weigh as feed. (b) Azolla in a plate for weighing purpose with analytic balance.

Figure E.10: Commercial pellet that had done weighed and packed for a week for feeding.

(a) (b)

Figure E.11: Preparation of sample for NPK analysis. (a) The apparatus and materials being used to take sample from both treatments for NPK analysis. (b) The sample from each treatment being transferred into bottles before analysis.

Figure E.12: The suspended of condition in hydroponic tanks during final week.