Effect of *Leucaena leucocephala* Seed Diet on Nile Tiapia (*Oreochromis niloticus*) Flesh Texture

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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I certify that the Report of this final year project entitled "Effect of *Leucaena leucocephala* Seed Diets On Nile Tilapia (*Oreochromis niloticus*) Flesh Texture" by Nurul Afizah Binti Muhammad Afiq Wong, matric number F14A0286 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Husbandry Science) with Honours,

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ABSTRACT

The quality of Nile tilapia fillet is significant for consumer preferences. Thus, it is important to supply the fish with good quality of feed which able to enhance the flesh texture quality. This research was conducted to determine the effect of *Leucaena leucocephala* seed diet on Nile tilapia (*Oreochromis niloticus*) flesh texture through texture profile analysis (TPA) and colour evaluation in producing the best quality of tilapia fillets. For texture evaluation, the value for hardness, cohesiveness, gumminess, chewiness and springiness of tilapia fillets were measured while for colour evaluation the L^*, a^* and b^* values were evaluated. Nile tilapia were raised within six weeks and were fed with different percentage of *L. leucocephala* seed diets. The feeds that were used in this experiment were; Control: formulated diet with 0% of *L. leucocephala* seed meals, Treatment 1: formulated diet with 25% of *L. leucocephala* seed meals and Treatment 2: formulated diet with 30% of *L. leucocephala* seed meals. The feed were given according to 5% of its average body weight. A total of 30 Nile tilapia fingerlings for each treatment were used. Each treatment was replicated three times. The results for texture analysis showing that tilapia fillets which fed with control diets show highest value for hardness, cohesiveness, gumminess and chewiness at 5899.60 g, 0.55, 3234.20 g, and 1346.92 mJ respectively. The value for springiness was highest for Treatment 2 at 42.81 mm followed by Treatment 1 at 42.66 mm and Control at 42.44 mm. For colour evaluation, the result obtained for a^* value which represent the redness of fish fillets showed significant difference and the highest a^* value was Control diets followed by Treatment 1 and Treatment 2 at 6.01, 4.23, and 3.33 respectively. In conclusion, control diets have show better quality of flesh texture that is preferable by consumers.

Keywords : Nile tilapia, *Leucaena leucocephala* seed diets, texture analysis, colour evaluation, fillet

Pengaruh Diet Benih *Leucaena leucocephala* Ke Atas Tekstur Isi Ikan Nil Tilapia (*Oreochromis niloticus*)

ABSTRAK

Kualiti filet Nil Tilapia penting bagi pilihan pengguna. Oleh itu, adalah penting untuk menyediakan makanan berkualiti kepada ikan yang boleh meningkatkan kualiti tekstur isi. Kajian ini dijalankan untuk menentukan kesan diet biji benih petai belalang ke atas tekstur isi ikan Nil tilapia *(Oreochromis niloticus*) melalui analisis profil tekstur (TPA) dan penilaian warna bagi menghasilkan filet ikan terbaik. Untuk penilaian tekstur, nilai bagi kekerasan, kepaduan, kegetahan, kekenyalan dan kelentingan filet tilapia telah diukur manakala bagi penilaian warna, nilai L^*, a^* dan b^* telah dinilai. Nil tilapia telah dipelihara selama enam minggu dan telah diberi makanan yang mempunyai peratusan tepung biji benih petai belalang yang berbeza. Makanan yang digunakan di dalam kajian ini adalah; Kawalan: diet formulasi dengan 0% tepung biji benih petai belalang, Rawatan 1: diet formulasi dengan 25% tepung biji benih petai belalang dan Rawatan 2: diet formulasi dengan 30% biji benih petai belalang. Jumlah makanan yang ditentukan melalui 5% dari purata berat badan. Sejumlah 30 anak ikan Nil tilapia bagi setiap rawatan telah digunakan. Setiap rawatan direplika tiga kali. Keputusan bagi analisis tekstur menunjukkan bahawa filet tilapia yang diberi makan dengan diet Kawalan menunjukkan nilai tertinggi bagi kekerasan, kepaduan, kegetahan, dan kekenyalan iaitu 5899.60 g, 0.55, 3234.20 g, dan 1346.92 mJ masing masing. Nilai bagi kelentingan adalah paling tinggi bagi diet Kawalan 2 pada 42.81 mm dan diikuti oleh Kawalan 1 pada 42.66 mm dan Kawalan pada 42.44 mm. Manakala, untuk penilaian warna, keputusan yang didapati untuk nilai a yang mewakili kemerahan filet ikan menunjukkan perbezaan signifikan dan nilai a tertinggi adalah diet Kawalan diikuti Rawatan 1 dan Rawatan 2 pada 6.01, 4.23 dan 3.33 masing-masing. Kesimpulannya, diet Kawalan menunjukkan kualiti yang lebih baik untuk tekstur filet yang dikehendaki oleh pelanggan.

Kata kunci: Nil tilapia, diet biji benih petai belalang, analisis tekstur, penilaian warna, filet

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	degree Celcius
a^*	red chromaticity
b^*	*yellow chromaticity*
DM	Dry Matter
FAO	Food and Agriculture Organization
g	gram
h	hours
L	Liter
L^*	lightness
LLSM	*Leucaena leucocephala* Seed Meals
MBD	Micro Bound Diet
mJ	milliJoule
mL	millilitre
mm	millimeter
NPN	Non Protein Nitrogen
T1	Treatment 1
T2	Treatment 2
TPA	Texture Profile Analysis

CHAPTER 1

INTRODUCTION

1.1 Research background

Tilapia has a sweet, mild taste with lean and a medium-firm, flaky texture. Texture can be described as the hardness, cohesiveness, chewiness, gumminess and springiness of the fish flesh. Fat, colour, texture and freshness are the major quality indicators for fresh fish (Vácha, Stejskal, Vesjada & Kouřil, 2014). The texture, odour and appearance of fish are of vital importance to customers. Texture can be determine and analysed either by sensory method or evaluation method.

Tilapia farming is becoming popular by day as demands for this fish are rising. Tilapia feed on a wide range of food making it a very favourable fish to grow. Tilapia that are raised in tank culture were provided with formulated feed. Fishmeal are one of the source of protein in fish diets. Due to high cost of fishmeal, researchers continue to conduct studies on plants for their ability in replacing fishmeal. *L. leucocephala* has been acknowledged to have the potential to reduce the total dependence of fish farming on fishmeal. This study helps to acknowledge people on the benefits of plants diets and the types of feed which show better fillet quality.

This research aims to determine the effect of *Leucaena leucocephala* seed diet on tilapia flesh texture in terms of hardness, springiness, cohesiveness, gumminess and chewiness.

1.2 Problem Statement

Tilapia is the worldwide third most essential fish in aquaculture as it has hearty taste, nutritious and inexpensive. Demands for tilapia flesh are high. Thus, the qualities of the flesh are important. Type of fish given is one of the factors that affect the colour of the flesh making it important for the producers to ensure that the feed given able to provide good flesh attributes towards the fish. This research was carried out to analyse the effectiveness of *Leucaena leucocephala* based raw material feed in producing better quality of tilapia flesh.

1.3 Hypothesis

H_0: Tilapia fed with *Leucaena leucocephala* based raw material feed have no effect on the flesh texture quality than of tilapia fillet.

$H_{A:}$ Tilapia fed with *Leucaena leucocephala* based raw material feed have better flesh texture quality than tilapia fed with formulated diet. .

1.4 Objective

1. To determine the effect of *Leucaena leucocephala* formulated feed on *Oreochromis niloticus* flesh texture.

1.5 Scope of Study

The scopes of this research are types of feed and tilapia flesh. Feeds used are commercial feed and *Leucaena leucocephala* diets. Tilapia flesh have been used to determine which feed is the best in quality in producing better texture of tilapia flesh in terms of hardness, cohesiveness, chewiness, springiness and guminess, and colour.

1.6 Significance of Study

Productions of good quality feed at low cost which have the ability to enhance the quality of tilapia flesh texture are important for both producers and consumers. Thus, it is significant to identify the best feed which able to produce good attribute of flesh texture.

CHAPTER 2

LITERATURE REVIEW

2.1 Tilapia

Tilapias are freshwater fish classified in the family Cichlidae. They are African native, but in the time of the second half of the 20th century, it was introduced into many temperate, tropical and subtropical regions (Pillay, 1990). The purposes of the introduction of tilapia into those regions were either for farming as food fish; recreational fishing; aquatic weed control; and research (El-Sayed, 2006). Tilapias expressed many aspects which make them an ultimate candidate for aquaculture industry, particularly in developing countries involving rapid growth, endurance towards a wide scale of environmental surroundings, resistance to stress and diseases, able to reproduce in a brief period of time and feeding on low tropic levels and toleration of artificial feeds right away after yolk-sac absorption.

The tilapia name was descended from the African Bushman word interpreting 'fish' (Trevawas, 1982). Smith (1840) was the first who described the genus of Tilapia. It was later split into two subgenera which are *Tilapia* (substrate spawners) and *Sarotherodon* (mouthbrooders) based on their breeding behaviour and feeding habits.

Tilapia is probably the oldest farm reared fish in the world and it is believed that tilapia culture have been originated since 4000 years ago, which is about 1000 years before carp was cultured in China (El-Sayed, 2006). Illustrations from Egyptian tombs suggested that this species have been cultured more than 3000 years ago. Stories from biblical scholars claiming that tilapia is the so-called 'St. Peter's Fish, which have been used by Jesus in order to provide the crowds at the Sea of Galilee (Popma & Masser, 1999). As reported by Food and Agriculture Organization (FAO), the aquaculture production statistics for 2004 indicated that about 100 countries implemented tilapia culture, since these countries reported tilapia production from aquaculture in 2002 (FAO, 2014).

Major technological inventions allowing the successful of all-male populations production via the application of sex-reversing hormone that give rise to higher returns from tilapia farming in 1970's (FAO, 2006). Tilapia culture involving only male population is preferable because of their rapid growth ability. Several techniques are used in producing monosex tilapia encompassing genetic manipulation, manual sexing, sex reversal, and hybridization (Beaven & Muposhi, 2012).

Nowadays, tilapia often farmed in polyculture as to overcome the problem of land limitation. This type of culture helps in optimising the financial return and prevents growth of harmful bacteria (Troell, 2009).

2.1.1 Nile tilapia (*Oreochromis niloticus*)

Out of all tilapia species, Nile tilapia or scientifically known as *Oreochromis niloticus* has been acknowledge as the most predominant identified species in capture fisheries. In 2002, the productions of that species come close to 253,871 Mt, globally representing 37 % of total production of tilapias (El-Sayed, 2006).

Oreochromis niloticus has fairly conventional, laterally compressed, deep-bodied shapes with cycloid scales which are not dislodged easily (Ross, 2000). The pelvic and pectoral fins are sizeable and more anterior in an advanced arrangement. This attribute provides the fish with excellent control as regards to swimming and manoeuvring. During breeding period, the *O. niloticus* often flushes red (Richardson et al., 2000).

Oreochromis niloticus are able to live more than 10 years (GISD, 2012). Availibility of food and temperature of water have seemed to be the limiting factors towards the growth of *O. niloticus* (Kapetsky & Nath, 1997). The optimal growth for *O. niloticus* is accomplished at the temperature ranging from 28 to 36 °C and deteriorates with declining temperature (Teichert-Coddington & Green, 1997). Variation in growth of *O. niloticus* may occur depending on the variation of feed intake (Bwanika, Murie & Chapman, 2007). In aquaculture ponds, *O. niloticus* are able to achieve sexual maturity as early as the age of 5 to 6 months and capable of growing up to 62 cm with the weight of 3.65 kg at an approximated nine years of age (FAO, 2014). The standard whole length of *O. niloticus* could be up to 20 cm (Bwanika, Makanga, Kizito, Chapman & Balirwa, 2004).

The fact that the *O. niloticus* eat a wide range of natural food organisms and have high tolerance on poor water quality showing that this species hold positive aqua cultural characteristics. *Oreochromis niloticus* have the ability in withstanding sustained water temperatures below 10 to 12 °C and reach early sexual maturity which lead into spawning before reaching market size (Popma & Masser, 1999).

There are various another possibility options for *O. niloticus* culture depending on their nature which is either seasonal or year-round production such as seasonal pond culture, seasonal cage cultures, thermally regulated intensive bio-secure recirculation systems in raceways, and tanks (Shipton, Tweddle & Watts, 2008).

Of all these culture, freshwater cage culture is reckoned to possess the greatest biosecurity risk such as the risk of escapement and transmitting of pathogens and diseases to wild populations. On the other hand, raceways or ponds culture represent a non-excessive biosecurity risk while the recirculating systems culture promotes a low biosecurity risk.

2.1.2 Feeding Habits of *Oreochromis niloticus*

Significantly, increasing in Tilapia culture in aquaculture production causes it compulsory to perceive the significance of their food selections and feeding routines in their natural habitats. These information are useful in order to formulate most appropriate diets for them and adopt favourable feeding routines under culture surroundings. Although tilapia devour food at low tropic levels and the feed costs are low-cost than other carnivorous fishes, tilapias are still reckon as a source of high-

quality protein well qualified for human consumption, at an amazingly economical price (El-Sayed, 2006).

Dietary selections and feeding routines of tilapia depend, between other elements, on the species and size of the fish, amount of daytime, photoperiod, depth of water, and geographical environment. Tilapia of the genus *Oreochromis* are typically microphagous which feeding mostly on detritus, periphyton, and phytoplankton. *Oreochromis Aureus*, *O. niloticus*, and *O. mossambicus* are the examples that belonged to this group. These types of species can productively devour the food sources as stated via 'filter-feeding'.

At first, tilapia fed on zooplankton throughout its larval stages especially the crustaceans (Bowen, 1976). The transition phase of tilapia from its planktivore stage diets to the regular, specialized diets is commonly taking a small amount of time (Bowen, 1982), but in some occasions it happens slowly over a prolonged period of a year or more (Whitefield & Blaber, 1978). The preferred food for juvenile and adult tilapias composes of abundant sorts of phytoplankton, aquatic vegetation, periphyton, zooplankton, and detritus from plant origin, depending on the species of tilapia.

Natural food efficiency in the water is presumably to differ at different deepness since that the penetration of light into the water column is influenced by turbidity and water depth. Hence, the feeding behaviours of tilapia may differ with different water masses. Tilapia able to change their food sources from one to another with only a small amount of alteration made in their compositions of diet (Moriarty & Moriarty, 1973).

It happened to be that tilapias have non-uniform feeding schemes, relying on the species and size of the fish, time of the day, photoperiod, season of the year, depth of water, geographical location, and type of habitat. It is essentially vital that tilapia farmers, farm managers, and researchers to chew over these factors in order to adopt the most suitable fertilization managements, organize the best fish diets and decide on the most suitable feeding systems in terms of feeding rate, timing and quantity.

2.1.3 Water quality management in Tilapia culture

Generally, under diverse culture and environmental circumstances, stocking densities and management approaches, Tilapias are cultured either semi-intensively or intensively. Quality of water is always to be concerned to constant alterations, mainly in either intensive or super-intensive culture systems (El-Sayed, 2006). Thus, management of water quality turn out to be a key aspect for successful aquaculture practices.

Temperature is one of the major significant aspects concerning the physiology, reproduction, growth and metabolism of tilapia. Tilapias are thermopiles and known to have high tolerance towards a broad scale of water temperatures. Wide-ranging researches have been performed regarding the effects of water temperature on the performance of tilapia (El-Sayed, 2006).

The range of temperature that is normal for the reproduction, development, and growth of tilapia is about 20 to 35 °C, depending on the species of fish, with a

most favourable range temperature around 25 to 30 °C (Balarin & Haller, 1982). Lower lethal extremes temperature for tilapia has been reported at 12 °C (Negroni, 2013). The upper critical temperature perimeters for tilapia may differ from one species to another, but it has been stated that most tilapias unable to put up with water temperatures beyond 40 to 42 °C for a long period (El-Sayed, 2006). It has also been fully aware that the greater the distance of the geographical location from the equator, the more tolerant are the *O. niloticus* strains towards cold (Sifa, Chenhong, Dey, Gagalac & Dunham, 2002).

Existing details signifies that tilapia species, size, adaptation period, environmental aspects and geographical locality are the main factors towards salt tolerance (Balarin & Haller, 1982). It have been reported that *O. mossambicus* possess the ability to tolerate up to 120 % water salinity and also able to mature normally and reproduce at water salinity level as low as 49% and their fry can endure and grow up quite well at 69 % (El-Sayed, 2006). However, *O. niloticus* are less tolerant towards salinity, but be capable of raising well at a salinity of up to 36 %, whereas reproduction take places at 29 % (El-Sayed, 2006).

Dissolved oxygen (DO) is one of the restraining environment aspects influencing fish growth, feeding and metabolism. Ambient DO levels generates the finest fish production, while low DO levels stunted the fish growth, respiration and other metabolic activities (Tsadik & Kutty, 1987). Tilapia are notable to possess the ability of resisting a very low levels of DO. Most of the tilapia species able to tolerate DO levels as low as 0.1 to 0.5 mg/l for different periods of time and can even stay alive at zero DO concentration, if access towards surface air is allowed (El-Sayed, 2006). Tilapia would normally suffer from high number of death if they did not

succeed in reaching the surface air. In contrast, it can put up with high oxygen super saturation conditions which are up to 400% that were frequently happening due to high photosynthesis arising from the blooming of phytoplankton and macrophytes (Morgan, 1972).

Some tilapias are recognized to tolerate water pH at a very broad such as the *Oreochromis alcalicus grahami* which able to survive successfully in Lake Magadi (Kenya), with a pH value of 10.5 (Randall et al., 1989). In another study, tilapia were reported to able to tolerate a pH range of 5 to 11 for at least 24 hours, however they reach mortality at pH ranging from lower than 3.5 and more than 12 (El-Sayed, 2006)

Wangead, Greater and Tansakul (1988), investigated the consequences of acidic water on the growth, behaviour, and survival rate of Nile tilapia fingerlings with the weight ranging from 0.4 to 1.0 g and adults with the weight up to 46.3 g. The result from the study showed that both Nile tilapia fingerlings and adults died within 1 to 3 days at pH 2 to 3. The result also stated that after 60 to 70 days, both size groups could successfully tolerated pH 4 to 5, and achieved survival and growth rates comparable to the control group which were raised in water pH of 7. Adult fish possess higher resistance towards low pH, with a survival rate up to 86% at pH as low as 4.

2.2 The use of fishmeal as protein source in Tilapia feed

Generally, fishmeal is included into animal foods in order to improve feed efficiency and growth all the way through effective feed palatability which have the ability to enhance the uptake of nutrients, absorption, and digestion (Miles & Chapman, 2010). Fishmeal is valuable because of the presence of perfect amount of amino acids that responsible in protein make up which are important for animal nutrition (Balois, 2003).

Fishmeal can be considered to be high in biological value as a feedstuff as it comprises of a high level of digestible essential amino acids such as methionine, leucine and lysine that cannot be easily obtain even though in exclusive grain feeds which are commonly use as typical base for most animal feeds (New & Wijktrom, 2002; Balios, 2003). In addition, fishmeal consists of low fibre content and is believed to be as a priceless source of vitamins B1, B2, B6 and B12 together with other essential elements such as calcium, magnesium, phosphorus, potassium, including iodine, zinc, iron, copper, selenium, cobalt, manganese, and fluorine (Hall, 1992).

However, the irregularity of supply, increasing demand and high price, causes the use of fishmeal to be limited and making the feed industry facing a greater pressure in discovering other alternative source of protein which are suitable for animal feed and most importantly economical for the farmers (Hardy, 2010).

2.2.2 Alternatives of fishmeal as protein sources for Tilapia feed

In choosing an alternative feed ingredient to fishmeal the following factors should be considered, the effect of the alternative feed ingredient on growth of the fish, survival rate, feed conversion ratio, resistance to disease, safety of aquaculture product and price (Schipp, 2008). Furthermore the alternative protein ingredient should not be environment degrading in terms of nitrogen and phosphorus (Tacon & De Silva, 1997). It has been demonstrated that fishmeal can be substantially replaced by alternative protein sources either from plant or animal sources (Schipp, 2008).

Animal based alternative protein ingredients that have been evaluated include grasshopper meal, earthworm meal, toad meal, termite meal, maggot meal, chicken offal meal, garden snail meal, fish waste, freshwater muzzle meal; silkworm pupa, crab meal and turkey meal (Nandeesha, Gangadhara, Varghese & Keshavanath, 2000).

In terms of protein content and digestibility of amino acid, plant proteins can be said to be likely similar to fishmeal. In recent study, cotton seed meal can successfully replaces 75 % of soybean meal without any negative effect on growth performance of tilapia fingerling (El-Saidy & Saad, 2010). A by Zhao et. al (2010), fish meal can be replaced with soy protein concentration by only rising the amount of *O. niloticus* feeding to less than 2 g.

The European seabass, *Dicentrarchus labrax*, maintained growth performance when 98 % of the fish meal was replaced by plant protein sources (Kaushik, Cauves, Dutto & Blanc, 2004). Another research by Monentcham et al. (2010), showed that combination of soybean and cotton seed meal can replace fish meal up to 50 %. Another study by Olivera-Novoa, Campos, Sabido and Palaclos (1990) reported that, the use of alfalfa leaf concentrate to replace 35 % of fishmeal in the diet of *O. Niloticus* fingerlings. It has been documented that 50 % of fishmeal can be substituted by guar seed meal in the diet of *O. niloticus* (Al-hafedh & Siddigui, 1998).

Plants protein are being used widely for tilapia farming although it has anti-nutritional factors and imbalanced amino acid profile that shows negative impact on fish growth performance (Francis, Makkar & Becker, 2001). These issues regarding anti-nutritional factors present in the plant protein can be control by employing various processing methods such as extraction, auto-cleaving, germination, boiling and toasting.

2.3 Leucaena leucocephala as a replacement of fishmeal

Leucaena leucocephala is a perrenial non-spiny, non-climbing shrub or tree. It now has been utilized as wood source, fodder, and reclamation species. *Leucaena leucocephala* which is also known as ‘alfalfa of the tropics’ is regarded as a weed in over 25 countries around the globe including Mexico, Phillipines and Indonesia (Walton, 2003).

The specific name *'leucocepahala'* derives from *'leu'* meaning white, and *'cephala'* which mean head that is referring to the flower. There are three subspecies of leaucaena such as *leucocephala*, *glabrataa* and *ixtahuana* (Orwa, Mutua, Kindt, Jamnadas & Anthony, 2009).

Leucaena leucocephala is basically a tropical species demanding warm temperature ranging from 25 °C to 30 °C for most favourable growth. It has less tolerance towards cold and extensively reduced in growth during cool winter months especially in subtropical areas. *Leucaena leucocephala* can grow up on a wide range of deep, well-drained fertile soils such as alkaline soils and volcanic soils (Walton, 2003).

Reproduction of *L. leucocephala* may occur by the spreading of seed by a number of vectors. Spreading method by vectors is usually time-consuming and mostly new plants were controlled either by ruminants or grass competition (Walton, 2003). The flowers are self fertile and most seed are resulting from self-pollination. As long as moisture available, flowering and fruiting will keep on occurring throughout the year (Orwa et al., 2009).

Leucaena leucocephala is propagated by seeds which remain viable for several months. These seeds have hard waxy seed coat which needs scarification before planting (Shelton & Brewbaker, 1998). Scarification is achieved by nicking the seed, soaking the seed in boiling water for 30 minutes or soaking in sulphuric acid for 15 minutes (Forest, farm And Community Network [FACT], 1997). Nursery grown seedling and bare root transplant have also been used to propagate *L. leucocephala*.

It has been reported that *L. leucocephala* takes 12 to 18 months to be fully established (FACT, 1997).

Leucaena leucocephala is popular for its high amount of nutritional value and the favourably same of chemical compound with alfalfa except for its higher tannins content and mimosine toxicity towards ruminant (Orwa et al., 2009).

Dried *L. leucocephala* leaves can be used in concentrate feeds for ruminant. The leaves firstly should be chopped down and afterwards made into dehydrated meals. *Leucaena leucocephala* leaves can be dried using conventional lucerne dryer or sun drying. (Ghosh & Bandyopadhyay, 2007).

The seeds of *L. leucocephala* are ovoid in shape with brown hulls and yellow kernels. The seed is low in oil content and have rich in protein. The proteins of *L. leucocephala* seeds contain high amount of essential amino acids such as leucine, isoleucine, histidine and phenylanine (Begum, 2014). High protein content of *Leucaena leucocephala* seeds enables it to replace the role of fish meal in providing protein for fish diet. The chemical constituents of *L. leucocephala* seeds can be described as in Table 2.1:

Table 2.1: The chemical constituents of *Leucaena leucocephala* seeds

Chemical constituents	Seeds
Potassium	137.3
Nitrogen	338.0
Magnesium	44.6
Calcium	44.4
Sodium	12.6
Manganese	52.6
Iron	642.4
Copper	55.0
Zinc	125.1
Fatty acid (%)	15
Saponification value	108.74
Iodine value	4.90
Acid value	1.08

The pods seeds and leaves of *L. leucocephala* have been used as food in some parts of Mexico (Simbaya, 2002). It has been documented that *L. leucocephala* was used in dry season feeding of ruminants, agro forestation, wind breakers, live fence, alley farming, roadside landscaping, fuel production, paper production, timber production and nitrogen fixation (Simbaya, 2002; Orwa et al., 2009)

2.4 Anti-nutritional factors in *Leucaena leucocephala*

Despite the high protein content and cost effectiveness of *L. leucocephala* seed meal in the feed of various animals and fishes, its usage in animal feed formulation is limited due to the presence of anti-nutritional factors. Anti-nutritional factors can be defined as materials which themselves or their metabolite products

that get in the way with utilization of food resulting in adverse effect towards the health and production of animals (Makkar, Francis & Becker, 2007). Most of the potential alternatives that were derived from plant nutrient sources were revealed to contain numerous amounts of anti-nutritional substances.

Anti-nutritional factors could be generally classified into four groups at which; the first are the factors affecting utilization and digestion of protein, such as protease inhibitors, tannins, lectins. The second are factors affecting utilization of mineral which including phytates, gossypol pigments, oxalates and glucosinolates. While the third is anti-vitamins and the fourth are miscellaneous substances such as mycotoxins, mimosine, cyanogens, nitrate, alkaloids and saponins (Francis et al., 2001).

The anti-nutritional compounds that can be found in *L. leucocephala* seeds are mimosine and tannins. The concentration of mimosine, the toxic, non-protein amino acid, in the seeds are higher than in the other parts contributing as much as 14.8% to the total amount of nitrogen content of *L. leucocephala* seeds (Begum, 2014). The seeds of *L. leucocephala* have low tannin content (1.2%) compared to the high levels of tannin in the dry pods and the bark (16.3%).

In cattle, sheep and goat, mimosine has been reported to cause loss of appetite, extreme salivation, enlargement of thyroid gland, reduced hair growth, goitre, and depressed growth (Shelton and Brewbaker, 1998; Monoj and Bandyopadhyay, 2007). In some ruminants mimosine is degraded to dihydropyridines which is less toxic by rumen bacteria (FACT, 1997). In fish high levels of mimosine

has been reported to cause fin erosion and stunted growth (Sogbesan, Adebisi, Faliyi & Okaeme, 2006). Reduction of mimosine will enhance usage of *L. leucocephala* seeds in fish feed.

According to Letts (1963), alopecia, loss of food desire, extreme salivation, in synchronization of association and enlarged thyroid gland in the calves of buffalo had been observed when fed with plant with high mimosine content. Male buffalo calves fed on 50% *Leucaena* reported zero motility in two of the buffalo bulls who secreted semen (Lohan, Singh, Kakar & Gupta, 1988).

2.4.1 Reduction of anti-nutritional factors

The amount of mimosine content in *Leucaena leucocephala* seeds can be reduced through various methods. Heat treatment of *L. leucocephala* seed and drying by exposure towards sunlight and high temperatures causes considerable reductions in mimosine (Akbar & Gupta, 1985). Moist heat treatments such as dipping seeds in hot water and autoclaving can also be adopted to reduce the content of mimosine.

Washing with water and soaking the seeds had a considerable outcome in lowering the contents of mimosine. Prolonged soaking for 48 hours in 30°C water was claimed to be the most effective technique in reducing virtually all the mimosine in the seeds (Wee & Wang, 1987).

One of the most effective reagents for extracting up to 95% mimosine is by using 0.05 N of sodium acetate (Tawata, Hango, Sungawa, Kawastima & Yoga, 1986). Research by Hossain, Mustafa, Alam and Khan (1991) have been carried out by treating *L. leucocephala* seeds with a multiple number of chemicals. Urea and sodium hydrogen carbonate is stated to able to completely removed mimosine. The protein content of the mimosine-free seed mass was reduced up to 80% after treated with urea and 88% after being treated with sodium hydrogen carbonate solution (Hossain et al., 1991).

2.5 Microbound Diet (MBD)

Recently, microbound diets (MBD) was found to have potential to be utilise as a replacement for or supplements to aquatic animals in order to provide more appropriate nutrition (Holme, Zeng & Southgate, 2006). Microbound diets (MBD) are the dietary components that are held within a gelled hydrocholloid matrix or binder. In previous study, it has been shown that microbound diets particles have been willingly ingested by *Seylla serrata* larvae (Genodepa, Zeng & Southgate, 2007).

A few numbers of binders could be used in preparing MBD and each imparts particular physical attributes to the resulting food particles. Factors such as nutrient leaching, water stability and settlement rate of MBD particles are to a great extent influenced by the properties of binders (Barrows & Lellis, 2000). The preferences of MBD particles by the fish larvae are primarily impacted by the kind of binder used during its preparation. Agar, alginate, carrageenan, gelatine or zein can be used as a binder for the microbound diets which share similar dietary compositions (Genodepa

et al., 2007). It have been previously suggested by Genodepa et al. (2007) that zein is the probably a more appropriate binder for MBD developed for *Scylla Serrata* larvae because of its consistency in lowering the rates of leaching.

Microbound diets particle can be produced by gelation of mixtures of components. The particles obtained are then dried and, grounded and sieved to produce the required granulation (Holme et al., 2006). Generally, growth and survival of larvae are favoured when microbound particles prepared by agglomeration is demonstrated together with a small amount of live feed such as algae and rotifers (Lazo, Dinis, Holt, Faulk & Arnold, 2000).

The main advantage of microbound particles is the low production cost and easy preparation (Holme et al., 2006). However, they have a low capacity for the controlled release of low molecular weight substances such as amino acids. It has been demonstrated that up to 91% of free amino acids included in the diets are released prematurely from agglomerates containing alginate, carragenate and zein, after being suspended in water for two minutes (López-Alvarado, Langdon, Teshima & Kanazawa, 1994). One method that can be adopted in order to improve control of the releases of particles and to reduce loss of components to the aquatic environment is to coat the preparations with lipid (López-Alvarado et al., 1994).

2.6 Fish freshness and quality analysis

Fish quality is a complicated theory concerning the welfare of consumers, nutritional quality, accessibility, convenience and integrity, freshness, eating quality

and physical attributes of the fish itself (Bremner, 2000). A progressive lost of food characteristics in terms of taste and a general concept of quality could be occurred resulted from either physically, chemically, biochemically and microbiological changes (Olafsdottir et al., 2004).

In fact, the rate of fish spoilage depends on various factors such as the species of fish, sanitary conditions implemented on board, and the amount of food in the guts of the fish. In order to point the state of freshness, an amount of sensorial inspection procedures have been introduced involving the use of sight, tactile and olfaction (Macagnano et al., 2005). These procedures which incorporated a number of trained panels are generally believed to be as an expensive and time consuming method. Thus, several instrumental methods have been introduced in order to satisfy the needs of quality measurement.

Recently, several instrumental methods in measuring the physical, chemical and biological parameters of fish such as spectrophotometers, texture meters, image analysers, colorimeters, surface electrical properties test devices and electronic noses have been introduced (Macagnano et al., 2005).

2.7 Texture Profile Analysis (TPA)

Appearance, texture, odour and taste are significant aspects for fish quality evaluations. Texture is about sensory interpretation and expression of the structure or

interior construction of products linked to their response to stress and haptic attributes (Coppes, Pavlisko & Vecchi, 2002).

Methods in analysing texture are related to sensory and instrumental measurements. In the industry, sensory measurements are generally using 'finger method' which depends on the firmness of the fillet (Coppes, Pavlisko & Vecchi, 2002. The evaluation of firmness is commonly performed by pressing on the fish fillet using finger. This method is largely depends on subjective assessments of the expert panel (Cheng, Sun, Han & Zeng, 2013).

It has been proved that textural measurements of fillet using instrumental methods are better and more precise than sensory evaluation. The four main instrumental techniques used to evaluate fish texture are the puncture, compression, shear and tension. Double compression capable of performing texture profile analysis (TPA) obtained from a force-time curve (Cheng et al., 2013).

In TPA methods, the texture is usually measured as mechanical properties, manifesting as performance of hardness, gumminess, firmness, cohesiveness, adhesiveness, and viscosity by human senses (Hagen, Solberg, Sirnes & Johnston, 2007). As suggested by Caine, Aalhus, Best, Dugan and Jeremiah (2003), the individual parameters of fish texture can be explained as in Table 2.2:

Table 2.2: Definition of texture profile analysis parameters

Parameter	Definition
Hardness (Ha)	The maximum force reached during the first compressive

	cycle.
Adhesion (Adh)	The force required to separate the sample surface from the compressive plate surface in contact with it.
Springiness (Spr)	An active deformation length in mm during the second compression divided by the sample height (Length 2/Length 1).
Cohesiveness (Co)	The ratio of energies in the second cycle relative to the first one.
Chewiness (Ch)	The energy required to chew a solid food to the point required for swallowing it [Ch = Gu x Spr = Ha x Co x Spr]
Gumminess (Gu),	Gu = Ha x Co, is characteristic for semi-solid foods with a low degree of hardness and a high degree of cohesion

Based on the previous study by Dhanapal et al. (2010), texture measurements of O*. niloticus* were obtained by carrying out a compression test by placing the sample on the base plate and compressed twice and the texture profile analysis (TPA) was measured with a Texture Analyzer with a cylindrical stainless steel probe of 5mm diameter for fresh and cooked fish steaks.

2.8 Colour Evaluation

Colour is a significant criterion in selecting fish products towards consumers. Colour of fish is highly depending on the concentration of myoglobin and the degree of its oxidation, as well as the structure of meat (Huidobro, Miguel, Blázquez, &

Onega, 2005). Colour are believed to be one of the important factors in purchasing decisions concerning meat ad meat products (Saláková, 1986).

In a previous research by Olafsdottir et al. (2004), a hand-held spectral colour meter was used in order to determine the whole fish colour by measuring the visible spectral range (400-700 nm) at intervals of 10nm. The CIE L^*a^* b^*(CIELAB) system which is a colour space that specified by the International Commision on Illumination (French Commission internationale de l'éclairage), was used to measure the colour of the fish samples (Olafsdottir et al., 2004).

The CIELAB colour system is one of the most commonly used colour spaces in measuring the colour object which defined colour as a point in three dimensional space in relation to coordinates L^*, a^* and b^*. In this system, *L** signifies the lightness of the colour. It is located on a vertical axis in space, and its value ranges on a 0-100 scale from black to white. The coordinates *a** and *b** represent the values from which saturation and hue of a colour can be calculated. They exist in a horizontal plane. An *a** is part of a spectrum of wavelengths corresponding to colours from green *(-a*)* to red *(+ a*), b** from blue *(-b*)* to yellow *(+b*)* (Saláková, 1986).

CHAPTER 3

METHODOLOGY

3.1 *Oreochromis niloticus* feed preparation

The feed preparation for *Oreochromis niloticus* was conducted in the Animal Laboratory of UMK Jeli campus.

Leucaena leucocephala seeds were soaked in cold water for 72 hours and afterwards was sundried for two days (Sotolu & Faturoti, 2008) to get rid of anti-nutritional factors. The seeds were subsequently mashed into fine powdery form and then were used in feed preparation.

Three diets with different percentage of *L. leucocephala* seed were made. As recommended by Sotolu and Faturoti (2011), the percentage of *L. leucocephala* seed for the diets of *O. niloticus* were 0%, 25%, 50%. The dietary ingredients of *Leucaena leucocephala* seed feed were presented in Table 3.1.

Table 3.1 Feed formulation of *Leucaena leucocephala* seed feed

	LISM Inclusion		
Ingredient **(g/100g/DM)**	Control (0%)	Diet 1 (25%)	Diet 2 (50%)
Fish meal	28.30	21.23	14.15
LLSM	-	13.49	26.99
Soybean meal	27.10	27.10	27.10
Rice bran	39.1	32.68	26.26
Vitamin premix	1.00	1.00	1.00
Mineral premix	1.00	1.00	1.00
Vitamin c	1.00	1.00	1.00
Tapioca	1.00	1.00	1.00
Vegetable oil	1.00	1.00	1.00

*LLSM: *Leucaena leucocephala* seed meal

Percentages of ingredients of formulated feed were referred from a previous study that had been conducted by Sotolu and Faturoti (2011).

3.2 Proximate Analysis of Ingredients

Proximate analysis for rice bran, fishmeal, soybean meal, tapioca, *and Leucaena leucocephala* seed meal was conducted at Faculty of Veterinary Medicine, UMK PC campus.

3.2.1 Moisture content

Moisture content was determined by using Moisture Analyser MS-70 (GPS Instrumentation Ltd.) at 160°C with the weight of 5 g of the sample on the sample pan. The instruction manual of using this moisture analyser can be referred to the website at A&D MS-70 Moisture balance. The value of moisture content of sample would appear on the screen of moisture analyser. The data was recorded.

3.2.2 Crude protein

Kjeldahl method can be utilized in determining crude protein. This can be determined by Kjeldahl nitrogen multiplied by 6.25 which have been developed by Johan Kjeldahl in 1883. This method was divided into three stages which are digestion, distillation and titration.

One gram of sample was weighed into a Kjeldahl flask. Then, two pieces of Kjeldahl tablet and 12 mL of sulphuric acid were added into the flask. Kjeldahl flask was then digested in the digester KJELDATHERM Block Heating System for 1 hour 30 minutes. After digestion process, the sample was cooled off in the fume hood for one hour before proceed with distillation process. Next, Kjeldahl distillation system Vapodest 30s was warm up for ten minutes and 30 mL of receiver in conical flask containing 4 % of boric acid, 1 mL bromocressol green, 0.7 mL methyl red and 100 mL of distilled water was connected to the distillation unit. After distillation process was completed, the sample in conical flask was titrated with 0.1 mL hydrochloric acid (HCl) until the sample turns greyish pink.

Crude protein was calculated as below (Kwikiriza, Tibenda, Wadunde, Abaho & Ondhoro, 2016):

$$\text{Kjeldahl Nitrogen, \%} = \frac{(V_s - V_b) \times M \times 14.01}{W \times 10}$$

$$\text{Crude protein, \%} = \text{\% Kjeldahl Nitrogen} \times F\ (6.25)$$

Where,

V_s = volume (mL) of standardized acid used to titrate a test

V_B = volume (mL) of standardized acid used to titrate reagent blank

M = molarity of standard HCl

W = weight (g)

F = factor to convert N to protein; 6.25

3.2.3 Crude fibre

Crude fibre was analysed using Fibre bag System 6. The fibre bag was dried for one hour at 105°C before the analysis begins. Then, the bag is left to cool off in the desiccator for 30 minutes. The weight of fibre bag is labelled as M_1. One gram of sample was weighed into the fibre bag and labelled as M_2. Glass spacer was inserted into the fibre bag in order to ensure good flow through of the reagents. Next, the fibre bag containing sample was washed in petroleum ether 40/60 (cold) for three times and dried for two minutes. Only six fibre bags can be put into a sample carousel per time. Then, the beaker was place on the hotplate.

The fibre bag undergoes two washing phase which; Phase I by boiling in 360 mL sulphuric acid for 30 minutes. After the acid starting to boil, the fibre bag was then washed three times with hot water in order to remove acids and Phase II was by boiling the fibre bag with 360 mL sodium hydroxide solution for 30 minute. After the alkaline solution started to boil, the fibre bag was then washed three times with hot water in order to remove alkali. The fibre bag was removed from carousel and dried for 4 hours at 105°C before it was been placed in the desiccator for 30 minutes.

The crucible used for incineration was heated in the oven at 600 °C for 30 minutes and was then placed in the drying chamber at 105 °C for 30 minutes in order to cool it off. Next, the crucible was placed in the desiccator for 30 minutes and the weight for both crucibles with fibre bag was recorded and labelled as M_3.

Last step, the fibre bag that had been incinerated for 4 hours at 600 °C was then placed in the drying chamber at 105 °C for 30 minutes. Next, fibre bag was placed in the desiccator for 30 minutes and the weight of crucible containing ash was recorded and labelled as M_4. The formula in determining crude fibre was as below and calculated using software:

$$\text{Crude fibre, \%} = \frac{[(M_3 - M_1 - M_4) - (B_3 - B_1 - B_4)] \times 100}{M_2}$$

$$\text{Blank value} = B_3 - B_1 - B_4$$

Where,

M_1 = Weight of fibre bag (g)

M_2 = Initial sample weight (g)

M_3 = Incinerating crucible and dried fibre bag after digestion (g)

M_4 = Incinerating crucible and ash (g)

B_1 = Blank value of empty fibre bag (g)

B_2 = Incinerating crucible and dried fibre bag blank value after digestion (g)

B_3 = Incinerating crucible and ash blank value (g)

3.2.4 Crude fat

The initial weights of aluminium cups were recorded. Then, one gram of sample was weighed in a fine powder into thimble. The thimble and sample was placed into the extraction units by attached them to the magnets. After that, 80 mL of petroleum ether which act as extracting solvent was added in the aluminium cups and placed into the extraction unit with the cup holder. This was assembled in the Foss Soxtec 2055 system. The sample contained in the thimble was extracted in the extracting solvent by boiling it at 135 °C for 15 minutes and rising, recovery and pre-drying for 20, 10 and 2 minutes respectively. Then, aluminium cups were dried into the oven at 105 °C for 30 minutes and then, and then was left cooled in desiccator at room temperature for 20 minutes. The final weights of aluminium cups were recorded. Fat content was calculated as below (Kwikiriza et al., 2016):

$$\text{Percentage of fat} = \frac{\text{Final weight of aluminium cup (g)} - \text{Initial weight of aluminium cup (g)}}{\text{Sample weight (g)}}$$

3.2.5 Ash

According to method that was previously used by Thiex, Novotny and Crawford (2012), 2 g of sample was weighed and placed into the porcelain crucible. All weights of the sample were recorded. Then, porcelain crucible containing sample were placed into temperature-controlled furnace. Furnace required more than one hour in order to let the temperature to rise up until reaching desired temperature. In this study, the temperature used was at 550 °C. The temperature of 550± 10 °C was hold for three hours. The furnace was allowed to cool down after three hours. Then, porcelain crucible was transferred into desiccators to cool down and weight within one hour. The final weights of porcelain crucibles were recorded. The ash content was calculated as below:

$$\text{Percentage of ash (\%)} = \frac{\text{Final weight of crucible} - \text{Initial weight of crucible}}{\text{Weight of sample}} \times 100$$

3.3 *Oreochromis niloticus* culture

Tilapia fingerlings were stocked in a 30 L rectangular aquarium tanks with water volume maintained at 25 L. An amount of 12 tanks were used to place in the fingerlings. 360 of fingerlings with the initial weight of 5±1 g were randomly divided into the aquarium tanks at the ratio of 30 fingerlings per tank (M'balaka, Kassam & Rusuwa, 2012). Feeds were given according to their feeding rate which is 5% from their average body weight twice a day for 60 days. The fingerlings were acclimatized for 1 week.

Water quality parameter such as dissolved oxygen, pH, ammonia, and temperature were maintained and monitored every morning and evening. *Oreochromis niloticus* in this experiment had been raised in the optimum water environment which is required for them. In this experiment, the temperature was maintained between 24.8 °C to 28.5 °C and considered to be in its optimum level as suggested by Balarin and Haller (1982) that the temperature for normal development of tilapia is ranging from 20 to 35 °C.

In this study, the tilapia fish was raised in the tank with level of dissolved oxygen (DO) ranging from 4.28 to 6.82 mg L^{-1}. Generally, tilapia is recommended to live in an environment with the concentration of DO at the range of 5.0 to 7.5 mg L^{-1} and it can still survive in chronically low DO concentration at below than 2.5 mg L^{-1} (DeLong, Losordo, & Rakocy, 2009). Another study by El-Sayed (2006) stated that, tilapia species can tolerate DO levels as low as 0.1 mg L^{-1} and can even stay alive at zero DO concentration. Thus, it can be claimed that the tilapia in this study was raised in an acceptable level for tilapia growth.

The pH of water is maintained in between 6.29 to 7.25 and considered to be preferable towards the tilapia culture. This statement can be supported by previous study by Vanessa, Lima, Cavalcante and Sa (2015) which claimed that the juveniles of Nile tilapia can survive at pH levels of 4 and were stated that pH 8 are an ideal condition for efficient excretion of ammonia by the fish. Leftover feeds and faeces were removed using siphon regularly in order to maintain water quality.

3.4 Texture Analysis

The texture of tilapia fillet was analysed via instrumental technique texture profile analysis (TPA) for hardness, springiness, cohesiveness, and chewiness (Vácha et al., 2014). According to the Brookfield manual, parameters of TPA can be defines as in Table 3.2:

Table 3.2: Definition of parameters of texture profile analysis

Parameters	Definition
Hardness	Force needed to press food between molars or described as forces require attaining a given deformation.
Springiness	Ratio of the height of the sample springs back after compression compared to maximum deformation.
Cohesiveness	Net work invested in non-recoverable deformations of the first and second chews.
Chewiness	The energy required to chew a solid food to the point required for swallowing it

In this experiment, the skinless fillets with the average weight of 2.81±0.5 g was analysed for its texture properties using a Brookfield CT3-1000 Texture Analyzer. The fillet was left for 12hours in the refrigerator at the temperature of 4°C before being analysed for TPA. After 12 hours, the fillet was defrosted in room temperature and was ready for TPA. For texture profile analysis the fillet size required will be 2x2 cm. Five measurements was taken for each sample. The result of TPA was recorded (Dhanapal et al., 2010).

3.5 Colour Evaluation

A hand-held Kinoca Minolta spectrophotometer was used in determining the tilapia fillet. The samples of fillet with weight ranging from 3 to 5 g were prepared and the values for L^*, a^* and b^* were taken by placing the round plate of the spectrophotometer on the fish fillets. Five readings were taken for each treatment. The readings of colour evaluation were recorded.

3.6 Statistical analysis

The texture profile analysis effect and colour evaluation of the fish fillet will be determined using One-way Analysis of Variance (ANOVA) and mean test followed by Duncan Multiple Range Test ($p<0.05$).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Weight loss determination

The initial and final weight of tilapia fillets before and after storage in refrigerator was presented in Table 4.1.

Table 4.1: Average initial and final weight of tilapia fillets before and after storage

Parameters	Initial weight (g)	Final weight (g)	Total weight loss (g)
Control	3.50	3.39	0.11
Treatment 1	2.48	2.31	0.17
Treatment 2	2.89	2.81	0.08

[1]Control: formulated diet without *Leucaena leucochephala* seed meals: Treatment 1: formulated diet with 25% of *Leucaena leucochephala* seed meals, Treatment 2: formulated diet with 30% *Leucaena leucochephala* seed meals

Based on Table 4.1, it shown that, the average weight of tilapia fillets for all treatments, were decreased after being stored at storage of 4 °C for 12 h. For Control samples, the total weight loss is 0.11 g while for the samples in Treatment 1 and 2, the total weight losses are 0.17 g and 0.08 g respectively. The reduction in weight of tilapia fillet is might be resulted from the effect of drip loss.

According to Gang (2013), long and chilled storage showed positive effect on the drip loss of fish fillets and thus, resulted in weight loss. This could be explained by partial degradation and breakdown of the muscle by bacteria and enzymes. There are some disadvantages on fish fillets that have been associated with frozen storage including freezer burn, product dehydration, rancidity, drip loss, and product bleaching which could show an overall effect on the quality (Arannilewa, Salawu, Sorungbe, & Ola-Salawu, 2006).

4.2 Texture profile analysis

The results for texture profile analysis of the hardness, cohesiveness, gumminess, springiness and chewiness values of each sample for different experimental diets were presented in Table 4.2.

Table 4.2: Hardness, cohesiveness, gumminess, springiness and chewiness values of each sample for different experimental diets

Parameter/ Treatment	Control	Treatment 1	Treatment 2
Hardness (g)	5899.60 ± 307.02[a]	5888.60 ± 252.56[a]	5812.20 ± 428.09[a]
Cohesiveness	0.55 ± 0.04[a]	0.53 ± 0.10[a]	0.51 ± 0.08[a]
Gumminess (g)	3234.20 ± 334.97[a]	3127.40 ± 702.13[a]	2978.20 ± 610.53[a]
Springiness (mm)	42.44 ± 0.48[a]	42.66 ± 0.37[a]	42.81 ± 0.38[a]
Chewiness (mJ)	1346.92 ± 149.07[a]	1315.32 ± 299.84[a]	1249.24 ± 251.35[a]

[1]Values are mean±SD. Values in the same row with same superscripts are not significantly different (p>0.05).
[2]Control: formulated diet without *Leucaena leucochephala* seed meals: Treatment 1: formulated diet with 25% of *Leucaena leucochephala* seed meals, Treatment 2: formulated diet with 30% *Leucaena leucochephala* seed meal.

Based on the result obtained, there were no significant differences among the hardness, cohesiveness, gumminess, springiness, and chewiness of the flesh texture of tilapia fillets fed on different levels of *Leucaena leucocephala* seed meals. The Control diets revealed to have the highest value for hardness, cohesiveness, gumminess, and chewiness at 5899.60 g, 3234.20 g, and 1346.92 mJ respectively but possess no significant difference. Tilapia that had been fed with Treatment 2 diets appeared to possess the lowest value of for hardness which is at 5812.20 g, cohesiveness at 0.51, gumminess at 2978.20 g, and chewiness at 1249.24mJ. The springiness value was the highest for Treatment 2 diets at 42.81 mm and the lowest for Control diets at 42.44 mm. Overall, there is no significant difference of texture profile analysis in this study.

The texture of fish texture is of importance in consumers' decision to purchase the product. Flesh from rainbow trout feeding on plant protein sources diets had higher hardness, less sweetness, less odour intensity, and lower juiciness compared to the rainbow trout fed on animal protein sources diet (Francisco et al., 2004). Based on previous study, it have been reported that the inclusion of high levels of lipid content in the diets of rainbow trout resulted in softer fillets compared to the fillets fed on less lipid diets (Andersen, Thomassen, & Bencze, 1997).

Webster, Thomson and Muzinic (2004), claimed that flesh quality of gilthead seabream *(Sparus aurata)* was not affected by partial replacement of anchovy oil with vegetable oils such as soybean oil, rapeseed oil, or linseed oil except that there was an obvious stronger smell and taste of fish fed on soybean oil diet compared to the fish fed on marine fish oil diets. There were no differences in texture analysis or

organoleptic analysis of sea bass that have been fed with different feed diets containing vegetable oils and marine fish oil (Webster et al., 2004). This study can be supported with other study by Rosenlund, Obach, Sandberg, Standal and Twelt (2001) which reported that the Atlantic salmon that were fed with different lipid sources show no difference in fillet texture and colour parameters.

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4.3 Colour evaluation

The results for colour evaluation by determination of the values of lightness *(L^*),* red chromaticity *(a^*)* and yellow chromaticity *(b^*)* of *Oreochromis niloticus* fillet were shown in Table 4.3.

Table 4.3: Colour evaluation of *Oreochromis niloticus* fillet

Parameters	L^*	a^*	b^*
Control	34.3 ±3.09[a]	6.01±1.74[b]	10.55±1.80[a]
Treatment 1	33.50±7.15[a]	4.23±1.84[ab]	11.15±2.81[a]
Treatment 2	31.82±2.90[a]	3.33±1.09[a]	9.57±0.85[a]

[1]Values are mean±SD. Values in the same column with different superscripts are significantly different ($p<0.05$).
[2]Control: formulated diet without *Leucaena leucochephala* seed meals: Treatment 1: formulated diet with 25% of *Leucaena leucochephala* seed meals, Treatment 2: formulated diet with 30% *Leucaena leucochephala* seed meals

Table 4.3 revealed that, there were no significant differences for the lightness *(L^*),* and yellow chromaticity *(b^*)* value for all experimental diet. Red chromaticity *(a^*)* values exhibit significant differences between Control diet and Treatment 2 diet but showed no significant differences when compared with Treatment 1 diet. These differences were might due to the thickness of the sample. It have been

experimentally proved that the sample thickness affects the colour of meat (Saláková, 1986).

According to Qiufen, Yong, and Shi (2012), low quantity and bad quality of fat and premix content in the diet resulting in abnormal transportation and absorption of pigment granules in fish body. Protein quality is also an important factor especially the non-protein nitrogen (NPN). Based on previous study, it was stated that the rainbow trout muscle fed on algae with yellow pigments causing high yellow chromaticity value on the fish muscle.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, Treatment 2 showed to have the highest value for springiness when compared with other diets. However, Control diets showed highest results in terms of hardness, cohesiveness, gumminess, and chewiness of the flesh texture of tilapia fillets compared to Treatment 1 diets and Treatment 2 diets.

On the other hand, for colour evaluation, it showed that Control possess the highest value for lightness *(L^*),* red chromaticity *(a^*)*. The value of yellow chromaticity *(b^*)* showed the highest for Treatment 1. This is due to the colour pigmentation of *L. leucocephala* which gives yellow colour for the fillet.

In a conclusion, the *Oreochromis niloticus* fillet that have been fed with Control diets adopted better quality than both Treatment 1 and Treatment 2 and good in producing more desirable tilapia fillets for consumer. Thus, H_0 is accepted.

5.2 Recommendations

In future study, it is suggested that different pretreatment methods by using chemical reagents such as urea will be used in removing antri-nutrients presence in *Leucaena leucocephala* seeds were used. The seeds of *L. leucocephala* seeds could also be utilized as a supplement in fish diet.

REFERENCES

Akbar, M. A. & Gupta, P. C. (1985). Nutrient composition of different cultivars of *Leucaena Leucocephala*. *Indian Journal Animal Science, 55,* 54-58.

Al-Hafedh, Y. S. & Siddiqui, A. Q. (1998). Evaluation of guar seed as a protein source in Nile tilapia, *Oreochromis niloticus* (L.), practical diets. *Aquaculture Research, 29(10),* 703-708.

Andersen, U., Thomassen, M., & Bencze R, A. (1997). Texture properties of farmed Rainbow trout (*Oncorhynchus mykiss*): effect of diet, muscle fat content and time of storage on ice. *Journal of Food Science and Agriculture, 74,* 347-353.

Arranilewa, S.T., Salawu, S. O., Sorungbe, A. A., & Ola-Salawu, B. B. (2006). Effect of frozen period on the chemical, microbiological and sensory quality of frozen Tilapia fish (*Sarotherodun galiaenus*). Nutrition *and Health (Berkhamsted, Hertfordshire), 18(2),* 185-192.

Balarin, J.D. & Haller, R.D. (1982). *The intensive culture of tilapia in tanks, raceways and cages*. USA: Westview Press. 265-355.

Balios, J. (2003). Nutritional value of fish by-products, and their utilization as fish silage in the nutrition of poultry. *International Conference on Environmental Science and Technology,* Lemnos Island, Greece. pp. 70-76.

Balois, J. (2003). Nutritional value of fish by-product and their utilization as fish silage in the nutrition of poultry. *Proceedings of the 8th International Conference on Environmental Science and Technology*, Lemnos Island, Greece. pp. 8-10.

Barrows F. T., & Lellis W. A. (2000). Microbound feeds. In: Stickney R. R. (eds.) *Encyclopedia of Aquaculture* (pp. 525-528). New York: John Wiley & Sons. 525-528.

Beaven, U. & Muposhi. V. K. (2012). Aspects of a Monosex Population of *Oreochromis niloticus* Fingerlings Produced Using 17-α Methyl *Testosterone Hormone. Journal of Aquaculture Research and Development 3(3)*.

Begum, T. (2014). Effects of *Leucaena leucocephala* (Lam. de Wit) leaves and seeds on *Channa punctatus* (Bloch and Schneider) and *Oreochromis niloticus* (Linnaeus). PhD Thesis, University of Dhaka, Bangladesh.

Bowen, S.H. (1976) Feeding ecology of the cichlid fish *Sarotherodon mossambicus* in Lake Sibaya, KwaZulu. PhD thesis, Rhodes University, Grahamstown, South Africa.

Bowen, S.H. (1982) Feeding, digestion and growth – qualitative considerations. In: Pullin, R.V.S. and Lowe-McConnell, R.H. (eds) *The Biology and Culture of Tilapias.* ICLARM Conference Proceedings No. 7 (pp. 141-156). Manila: Phillipines.

Bremner, H. A. (2000). Toward practical definitions of quality for food science. *Critical Reviews in Food Science and Nutrition, 40,* 83–90.

Bwanika, G. N., Makanga, B., Kizito, Y., Chapman, L. J., & Balirwa, J. (2004). Observations on the biology of Nile tilapia, *Oreochromis niloticus* L., in two Ugandan crater lakes. *African Journal of Ecology, 42(1),* 93-101.

Bwanika, G. N., Murie, D. J., & Chapman, L. J. (2007). Comparative age and growth of Nile tilapia (*Oreochromis niloticus L.)* in Lakes Nabugabo and Wamala, Uganda. *Hydrobiologia, 589(1),* 287-301.

Caine, W. R., Aalhus, J.L., Best, D. R., Dugan, M. E. R. & Jeremiah, L. E. (2003). Relationship of texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks. *Meat Science, 64,* 333–339.

Cheng, J. H., Sun, D. W., Han, Z., & Zeng, X. A. (2013). Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: A review. *Comprehensive Reviews in Food Science and Food Safety*, *13(1)*, 52-61.

Coppes Z., Pavlisko. A., & Vecchi. S.D., (2002). Texture measurements in fish and fish products. *Journal Aquatic Food Prod Technology ,11(1)*, 89–105.

DeLong, D.P., Losordo, T. M., & Rakocy, J. E. (2009). Tank Culture of tilapia. Southern Regional Aquaculture Centre, 17(282). Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7836875

Dhanapal, K., Reddy, G. V. S., Naik, B. B., Venkateswarlu, G., Reddy, A. D., Basu, S. (2012). Effect of cooking on physical, biochemical, bacteriological characteristics and fatty acid profile of Tilapia (*Oreochromis mossambicus*) fish steaks. *Archive Applied Sciences Research, 4(2),* 1142-1149.

El-Saidy, D. M. S. D. & Saad, S.A., (2010). Effects of partial and complete replacement of soybean meal with cottonseed meal on growth, feed utilization and haematological indexes for mono-sex male Nile tilapia *Oreochromis niloticus(L.)* Fingerlings. *Aquaculture Research, 42(3)*, 351-359.

El-Sayed, A. F. M. (2006). Tilapia Culture in salt water: environmental requirements, nutritional implications and economic potentials. *Avances En Nutrición Acuícola, VII*, 95–106.

Food and Agriculture Organization [FAO]. (2006). *Oreochromis niloticus* (Linnaeus, 1758). Retrieved on November 15, 2007 from the Fisheries and Aquaculture Department website: http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en

Food and Agriculture Organization [FAO]. (2014). *The State of World Fisheries and Aquaculture.* Italy: Food and Agriculture Organization of The United Nations. 39-55

Forest, Farm, and Community Tree Network [FACT]. (1997). *Leucaena leucocephala*: A Versatile nitrogen fixing tree. FACT sheet. Retrieved October 20, 2017 from the FACT Net Website: http://thegrownetwork.com/wp-content/uploads/2015/07/Leucaena_leucocephala_A_Versitile_Nitrogen_Fixing_Tree.pdf

Francis, G., Makkar, H. P., & Becker, K. (2001). Anti- nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture, 199,* 197-227.

Francisco, M., Parisi, G., Médale, F., Lupi, P., Kaushik, S. J. & Poli, B. M. (2004). Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 236(1-4),* 413-429.

Gang, M. (2013). Changes in the quality and yield of fish fillets due to temperature fluctuations during processing. Final Project. Dalian Ocean University, China.

Genodepa, J., Zeng, C., & Southgate, P. C. (2007). Influence of binder type on leaching rate and ingestion of microbound diets by mud crab, *Scylla serrata* (Forsskål), larvae. *Aquaculture Research*, *38*, 1486–1494

Ghosh, M. K. & Bandyopadhyay, S. (2007). Mimosine Toxicity-A Problem of *Leucaena* Feeding in Ruminants . *Asian Journal of Animal and Veterinary Advances, 2,* 63-73.

Global Invasive Species Database [GISD]. (2012). Species profile: *Oreochromis.* Retrieved April 17, 2007 from the Invasive Species Specialist Group website: http://www.iucngisd.org/gisd/speciesname/*Oreochromis*

Hagen Ø., Solberg C., Sirnes E, & Johnston I. A. (2007). Biochemical and structural factors contributing to seasonal variation in the texture of farmed Atlantic Halibut (*Hippoglossus hippoglosus* L.) flesh. *Journal of Agricultural and Food Chemistry*. *55(14)*. 5803-5808.

Hall, G.M. (1992). Fish processing technology. In: Ockerman, H.W. (eds*). Fishery byproducts*, (pp. 155-192). New York: VCH Publishers.

Harbott, B.J. (1982) Studies on the feeding activity of *Sarotherodon niloticus* (L.) in Lake Turkana. In: *A Report on the Findings of the Lake Turkana Project, 1972–1975*, Vol. 5. Government of Kenya and the Ministry of Overseas Development, London, pp. 1357–1368.

Hardy, R. W. (2010). Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquaculture Research, 41*, 770-776.

Holme, M.-H., Zeng, C., & Southgate, P. C. (2006). Use of microbound diets for larval culture of the mud crab, *Scylla serrata*. Aquaculture, *257*, 482–490.

Hossain, M. A., Mustafa, A.I., Alam, &, Khan, M. Z. A. (1991). Studies on the removal of mimosine from ipil-ipil (*Leucaena leucocephala*) seed. *Journal Bangladesh Chemical Sociology, 4,* 83-5.

Huidobro, F. R., Miguel, E., Blázquez, B. & Onega, E. (2005). A comparison between two methods (Warner-Bratzler and texture profile analysis) for testing either raw meat or cooked meat. *Meat Sciene, 69(3),* 527-536.

Kapetsky, J. M., & Nath, S. S. (1997). A strategic assessment of the potential for freshwater fish farming in Latin America. *COPESCAL Technical Paper No 10*, Rome. pp. 128.

Kaushik, S. J., Coves, D., Dutto, G., & Blanc, D. (2004). Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax.Aquaculture, 230*, 391-404.

Kwikiriza, G., Tibenda, V. N., Wadunde, A. O., Abaho, I., & Ondhoro, C. C. (2016). Proximate nutrient composition and cost of the selected potential fish feed ingredients in Lake Victoria basin, Uganda. *International Journal of Fisheries and Aquatic Studies*, *4*(3), 611–615.

Lazo, J. P., Dinis, M. T., Holt, G. J., Faulk, C. & Arnold, C. R. (2000). Co-feeding microparticulate diets with algae: towards eliminating the need of zooplankton at first feeding in larval red drum (*Sciaenops ocellatus), Aquaculture, 188*, 339-351.

Letts, G. A., (1963). *Leucaena glauca* and ruminants. *Australia Veterinary Journal, 39,* 287-288.

Lohan, O. P., Singh, S.P., Kakar, M. L. and Gupta, P. C. (1986). Effect of Subabul (*Leucaena leucocephala*) feeding on reproductive performance and hormonal profile in Murrah males. *Indian Journal Dairy Science, 31*, 404-405.

López-Alvarado, J., Langdon, C. J., Teshima, S. I., & Kanazawa, A. (1994). Effects of coating and encapsulation of crystalline amino acids on leaching in larval feeds. *Aquaculture*, *122*, 335–346.

M'balaka, M., Kassam, D., & Rusuwa, B. (2012). The effect of stocking density on the growth and survival of improved and unimproved strains of *Oreochromis shiranus. Egyptian Journal of Aquatic Research*, *38(3),* 205–211.

Macagnano, A., Careche, M., Herrero, A., Paolesse, R., Martinelli, E., Pennazza, G., Carmona, P., Amico, A. D., Natale, C. D. (2005). A model to predict fish quality from instrumental features. *Sensors and Actuators B, 111-112(2005),* 293-298.

Makkar, H. P. S., Francis, G. & Becker, K. (2007). Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal, 1(9),* 1371–1391.

Miles, R. D. & Chapman, F. A. (2010). The benefits of fish meal in aquaculture diets. *Fisheries and Aquatic Sciences, 122(1).*

Monentcham, S. E., Kouam, J., Chuba, D., Wathelet, B., Pouomogne, V., & Kestemont, P. (2010). Partial substitution of fish meal with soybean and cotton seed meals in diets of African bonytongue, *Heteroclarias niloticus* (Cuvier, 1829) fingerlings: effects on growth, feed efficiency, and body composition. *Aquaculture Research, 41(10),* 385-392.

Monoj, K.G. and Bandyopadhyay, S. (2007). Mimosine toxicity - A problem of *Leucaena* feeding in ruminants. *Asian Journal of Animal and Veterinary advances*, *2(2),* 63-73.

Morgan, P. R. (1972) Causes of mortality in the endemic tilapia of Lake Chilwa (Malawi). *Hydrobiologia,* 40, 101–119.

Moriarty, C.M. & Moriarty, D.J.W. (1973). Quantitative estimation of daily ingestion of phytoplankton by *Tilapia nilotica* and *Haplochromis nigripinnis* in Lake George, Uganda. *Journal of Zoology, 171*, 15–24.

Nandeesha, M.C., Gangadhara, B., Varghese, T.J. and Keshavanath, P. (2000). Growth responses and flesh quality of common carp, Cyprinus carpio fed with high levels of non-defatted silkworm pupa. *Asian Fisheries Science*, *13*, 235-242.

Negroni, G. (2013). Tilapia farming guide. *Tilapia farming introduction, (2*), 23-34.

New, M. B. & Wijkstrom, U. N. (2002). Use of fishmeal and fish oil in aquafeeds: further thoughts on the fishmeal trap. *FAO Fisheries Circular, 95*, 7-55.

Olafsdottir, G., Nesvadba, P., Natale, C. D., Careche, M., Oehlenschläger, J., Tryggvadóttir, S. V., Schubring, R., Kroeger, M., Heia, K., Esaiassen, M., Macagnano, A., Jørgensen, B. M. (2004). Multisensor for fish quality determination. *Trends in Food and Science Technology, 15(2004),* 86-93.

Olvera-Novoa, M. A., Campos, G. S., Sabido, M. G., & Palacios, C. A. M., (1990). The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oreochromis mossambicus*). *Aquaculture, 90*, 291-302.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Anthony, S. (2009). Leucaena leucocephala. Retrieved on April 15, 2017 from the Agroforestree Database Website: http://www.worldagroforestry.org/treedb/AFTPDFS/Leucaena_leucocephala.PDF

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Simons, A. (2009*). Leucaena leucocephala.* Retrieved October 27, 2017 from Agroforestry Database Website: http://www.worldagroforestry.org/treedb/AFTPDFS/Leucaena_leucocephala.PDF

Pillay, T. V. R. (1990). *Aquaculture: principles and practices*. UK: Fishing New Books. 575.

Popma, T., & Masser, M. (1999). Tilapia Life History and Biology. *South Regional Aquaculture Center*, *283*, 1–4.

Qiufen, D., Yong, Y., & Shi, S. (2012). Nutrition and changes in fish body colouration in catfish. *Aquaculture Asia Pacific Magazine*, *(2),* 26–28.

Randall, D.J., Wood, C.M., Perry, S.F., Bergman, H., Maloiy, G.M.O., Mommsen, T.P. & Wright, P.A. (1989). Urea excretion as a strategy for survival in a fish living in a very alkaline environment. *Nature,* 337, 165–166.

Randall, D.J., Wood, C.M., Perry, S.F., Bergman, H.,Maloiy, G.M.O., Mommsen, T.P. and Wright, P.A. (1989). Urea excretion as a strategy for survival in a fish living in a very alkaline environment. *Nature, 337*, 165–166.

Reite, O.B., Maloiy, G.M.O. and Aasehaug, B. (1974). pH, salinity and temperature tolerance of Lake Magadi *Tilapia*. *Nature, 247*, 315.

Richardson, D. M., Bond, W. J., Dean, W. R. J., Higgens, S. I., Midgley, G. F., Milton, S. J., Powrie, L. W., Rutherford, M. C., Samways, M. J. & Schulze, R. E. (2000). Invasive alien species and global change: a South African perspective. In: Mooney, H. A. & Hobbs, R. J. (eds), *Invasive species in a changing world* (pp. 303-349). Washington D. C: Island Press.

Rosenlund, G., Obach, A., Sandberg, M. G., Standal, H. & Twelt, K. (2001). Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar* L.). Aquaculture Research, 32(1), 323-328.

Ross, L.G. (2000) Environmental physiology and energetics. In: Beveridge, M.C.M. and McAndrew, B.J. (eds) *Tilapias: Biology and Exploitation* (pp. 89-128). Dordrecht, Boston, London: Kluwer Academic Publishers.

Saláková, A. (1986). Instrumental measurement of texture and colour of meat and meat products. *Journal of the University of Veterinary and Pharmaceutical Sciences in Brno, Czech Republic*. *Vol 2012*, 107-104.

Schipp, G. (2008). *Is the Use of Fishmeal and Fish Oil in Aquaculture Diets Sustainable?.* Australia: Northern Territory Government.

Shelton, H.M. & Brewbaker, J.L. (1998). *Leucaena leucocephala* – the Most Widely Used Forage Tree Legume Species. In: Gutteridge, R. C. & Shelton, H. M (pp. 15-29). London: CAB International.

Shipton, T., Tweddle, D. & Watts, M. (2008). Introduction of The Nile Tilapia (*Oreochromis niloticus)* into the Eastern Cape. *Eastern Cape Development Corporation*, *30.*

Sifa, L., Chenhong, L., Dey, M., Gagalac, F. and Dunham, R. (2002). Cold tolerance of three strains of Nile tilapia, *Oreochromis niloticus*, in China. *Aquaculture, 213*, 123–129.

Simbaya, J. (2002). Potentials of fodder tree, shrub, and legumes as feed resource for dry season supplementation of small-holder ruminant animals. *IAEA Technical Cooperation Regional AFRA Project*, FAO, Cairo.pp. 69-76.

Smith, A. (1840). *Illustration of the Zoology of South Africa*, London: Smith, Elder and Co.Cornhill. 5.

Sogbesan, A. O., Adebisi, A. A., Falayi, B .A. & Okaeme, A. N. (2006). Some aspects of dietary protein deficiency pathology in the culture systems of tropical fishes. *Journal of Arid Zone Fisheries*, *2(1),* 80-89.

Sotolu, A. O. & Faturoti, E. O. (2008). Digestibility and nutritional values of differently Processed *Leucaena leucocephala* (Lam de wit) seedmeal in the diet of African catfish (*Clarias gariepinus*). *Middle-East Journal of Scientific Research. 3(4),* 190-199.

Sotolu, A. O. & Faturoti, E. O. (2011). Growth performance and hematological effects of varying dietary processed *Leucaena leucocephala* seed meal in *Clarias gariepinus* (burchell, 1822) juveniles. *African Journal of Food, Agriculture, Nutrition and Development, 11(1).*

Tacon A.G.J. & De Silva S.S. (1997). Feed preparation and feed management strategies within semi-intensive fish farming systems in the tropics. *Aquaculture 151*, 379-404.

Tawata, S., Hongo F., Sungawa, K., Kawastima, Y. & Yoga, S. (1986). A simple reduction method of mimosine in the tropical plant *Leucaena*. *Science Bulletin College Agriculture University of Rykyus, 33,* 87-93.

Teichert-Coddington, D. and Green, B.W. (1997) Tilapia yield improvement through maintenance of minimal oxygen concentrations in experimental grow-out ponds in Honduras. *Aquaculture, 118*, 63–71.

Thiex, N., Novotny, L., & Crawford, A. (2012). Determination of Ash in animal feed: AOAC Official Method 942.05 revisited. *Journal of AOAC International*, *95(5)*, 1392–1397.

Trewavas, E. (1982) Tilapias: taxonomy and speciation. *The Biology and Culture of Tilapias ICLARM Conference Proceedings No. 7*, ICLARM, Manila, Philippines, pp. 3–13.

Troell, M. (2009). Integrated marine and brackish water aquaculture in tropical regions: research, implementation and prospects. *FAO Fisheries and Aquaculture Technical Paper*. *(529),* 56-70.

Tsadik, G. G. and Kutty, M.N. (1987). Influence of Ambient Oxygen on Feeding and Growth of the Tilapia, *Oreochromis niloticus* (Linnaeus). UNDP/ FAO/NIOMR, Port Harcourt, Nigeria, pp 13.

Vácha, F., Stejskal, V., Vejsada, P. & Kouřil, J.D.H. (2014). Texture profile analyses in tench (*Tinca tinca L*., 1758) from extensive and intensive culture. *Acta Veterinaria Brno*, *82(4)*, 421–425.

Vanessa, T. R., Lima, F. R. D. S., Cavalcante, D. D. H., Sa, V. D. C. (2015). Tolerance of Nile tilapia juveniles to highly acidic rearing water. Acta *Sciencetiarum Animal Sciences, 37(3),* 227-233.

Walton, C.S. (2003). *Leucaena (Leucaena leucocephala)* in Queensland: Pest Status Review Series-Land Protection. Brisbane: Department of Natural resources and Mines of Queensland. 5-38.

Wangead, C., Greater, A. & Tansakul, R. (1988) Effects of acid water on survival and growth rate of Nile tilapia (*Oreochromis niloticus*). *Proceedings of the Second International Symposium on Tilapia in Aquaculture ICLARM Conference Proceedings No. 15*, Department of Fisheries, Bangkok, Thailand, and ICLARM, Manila, Philippines, pp. 433–438.

Webster, C. D., Thompson, K. R. & Muzinic, L. A. (2004). *57th American Meat Science Association Reciprocal Meat Conference*, Lexington, Kentucky. pp. 49-55.

Wee, K. L. & Wang, S. S. (1987). Nutritive value of *Leucaena* leaf meal in pelleted feed for Nile tilapia. *Aquaculture, 62*, 97-108.

Whitefield, A. K., & Blaber, S. J. M. (1979). The distribution of the freshwater cichlid *Sarotherodon mossambicus* in estuarine systems. *Environmental Biology of Fishes*, *(4),* 77–81.

Whitefield, A.K. & Blaber, S.J.M. (1978). Resource segregation among iliophagous fish in Lake St Lucia, Zululand. *Environmental Biology of Fishes, 3*, 293–296.

Zhou, H., Jiang, R., Xue, M., Xie, S., Wu, X., & Guo, L. (2010). Fishmeal can be completely replaced by soy protein concentrate by increasing feeding frequency in Nile tilapia (*Oreochromis niloticus* GIFT strain) less than 2 g. *Aquaculture nutrition, 16(6),* 648-653.

APPENDIXES

A.1 One-way ANOVA

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
initialweight	Between Groups	2.610	2	1.305	1.856	.199
	Within Groups	8.440	12	.703		
	Total	11.050	14			
finalweight	Between Groups	2.890	2	1.445	2.117	.163
	Within Groups	8.189	12	.682		
	Total	11.079	14			
hardness	Between Groups	22238.533	2	11119.267	.098	.908
	Within Groups	1365227.200	12	113768.933		
	Total	1387465.733	14			
cohesiveness	Between Groups	.004	2	.002	.302	.744
	Within Groups	.072	12	.006		
	Total	.075	14			
springiness	Between Groups	.336	2	.168	.990	.400
	Within Groups	2.036	12	.170		
	Total	2.372	14			
gumminess	Between Groups	165338.133	2	82669.067	.254	.780
	Within Groups	3911744.800	12	325978.733		
	Total	4077082.933	14			
chewiness	Between Groups	24844.181	2	12422.091	.213	.811
	Within Groups	701207.528	12	58433.961		
	Total	726051.709	14			
L	Between Groups	16.087	2	8.044	.349	.712
	Within Groups	276.290	12	23.024		
	Total	292.377	14			

A	Between Groups	18.583	2	9.292	3.651	.058
	Within Groups	30.540	12	2.545		
	Total	49.124	14			
B	Between Groups	6.364	2	3.182	.805	.470
	Within Groups	47.429	12	3.952		
	Total	53.792	14			

A.2 Post Hoc Analysis of Duncan Multiple Test for Initial Weight

Initial weight

Duncan[a]

treatment	N	Subset for alpha = 0.05
		1
treatment1	5	2.4800
treatment 2	5	2.8940
control	5	3.4960
Sig.		.093

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 5.000.

A.3 Post Hoc Analysis of Duncan Multiple Test for Final Weight

Final weight

Duncan[a]

treatment	N	Subset for alpha = 0.05
		1
treatment1	5	2.3120
treatment 2	5	2.8060
control	5	3.3860
Sig.		.073

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A.4 Post Hoc Analysis of Duncan Multiple Test for Hardness

Hardness

Duncan[a]

treatment	N	Subset for alpha = 0.05
		1
treatment 2	5	5812.2000
treatment1	5	5886.6000
Control	5	5899.6000
Sig.		.704

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A.5 Post Hoc Analysis of Duncan Multiple Test for Cohesiveness

Cohesiveness

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
treatment 2	5	.5100
treatment1	5	.5300
Control	5	.5480
Sig.		.474

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A.6 Post Hoc Analysis of Duncan Multiple Test for Springiness

Springiness

Duncan[a]

treatment	N	Subset for alpha = 0.05
		1
control	5	42.4440
treatment1	5	42.6640
treatment 2	5	42.8080
Sig.		.208

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A.7 Post Hoc Analysis of Duncan Multiple Test for Gumminess

Gumminess

Duncan[a]

treatment	N	Subset for alpha = 0.05
		1
treatment 2	5	2978.2000
treatment1	5	3127.4000
control	5	3234.2000
Sig.		.513

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A.8 Post Hoc Analysis of Duncan Multiple Test for Chewiness

Chewiness

Duncan[a]

treatment	N	Subset for alpha = 0.05
		1
treatment 2	5	1249.2400
treatment1	5	1315.3200
control	5	1346.9200
Sig.		.555

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A.9 Post Hoc Analysis of Duncan Multiple Test for L^*

L*

Duncan[a]

treatment	N	Subset for alpha = 0.05
		1
treatment 2	5	31.8180
treatment1	5	33.4980
control	5	34.3040
Sig.		.451

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A.10 Post Hoc Analysis of Duncan Multiple Test for *a*[*]

a*

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
treatment 2	5	3.3340	
treatment1	5	4.2300	4.2300
Control	5		6.0120
Sig.		.392	.103

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A.11 Post Hoc Analysis of Duncan Multiple Test for *b*[*]

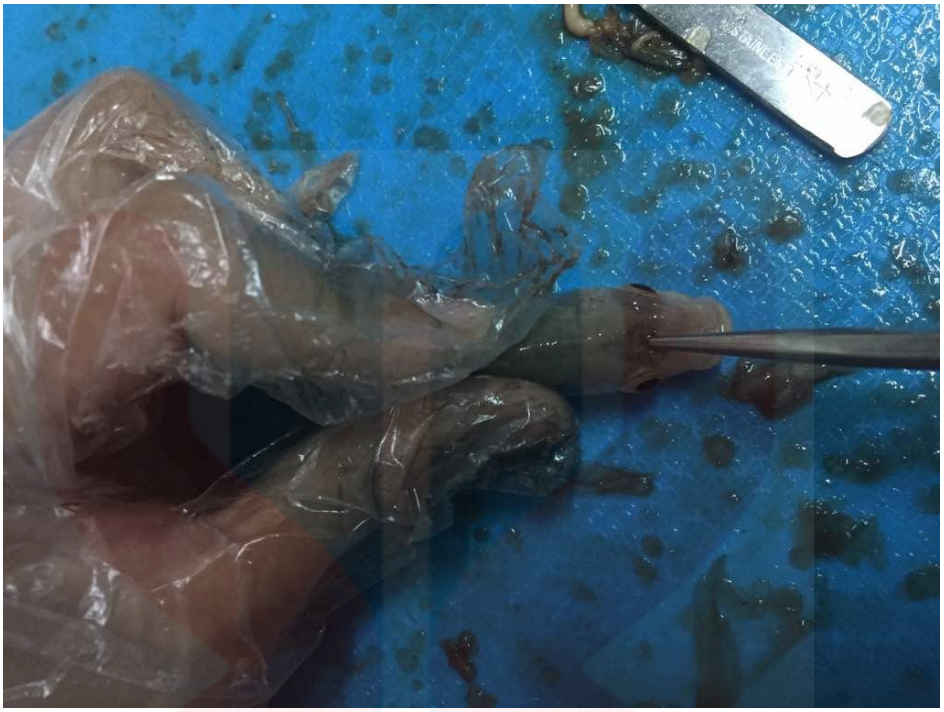
b*

Duncan[a]

treatment	N	Subset for alpha = 0.05
		1
treatment 2	5	9.5660
control	5	10.5480
treatment1	5	11.1460
Sig.		.255

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.



A.12: Pitting method is used to paralyse the tilapia fish.

A.13: The tilapia was cut into fillet.

A.14: Texture profile analysis of tilapia fillet.