Partial Replacement of Fish meal with *Leucaena leucocephala* Seeds Meal on Nile Tilapia (*Oreochromic niloticus* Linnaeus 1757) Fingerlings

By

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A report submitted in fulfilment of the requirement for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours

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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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Student

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I certify that the report of this final year project entitled “Partial Replacement of Fish meal with *Leucaena leucocephala* Seeds Meal on Nile Tilapia (*Oreochromic niloticus,* Linnaeus 1757) Fingerlings by Nurul Nabilah Binti Abdul Rahman Adrin, matric number F14A0311 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 Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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PARTIAL REPLACEMENT OF FISH MEAL WITH *LEUCAENA LEUCOCEPHALA* SEEDS MEAL ON NILE TILAPIA (*OREOCHROMIC NILOTICUS*, LINNAEUS 1757) FINGERLINGS

ABSTRACT

This study was conducted on partial replacement of fish meal with different percentage of *Leucaena luecocephala* seed meal (LSM) which are Diet 1 = 0 % LSM, Diet 2 = 25 % LSM and Diet 3 = 30 % LSM on Nile tilapia (*Oreochromic niloticus*) fingerling. The used of *L. leucocephala* seed were expected to give higher nutrition value for fish dietary as well as it come from legume plant that are easily growth and cheaper than fish meal. The diet were fed to the Nile tilapia (*O. niloticus)* fingerlings for six weeks in order to determine their growth performance. Data were collected on fish growth, feed consumption and survival rate. Different percentage of the *L. leucocephala* seed meal were produced different growth rate performance. At the end of the experiment, all the treatment diet showed positive growth effect. Diet 2 containing 25 % of *L. leucocpehala* seed meal showing the best growth performance with the best weight gain 6.35 g/fish, specific growth rate (SGR) 0.90 %, and feed conversion ratio (FCR) that is 2.45 among other but lowest on the survival rate that is 85.56 %. Nevertheless, there is no significant difference between weight gains, SGR, FCR and survival rate from all the treatment diets.

Keywoods: *Leucaena leucocephala* seed meal, fish meal, tilapia fingerling.

PERTUKARAN TEPUNG IKAN DENGAN MENGGUNAKAN TEPUNG BIJI BENIH *LEUCAENA LEUCOCEPHALA* YANG TELAH DIPROSES KE ATAS ANAK IKAN TILAPIA HITAM (*OREOCHROMIC NILOTICUS*, LINNAEUS 1757)

ABSTRAK

Kajian ini keatas penukaran sebahagian tepung ikan dengan tepung biji benih *Leucaena leucocephala* (LSM) dalam peratus yang berbeza-beza seperti Diet 1 = 0 % LSM, Diet 2 = 25 % LSM dan Diet 3 = 30 % LSM pada anak ikan Tilapia Hitam (*Oreochromis niloticus*). Penggunaan tepung biji benih *L. lleucocpehala* dijangka memberikan nilai nutrisi yang tinggi dan juga berasal dari tumbuhan kekacang yang mudah tumbuh dan murah dari tepung ikan. Diet ini telah diberi kepada anak ikan Tilapia Hitam (*O. niloticus)* selama enam minggu untuk menentukan kadar pertumbuhan berat mereka. Data dikumpul untuk pembesaran ikan, kadar pemakanan dan kadar hidup. Perbezaan peratusan daripada tepung biji benih *L. leucocephala* menghasilkan kadar pembesaran yang berlainan. Pada akhir kajian, semua diet menunjukkan kadar pembesaran yang positif. Diet 2 yang mengandungi 25 % tepung biji benih *L. leucocephala* menunjukkan prestasi pertumbuhan terbaik dengan kenaikan berat badan (WG) iaitu 6.35 g/ikan, kadar pertumbuhan tertentu (SGR) 0.90 %/hari dan nisbah penukaran makanan (FCR) iaitu 2.45 berbanding yang lain tetapi rendah pada kadar hidup iaitu hanya 85.56 %. Walaubagaimanapun, tiada perbezaan signifikan antara kenaikan berat badan, SGR, FCR dan kadar kelangsungan hidup dari semua Diet.

Kata kunci: Tepung biji benih *Leucana leucocephala,* tepung ikan, anak ikan tilapia hitam

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
˚C	Degree Celcius
<	Less than
>	More than
Al^{3+}	Aluminium
AMBD	Agar Microbound Diets
CF	Crude Fibre
CP	Crude Protein
DO	Dissolved Oxygen
EE	Ether Extract
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
FE	Feed efficiency
Fe^{2+}	Iron
g	Gram
$ha^{-1}yr^{-1}$	Per hectare per year
HCl	Hydrochloric acid
LSM	*Leucaena* Seeds Meal
MBD	Microbound diet
MED	Microencapsulated diets
mg/l	Milligram per litre
MWG	Mean Weight Gain
NFE	Nitrogen free-extract
pH	Potential of hydrogen

SGR	Specific Growth Rate
WG	Weight Gain
Zn^{2+}	Zinc

CHAPTER 1

INTRODUCTION

1.0 Research Background

It is generally well known that fish and fishery product create really important source of protein and nutritional security for many people all over the world (FAO, 2012). The farms proprietors are overwhelmingly male that range from 41 and 60 years and around 70 % aquaculture was recorded as their real wellspring of salary in Malaysia. There are 54 % of the farmer had their own particular land however these are basically small and medium scale producers. The typical feeding costs of surveyed farms were almost 63 % of the production cost. The utilized of business tilapia sustain in more than 90 % of the farms overview was lead to high production cost. There are a considerable measure of supplementary encourage sources of info, for example cattle and poultry pellet nourish, farm-made feeds, copra meal, palm kernel cake, poultry intestines, animal carcasses and kitchen waste were utilized by little and medium creators to cut sustaining expense to the raising animal (Ng, Teh, Chowdhury, & Bureau, 2013).

This study is conduct to determine the effect of partial replacing fish meal with *Leuceana leucocephala* seed meal in order to improve growth performance of Nile tilapia fingerlings. Fish meal is characterized as a strong product found by eliminating large portion of water and few or the majority oil from fish or fish leftover. Commonly, fish meal is sold as powder form, and usually used in compound food for poultry, pigs, and farmed fish (Windsor, 2001). Adding of fish meal in animal diet may increasing

growth through better sustenance attractiveness, upgrades supplement take-up, assimilation and digestion. Nutrient in feed meal also help in battling illness by boosting and keeping a strong practical immune system (Miles & Chapman, 2006).

Other alternative using plant protein were used in order to replacing fish meal because of the high cost of production and availability of fish meal (Akeem, 2011). Studies had revealed that the vegetable protein sources have great capabilities for supplying fish with their requirement protein essential for all-out productivity after been accurately processed. . If vegetable protein sources are not accurately processed, it will result in mortality and poor growth performance. Toxicity in the vegetable protein sources that was called anti nutritional factor causing the negative result of productivity in livestock. So it is important to process the vegetable protein source before use in practical fish feeding (Sotolu & Faturoti, 2008).

1.1 Problem Statement

For growth of Nile tilapia *Oreochromic niloticus* fingerling, there are need to give higher protein source of pellet. In market, higher usage of fish meal as main ingredient for the pellet making the pellet are highly in cost and uneasy to get from the supplier. There are a lot of alternative ways to replace fish meal in order to reduce the production cost of the pellets. Plant base protein such as *Leucaena leucacophala* seed can be used as alternative or partially replacement to fish meal. Studies has shown that the plant protein source has high possible for providing fish with protein need for productivity after it has been properly process. So, this research were found to replacement the fish meal in order to increase growth performance of tilapia fish as well as to reduce the cost of feed production.

1.2 Hypothesis

High nutrition value on feed are needed in order to boost up growth performance of tilapia fingerlings. The feed with high nutrition value using different levels of processed *Luecaena leucocephala* seeds meal at 0 %, 25 % and 30 % properties as partial replacement of fish meal were used and it is predicted to increase growth performance of tilapia fingerlings.

H_0: Different dietary level of *Leucaena leucocephala* seed will not affect growth performance of Nile tilapia fingerlings.

H_A: Different dietary level of *Leucaena leucocephala* seed will affect growth performance of Nile tilapia fingerlings.

If p-value > 0.05, H_0 will be accepted

If p-value < 0.05, H_0 will be rejected.

1.3 Objective

1. To determine the effect of different level of *Leucaena leucocephala* seed meal on growth performance of tilapia fingerlings.

1.4 Scope of Study

This study was focus on the best types of feed used to feed tilapias fingerling. The feed used in this study was AMBD diets with different level of *Leucaena leucocephala* seeds meal (LSM) as ingredient on formulated diet (fish meal, soybean meal, rice bran, vegetable oil, vitamin premix, mineral premix, vitamin c, and tapioca flour). This study is also to evaluate the best quality of feed in different level of Leucaena leucocephala seeds meal that were produce higher weight gain.

1.5 Significance of Study

Currently, fish meal was having high cost of production due to the high demand in feedstuff for not only aquaculture but also other animal feed production. In order to having low cost of feed with higher protein value, fish meal that used in feed formulation could be partial replaced with other source of protein such a plant based protein.

One of the plant based protein that usually used as alternative to replace fish meal was legume because it contain higher protein sources compared to other plants. *Leucaena leucocephala* seeds was used in fish diets as plant protein sources to replace fish meal because of its high protein content, cheap and easy to get. The partial replacement of fish meal with this plant based protein helps in making cheap and affordable feed especially for small scale farmers in order to increase their farm production.

CHAPTER 2

LITERATURE REVIEW

2.1 *Leucaena leucocephala*

Leucaena leucocephala or may know as Ipil-ipil is a little, fluidly shrubby and exceptionally branched to medium-sized tree with a short, upright angular branching and thin open crown. *Leuceana leucocephala* also known as one of the fastest-growing leguminous trees (Sethi & Kulkarni, 1995). Scientific name *Leucaena* come from 'leu' that meaning white while 'cephala' meaning head, referring to the blossoms. During early 1970 and early 1980, this forage is known as the 'miracle tree' due to its overall accomplishment as an extensive exceptionally nutritious forage tree (20-30%) and its awesome assortment of different uses. It also enriched in protein (25-35% CP) and other nutritional components (Ghosh & Bandyopadhyay, 2007). *Leucaena* was beginning point in Central America and the Yucatan Peninsula of Mexico where its fodder was sorted out more than 400 years back by the Spanish conquistadores who conveyed *leucaena* feed and seed on their ships to the Phillippines to bolster their stock (Shelton & Brewbaker, 2017).

Leucaena leucocephala is basically a tropical species requiring warm temperature for ideal development and with poor chilly resistance and fundamentally decreased development during cool winter long time in subtropical areas (Orwa, Mutua, Kindt, Jamnadass, & Anthony, 2009). Research shown that, *L. leucocephala* has been depicted as quickly developing and lack resistant tropical legumes tree, which offers the broadest arrangement of employments among different legumes (Akeem,

2011). *Leucaena leucocephala* can regrow in the wake of being scorched to the crown by slower fires and can make it tolerates fast fires (Orwa et al., 2009).

For chemical composition and nutritive value for *Leucaena* seed, study had shown that the protein of *L. leucocephala* seeds are genuinely rich in the fundamental amino acid, for example isoleucine, leucine, phenylalanine, and histidine. The leaves and the seeds contain lipids, crude protein and carbohydrates (Devi, Ariharan, & Prasad, 2013). *Leucaena* is well-known with its high nourishing quality and for the closeness of its chemical composition with that of alfalfa. This forage also can be little in sodium and iodine yet high in β-carotene (Shelton & Brewbaker, 1990).

This chemical composition of *L. leucocephala* depends on several factors such as location, age of plants, variety, soil type, season and drying methods (Ghosh & Bandyopadhyay, 2007). Based on analysed nutrient composition it was claimed that *Leucaena* seeds had potential wellspring of protein and vitality. Protein content was 31.1 % and the ascertained metabolic vitality of seeds was 2573.26 kcal/kg. The amino acid of *Luecaena* seeds were 1.39 %, 0.36% methionine, 0.35 % cysteine, 2.62 % arginine, 4.63 % glutamic acid, 0.87 % threonine, 1.38 % glycine, 1.11 % ananine, 1.11 % valine, 0.93 % isoleucine, 1.81 % leucine and 0.71 % methionine + cysteine (Ahmed & Abdelati, 2009).

Total of oligosaccharide substance of *L. luecocephala* was 3.5 to 3.6 %, with sucrose 1.9 to 2.0 %, raffinose 0.7 to 0.8 % and stachyose 0.7 to 0.8 %. Mimosine contribute 60 % of total free amino acid in *L. leucocephala* seeds (Sethi & Kulkarni, 1995). According to Sotolu and Faturoti (2008), previous study reported that *L.*

leucocephala create around 3-5 tonnes seed ha^{-1} yr^{-1} and great crude protein esteem that is 28 to 45 %. The nutrient composition of *Leucaena* fodder are consider various during different month of years. The range of nutrient value like dry matter (DM) are 24.98 to 36.39, crude protein (CP) 18.9 to 27.57, crude fibre (CF) 10.16 to 17.23, ether extract (EE) 2.59 to 5.88, Nitrogen free-extract (NFE) 46.70 to 59.91 and ash 7.49 to 10.90% (Ghosh & Bandyopadhyay, 2007).

For the chemical composition, study found that there were different constituents in the leaf and seed (Devi et al., 2013). The Table 2.1 below showed the different between chemical constituents of leaves and seed of *L. luecocephala*.

Table 2.1: The chemical constituents of *Leucaena luecocephala* leaves and seeds.

Chemical constituents	Leaves	Seeds
Crude protein %	25.90	46.00
Carbohydrate %	40.00	45.00
Tannin%	4.00	1.20
Mimosine%	7.17	10.00
Total ash%	11.00	3.79

Source: (Devi et al., 2013)

Akeem (2011) reported that there is different proximate composition between unprocessed *Leucaena* seed meals and processed *Leucaena* seeds meal. Table 2.2 below showed the different between this two different types of meal that is *Leucaena* seed meal that were processed and unprocessed.

Table 2.2: Proximate composition of *Leucaena leucocephala* seed meal.

***Leucaena* seed meal**	**Crude protein (%)**	**Crude fibre (%)**	**Fat (%)**	**Ash (%)**	**Moisture (%)**	**Energy (Kcal kg)**
Processed	36.01	7.11	5.18	3.74	12.56	2899.76
Unprocessed	22.75	11.38	6.12	5.98	15.14	2833.50

Source: (Akeem, 2011)

According to Sotolu & Faturoti (2008), there were different nutritional value when processing with different method of making it into a *Leucaena* seed meal. Table 2.3 above showing the different composition of nutritional value with different types of processed *Leucaena* seed meal such as initial, sundrying, toasting, soaking in water and soaked in alkaline and sundrying.

Table 2.3: Different nutritional value on different process of *Leucaena leucocephala* seed meal

Different process *Leucaena* seed meal	**Crude protein (%)**	**Crude fibre (%)**	**Ash (%)**	**Moist (%)**	**Crude fat (%)**
Initial	62.08	1.46	11.60	14.52	4.27
Sundrying	62.32	1.44	11.74	13.96	4.30
Toasting	62.11	1.46	11.69	14.44	4.31
Soaking in water	63.34	1.42	11.56	14.41	4.33
Soaking in alkaline and sundry	62.54	1.41	11.59	14.04	4.31

Source: (Sotolu & Faturoti 2008)

As it popular to be well known to be ‘miracle tree’, it has numerous parts that prompted the overall worldwide notoriety (Ghosh & Bandyopadhyay, 2007). It has really much of benefits in this tree. The leaves of *Leucaena* were exceedingly nutritious for ruminants and numerous magnificent animal production that been distributed affirming the fodder estimation of *Leucaena*. This forage also be utilized for posts, props and casings for assortment climbing crops. The low seedling assortments are utilized to give shade for cacao and coffee and bolster climbers, for example pepper and vanilla. Other used of this forage are it also can make a necklaces from seeds and used as vegetable for human consumption from young leaves and seed. This can be eaten only in small portion because of the presence of toxic amino acid mimosine in the seed (Shelton & Brewbaker, 2017).

Figure 2.1: *Leucaena leucocephala* from UMK Jeli, Kelantan.

2.1.1 *Leucaena leucocephala* Taxonomy

According to ITIS (2017), the taxonomy of *Leucaena leucocephala* starting from kingdom, subkingdom, infrakingdom, superdivision, division, subdivision, class, superorder, order, family, genus, species and taxonomic status are shown below.

Kingdom:	Plantae
Subkingdom:	Viridiplantae
Infrakingdom:	Streptophyta
Superdivision:	Embryophyta
Division:	Tracheophyta
Subdivision:	Spermatophytina
Class:	Magnoliopsida
Superorder:	Rosanae
Order:	Fabales
Family:	Fabaceae
Genus:	*Leucaena*
Species:	*Leucaena leucocephala* (Lam.) de Wit – white leadtree, koa haole, lead tree

2.1.2 Anti-nutritional Factor

Every legume plant having anti-nutritional factor including *Luecaena luecocephala*. Mimosine is the anti-nutritional factor for *L. leucocephala* seed. Mimosine, is a non-protein amino acid corrosive basically like tyrosine, happens in a couple of types of mimosa and all types of firmly associated genus *Leucaena*. In the *L. leucocephala* leaf, the level of mimosine is about 2-6 % (Kumar, 1991). Mimosine also provides 14.8 % to the nitrogen content of *L. leucophala* kernels (Chanchay & Poosaran, 2009). The mimosine has higher concentration in the seed with different parts of plant, second just to the youthful tender leaves. The mimosine content range from 2.2 to 10 %. Tannins content is lower in seeds (1.2 %) and different with high level of dry pods and the bark (16.3%) (Sethi & Kulkarni, 1995).

Study by Sethi & Kulkarni (1995) stated that, the possible solution to the mimosine activity is washing with water and stocking the leaves and seeds that can reduced their mimosine content. Soaking for 48 hours in 30℃ water demonstrating best approaches todecreasing practically all the mimosine in leaves. Other study found that the effect of *Leucaena* and mimosine can be diminished by heat treatment, by supplementation with amino acids or with metal ion such as Fe^{2+},Al^{3+} and Zn^{2+} (Kumar, 1991). Based on Francis, Makkar, & Becker, (2001), after soaking of the seed in water for 2 days will enhanced execution of *L. leucocephala* meal in fish generation and it also useful to removing up to 90 % of anti nutritional factor that is mimosine that present in the seeds. Chanchay & Poosaran (2009) stated that, doused *Leucaena* leaf meal gave better development execution of Nile tilapia contrast with sundried or commercial leaf meal.

2.1.3 *Leucaena leucocephala* as Feed

Leucaena leucocephala is being labels as the 'alfafa of the tropics' because it is the most palatable and highest quality fodder trees of the tropics (Devi et al., 2013). As an animal feedstuff, *Leucaena* give attractive, digestive and nutrition forage for ruminant and increment milk production in both the monosoonal and humid tropics. Previous study shown that in India, cow and buffaloes milk yield was 20 % higher when fed with *L. luecocephala* foliage at 10 % of their diet. Sheep can stand *L. leucocephala* consumption parallel to just 0.14 g mimosine/kg body weight while cattle can endure 0.18 g/kg without serious toxic symptoms (Sethi & Kulkarni, 1995).

On other hand, decrease in weight pick up, cataract in young animals, goitre, most striking feature loss of hair, and infertility are symptom of mimosine toxicity. Other study shown in Australia, the cattle may lose some of their coarse hairs when fed completely on *Leucaena* but they will not die (Masafu, 2006). Until some solution to toxicity is found, *Leuceana* can be sustained securely just as a supplement (< 3 %) to roughage diet, as opposed to as noteworthy dietary component (Sethi & Kulkarni, 1995). It also supported that, *Leucaena* offers nutritious and high protein forage for ruminant such as water buffalo, cattle, goats and sheep which increases milk production and protein supplements fed for dairy cows (Devi et al., 2013).

For non-ruminant, previous study shown *Leucaena* ought not to be a main part of the eating regimen since they are less ready to endure mimosine than ruminant. Mimosine causes poor development, alopecia, eye cataracts and regenerative problem for non-ruminant animals. According to Kumar (1991), poor result in animal performance such as poultry, rabbit and swine on level of *Leucaena* meal above 5-

10%. Study had found that vegetable protein sources have high possibilities for providing fish with their protein requirement for most extreme productivity after been properly processed (Sotolu & Faturoti, 2008). Study from Devi et al., (2013) stated that, it has very good result on the performance of growing and fattening pigs. Most animal feed used its leaves as it contain high nutritional value. It is also reported that fish, rodents and poultry may also be raised on diets supplements with *L. leucocephala* leaves makes a decent potential, less expensive plant protein source with high nutritive esteem (Masafu, 2006).

Oreochromis mossambicus and *O. niloticus* showing improved growth responses when feeding on diet that contain 100% *Leucaena* leaf meal (Osman, Omar, & Nour, 2004). An amount of 25% of *Leucaena* leaf meal added in fed for *O. mossambicus* showing very poor growth response (Akeem, 2011). Increasing dietary *Leucaena* leaf meal in bolstered for Nile tilapia indicating diminished development execution performance and feed consumption efficiency (Wee & Wang, 2003). Previous study found that survival rate and digestibility value was high when fish are fed on processed *L. leucocephala* seed meal (Akeem, 2011).

Previous study reported mollies and topminnows *(Poecilia* spp.) and freshwater prawn, *Macrobrachium rosenbergii* used *Leucaena* as feed ingredient whereas it reported as capability in raising chicken broiler (Ahmed & Abdelati, 2011). Using 33 % to 100 % of the *Leucaena* leaf meal to *O. niloticus* fingerlings in cages in Laguna lake as a component of feed developed the growth rate (Sotolu & Faturoti, 2008).

Akeem (2011) found that, 25% of LSM in encouraged nourishing of *Clarias gariepinus* (catfish) delivered best development rate while 50% requires additionally contemplate in order to rise its operation proficiency in fish generation. According to Michael (2002), increasing in cost and vulnerabilities about quality and accessibility of fish meal making nutritionist and sustain makes to utilize cheap and promptly accessible plant material (such as *L. leucocephala*) as an option to exceedingly cost fishmeal (Islam, Nahar, & Islam, 1995).

2.2 Nile Tilapia *(Oreochromis niloticus*)

Nile tilapia, *Oreochormis niloticus* happened amid 1950s, spreading of the more alluring of Nile tilapia amid 1960s up to the 1980s. Nile tilapia that from Japan were acquainted with Thailand in 1965, and that it was sent to Philippines. Nile tilapia was acquainted with China in 1978 making prompts to the universe of tilapia production and reliably create the greater part of worldwide production in consistently from 1992 to 2003 (El-Sayed, 2006).

Nile tilapia was one of the main fish species refined. It was stated by illustration from Egyptian tombs that Nile tilapia were refined over 3,000 years back (Popma & Masser, 1999). Tilapia are generally refined in both tropical and subtropical district of the world and contribute the third biggest gathering of cultivated finfish after carps and salmonids, with yearly development rate around 11.5% (Fattah & El-sayed, 1999). For all tilapia species, Nile tilapia is considered the most essential species for aquaculture throughout the world, accounting for over 70% of cultured tilapia (Fitzsimmons, 2004).

The attractants of this aquaculture attributes of tilapia was their resistance to poor water quality and the way that they eat an extensive variety of natural food organisms. “Tilapia” is originated from non-specific name of a group of cichlids endemic to Africa (Popma & Masser, 1999). The name of “tilapia” was gotten from the African Bushman words meaning ‘fish’ (El-Sayed, 2006). Those the greater part of the tilapia species were fertilized eggs, nest builders are guarded in the nest by brood parent. Only for *Oreochromis* species the females practice mouth brooding whereas for *Sarotherodon* species either male or both male and female are mouth brooders. There are some confusion on scientific name of tilapia over last 30 years. The scientific name that had been given for Nile tilapia as *Tilapia niloticus*, *Sarotherodon niloticus* and currently until now using *O. niloticus* (Popma & Masser, 1999).

As for the physical characteristic, tilapia having shaped as much prefer sunfish or crappie yet can be effortlessly perceived by an intruded on of line normal for the Cichlid family of fish. Tilapia horizontally compacted and profound with long dorsal fins. The forward segment of the dorsal fin is intensely spined. Spines are likewise found in the pelvis and butt-centric fins. There are generally wide vertical bars down the sides of fry, fingerlings and now and then grown-ups. Nile tilapia having strong vertical bands (Popma & Masser, 1999).

Tilapia was consider as channel feeders as a result of efficiently harvest plankton from the water yet they don’t physically channel water however out gill rakers such as gizzard shad and silver crap. Tilapia don’t distract the lake base as aggressive as regular carp when feeding time (Popma & Masser, 1999). Tilapia bodies were commonly characterized by vertical bars, with relatively subdued colours and with little contrast over the body colours. This fish colour was changed due to response in stress

by controlling skin chromatophores. The eyes of this fish also quite large, giving the fish with an outstanding visual capability (El-Sayed A., 2006).

Figure 2.2: *Oreochromis niloticus* from Kg. Gual Ipoh, Tanah Merah, Kelantan.

2.2.1 Taxonomy of Nile Tilapia

The taxonomy of Nile tilapia (*Oreochromis niloticus*) below starting from kingdom, subkingdom, phylum, subphylum, infraphylum, superclass, class, subclass, infraclass, superorder, order, suborder, family, genus, species and lastly taxonomic status were stated by ITIS (2017).

Kingdom:	Animalia
Subkingdom:	Bilateria
Phylum:	Deuterostomia
Subphylum:	Chordate
Infraphylum:	Vertebrata

Superclass:	Gnathosmata
Class:	Osteichthyes
Subclass:	Actinopterygii
Infraclass:	Neopterygii
Superorder:	Teleostel
Order:	Acanthopterygii
Suborder:	Labroidei
Family:	Cichlidae
Genus:	*Oreochromis*
Species:	*Oreochromis niloticus* (Linnaeus, 1758) – Nile mouthbreeder, tilapia del Nilo, Nile tilapia

2.2.2 Fish Nutrition, Feeds and Feeding

Most of fish diet were having all the required protein (18-50%), lipids (10-25%), carbohydrate (15-20%), ash (<8.5%), phosphorus (<1.5%), water (<10%) and trace amount of vitamin and minerals. At the point fish were rearing with higher stocking density, it requires high quality, healthfully total, adjusted diets to develop quickly to stay healthy. For fish growth, protein is utilized if satisfactory levels of fats and sugars are available in the eating regimes. Up to 65% of the protein might be lost to nature despite the fact that fish are fit for utilizing a high protein diet (Davis, 2009).

The protein level in in aquaculture feeds for tilapia normally average from 32-38%. Its shows that actually protein requirement are lower from the herbivorous fish and omnivorous fish compare to carnivorous fish (Davis, 2009). During larvae stage, Nile tilapia require around 35-45% dietary protein for greatest development growth performance while during juvenile, tilapia requires protein ranges from 30-40%. Adult tilapia need less amount of dietary protein that is 20-30% only for optimum performance. For broodstock tilapia, it require 35-45% dietary protein for ideal reproductive, spawning efficiency and larval existence and development (El-Sayed, 2006).

Feeding by formulated feeds, tilapia can grows rapidly with bring down protein levels and endure higher starch levels than numerous carnivorous farmed species. Tilapia also can feed with more level of plant protein making its simple to breed and culture them seriously and financially. This fish are quite impervious to disease and lowly water quality. Tilapia move into mainstream seafood markets for these countries as the production technique improved and off-flavours are collected. Tilapia prompted the commercialization creation more than 100 nations because of the toughness and flexibility to an extensive variety of culture framework (El-Sayed, 2006).

There are many factor affecting the sustaining rates of the fish for example like time of day, season, water temperature, dissolved oxygen levels and other water quality factors. Farmers need to figure ideal feeding rates in light of the normal size long or weight and the quantity of fish in the tank, raceway or pond (Davis, 2009). The feeding regularity for Nile tilapia amid ideal developing temperature will differ as indicated by the size or the phase of their life cycle. As for the recently hatched fry from

to 12 times each day, with respect to the fingerlings is 3 to 4 times and 2 to 3 times each day for develop out fish and a day for brood fish (Schmittou, 2006).

Tilapia having small stomach and are categorised by non-stop feeding behaviour, greater normality encouraging would be proper for them. When size of the fish increasing, the feeding levels and frequency of tilapia will be decreased. Fish fingerlings needs 3-54% of body weight, apportioned three to four times dairy. It additionally found that expanding feeding levels above fish necessities may diminish feed digestibility and use effectiveness. Study also found that nourishing three to four times each day brings about better growth development and feed conversion ratio (FCR) compare feeding two times each day (El-Sayed, 2006).

It is not a problem when feeding small fish in excess compare to bigger fish on account of the little fish need just little amount of feed in respect to volume of water in culture framework (Davis, 2009). The age and size is one of the factor that make the protein requirement decline. Dietary crude protein concentration are higher for fry that is 30-56% while for juvenile are 30-40% and for the larger tilapia are 28-30%. Tilapia can successfully consume starch up to 30 to 40% in the eating routine, which can impressively more than most refined fish (Amos, 2013).

Nile tilapia fingerlings and juveniles raised in floating cages fed well on crushed pellet compared to normal pellet feed whereas adult fish preferred pelleted diet. Tilapia likewise consume both floating and sinking pellets very efficiency. Blue tilapia were preferred to consume sinking pellets over unpelleted feed. At the point when fish were fed moist or dry pellets in cages, moist feed was consumed slightly better than dry feed but the leaching rate was higher in moist than the dry feed (El-Sayed, 2006).

In fish feeding, both protein and energy is important for imperative for upkeep, development and production. Their suitable supply in both quality and amount empowers fish to understand their potential. Fishmeal and cereal have been utilized as energy and protein source (Madalla, 2008). Protein were known as a part in total fish feeds and commonly is the most costly segment representing for more than 50 % add up to feed cost in concentrated aquaculture (Thompson, 2005). Study by Winson (2002), protein intake is importance to the fish to give the amino acids required to union of new tissues and supplanting harmed protein. Protein likewise significant organic material in fish tissue making up 65-75 % of aggregate weight on a dry matter basis (Winson, 2002).

Fish meal had been traditionally utilized as primary protein source in the aqua nourish industry due to its great protein content and adjusted essential amino acid (El-Sayed, 2006). Fish meal mainly very tasty, very absorbable and high in essential amino acids, energy and minerals (Ogunji, 2004). As the increases demand for this protein source, it create sharp rivalry for its utilization by animal feed industry and as the results it's become the most costly protein product in animal and aquaculture feeds in recent years (El-Sayed, 2006). Unsteadiness of the supply of fishmeal has led to higher cost past the compass of numerous asset poor fish farmer (Madalla, 2008).

Researched discovered other alternative to replace fish meal as main source of the feed for aquaculture feeding. Study by Fattah and El-Sayed (1999) reported to include leguminous or grain plants and by product as replacement for fish meal in tilapia feeds. *Leucaena* leaf meal that has been 30 % crude protein has assessed as a protein source for tilapia, with somewhat clashing outcomes. It been accounted for that cooked or sun-dried *L. leucopehala* meal delivered preffered development of Nile

tilapia over did sodium hydroxide-treated or rumen liquid-incubated *L. leucocephala* meal (Fattah & El-Sayed, 1999). Study by Madalla (2008) stated that, consideration of plant materials in fish feeds hints to decreased execution much of the time. This is ascribed to a several of variables including poor palatability, poor digestibility, high fibre content, anti-nutritional factors and poor protein profile in terms of low protein content and poor amino acid profile.

Feed are seem to be more expensive because of the high demand from aquaculture sector. So, other alternative to make sure that the feed are not being wastes easily, feed conversion ratio (FCR) or feed efficiency (FE) were vital to analyse so that the feed were being utilized as effectiveness as could be expected under the circumstances. FCR were calculated as the heaviness of the feed fed to the fish divided by the heaviness of the fish growing. Final reading of FCR that range from 1.5-2.0 are considered as great development for most species. Calculation of FE are essentially the equal of FCR (1/FCR). FE were consider great development for fish when it's greater than 50%. FCR are different among species, sizes and action level of fish, ecological parameters and culture system utilized (Davis, 2009).

2.2.3 Stocking Density

Stocking density is very important to influence feed consumption and growth rate of the fish. The period of nursery is usually five to thirteen weeks, reliant to desired final size in tropical countries. Fingerlings, can reach weight of 10 - 15 g in five to six weeks and 25 - 30 g in eight to 10 weeks with a pretty good diet and water temperature above 25 ˚C. Appetite and growth of tilapia fingerlings may influence by sub-optimal water temperature. Greater stocking density for the most part result in higher fish

yields, yet singular fish development is regularly sacrificed. Producer has to find other carefully optimum stocking rate to deliver high yields with fast fish development to wanted size. There are three options to reduce the grow out cycle such as reduce stocking density, improved feed quality, and modified ecological condition with aeration and or water exchanges to warranty higher feeding rates (Popma & Lovshin, 1995).

The optimum stocking density will guarantee a good production, income, proper utilization of feed, sound environment and health (Alam et al., 2014). As reported by Osofero, Otobusin, & Daramola (2009), the effect of stoking density on Nile tilapia growth was generally decrease with rise in stocking density. When condition is crowded, fish feel stress and as the result it will be hostile feeding communication and poor eating, resulting in development delay (Bjornsson, 1994). As the density increase, both water quality and feed get to decline and it will constrain the production performance through its impact on water quality and feed access (Schmittou, 2006).

There is research reported that negative connection amongst growth and feed productivity in Nile tilapia provided at various densities (El-Sayed A. , 2002). When there is increase in density, feed loss potential will increase because of increase fish-induced water turbulence at feeding time (Schmittou, 2006). There is also effect of stocking density were it was significantly on the growth and profitability of Nile tilapia in terms of daily weight gain, food utilization, condition factor, specific growth rate and yield (Amos, 2013).

2.2.4 Water Quality

Water quality can be describe as the environmental condition for the fish to grow. Fish are absolutely subject on water to breathe, feed and develop, excrete wastes, keep up salt balance and reproduce. Temperature, dissolved oxygen (DO) and hydrogen-ion concentration (pH) are the main thing to see when checking water quality. Other parameter that are also take part in water quality is ammonia, nitrates, phosphates, alkalinity and hardness that also take part on impact in aquaculture ecosystems (Abolude, 2007).

Temperature is one of the factor that influence the growth, reproduction and digestion of the fish. Ideally temperature surrounding water should be about 26 to 28 ˚C and within optimum range of about 23 to 30 ˚C (Amos, 2013). For the normal development, reproduction and growth, the temperature that are suitable range from 20 to 35˚C reliant on the fish species with the ideal range about 25 to 30˚C (El-Sayed, 2006). Other study reported that Nile tilapia shows optimum food consumption and growth at temperature ranging between 31 to 36 ˚C (Amos, 2013).

When temperature go beyond 37 or 38 ˚C, stress-induced disease and mortality will be problem to the fish. Lower temperature resulted in stress-induced trauma and in mortality at temperature lower than 17 or 18 ˚C (Schmittou, 2006). Other study by El-Sayed (2006) stated that, tilapia can endure temperature as low as 7 to 10 ˚C but only for short-term periods. It was sharply reduced on tilapia feeding when the temperature are below 20 ˚C and they end nourishing about the temperature of 16 ˚C whereas some mortality occurs at temperature of 12 ˚C.

As for the little fingerling tilapia, they were more disposed to low temperature compare to bigger fish (Hofer & Watts, 2002). It also had been reported that big fingerlings were preferred for shipping and culture if water temperature was predictable to drop because of their better tolerance to cold temperature. Some fish can accept temperature as low as 6 to 7 ˚C for first few hours. Long terms experience to such low temperature make it the fish incapable to keep their body position (El-Sayed, 2006).

As for the dissolved oxygen (DO), there is main environment needed because fish consume oxygen large quantities as well as for growing and affect the fish feeding (El-Sayed, 2006) Fish required dissolved oxygen as it need for aerobic metabolism. Low dissolved oxygen levels might kill the fish, either straightforwardly or indirectly than different problems combined (Schmittou, 2006). The concentration of dissolved oxygen are obtained at concentration greater than 3 mg/l at optimum growth for *Oreochoromis niloticus* (Ross, 2000). Low dissolved oxygen is associated with increase ammonia, high in free carbon monoxide, decrease pH, increase nitrate, increase fish metabolism, increase water temperature, abundant gill parasites and numerous other factors, which when combined can significantly reduce fish performance (Schmittou, 2006).

It was widespread that growing water temperature can reduce the rate of dissolved oxygen in the water. This is hints to increasing breathing rate and oxygen utilized in tilapia because of under increase water temperature the amount of metabolism and in turn, the tissue request for oxygen will be increases. Dissolved oxygen was continued under 20 % saturation for more than 2 to 3 days (El-Sayed, 2006).

For the effect of hydrogen ion concentration (pH), regular monitoring is a part of the operation of intensive firewater-fish culture systems. *Oreochomis niloticus* can tolerate to lower pH to approximately 5 however it having best growth between pH 7 to 9 (Ross, 2000). Study had establish that the both fingerling and grown-ups tilapia died at pH 2-3 at the range periods within 1-3 days. Nevertheless, adults tilapia were extra enduring to low pH with survival rate of 86.6 and 100 % at pH 4,5 and 7 while for the survival of fingerlings was 57.8, 82.2 and 84.5 % at the same pH values (El-Sayed, 2006).

Ammonia toxicity actually closely related to pH and to smaller amount, water temperature and dissolved oxygen absorption. Ammonia toxicity increase because of low dissolved oxygen, however this is basically stable by decreases toxicity created by growing carbon monoxide concentration which drops pH (Schmittou, 2006). Nile tilapia that unprotected to ammonia had lesser amount of red blood cells and haemolytic anaemia, making to a major decrease in blood oxygen content which develop ammonia toxicity (El-Sayed, 2006).

2.3 Artificial Diet

In past decades, there is major progress that been reached in culture of larvae through the development of microparticulate diets that is characterized as either microbound, microencapsulated or microcoated. The common of artificial diets explored tentatively for finfish larvae have been obtainable as either microencapsulated diets (MED) or microbound diets (MBD). Microbound diets shows the simplest and regularly used for microdiet (National Research Council, 2011). Microbound diet is consider a dietary parts held inside a gelled hydrocolloid matrix or

binder and differ from microencapsulated diet because of the lack of a capsule wall. Microbound diets look like more reasonable than microencapsulated diets for presenting artificial diets to marine fish larvae as of it lack of a capsule wall that were improves digestion of the artificial food particles (Silva, 1998).

Microbound diet were economical, easy to produce and are reported to have been used positively success in laboratory and hatcheries (Kumlu, 1999). There are several dissimilar binders that have utilize in MBD which differ considerate in their source, belongings and nutritional value. Study by Lucas & Southgate (2012), microbound diets nutrients that are both broken down are bound inside a particle matrix consiting of binding material such as agar, gelatin, alginate, carrageenan or zein. Agar, alganite and carrageenan were polysaccharides from seaweeds whereas gelatin and zein are protein that came from corn and swine respectively (Genodepa, Zeng, & Southgate, 2007). There is also binders such as carboxymethyl cellulose (Kumlu, 1999).

This diet were formed by mixing the nutritional ingredients thoughly with binders. The mixture then were heat by oven or freeze-dried (Kumlu, 1999). Binder were actually triggered either by a chemical reaction or temperature. This diets is generally dried before it ground and sieved to the desired size for feeding. In the use of binders, care must be seen as important things although physical integrity is achived but the nutrient utilization may be correspondingly reduced (National Research Council, 2011).

It has be proved that the most successful fed to larvae of penaeid shrimp are with microbound diets. Study shows that the desirability of the microbound diet were not purposely affected by the kind of binder utilized to prepared (Genodepa et al., 2007). It reported that existence and development was alike when larvae of *Penaeus japonicus* (post zoea) were fed a carrageenan-based microbound diet having casein as the major protein source to that larvae fed live diatoms and *Artemia* nauplii (Kovalenko, D'Abramo, Ohs & Buddington, 2002). The struggles to culture larvae of *Macrobrachium rosenbergii* using microbound diet were made in a multiplicity of ways proved unsuccesful (National Research Council, 2011).

Several study had showns that microbound diets ready with binders presenting greater rate of leaching were not applied to better degree by *Scylla serrata* larvae. It had been shows that dietary nutrients were wasted because of unnecessary leaching from microbound diets and it likely to effect the water quality, near to lowly raising outcomes. Zein is perhaps more appropriate binder for microbound diets developed for *S. serrata* larvae since its had properties having minor leaching amounts compare to agar, alginate, carrageena and gelatin (Genodepa et al., 2007).

CHAPTER 3

METHODOLOGY

3.1 Proximate Analysis

Proximate analysis were carried out in Animal Laboratory at UMK Jeli Campus and UMK Pengkalan Chepa Campus. This proximate analysis were carried out for testing moisture content, crude protein, crude fibre, crude fat and ash on raw ingredient such as *Leucaena leucocephala* seed meal, fish meal, soybean meal, rice bran and tapioca.

3.1.1 Moisture Content

Moisture content was conducted after weighing each sample on the sample plate by using Moisture Analyser MS-70 (GPS Instrumentation Ltd.) at different temperature according to the instruction on manual book given. The manual can be referred at the manual book given or referred to website A&D MS-70 Moisture balance. When the sample was ended run, the reading would appear run on the screen of moisture analyser. Then, the data was recorded.

3.1.2 Crude Protein

Kjeldahl method is used in determining crude protein. This method was developed by Johan Kjeldal in 1883 using the equation Kjeldahl nitrogen multiplied by 6.25. This method was isolated into three phases which are digestion, distillation and titration.

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

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

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

Crude protein, % = % Kjedhal Nitrogen × 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

14.01 = atomic weight of N

W = weight (g) of sample

10 = factor to convert mg/g to percent

F = factor to convert N to protein; 6.25

3.1.3 Crude Fibre

Crude fibre was analysed using Fibre bag System 6. Fibre bag was drying for one hour at 105°C before analysis was started. After that, let it cool in the desiccator for 30 minutes. The weight of fibre bag was labelled as M_1. One gram of sample was weighed into the fibre bag and labelled as M_2. Glass spacer was embedded into the fibre bag in order to confirm 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. Just six fibre bags can be put into a sample carousel. At that point, the beaker was place on the hotplate.

The fibre bag would experiences two washing stage which was for the Phase I, boiling in 360 mL sulphuric acid for 30 minutes after the acid starting to boiled and then to removed acids needed to washed three times with hot water. Step for Phase II was boiling with 360 mL sodium hydroxide solution for 30 minutes after alkali starting to boiled and then to remove alkali needed to washed for three times with hot. The fibre bag was removed from carousel and dried for 4 hours at 105°C before placed it in the desiccator for 30 minutes.

Next step was preparation of crucible for incineration was heated in the oven at 600°C for 30 minutes. After that in order to cool it off, placed in the drying chamber at 105°C for 30 minutes. Then, the crucible was placed in the desiccator for 30 minutes and the weighed of crucible with fibre bag was recorded and labelled as M_3.

For the last step, fibre bag had been incinerated for 4 hours at 600°C. After that it was placed in the drying chamber at 105°C for 30 minutes. Then, the fibre bag was placed in the desiccator for 30 minutes and the weighed of crucible containing ash was recorded and labelled as M_4. The formula using in determining the crude fibre was calculated using software:

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

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)

3.1.4 Crude Fat

The initial weights of aluminium cups was recorded. After that, 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. As extracting solvent, 80 mL of petroleum ether was added in the aluminium cups and placed into the extraction unit with the cup holder. This was collected in the Foss Soxtec 2055 system. The sample containing in the thimble was extracted in the extracting solvent by boiling at 135°C for 15 minutes and rising, recovery and pre-drying for 20, 10 and 2 minutes separately. Then, aluminium cups were dried into the oven at 105 °C for 30 minutes and before cooled it in the desiccator for 20 minutes at room temperature. The final weights of aluminium cups were recorded. Fat content was calculated using formula as below (Kwikiriza et al., 2016).

Percentage of fat:

$$= \frac{\text{[Final weight of aluminium cup (g) – Initial weight of aluminium cup (g)]}}{\text{Sample weight (g)]} \times 100}$$

3.1.5 Ash

According to method did by Thiex, Novotny, & Crawford (2012), all the sample was weighed for 2 g each and placed into porcelain crucible. The weights for all sample were recorded. Then, porcelain crucible that contained sample were placed into temperature-controlled furnace. In order to rise the temperature until 550°C, Furnace required more than one hour. Temperature at 550± 10°C was embrace for three hours. Next after three hours, furnace were allowed to cool to below than 200°C. After that, the porcelain crucible were transferred into desiccator and then let it cold cool before weight within one hour. The final weights of porcelain crucibles were recorded. The ash content in all sample were calculated using formula as below:

$$\text{Ash} = \frac{W_3 - W_1}{W_2} \times 100$$

Where:

W_1 = empty crucible

W_2 = weight of sample (2g sample)

W_3 = weight of sample after dry

Table 3.1: Proximate analysis for all of the feed stuff

Ingredient	Crude protein (%)	Crude fibre (%)	Crude fat (%)	Ash (%)	Moisture (%)
LSM	24.68	17.66	2.69	9.08	10.68
FM	27.35	0.28	27.2	64.52	7.07
SBM	52.55	3.95	0.16	9.95	14.60
RB	19.48	-	10.70	11.85	15.03
Tapioca	2.31	0.24	5.49	0.244	13.36

LSM= *Leucaena* seed meal, FM= Fish meal, SBM= Soybean meal, RB= Rice bran

3.2 Diet Formulation

The *Leucaena leucocephala* seeds were collected around Kelantan. The dry matter of *Leucaena* seed were needed around 2 kg. According to Sotolu & Faturoti (2008), the seeds of *Leucaena* were processed by soaking in cold water for 72 hours. After soaking, treated seed were toasted to remove the water though sieved and finally sundrying for two days. Then, the seeds later mashed into fine powder and were stored it in the close container (Akeem, 2011). This *Leucaena* seed meal were formulate with partial replacement of fish meal at different level that is Diet 1= 0%, Diet 2= 25% and Diet 3= 30%.

All the ingredient then were undergo proximate analysis in order to find the crude protein, crude fat, crude fibre, ash, moisture and crude fibre in each of the ingredient used while making this feed. The feed ingredient that formulated are *Leucaena* seed meal, fish meal, soybean meal, rice bran, vitamin premix, mineral premix, vitamin c, tapioca and vegetable oil using Winfeed 2.8 software.

Table 3.2: The *Leucaena Leucocephala* seed meal inclusion in dietary feed for 100g.

Ingredient (g/100g/DM)	**Diet 1 (Control)**	**Diet 2**	**Diet 3**
LSM	0	25.00	30.00
FM	45.97	20.96	15.95
SBM	30.00	30.00	30.00
RB	0.03	0.04	0.05
Tapioca	20.00	20.00	20.00
Vitamin premix	1.00	1.00	1.00
Mineral premix	1.00	1.00	1.00
Vitamin c	1.00	1.00	1.00
Vegetable oil	1.00	1.00	1.00

LSM= *Leucaena* seed meal, FM=Fish meal, SBM= Soybean meal, RB= Rice bran

For the process of making feed, the ingredient were weight for total of 1kg for each of the diet feed. After weighing, the feed were mixed using mixture machine. After the feed are being mixed well, the mixed ingredient of each diet were keep in the container.

3.3 Microbound Diet (AMBD) Method

The process of making microbound diet (MBD) were firstly setup the apparatus such as hot plate, beaker and glass rod. The ingredient for making this MBD are 97g of diet feed, 3g of agar powder and 100mL of distilled water. The hot plate was setup for 100°C. The feed diet was mixed with the agar powder on the hot plate. After mixed well, the distilled water were added. Then, the mixture were poured into an aluminium foil tray. Then, the "pudding" was cutter into cubes. The cubes was cooled and oven dried at 30-40°C. Finally, the cubes were grinded and sieved to required size.

Figure 3.1: The apparatus were set up

Figure 3.2: The AMBD diets

3.4 Fish Culture

For the acclimatization process, 300 tilapia fingerling fish that had been taken in the Gual Ipoh, Tanah Merah were rearing for about a five day in a cement tank in UMK Jeli Campus. The cement tank were filled with water, some anti-chlorine and the aerator for around two day before the fish safety arrived. The tilapia fingerling fish were fed with commercial pellet for five day in the cement tank. After five days in the cement

tank, the tilapia fingerling fish were stocked in the experiment tank. The fish were fasting for a day before experiment was stated.

The tilapia fingerling were placed into nine tanks and each filled with 30 litre water per tank. Total of 270 fingerling of Nile tilapia (*O. niloticus*) that have average weight of 5.0 $\pm$ 1.0 g were randomly places in nine tanks. There were three formulated diet. For each of the diet consist of three replicated of each tanks. Each tank were filled with water capacity of 25 littler per tank. Stocking density for Nile tilapia fingerling was 5 fish/m^3 (M'balaka, Kassam, & Rusuwa, 2013). Each tank consist of 30 fingerling. The tank were set with aerator and air stone, anti-chlorine and fresh water.

The fingerling fish were fed three times a day as recommended by Schmittou (2006) and the quantity of the fed that were given are recorded daily. Percentages feed given to the fingerling were about 5 % of body weight of the fingerlings fish according to Popma & Lovshin (1995); Riche & Garling (2003). Water quality parameter which included dissolved oxygen (DO), temperature, Ammonia, and pH were monitor every morning. The waste product of the fish were collected and eliminated from the tank each of the time before feeding the fish. The waste product and uneaten fed were remove using a small net. The water were exchange every two days. This study were conducted for six weeks and the fish were weighed every weeks in order to determine the growth performance.

3.5 Calculation of Fish Growth Performance

According to Akeem (2011), the fish growth and performance were determined as follows:

1. Weight gain (MWG) = $W_2 - W_1$

   Where:

   W_2 = final weight of fish,

   W_1 = initial weight (g) of fish,

2. Specific Growth Rate (SGR) = (Log W_2 – Log $W_1/T_2 - T_1$) x 100

   Where:

   T_2 = end of experiment,

   T_1 = beginning of experiment (days)

3. Feed conversion ratio (FCR) = Total feed intake/Weight gain

4. Survival rate (%) = (initial no. of fish stocked - mortality)/initial no. of fish x 100

3.6 Statistical Analysis

All data collected during the experiment were subjected to the one-ways analysis of variable (ANOVA) and were tested by Duncan multiple range test with ($P<0.05$) using SPSS Software Version 22.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Weight Gain of Nile Tilapia

Table 4.1 shown the initial weight, final weight and weight gain of Nile tilapia fingerling after six weeks of feeding trial based on different treatment that were Diet 1, Diet 2 and Diet 3.

Table 4.1: Initial weight, final weight and weight gain of Nile tilapia.

Treatment	Initial Weight (g/fish)	Final Weight (g/fish)	Weight Gain/fish (g/fish)
Diet 1	4.55±0.14	10.11±0.29	5.56±0.42[a]
Diet 2	4.58±0.64	10.93±0.17	6.35±0.47[a]
Diet 3	5.18±0.94	11.41±0.25	6.24±0.32[a]

*Value are mean±SE of three replicates. Values in the same column with different superscripts are not significantly different ($p<0.05$)

*Diet 1= 0 % LSM, Diet 2= 25 % LSM, Diet 3= 30 % LSM

Based on initial weight gain in all diet recorded, even there is slightly different in weight between all the data but there is no significant different in all treatment. Data for highest final weight gain came from Diet 3 that was 11.41g per fish. Diet 1 that was control was the lowest for final weight gain that was 10.11g per fish. There was also no significant different between all the Diet for final weight gain.

Based on data that had been calculated, weight gain from Diet 2 which is has 25 % of LSM on the diet showing the highest gain weight with 6.35 g per fish. Then it was followed by Diet 3 that has 30 % of LSM with gain weight of 6.24 g per fish. The lowest weight gain of Nile tilapia was from diet control that is 5.56 g per fish. Weight gain and specific weight gain showing that there was no significant different between all the treatments.

This is because poor utilization of the LSM by fish. It was identified that the efficiency of feed utilization and subsequent growth performance was affect by dietary protein. Increasing the LSM level were reduce the feed utilization of efficiencies.

This is also happen on previous study by Akeem (2011) that there is no significant difference between gain weights of catfish fed on 0 % and 25 % LSM diets ($p>0.05$) but fish fed on 0 % and 25 % LSM had extra weight gain compare to 50 %, 70 % and 100 % of LSM addition. The slow growth of weight gains might be attributed to the fact that fish were fed exclusively on the formulated feeds with no access to natural feed as may be found in pond or riverine condition (Amisah et al., 2009).

4.2 Specific Growth Rate (SGR)

Table 4.2 shown the percentages of specific growth per day of Nile tilapia for different treatment that are Diet 1, Diet 2 and Diet 3.

Table 4.2: Specific growth rate (SGR) per day of Nile tilapia

Treatment	Specific growth rate (%/day)
Diet 1	0.83 ± 0.08^{a}
Diet 2	0.90 ± 0.06^{a}
Diet 3	0.82 ± 0.02^{a}

*Value are mean±SE of three replicates. Values in the same column with different superscripts are not significantly different ($p<0.05$)

*Diet 1= 0 % LSM, Diet 2= 25 % LSM, Diet 3= 30 % LSM

Based on Table 4.2, the maximum SGR presenting at Diet 2 with value of 0.90 % compare to control with 0.83 % SGR and Diet 2 that is 0.82 %. This result was similar to previous study that use different fish species that is catfish. It said that catfish can used LSM to achieve a parallel growth design without significant difference as it has slightly dissimilar values of SGR at 50 % LSM inclusion associated with control and 25 % inclusion level. There was best growth rate produced when inclusion LSM at 25 % in catfish diet compare to 50 %, 75 % and 100 % of LSM addition (Akeem, 2011).

The specific growth rate were increase if the accepted of the feed was in good condition. Based on Amisah, Oteng & Ofori, (2009), there was common problems encountered the acceptability of feed by fish is using alternative feed sources that was from plant protein and this frequently related to palatability of the diets. Increasing LSM

in dietary level leading to low acceptability of the feed by fish. Specific growth also were affect if the fish having problem to accept the feed from plant protein source.

4.3 Feed Conversion Ratio (FCR)

Table 4.3 shown, feed conversion ratio of Nile tilapia for three different treatment that are Diet 1, Diet 2and Diet 3.

Table 4.3: Feed conversion ratio (FCR) of Nile tilapia.

Treatment	Feed conversion ratio
Diet 1	2.53±0.21[a]
Diet 2	2.45±0.12[a]
Diet 3	2.61±0.08[a]

*Value are mean±SE of three replicates. Values in the same column with different superscripts are not significantly different ($p<0.05$)

*Diet 1= 0 % LSM, Diet 2= 25 % LSM, Diet 3= 30 % LSM

From the Table 4.3, the FCR value were slightly different between Diet 1, Diet 2 and Diet 3. The result showed Diet 3 has the highest value of FCR that was 2.61 compare to Diet 1 that is 2.53 and the lowest value was record in Diet 2 with FCR of 2.45. The result that recorded in this study showing it has no significant difference for all the treatment. The result was same like prior study showing there is no significant difference between FCR of catfish fed for only on 0 % and 25 % LSM diets but there is advanced FCR on catfish fed on 0 % and 25 % of LSM diets compare to greater LSM quantity (>50 %) replacing on fish meal diet (Akeem, 2011).

The data shown all the diet having FCR more than 2.00. This is because improper nutrient and feed supply to the fish. Feed that coating with agar microbound diet were having loss in nutrient when immersed in water tank. It was supported by Genodepa et al. (2007) that revealed microbound diets that prepared with binders showing higher rate of leaching and make the dietary nutrients were waste leading to lower rearing outcomes.

4.4 Survival Rate

Figure 4.1 shows the survival rate of 30 fingerlings Nile tilapia between three different treatment, Diet 1, Diet 2 and Diet 3 for six weeks.

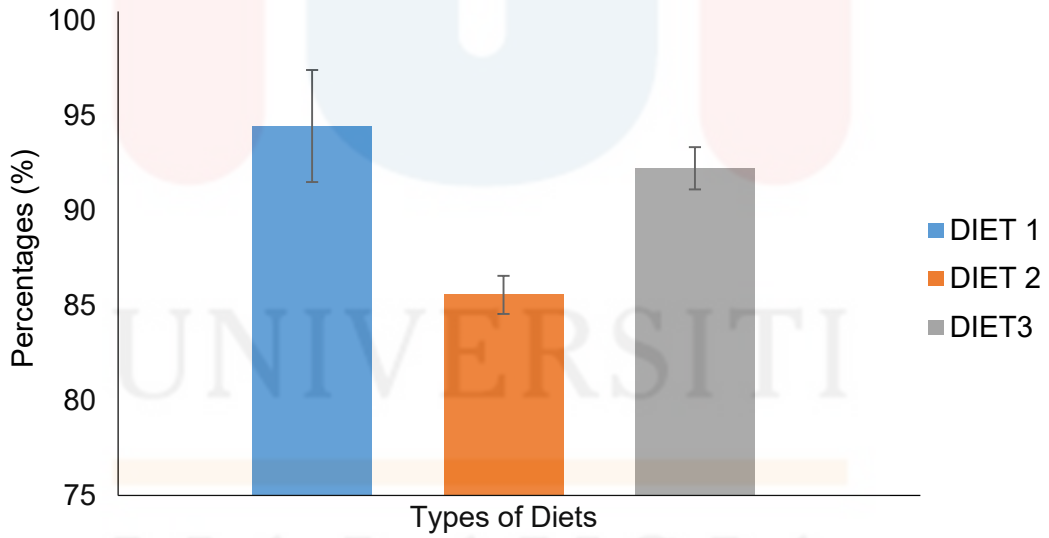

Figure 4.1: Percentages of survival rate

Based on Figure 4.1, the survival rate of the Nile tilapia showed that there is advanced survival rate on the Diet 1 that was 94.44 % compare to Diet 3 with survival rate of 92.22 %. The bottom value of survival rate was from Diet 2 with 85.56 %. From the data recorded, there had superior in weight gain, SGR and FCR from the Diet 2 but there was lack of survival rate. This is maybe because of the mimosine toxicity on the *Leucaena* seeds. There is no significant between all the treatments for survival rate in this study. On other hand, other study had found 100 % of survival rate on catfish was recorded in all treatment because of the enhanced performance of LSM in fish manufacture after soaking seeds in water for 2 days (Akeem, 2011).

Other than that, the survival rate was not 100% because of the microbound diet itself. Effect from the dietary nutrients were waste, the fish were had higher competition when feeding and it leads to struggle to get the feed. There were also cannibalism behaviour that lead to lower survivalist rate. According to Fessehaye, Kabir, Bovenhuis & Komen, (2006), food availability, population density, refuge, water clarity, light intensity, feeding frequency and frequency at which alternative prey is presented were the factor that affect behaviour of cannibalism.

The present of anti-nutritional factor in *Leucaena* seeds meal that was mimosine in these two diets (Diet 2 and Diet 3) could also led to poorer growth response and survival rate. Moreover, an increasing in level of *Leucaena* seeds meal incorporation led to increases in dietary mimosine concentration and making the more toxic to the fish and lead to low survival rate (Sotulo & Faturoti, 2008).

4.5 Environmental Condition

Table 4.4: Water parameter in all tank of Nile tilapia fingerlings.

Water parameter	Diet 1	Diet 2	Diet 3
Temperature (˚C)	26.29	26.74	26.78
Dissolved oxygen (mg/l)	5.60	5.40	5.19
pH	7.00	7.17	7.16

Water parameter also take part as factor on survival rate of Nile tilapia. From Table 4.5, the temperature of all aquarium diets that range from 26.29 to 26.78˚C was suitable for the growth of fish. Amos (2013) also stated that, the ideal temperature surrounding water should be about 26 to 28˚C. The dissolve oxygen of all aquarium diets were range from 5.19 to 5.59 mg/l showing ideal condition for growth of tilapia fingerlings. Dissolved oxygen that was greater than 3 mg/l had optimum growth for Nile tilapia (Ross, 2000). As mention by Ross (2000), the best growth of Nile tilapia were between pH 7 to 9 and from this study, the pH that were monitoring was 7.00 to 7.17.

CHAPTER 5

CONCLUSION AND RECOMENDATION

5.1 Conclusion

As for conclusion, among all the diets tested in the present study, Diet 2 that contain 25 % of *Leucaena* seeds meal (LSM) showed the best growth performance with the best weight gain which was 6.35 g/fish, specific growth rate (SGR) of 0.90 %/day, and feed conversion ratio (FCR) which was 2.45 among others. For the survival rate, the treatment control that contain 0 % of *Leucaena* seeds meal (LSM) showed the best result which was 94.44 %. Therefore, Diet 2 that having 25% of *Leucaena* seeds meal plant protein to partial replacing fish meal shown the best diet consumed by Nile tilapia fingerlings compared to other diet treatments. Even though there is slightly different in data observation collected, however statistically there was not significant in all diet in growth performance. Hence, hypothesis H_0 of this study was accepted.

5.2 Recommendation

For the better growth performance of Nile tilapia, further study for suitable feed of Nile tilapia is necessity because fish feed need contains high nutritional value to support their growth. The feed must contain high level of protein because protein is the supreme luxurious portion of fish feed so it is vital to determine the protein requirement for each species and size cultured fish. For this study, the method to reduce fish meal was partial replacement using processed *Leucaena* seed meals (LSM). For further study, the method of partial replacement might be change to extraction of *Leucaena leucocephala* as a supplement added to feed so that it will improve growth performance of the fish. The method of extraction also can be variety such as Soxhlet extraction, maceration, ultrasound-assisted extraction and other. Comparing different method will give more value of protein level in the feed and the acceptation of the fish to feed the diet will also increase.

In this study, handling time when taking sampling method need to do carefully in order to get good result. The size of Nile tilapia are not uniform in each of the tank because there are competition in each of the tank when came to feeding. As the result some fish were having more weight compare to other fish. For further study, choosing the fish that are larger can improve the method of sampling the fish. For the stocking density, fish were having great growth performance if the space for them in the tank wider. The fish need more space so that the population in the tank were having less stress and less cannibalism detected. So for the further study, rearing the fish with larger tank will result in good growth performance as it will result in good survival rate also. Rearing the fish for longer duration of time also will improve the growth performance of the fish.

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APPENDIX A

Table A.1: One-way ANOVA

				Sum of Squares	df	Mean Square	F	Sig.
SGR	Between Groups	(Combined)		.012	2	.006	.567	.595
		Linear Term	Contrast	.000	1	.000	.019	.894
			Deviation	.012	1	.012	1.114	.332
	Within Groups			.063	6	.011		
	Total			.075	8			
SurvivalRate	Between Groups	(Combined)		128.395	2	64.198	1.020	.416
		Linear Term	Contrast	7.407	1	7.407	.118	.743
			Deviation	120.988	1	120.988	1.922	.215
	Within Groups			377.778	6	62.963		
	Total			506.173	8			
FCR	Between Groups	(Combined)		.042	2	.021	.332	.730
		Linear Term	Contrast	.011	1	.011	.177	.689
			Deviation	.031	1	.031	.488	.511
	Within Groups			.383	6	.064		
	Total			.426	8			
Mean Weight Gain	Between Groups	(Combined)		.031	2	.015	1.124	.385
		Linear Term	Contrast	.019	1	.019	1.410	.280
			Deviation	.011	1	.011	.838	.395
	Within Groups			.082	6	.014		
	Total			.112	8			
Weight Gain	Between Groups	(Combined)		1.101	2	.551	1.124	.385
		Linear Term	Contrast	.691	1	.691	1.410	.280
			Deviation	.411	1	.411	.838	.395
	Within Groups			2.939	6	.490		
	Total			4.041	8			

Final Weight	Between Groups	2.602	2	1.301	2.902	.131
	Within Groups	2.690	6	.448		
	Total	5.292	8			
Initial Weight	Between Groups	.749	2	.375	2.189	.193
	Within Groups	1.027	6	.171		
	Total	1.776	8			

Table A.2: Post Hoc Analysis using Duncan Multiple Test for Weight Gain

Duncan[a]

		Subset for alpha = 0.05
Treatments	N	1
Control	3	5.5564
Treatment2	3	6.2350
Treatment1	3	6.3489
Sig.		.228

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.3: Post Hoc Analysis using Duncan Multiple Test for Mean Weight Gain

Duncan[a]

		Subset for alpha = 0.05
Treatments	N	1
Control	3	.9261
Treatment2	3	1.0392
Treatment1	3	1.0581
Sig.		.228

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.4: Post Hoc Analysis using Duncan Multiple Test for SGR

Duncan[a]

Treatments	N	Subset for alpha = 0.05
		1
Treatment2	3	.817370
Control	3	.828984
Treatment1	3	.899805
Sig.		.378

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.5: Post Hoc Analysis using Duncan Multiple Test for FCR

Duncan[a]

Treatments	N	Subset for alpha = 0.05
		1
Treatment1	3	2.445572
Control	3	2.526990
Treatment2	3	2.613792
Sig.		.460

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.6: Post Hoc Analysis using Duncan Multiple Test for Survival Rate

Duncan[a]

Treatments	N	Subset for alpha = 0.05
		1
Treatment1	3	85.555556
Treatment2	3	92.222222
Control	3	94.444444
Sig.		.233

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.7: Post Hoc Analysis using Duncan Multiple Test for Initial Weight

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
Control	3	4.552222
Treatment 1	3	4.577778
Treatment 2	3	5.176667
Sig.		.124

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.8: Post Hoc Analysis using Duncan Multiple Test for Final Weight

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
Control	3	10.108667
Treatment 1	3	10.926667
Treatment 2	3	11.411667
Sig.		.061

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX B

Figure B.1: Moisture analyser.

Figure B.2: Proximate analysis for ash.

Figure B.3: Furnace for ash analysis.

Figure B.4: Fresh unprocessed *Leucaena leucocephala* seeds.

Figure B.5: Sundrying of *Leucaena leucocephala*.

Figure B.6: The treatment seeds.

Figure B.7: Mixing process.

Figure B. 8 : Oven drying of microbound diet (AMBD).

Figure B.9: Experiment tanks.

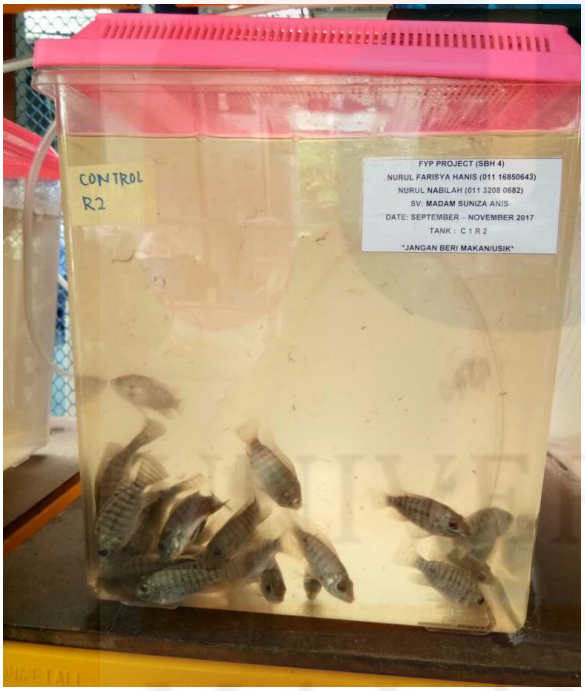

Figure B.10: Experimental fish.

Figure B.11: Weighing procedure.

Figure B.12: Cleaning process.