Use of Mung Bean Seeds (*Vigna radiata* L.) as Partial Replacement of Soybean Meal Protein in the Diets for Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1757) Fingerlings

By

Nurul Farisya Hanis Binti Zakir

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

Faculty of Agro Based Industry

UNIVERSITI MALAYSIA KELANTAN

2018

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.

_____

Student

Name: Nurul Farisya Hanis Binti Zakir

Date:

I certify that the report of this final year project entitled “Use of Mungbean Seeds (*Vigna radiata* L.) As Partial Replacement Of Soybean Meal Protein In The Diets For Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1757) Fingerlings” by Nurul Farisya Hanis Binti Zakir, matric number F14A0305 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.

Approved by:

_____

Supervisor:

Name: Dr. Suniza Anis Binti Mohamad Sukri

Date:

ACKNOWLEDGEMENT

The completion of this thesis undertaking could not had been possible without the participation and assistance of so many people whose names may not all be numerated. Their contributions are sincerely appreciated and gratefully acknowledged.

First of all, I am deeply grateful to Allah. I would like to express my gratitude towards my parents, Mr. Zakir Mokri and Mrs Nourral Loushbhy Ideris Shah, sisters and brother who never stop praying and supporting me in completing this study.

I am deeply grateful and I wish to express my deepest gratitude to my supervisor, Dr. Suniza Anis Mohamad Sukri for her supervision of the work, invaluable guidance, tremendous effort to offer every possible help and time to consult with me about this thesis. It was a great honour to finish this study under her supervision.

My thankfulness is also to the staff especially lab assistant in Faculty of Agro-Based Industry and Faculty of Veterinary Medicines for their great support, kind help and co-operation throughout my experimental period. Special thanks go to my friends and teammates, Muhammad Hafiy Hamizan Md Rife, Nurul Afizah Muhammad Afiq Wong, Zuraini Zainalabidin, Siti Alawiyah Jamal Abdul Naser, Nurnadhirah Mohd Saufi and other teammates who help, gave moral support, suggestion and encouragement during experimental period.

USE OF MUNG BEAN SEEDS (*Vigna radiata* L.) AS PARTIAL REPLACEMENT OF SOYBEAN MEAL PROTEIN IN THE DIETS FOR NILE TILAPIA (*Oreochromis niloticus*, Linnaeus 1757) FINGERLINGS

ABSTRACT

Nile tilapia (*Oreochromis niloticus*) fingerlings with average weight of 4.0±1.0 g were used to investigate the growth performance of tilapia fingerlings fed with partially replacement of raw and boiled mung bean seeds meal (*Vigna radiata*) within six weeks of feeding trial. Three practical diets containing with or without raw and boiled mung bean seeds meal were formulated to replace soybean meal (SBM). The dietary treatment consists of Diet 1: control contained 30 % SBM; Diet 2: raw mung bean seeds meal (RwMB) contained 21 % SBM with 25 % RwMB and Diet 3: boiled mung bean seeds meal (BoMB) contained 21 % SBM with 25 % BoMB. Thirty tilapia fingerlings were randomly distributed into nine aquariums filled with 32 litres of dechlorinated tap water with double aeration. Fish were fed at 5 % of average body weight for three times daily. The best growth performance and feed conversion ratio (FCR) were observed with tilapia fed Diet 3 (14.97 g; 2.00), Diet 2 (13.47 g; 2.08) and Diet 1 (10.11 g; 2.54). Similar result were also obtained for specific growth rate (SGR) where Diet 3 had higher SGR (2.76 % day^{-1}) compared to Diet 2 (2.62 % day^{-1}) and Diet 1 (1.91 % day^{-1}). Diet 2 contained boiled mung bean seeds meal had the best growth performance were compared with fish fed on control and raw mung bean seeds meal diet. Combinations of two or more techniques were required in inactivation of some anti-nutritional factors in legumes.

Keyword: Tilapia, mung bean seeds, raw, boiled, growth performance

PENGGUNAAN KACANG HIJAU (*Vigna radiata* L.) SEBAGAI SEPARA PENGGANTIAN PROTEIN KACANG SOYA DI DALAM DIET ANAK IKAN NIL TILAPIA (*Oreochromis niloticus*, Linnaeus 1757)

ABSTRAK

Anak ikan Nil tilapia (*Oreochromis niloticus*) dengan purata berat 4.0±1.0 g telah digunakan untuk menyelidik prestasi pertumbuhan anak ikan tilapia diberi makan dengan separa penggantian kacang hijau (*Vigna radiata*) mentah dan rebus selama enam minggu percubaan makanan. Tiga diet praktikal yang mengandungi atau tanpa mengandungi kacang hijau mentah dan rebus telah dirumuskan bagi menggantikan kacang soya (SBM). Rawatan permakanan terdiri daripada Diet 1: kawalan mengandungi 30 % kacang soya; Diet 2: kacang hijau mentah mengandungi 21 % kacang soya bersama kacang hijau mentah dan Diet 3: kacang hijau rebus mengandungi 21 % kacang soya bersama kacang hijau rebus. Tiga puluh ekor anak ikan tilapia diagihkan secara rawak ke dalam sembilan akuarium yang dipenuhi 32 liter air paip yang dinyahklorin dan pengudaraan berganda. Anak ikan tilapia diberi makan pada kadar 5% daripada berat badan purata sebanyak tiga kali sehari. Pemerhatian prestasi pertumbuhan dan nisbah penukaran makanan (FCR) yang terbaik oleh ikan dalam Diet 3 (14.97 g; 2.00), diikuti Diet 2 (13.47 g; 2.08) dan Diet 1 (10.11 g; 2.54). Keputusan yang sama juga diperolehi bagi prestasi pertumbuhan tertentu (SGR) dimana Diet 3 (2.76 % sehari) mempunyai prestasi pertumbuhan tertentu (SGR) lebih tinggi berbanding Diet 2 (2.62 % sehari) dan Diet 1 (1.91 % sehari). Diet 2 yang mengandungi kacang hijau rebus memperolehi prestasi pertumbuhan yang lebih baik berbanding ikan yang diberi makanan kawalan dan kacang hijau mentah. Pengunaan lebih daripada dua cara diperlukan untuk mengnyahaktifkan beberapa faktor anti-pemakanan dalam kekacang.

Kata kunci: Tilapia, kacang hijau, mentah, rebus, prestasi pertumbuhan

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION AND SYMBOLS

% day^{-1}	percentage per day
%	percentage
°C	degree Celcius
am	morning
BL	body length
BoMB	boiled mung bean
BW	body weight
CF	crude fibre
CFM	*Cassia fistula* meal
CP	crude protein
DO	dissolved oxygen
EFA	Essential Fatty Acid
FCR	Feed Conversion Ratio
FFSB	Full-fat soybean
FM	Fishmeal
g	gram
kg	kilogram
L	litres
MBD	Microbound diets
mg L^{-1}	milligram per litre
P	phosphorus
pH	potential of hydrogen

pm	evening
ppm	part per million
ppt	part per thousand
RLSM	Raw linseed meal
ROLSM	Roasted linseed meal
SBM	Soybean meal
SGR	Specific growth rate
UV	Ultraviolet
w/v	weight/volume
WG	Weight gain

CHAPTER 1

INTRODUCTION

1.1 Research Background

Plant-based sources are other materials rich with crude protein that can be used in fish diets. Commonly, manufacturer used fish meal and soybean meal as main ingredients in feed. Fish meals was widely used in feed due to its digestibility, palatability and contain a lot of nutrients such as amino acid and energy (Jahan, Hussain, & Islam, 2007; Ganzon-naret, 2014). Few studies in marine fish and shrimps use soybean meal as partially or completely replace fish meal in fed for poultry and fish due to presence of good crude protein (CP) and amino acid and availability (Ganzon-naret, 2014).

Soybean that is belongs to the family '*Leguminosae*' and contains anti-nutritional factors that would influence fish performance. Protein is important nutrient required by fish in order to develop growth and provides energy especially during fry and fingerlings stage (Ganzon-naret, 2014). Since fish and soybean meal were in limited stock and widely used, it was necessary to use other alternative plant protein sources such as cottonseed and mung bean seeds that contain high protein content with low cost (Yue & Zhou, 2008). Based on previous study, replacement of fish meal with soybean meal or combination of soybean meal with cottonseed meal in 32 % protein content in catfish feed is reasonable for economic reason and improvement of

palatability (Robinson & Li, 1994). Replacing soybean meal with plant protein sources able to minimize feed cost by 10 to 30 dollars per metric tonnes of feed in helping aquaculture industry (Robinson & Li, 1994; Yue & Zhou, 2008).

In this study, Nile tilapia (*Oreochromis niloticus*) fingerlings were used as experimental subjects because able to utilize artificial diets and tolerate against adverse environment, resistance against disease and grow well when fed with low cost of feed (El-Sherif & El-Feky, 2009a; Winfree & Stickney, 2015). Mung bean seeds is one of the legume that contain high protein content and presence of anti-nutrients that may affect protein digestibility and digestion of fish (Francis, Makkar, & Becker, 2001). Tilapia fingerlings were fed with mung bean seeds meal that is partially replacing soybean meal in fish diets. Perhaps that by adding mung bean seeds meal in fish diets could increase protein content and reduce cost. Previous study by Robinson and Li (1994) reported that, combination of soybean meal with cottonseed meal can be used to fully replace feeds of catfish containing animal protein as there were no effects on weight gain, feed conversion ratio (FCR) or body composition when consumed plant protein feeds.

There were limited studies on replacing fish meal or soybean meal with other plant-protein sources in fish diets such as mung bean and kidney bean due to presence of anti-nutritional factors. Thus, aim of this study was to investigate the effect of partial replacing soybean meal with raw and boiled mung bean seeds meal on growth performance of tilapia fingerlings.

1.2 Problem Statement

Malaysia need to import raw ingredients due to lack of good quality raw ingredients since local supply is too low and unable to meet the demand. Insufficient stock of good quality raw ingredients result in increases of local production in feed (Talib, 1993). Ng and Chen (2002) and Soltan (2005) reported that, price of soybean meal increases related with increases of global demand from animal diet industry thus, cost of imported soybean rise since soybean are not grown in topical countries. According to Gupta and Sahu (2017), price of soybean meal was expected to become expensive due to high demand from the customers that encouraged exports of soybean meal and suppliers became diminishing. Perhaps by substituting another plant protein sources other than soybean meal could meet protein requirements by fish and help in reducing feed cost. Plant protein sources such as legume seeds contain anti-nutrients that would limit usage of it. Hence, further methods in order to eliminate it are necessary.

According to Hardy (2010), high percentages of fishmeal used in aquaculture industry. Fry and fingerlings of carp, catfish, tilapia and other omnivorous species used 21 % of fishmeal. Since 2006, price of fishmeal was increased and remain above USD 1100. When price of fishmeal and soybean meal are increased, this would create opportunity for other plant proteins sources to replace these main ingredients in feed.

1.3 Hypothesis

H_O = Different methods of processing mung bean seeds does not effect on growth performance of Nile tilapia fingerlings

H_A = Different methods of processing mung bean seeds effect on growth performance of Nile tilapia fingerlings.

H_O is rejected when p-value is less than 0.05 ($p<0.05$).

1.4 Objective

1. To determine the effect of mung bean seeds usage as partial replacement of soybean meal protein in fish diets towards tilapia fingerlings growth performance.

1.5 Scope of Study

The scopes of study involved feed preparation in which commercial feed as control diet. While, experimental diets containing mung bean seeds were undergoes two different methods which are raw mung bean seeds undergo soaking without experience any heat treatment and boiled mung bean seeds undergoes soaking and boiling process.

1.6 Significance of Study

Main feedstuffs such as fish meal and soybean meal had been used at rate 50 % in fish diets. Feeds for herbivores and omnivorous fish species and crustaceans commonly contain 15 to 30 % of soybean meal. In 2007, aquaculture sectors used 6.8 million tonnes of soybean meal. Fish feed and feed ingredients were continuously increasing due to high demand as increase in population, cost of production and transportation (Tacon, Hasan, & Metian, 2011).

Increasing demand of soybean meal from animal diet industry and limited stock of soybean meal as soybean are not grown in tropical countries effect on price of soybean meal in global market (Ng & Chen, 2002; Soltan, 2005). This factors cause concern on the availability of soybean meal in fish diets. Thus it is required to find alternatives sources of protein from plant such as mung bean seeds in order to fully or partially replace soybean meal in fish diets due to high cost of soybean meal, availability and unpredictable market (Azaza et al., 2009).

Mung bean seed was used in fish diets as plant protein sources in partial replacing soybean meal due to high protein content, cheap and widely available in local market. Presence of anti-nutrients in legumes required further process in order to eliminate anti-nutrients.

Shimelis and Rakshit (2007) stated that, presence of anti-nutrients such tannins, phytic acid and saponins were necessary to be eliminate as anti-nutrients would resulted in poor protein and carbohydrate utilization. Anti-nutrients also could

disturb the ability of fish to digest and absorb nutrients from feed efficiently. Techniques that can be applied in reducing anti-nutrients from legumes were soaking, de-hulling and heat treatments such as boiling process.

CHAPTER 2

LITERATURE REVIEW

2.1 Mung bean Seeds

Mung bean known as (*Vigna radiata* L.) belong to '*Leguminosae*' family (Ganzon-naret, 2014). This legume grows in short-season, summer-growing and grown widely in tropic and subtropics regions such as India, China, Myanmar and Thailand. Moreover, mung bean achieve maturity within short period, high tolerance against drought and able to grow during poor soil condition. The annual production of mung bean is 6 million tonnes (Tang, Dong, Ren, Li, & He, 2014; Chen et al., 2015).

It is categorized into legume seeds that contain high amount of protein, calories, minerals such as iron, zinc, potassium, vitamin A and B (Khattab & Arntfield, 2009;Chen et al., 2015). Legumes can be divided into two categories which are oilseeds that grow for protein and oil content such as soybean and grain legumes that grow as protein sources such as cowpeas (Venter & Eyssen, 2001). According to Chau and Cheung (1997) and Mubarak (2005), legumes contain low sulphur and protein digestibility due to presence of anti-nutrients. Other than that, legumes are known for their toxic effects on poultry caused by tannins and haemagglutinins when ingested at raw state (Ologhobo, 1992).

Mung bean seeds contain 20.97 to 31.32 % of protein and amino acid except methionine, cysteine and tryptophan and safe to be consumed by animals (Anwar, Latif, Przybylski, Sultana & Ashraf, 2007; Tang et al., 2014). Protein of mung bean seeds rich with essential amino acids such as leucine, isoleucine and valine but deficient in lysine and threonine. It is also content high value of carbohydrate at 50 to 60% compared to soybean (Tang et al., 2014).

In previous study by Ganzon-naret (2014), soybean meal protein in diets of sea bass, *Lates calcarife*r fingerlings was replaced with raw and heat-treated mung bean seeds. Nutritional composition of mung bean seeds was 23.7 % crude protein, 19.7 % crude fat, minerals, vitamins and essential amino acids. This study had three experimental diets which are Diet 1, Diet 2 and Diet 3 that contained commercial feed, raw and boiled mung bean seeds respectively. Based on the result, sea bass with best growth performance and feed conversion ratio (FCR) were observed in Diet 3 (8.33 g, 2.06) followed by Diet 1 (8.26 g, 2.08) and Diet 2 (6.59 g, 2.44).

2.2 Benefits of legumes in diets

Mung bean had consumed as processed dry grain, sprouts or green vegetable. Mung bean had consumed as dhal in South Asia. While in Eastern China, noodles made from mung bean starch had prepared using machine or manually by hand. Mung bean was easier to be digested compared to other legumes. Use of mung bean based oral rehydration able to treat acute diarrhoea and dehydration that attack kids between age of three months old to five years old (Nair et al., 2013).

According to Tang et al. (2014) stated that, mung bean were consumed by people in China for more than 2000 years as common food in their daily life. It known in detoxification activities, refreshes mentality, relieve heat stroke and reduce swelling. Risk of osteoporosis and urinary calcium excretion can be minimised when vegetable was used to substitute animal protein. Other than that, minerals present in legumes could reduce risk of hypertension (Venter & Eyssen, 2001).

Mung bean was used in folk remedies that functioning to cure toxic poisoning, heat stroke that related with thirst, irritability, fever and inflammatory response (Lee et al., 2011). Previous study by Randhir, Lin, and Shetty (2004) stated that, polyphenol extracts from mung bean sprout act as antimicrobial and antifungal had shown activity against *Helicobacter pylori*, which is common bacterial infections in human and cause gastroduodenal disease.

Regular intake of mung bean able to regulate the flora of enterobacteria, reduce risk of coronary heart disease and hypercholesterolemia when absorption of toxic substances can be controlled, thus helps in preventing cancer (Kruawan, Tongyonk & Kangsadalampai, 2012).

2.3 Replacement of soybean meal with other legumes in fish diets

Legumes such as kidney bean, mung bean, cowpea, cottonseed meal had possibility to replace soybean and fish meal at adequate amount without adverse growth performance of fish.

Study conducted by Balogun and Ologhobo (1989), were to investigated growth performance of *Clarias gariepinus* (Burchell) fingerlings when fed with raw and cooked soybean diets. From the result, growth response of fish fed with raw soybean meal (SBM) was lower ($p<0.05$) than fish fed with control and cooked soybean meal. Moreover, percentage of weight gains of fish fed at different levels 25, 50, 75 and 100 % of cooked SBM were higher than fish fed on raw SBM. This situation happens because absence of toxic principle that could inhibit fish growth which consumed cooked SBM.

In another study by Adebayo, Fagbenro and Jegede (2004), growth depression were observed in fish feed on diets in which soybean meal substituted with *Cassia fistula* meal (CFM) at 500 to 1000 g kg^{-1}. This is due to presence of high fibre content that cause reduction in digestibility. This proved inappropriate to completely replaced SBM with CFM in *Oreochromis niloticus* fingerlings.

Based on the data presented by Soltan (2005), body weight (BW) or body length (BL) of Nile tilapia were not affected when 25 % of soybean meal protein was replaced with raw linseed meal protein. However body length (BL), body weight (BW), weight gain (WG) and specific growth rate (SGR) were decreased at higher levels of

50, 75 and 100 % replacing soybean meal (SBM). The result indicated that, there was possibility of replacing only 25 % of soybean meal by reasonable price raw linseed meal (RLSM) in Nile tilapia. Furthermore, the best feed conversion ratio (FCR) of fish was obtained at 75 % of replacing soybean meal with roasted linseed meal (ROLSM) which means absence of adverse influence on growth performance parameter (BW, BL, WG and SGR) of Nile tilapia but completely replacement of soybean meal effect on these four parameters. High potential of heat-treated linseed meal such as roasting and autoclaving as SBM substitute in tilapia diets due to heat treatment had capability to reduce anti-nutritional factors and improve nutritional value of feed ingredients instead of 25 % of raw linseed meal (RLSM).

2.4 Presence of Anti-nutrients

Francis et al. (2001) stated that, used of legume seeds as protein sources in feed ingredients were inadequate due to presence of anti-nutritional factors. Anti-nutrients that commonly present in legume seeds are tannins, phytate, raffinose, saponins and haemaglutinnins (lectins) that will reduce nutritional quality of protein (Alonso, Orúe, & Marzo, 1998; Soetan & Oyewole, 2009). Presence of anti-nutritional factors in feeds could decrease animal productivity and become toxic when consumed by animals in large quantities (Kumar,1991). Example of anti-nutrients are tannins, oxalate and phytate could be harmful when consumed in an unprocessed food (Ojiako & Igwe, 2008). Oligosaccharides that present in raw or poorly processed legumes are able to dissolve in water and can be removed through adequate soaking, germination or fermentation (Tang et al., 2014).

According to Eusebio and Coloso (2000), soybean meal has high protein digestibility. Low level of apparent protein digestibility (APD) in leguminous seed meal and leaf meal cause presence of anti-nutrients in meal samples. Raw legumes and oilseeds contain higher levels of anti-nutritional factors compared than their processed forms hence further processing are required before consumed by animals (Hajos & Osagie, 2004).

2.4.1 Phytates

Phytic acid or known as phytates when in salt form is principal storage form of phosphorus (P) in plant tissue (Kumar, Sinha, Makkar, & Becker, 2010). The growth rate of fish become slower when fed with high content of phytates as it has ability to reduce absorption of nutrients, bioavailability of minerals, protein digestibility and utilisation efficiency due to damage of the pyloric cecal region in intestine (Udensi, Ekwu, & Isinguzo, 2007; Kumar et al., 2010; Bora, 2014). This happen when phytic acid has ability to interacts with seed proteins in order to form protein-cation-phytate complexes which influence protein digestibility.

2.4.2 Saponins

Saponins recognised as anti-nutrients that could bring negative effect towards fish. According to Shi et al. (2004), heat-stable and glycosidic compounds that naturally exist in varieties of food plant. Structures of saponins are depends on types of plant, quantity of sugar and composition of steroid ring. Saponins are extremely poisonous towards fish and cold-blooded animals. Saponins could affect

the growth of fish when endogenous saponins reduce protein digestibility of soybean by chymotrypsin. High content of saponins with seven percent to eight percent in tilapia diets shows death within five to six hours. Saponins content below one g/kg of diet did not show negative effect on growth performance of common culture fish (Francis et al., 2001).

Soetan and Oyewole (2009) stated that, saponins could disturb absorption of cholesterol called as hypocholesterolaemia. Saponins able to reduce bioavailability of nutrients and enzyme activity when previous researcher did investigated effect of saponins in sheep (Shi et al., 2004). Legume that contain saponins can be used in diet at moderate concentration as high concentration of saponins result in high toxicity (Bora, 2014). High concentrations of saponin would cause unpleasant taste and astringency in dietary plants, thus it cause limitation and low intake of saponin by animals and humans (Shi et al., 2004).

2.4.3 Tannins

Presence of tannins in animal diets could decrease feed consumption in animals as able to reduce nutritive value and biological availability of macromolecules such as protein and carbohydrates, thus reduce palatability and growth rate of animal (Eusebio & Coloso, 2000; Soetan & Oyewole, 2009). Based on Francis et al. (2001), tannins able to inhibit the digestive enzymes and reduce protein and carbohydrate digestibility. Poel, Gravendeel, and Boer (1991) also stated that, tannin in faba bean (*Vicia faba* L.) could reduce in vitro digestibility of proteins and carbohydrates and biological availability. Tannins in fish diets could reduce absorption of vitamin such as

vitamin B_{12}. Condensed tannin in copra at level 2.4 % shows growth depression in tilapia and rohu (*Labeo rohita*) fingerlings that indicate fish are sensitive against tannins (Francis et al., 2001).

Tannin content could be reduce by using water, acid, bases or salts (Eusebio & Coloso, 2000). Other than that, de-hulling process able to decrease tannin contents in sample by removing seed coat of legumes (Poel et al., 1991).

2.5 Elimination of anti-nutritional factors

The following protocols were used by Ganzon-naret (2014), in study involves mung bean seeds in fish diets. Raw mung bean seeds were washed to remove dirt and impurities. Then, mung bean seeds were soaked in tap water at ratio of 1:10 (v/w) for 10 hours at room temperature. After that, seeds were drained and rinsed for six times with distilled water. After soaking, rinsed mung bean seeds were divided into three portions that undergo different method of preparation. One-third of rinsed mung bean seeds were mashed, dried in the oven and ground into meal. Another 2/3 of rinsed mung bean seeds were conducted through heat-treatments which were boiling and autoclaving.

The 1/3 of rinsed mung bean seeds were boiled in a hot plate at 100°C for one hour until soft. Then, cooked mung bean seeds were mashed, dried and ground to fine powder through sieve. Another 1/3 of rinsed mung bean seeds were autoclaved for 30 minutes at 121 °C. After 30 minutes, the seeds were mashed,

grounded and sieve to become powdered meal. This study had four experimental diets which were Diet 1 as control diet without raw, boiled or autoclaved mung bean seeds, Diet 2, Diet 3 and Diet 4 contained raw, boiled and autoclaved mung bean seeds respectively that replaced soybean meal protein.

Crude protein, lipid, ash and moisture content in mung bean seeds after processed by soaking and heat-treatments were presented in Table 2.1.

Table 2.1: Proximate analysis of raw and heat-treated mung bean seed meals (% dry weight)

Components	Raw mung bean seeds meal (RwMB)	Boiled mung bean seeds meal (BoMB)	Autoclaved mung bean seeds meal (AcMB)
Dry matter	93.43	93.40	93.42
Crude protein	23.96	23.06	22.98
Crude lipid	1.20	1.18	1.18
Crude ash	3.88	3.64	3.62
Crude fibre	3.28	3.16	3.26
Nitrogen Free Extract	67.68	68.96	68.96

Table 2.1 showed that, boiled and autoclaved mung bean seeds meal showed slightly decreased in crude protein (CP) and ash content compared to raw mung bean seeds meal with 23.96 % of crude protein and 3.88 % crude ash.

Study by Mubarak (2005), traditional processes in reducing anti-nutrients of mung bean seeds were soaking in distilled water, de-hulling in order to remove hull, boiling in tap water at 90 °C until soft, autoclaving at 121 °C, microwave oven and germination. Increase of time and temperature during processing able to reduce nutrition value and lysine in legumes. Chemical composition of mung bean seeds after undergoes traditional methods were presented in Table 2.2.

Table 2.2: Chemical composition of mung bean seeds after process by traditional methods (g/100 g dry weight basis)

Components	Raw	De-hulling	Soaking	Boiling
Crude protein	27.5	27.6	27.0	26.80
Crude fat	1.85	1.82	1.83	1.82
Crude fibre	4.63	4.10	4.45	4.50
Ash	3.76	3.60	3.32	3.55
Total carbohydrate	62.3	62.9	63.4	63.3
Moisture	9.75	10.10	10.50	10.13

According to Francis et al. (2001), phytate content can be reduced through milling, fermentation and heat-treatment such as autoclaving. Level of phytates should be maintained below five g/kg in feeds. While, tannins content can be eliminate through de-hulling, autoclaving and alkali treatment. Sesame seed meal shows reduction of tannin content from 20 to 10 g/kg after being ferment with lactic acid bacteria. Previous study by Alonso et al. (1998) stated that, the removal of seed coats could increase the protein content.

Soaking technique is common practice that able make texture of legumes become softer, reduced processing time and toxic content of edible beans. Commonly, dry beans were soaked for 12 to 18 hours at room temperature. Increase soaking duration would decrease hardness of legumes and cause great reduction of saponins. The reduction of saponins happens when anti-nutrients were leached out into the soaking media through simple diffusion. The volume of beans is increase triples during cooking and soaking as raw beans gain 80 % in weight due to water inhibition and 53 to 57 % moisture content (Shi et al., 2004).

Extensive soaking period prior fermentation or germination leads to reduction in phytate content (Duhan, Khetarpaul, & Bishnoi, 2002). Duhan, Chauhan, Punia, and Kapoor (1989) reported that, phytic acid contents in chickpea (*Cicer arietinum*) and black gram (*Vigna mungo*) were reduced through soaking the seeds for 12 hours when anti-nutrients leached out into soaking water. In legume seeds, phytates content related with protein bodies as phytate level would increase when protein content increases (Maga, 1982; Reddy & Sathe, 2002). Previous study by Vijayakumari, Siddhuraju, and Janardhanan (1996) stated that, soaking process in distilled water could affect oligosaccharides and phytic acid contents in legumes as

percentage loss of these anti-nutrients was higher when soaking in distilled water compared with soaking in salt water.

Wiryawan and Dingle (1999) stated that, heat treatments causes denaturation of proteinaceous inhibitors in decreasing lectins activities, trypsin and chymotrypsin inhibitors. The improvement of nutrition value may associate with decrease in urease and trypsin inhibitor activities (Araba & Dale, 1990). Temperature and duration of processing grain legumes need to be carefully controlled as excessive heating would cause reduction of protein solubility and certain amino acid such as lysine (Araba & Dale, 1990; Kratzer, Bersch, Vohra, & Ernst, 1990; Poel, Mollee, Huisman, & Liener, 1990; Wiryawan & Dingle, 1999). After thermal treatments, digestibility would increase due to interruption of protein structures and cell wall encapsulated starch, starch gelatinization and physical disintegration of legume seeds (Tovar, Francisco, Björck, & Asp, 1991). Other previous study by Kataria, Chauhan, and Punia (1989) stated that, heat treatments caused thermal degradation, denaturation of anti-nutrients and formation of insoluble complexes thus, this would cause reduction of anti-nutrients.

Cooking also effective in deactivate tannins, phytic acid and trypsin inhibitors in faba bean seeds and helps in improving protein digestibility (Saikia, Sarkar, & Borua, 1999; Elsheikh, Fadul, & El Tinay, 2000). Previous study by Clemente, Śanchez-Vioque, Vioque, Bautista, and Millán (1998) and Zia-ur-Rehman and Shah (2005) reported that, cooking after soaking technique could increase protein digestibility from 7 to 12 % improvement. Cooking process provide more protein digestibility than soaking in *Vigna aconitifolia* and *Vigna sinensis* with 9.35 % and 11.07 % improvement respectively. Cooking of legumes reduces the amount of

saponin by 7 to 53 % (Vijayakumari et al., 1996). Based on Shi et al. (2004), cooking treatment able to lower saponin content in chick pea and black gram and faba beans by 7 to 17 % and 35 % respectively. Greater losses of saponin when undergoes pressure cooking compared to ordinary cooking method.

Improvement of protein quality after heat treatment due to increased availability of the protein to enzymatic attack and structural disintegration of enzyme inhibitors (Wu et al., 1994; Vijayakumari, Siddhuraju, & Janardhanan, 1995). Autoclaving at 121 °C for 10 minutes was the most effective method in reducing anti-nutrients and improve protein and starch digestibility (Vijayakumari et al., 1996; Zia-ur-Rehman & Shah, 2005).

2.6 Nile Tilapia (*Oreochromis niloticus*)

Nile tilapia known as *Oreochromis niloticus* are able to tolerate against water temperature, salinity, pH, dissolved oxygen (DO) and photoperiod (El-Sayed & Kawanna, 2004). According to Wei and Wee (2010), Nile tilapia able to withstand at temperature ranged from 13.5 to 33 °C. While other previous study by El-Sheriff and El-Feky (2009b) stated that, suitable water temperature for Nile tilapia fingerlings between 25 to 30 °C. Previous study by El-Sheriff and El-Feky (2009a) reported that, Nile tilapia fingerlings more favourable to culture at pH level 7 to 8 in order to gain optimum growth performance and survival rate.

Tilapia also able to withstand at very low levels of dissolved oxygen (DO) thus, *O. niloticus* can tolerate DO levels as low as 0.1 ppm (Wei & Wee, 2010). El-Shafai, El-Gohary, Nasr, Steen, and Gijzen (2004) stated that, Nile tilapia showed best growth rate when rearing at dissolved oxygen ranged 7.0 to 8.3 mg L^{-1}. Appropriate dissolved oxygen during rearing tilapia at ranged from 6.17±1.64 mg L^{-1} (Lara-flores, Olvera-Novoa, Guzmán-Méndez, & López-Madrid, 2003).

According to Abdel-Tawwab, Hagras, Elbaghdady, and Monier (2015) from the effect of dissolved oxygen and size on Nile tilapia, *O. niloticus* towards their growth performance showed that, fish under normal dissolved oxygen (DO) conditions had better growth compared to other treatment due to well feed intake and nutrient digestibility. Normal, medium and low dissolved oxygen level was aerated with four, two and one air stones respectively. Low dissolved oxygen (DO) level effect on growth and feed utilization of fish due to shortage in oxygen availability for fish to growth. Fish shows good feed efficiency when provide with adequate dissolved oxygen in water as growth and feed efficiency of fish were affected by DO availability. Fish should be maintained at adequate dissolved oxygen level in order to improve fish performance and health.

Previous study by Iqbal et al. (2012) stated that, different salinity at 800, 1600, 2400, 3200 and 4000 ppm effect on survivability and growth performance of Nile tilapia (*O. niloticus*) reared in cement tank showed, better growth performance of tilapia was in treatment at highest salinity level at 4000 ppm or 4 ppt. Moreover, feed intake was lowest at 800 ppm salinity level and feed intake increased as salinity level increases. Likongwe (2002) reported that, temperature influence on tolerant of salinity change together in nature and the changes may give positive or negative result on

growth of fish. It can conclude that *O. niloticus* able to survive and grow well at salinities above 4000 ppm (Iqbal et al., 2012).

Tilapia fish with 5 to 25 g required 25 to 35 % of protein while 1 to 5 g fish needs 30 to 40 % of protein (Abdel-Tawwab, Ahmad, Khattab, & Shalaby, 2010). Previous study by El-Sayed (2004) stated that, protein requirement in order to obtain maximum performance of tilapia during larval stages was high which between 35 to 50 % and decreased when increasing in fish size. Protein requirement for tilapia juvenile is from 30 to 40 % while adult required 20 to 30 % protein in diets. Tilapia broodstock need 35 to 45 % protein for reproduction, spawning efficiency and larval growth and survival (El-Sayed, 2004). Based on Ogunji and Wirth (2000), highest growth rate of *O. niloticus* with initial weight 4 to 5 g at dietary protein content of 33.32 % (dry matter).

According to Inayat and Salim (2005), feed conversion ratio (FCR) was the best parameter in order to judge the acceptability and suitability of feed towards fish. FCR had been termed as food coefficient. Feed digestibility play role in lesser FCR through effective feed consumption. Good FCR might due to the proper feed supply and availability of some nutrient of feed in water, suitable water temperature and dissolved oxygen (Daudpota et al., 2016).

Previous study by Goda, Wafa, El-Haroun, and Chowdhury (2007), feed conversion ratio (FCR) of *O. niloticus* and *Sarotherodon galilaeus* fed with full-fat soybean (FFSB) diets had worst FCR at 2.0 and 2.1 respectively. While, *O. niloticus* and *S. galilaeus* fed with diets containing soybean meal (SBM) had the best FCR

($p<0.05$) at 1.7 and 1.9 respectively. Increased weight of fish caused FCR increases while decreased when increased dietary protein level.

According to El-Sayed (2004), in order to gain maximum growth performance, tilapia required 10 to 15 % of dietary lipids as tilapia able to utilize dietary lipids very efficient. Growth performance of Nile Tilapia fed with fish oil contain diet rich in n-3 essential fatty acid (EFA) was decreased compared with other fish that fed with soybean oil or maize oil called as vegetable oil that rich in n-6 EFA. Tilapia which is herbivorous fish able to utilize dietary carbohydrates more efficient compared to carnivorous fish. Wheat bran, rice bran and maize were used- as carbohydrate sources. Carbohydrate utilization by tilapia was affected by daily feeding frequency as previous study by Tung and Shiau (1991) reported that, increasing feeding frequency able to improved carbohydrate utilization, growth rates and protein sparing. Tilapia able to consume floating and sinking pellets very efficient (El-Sayed, 2004).

Previous study by Yousif (2002), juvenile Nile Tilapia, *O. niloticus* L. stocked at 0.5 fish/L had the best average body weight of 14.40 g compared to fish stocked at 2 fish/L had lowest average body weight of 9.15 g.

2.7 Use of Microbound diets (MBD)

Recently, microbound diets (MBD) were famous to be used in shrimp industry and accepted by farmers as rearing and handling of live food for production of shrimp post-larvae is quiet costly and live food does not offer all essential nutrients required by this species. New effort had been developed in order to reduce cost in shrimp post-larvae industry by producing microparticulate which is microencapsulation, microbound and microcoated diets that able to deliver necessary nutrients needs by larvae (Gallardo et al., 2002; Luzardo-Alvarez, Otero-Espinar, & Blanco-Méndez, 2010). Microbound particles lack of distinct wall and consist of polymer matrix where nutrients are bound and form feed that stable in water (Langdon, 2003).

Benefits of microparticulate in aquaculture industry are low cost of production as it is economic to be produce, easy preparation and does not use of any toxic ingredients that would affect health condition of aquaculture itself (Langdon, 2003; Luzardo-Alvarez et al., 2010). These diets also act as nutrients protection by providing wall that functioning as coating until it reach digestive system of fish and available for digestion and absorption.

According to Luzardo-Alvarez et al. (2010), aquatic species experience difficulties in digesting some type of MBD. Zein, alginate, agar, gelatin, carragenates, starch and carboxymethylcellulose that used as agglutinants in order to prepare MBD are effect on nutrient release, acceptance by aquatic species and digestion of production is vary in different species. Some agglutinants are not favourable to be

used in all aquatic species. Agar, carrageenan and alginate are polysaccharides from seaweed (Genodepa, Zeng, & Southgate, 2007).

Previous studies by Ruyet, Alexandre, Thébaud, and Mugnier (1993) and Partridge and Southgate (1999) stated that, gelatin and carragenate are more suitable for barramundi larvae (*Lates calcarifer*) while sea bass (*Dicentrarchus labrax*) are more suitable with alginate and zein respectively. Effect of binder composition on ingestion and assimilation of MBD by barramundi *Lates calcarifer* Bloch larvae showed that, there were no significantly differences between gelatin-bound MBD with lowest assimilation rate (1.60 $\mu g\ mg^{-1}\ h^{-1}$) and agar-bound MBD (2.11 $\mu g\ mg^{-1}\ h^{-1}$) or carrageenan-bound MBD. The best binder with low rate of ingestion and assimilation efficiency at 49 % is gelatin-bound MBD. Gelatin- or carrageenan-bound MBD are suitable for barramundi larvae due to higher assimilation efficiency (Partridge & Southgate, 1999).

Ingestion indicates palatability of diet and determines which nutrients are available to be digesting. Type and strength of binder, diet colour, appearance and texture of diets influenced attractiveness, digestibility and acceptance towards MBD thus resulted on growth performance and mortality rate of larval (Genodepa et al., 2007).

Study by Genodepa et al. (2007) reported that, diets with low concentration of binder cause excessive leaching maybe inadequate water stable thus result in loss of valuable dietary nutrients and negative impact on water quality. This was supported with proper result from Partridge and Southgate (1999) said, diet with 1 %

alginate is more leached (12.00 %) compared to the MBD contained 3 % alginate (9.28 %).

Other study did by Genodepa et al. (2007), zein-bound MBD which zein act as protein source showed lowest rate of leaching in three different immersion duration which are 30, 60 and 240 minutes compared to agar, alginate and carrageenan-bound MBD. Carnivorous animals such as mud crab larvae are more appropriate to use protein source as binder such as zein or gelation compared to polysaccharides.

According to Langdon (2003), amino acid and other water soluble nutrients were rapidly discharged from microbound particles. Study by López-Alvarado, Langdon, Teshima, and Kanazawa (1994), shape of microbound particles were irregular when examined under microscope. Irregular shape of this diet caused greater area of microbound diets contact with water and leakage of soluble nutrients was facilitated. 81 and 85 % of free amino acids were discharged from alginate and carrageenan-microbound particles after two minutes aqueous suspension. While, study by Partridge and Southgate (1999) showed that, 1 % binder in diets experience great losses of dietary nutrients compared to diet bound with 2 to 3 % binder. This situation were affected by type and concentration of binder used in MBD preparation. Performance of MBD can be improved by using suitable concentration of binder in feed preparation.

Formulation of microparticulate diets using binder is made by gelling mixture of dietary components with binder, then followed with drying, grinding and sieving the mixture in order to obtain desired size (Langdon, 2003).

According to Holme, Zeng, and Southgate (2006), microbound diets for larval culture of the mud crab *Scylla serrata* were prepared by combining all dry and moist ingredients in separate containers. Then, dry and moist ingredients were mixed thoroughly before binder (zein) was dissolved in 70 % alcohol and homogenized with diet mixture. Homogenous paste was spread thinly in aluminium dishes and dried at 50 °C for 48 hours. After that, mortar and pestle was used to ground dry diets and sieve in order to obtain desired size. The composition (% dry weight) of the basal experiment diets used in feeding experiments with *S. serrata* larvae were presented in Table 2.3.

Table 2.3: Composition (% dry weight) of the basal experiment diets used in feeding experiments with *Scylla serrata* larvae

Ingredients	Dry weight (%)
Squid meal	39.7
Dried rotifer	39.7
Fish oil	5
Corn oil	1
Lecithin	3
Cholesterol	1
Dibasic calcium phosphate (DCP)	0.6
Vitamin mix	4
Mineral mix	3
Zein (binder)	3
Total	100

Microbound diets that prepared by Partridge and Southgate (1999), were by adding dry ingredients and mixing with oil and lecithin. All binders which are agar (Swallow Globe Brand), carrageenan, alginate and gelatin were dissolved in hot water before mix with other ingredients. Then, diets were dried at 45 °C for 72 hours. Composition (% dry weight) of experimental MBD was presented in barramundi *Lates calcarifer* Bloch larvae diet Table 2.4.

Table 2.4: Composition (% dry weight) of experimental MBD in barramundi *Lates calcarifer* Bloch larvae diet

Ingredient	Radioactive diet	Non-radioactive diet
^{14}C-labelled rotifers	50	-
Squid powder	36.25	86.25
Fish oil	4	4
Lecithin	2	2
Pancreatin	0.05	0.05
Choline chloride	1	1
$CaHPO_4.2H_2O$	0.6	0.6
Mineral mix	1	1
Vitamin mix	1	1
Vitamin C	1	1
Vitamin E	0.1	0.1
Binder	3	3
Total	100	100

Another method in preparation of microbound diets by Gallardo et al. (2002), dry ingredients consist of anchovy (*Engraulis mordax*) (*Loligo spp*), squid and shrimp powder were mixed for 15 minutes. Then, oil was added into mixed ingredients and continuous mixing for another 15 minutes. Water was added until homogenous paste was formed. The paste was dried at 60 °C in order to obtain constant weight (48 hours) and ground to produce desirable size.

All ingredients were blend using mechanical mixer. Then, binder (gelatine) was dissolved in hot water (80 °C) and added into mixed ingredients. Additional cold water was added in order to obtain uniform paste. Next, paste was extruded and dried in an oven at 45 °C for 48 hours (Kolkovski, Curnow, & King, 2010).

CHAPTER 3

MATERIALS AND METHODS

3.1 Location

Mung bean seeds (*Vigna radiata*) were obtained from local market in Kelantan. The study was conducted at Aquaculture Laboratory and Animal Laboratory, Faculty of Agro Based Industry, University Malaysia Jeli Campus, Kelantan.

3.2 Proximate Analysis

Proximate analysis for feedstuffs (fish meal, soybean meal, rice bran, tapioca, raw and boiled mung bean seeds meal) was conducted at Animal Nutrition Laboratory, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan Pengkalan Chepa Campus, Kelantan.

3.2.1 Moisture content

Moisture content was determined by Moisture Analyser MX-50 (A&D Company, Limited) that used heating and drying method which able to compare weight before and after heating and drying of sample. Five grams of sample was weighed and placed on the sample pan. Then, button start was pressed in order to run the analysis. During heating process, sample was heated for a period of time at 160 °C in order to dry the sample and evaporate its water. After few minutes, value of moisture content of sample would appear on the screen of machine analyser. The data was recorded.

3.2.2 Crude protein

Kjeldahl method was used in determining crude protein and determined by Kjeldahl nitrogen multiplied by 6.25 which 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 (Gerhardt Analytical Systems) for 1 hour 30 minutes. After digestion process, the sample was cooled in the fume hood for one hour before proceed with distillation process. Next, Kjeldahl distillation system Vapodest 30s (Gerhardt Analytical Systems) was warm up for ten minutes. 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, sample in conical flask was titrated with 0.1 mL hydrochloric acid (HCl) until the sample turns greyish pink.

Figure 3.1: After titration process, samples turns to 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

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

3.2.3 Crude fibre

Crude fibre was analysed using Manual Fiberbag System (Gerhardt Analytical Systems) where condenser ball was used to cover the beaker that placed on hot plate. Before analysis, fibre bag was drying for one hour at 105 °C then, 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 named 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 placed into a sample carousel. Then, the beaker was place on the hotplate.

The fibre bag would undergoes two washing phase which Phase I by boiling in 360 mL sulphuric acid for 30 minutes after the acid starting to boiled then, three times washing with hot water in order to remove acids and Phase II by boiling with 360 mL sodium hydroxide solution for 30 minutes after alkali starting to boiled then three times washing with hot water in order to remove alkali. 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 then placed in the drying chamber at 105 °C for 30 minutes in order to cool it off. Next, crucible was placed in the desiccator for 30 minutes and weighed of crucible with fibre bag was recorded and named as M_3.

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

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

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, 1 g 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. Eighty millilitres of petroleum ether 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 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, cooled in the 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):

$$\text{Percentage of fat (\%)} = \frac{\text{Final weight of aluminium cup (g)} - \text{Initial weight of aluminium cup (g)}}{\text{Sample weight (g)}} \times 100$$

3.2.5 Ash

According to method did by Thiex, Novotny, and Crawford (2012), 2 g of sample was weighed and placed into porcelain crucible. All weights of sample were recorded. Then, porcelain crucible containing sample were placed into temperature-controlled furnace. Furnace required more than one hour in order to increase the temperature until 550 °C. Temperature at 550±10 °C was hold for three hours. Next after three hours, furnace was allowed to cool to below than 200 °C. Then, porcelain crucible were transferred into desiccator, cool and weight within one hour. The final weights of porcelain crucibles were recorded. The ash content was calculated as below:

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

3.3 Nutritional composition

Nutritional composition of feedstuffs (fish meal, soybean meal, rice bran, tapioca, raw and boiled mung bean seeds meal) used in fish diets was presented in Table 3.1.

Table 3.1: Nutritional composition of feedstuffs (% g)

Components	Crude protein	Crude fat	Crude fibre	Ash	Moisture
Fish meal (FM)	27.35 %	2.72 %	0.28 %	64.52 %	7.07 %
Soybean meal (SBM)	52.55 %	0.16 %	3.95 %	9.95 %	14.60 %
Tapioca	2.31 %	5.49 %	0.24 %	0.24 %	13.36 %
Rice bran	19.48 %	10.70 %	-	11.85 %	15.03 %
Raw mung bean meal (RwMB)	28.20 %	0.72 %	8.07 %	7.65 %	8.55 %
Boiled mung bean meal (BoMB)	26.14 %	1.30 %	5.87 %	11.87 %	8.77 %

3.4 Diet preparation

Raw mung bean seeds were washed in order remove dirt and other foreign substances.

3.4.1 Soaking process

Mung bean seeds were soaked in distilled water at ratio of 1:10 (w/v) for 12 hours. Then, soaked mung bean seeds were drained and rinsed for three times with distilled water. After that, 1/2 of rinsed mung bean seeds were dried in oven at 50 °C for 20 hours. After 20 hours of drying, mung bean seeds were mashed and ground to fine powder passing through sieve.

3.4.2 Boiling process

Another 1/2 of rinsed mung bean seeds were boiled with tap water (100 °C) at ratio of 1:10 (w/v) on portable stove until soft and able to felt with fingers. Boiled mung bean seeds were dried in the oven at 50 °C for 20 hours. After 20 hours of drying, mung bean seeds were mashed and ground to fine powder passing through sieve.

All these powdered mung bean meal from raw and boiled process were stored in the closed container for further analysis.

3.4.3 Diet formulation

The basal diet formulation contains fish meal, soybean meal, rice bran, tapioca, vegetable oil, vitamin and mineral premix and vitamin C. The control diet (Diet 1) contained SBM without raw and boiled mung bean seed meals. Diet 2 and Diet 3 contained raw and boiled mung bean seed meals respectively. The formulations of every experimental diet were presented in Table 3.2.

Table 3.2: Formulation of control and experimental diets (g/100 g dry weight)

Ingredients	Diets		
	Diet 1	Diet 2	Diet 3
Fish meal	45.97	29.90	29.91
Soybean meal	30.00	21.00	21.00
Raw mung bean meal	-	25.00	-
Boiled mung bean meal	-	-	25.00
Rice bran	0.03	0.1	0.09
Tapioca	20.00	20.00	20.00
Vegetable oil	1.00	1.00	1.00
Vitamin C	1.00	1.00	1.00
Vitamin and mineral premix	2.00	2.00	2.00

[1]Diet 1: control without raw and boiled mung bean seeds meal; Diet 2: raw mung bean seeds meal; Diet 3: boiled mung bean seeds meal

Preparation of control diets prior addition of agar powder (Swallow Globe Brand), vitamin and mineral premix, rice bran, tapioca, soybean meal and fishmeal were homogeneously mixed in the heavy duty mixer. Then, vegetable oil was poured slowly into the mixture. The mixture was stored in the tight container. Preparations of experimental diets were similar with the preparation of control diets but with addition of raw and boiled mung bean seeds meal for Diet 2 and Diet 3 respectively.

Microbound diet (MBD) were prepared using the method described by Partridge and Southgate (1999), Gallardo et al. (2002) and Kolkovski et al. (2010). The method was modified by using agar powder and diet mixture. Three grams of agar powder (Swallow Globe Brand) was added into 97 grams of diet mixture. The mixture was homogenously mixed on hot plate (Thermo Scientific Cimarec Ceramic Stirring Hot Plate) at 100 °C with addition of adequate amount of distilled water. The mixed ingredients were continuous mixed until agar melts and distilled water were completely dissolved with mixture. Then, the complete diet mixture was spread into an aluminium foil tray. The mixture is formed in rectangular shape before cut into cubic shaped. Cubes were placed in the oven for 12 hours at 40 to 50 °C. Next, dry MBD were ground using mortar and pestle before feed to the fish.

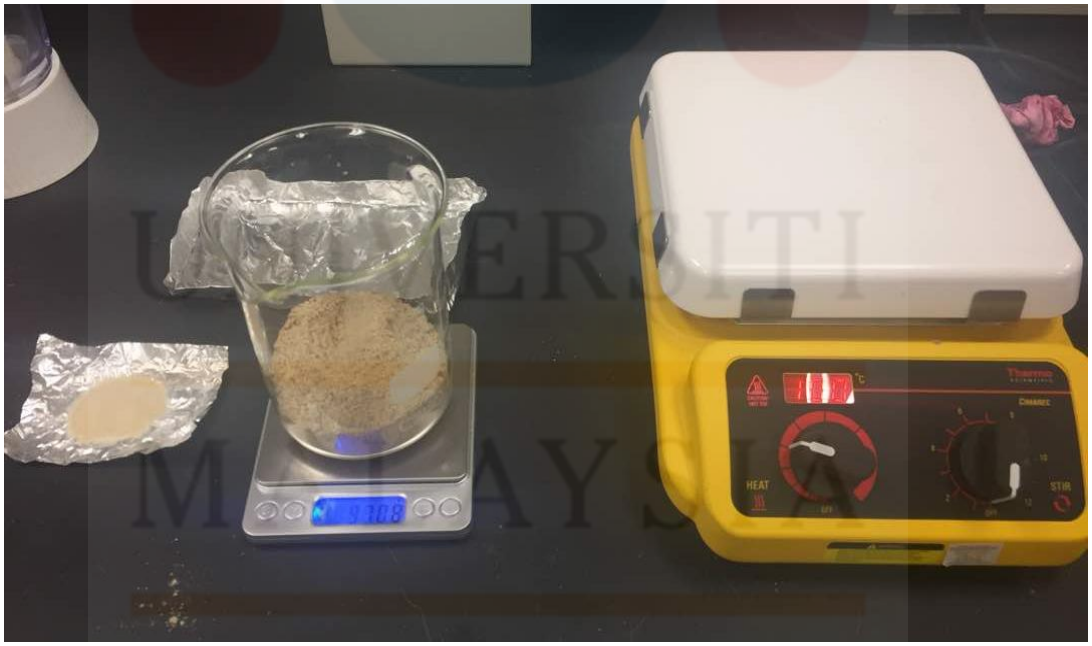

Figure 3.2: AMBD diet preparation

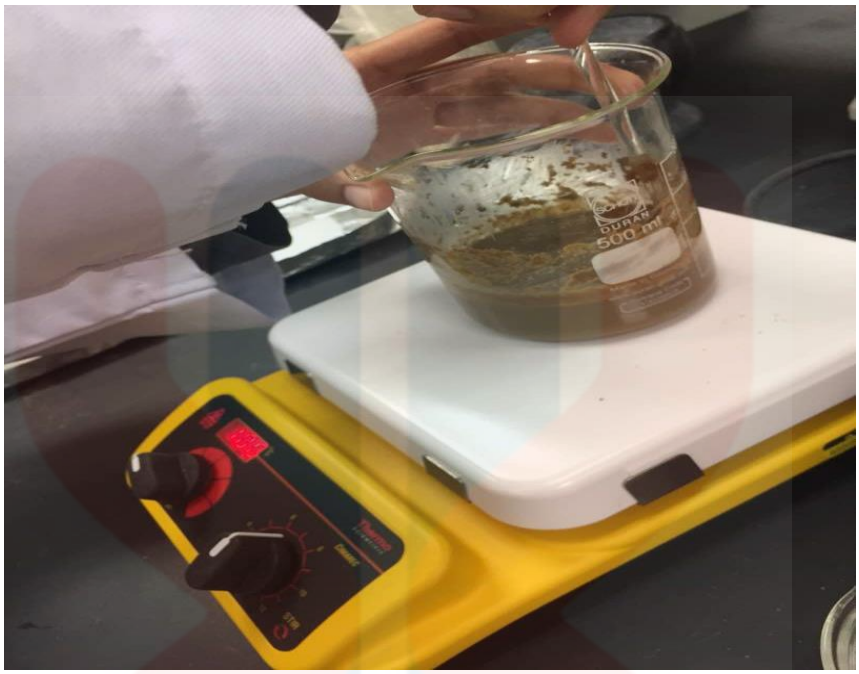

Figure 3.3: AMBD preparation process

Figure 3.4: The AMBD for oven-dried.

3.5 Fish culture

Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from local farmer in Gual Ipoh, Kelantan. *Oreochromis niloticus* fingerlings with weight 3.0±1.0 g were acclimatized in the cement tank for seven days while being fed with commercial diet (Star feed from CP Sdn. Bhd). After one week of fish acclimation, 30 experimental tilapia fingerlings of similar sizes with initial weight 4.0±1.0 g were randomly distributed into nine aquariums filled with 32 litres of dechlorinated tap water and aeration. Each aquarium tank was aerated with two air stones. Each of the experimental diets was assigned to each aquarium tank at three replicates.

Culture water in the tanks changed once per two day. Fish in each treatment were fed at rate of 5 % of their total body weight with experimental diets three times a day. Feed was provided three times a day at 8:00 am, 12.00 pm and 5.00 pm (Ogunji & Wirth, 2000;El-Sayed & Kawanna, 2004). The amounts of feed given were recorded. Five fish were picked randomly from each tank and weighed for every seven days and then, released back to the experimental aquarium tank. The average body weight of fish in each aquarium tank was recorded. Mortality was monitored daily and recorded. Amount of daily diet was adjusted accordingly. Feeding trial was lasted for seven weeks.

3.6 Growth parameters

Growth performances were evaluated by calculating weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate using the following formulae (Ganzon-naret, 2014):

a) Weight gain (g) = Final weight (g) – Initial weight (g)

b) $$\text{Specific Growth Rate (SGR)} = \frac{\ln \text{final weight (g)} - \ln \text{Initial Weight (g)}}{\text{Time (days)}} \times 100$$

c) $$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed Intake (g)}}{\text{Weight Gain (g)}}$$

c) $$\text{Survival rate (\%)} = \frac{\text{Number of fish survived}}{\text{Number of fish stocked}} \times 100$$

3.7 Water quality parameters

Freshwater conditions were monitored daily at 9 am using YSI Pro Plus Water Quality Meter. Culture water were monitored for optimum condition with 27±1 °C, pH 7 to 8 and dissolved oxygen (DO) of 7.1 to 8.1 mg L^{-1} (El-Sherif & El-Feky, 2009a; El-Sherif & El-Feky, 2009b). Optimum water quality parameter according El-Sherif and El-Feky, 2009a and El-Sherif and El-Feky, 2009b was presented in Table 3.3.

Table 3.3: Water quality parameter

Water quality parameter	Standard
Temperature (°C)	27 ± 1
pH	7 to 8
Dissolved oxygen (DO) (mg L^{-1})	7.1 to 8.1

3.8 Statistical analysis

All the data were subjected to One-way Analysis of variance (ANOVA) followed by Duncan Multiple Range Test with significance ($p<0.05$) using SPSS Software Programs for Windows, Version 22.0.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Proximate Analysis

Mung bean seeds in de-hulling process were undergoes both soaking and removal of seed coats while in boiling process, mung bean seeds were undergoes soaking, removal of seed coats and boiled in water. The nutrition value of raw and boiled mung bean seeds meal used in experimental diet was presented in Table 4.1.

Table 4.1: Proximate analysis of raw and boiled mung bean seeds meals (% dry weight) used in experiment

Components	Crude protein (%)	Crude fat (%)	Crude fibre (%)
Raw mung bean meal (RwMB)	28.20	0.72	8.07
Boiled mung bean meal (BoMB)	26.14	1.30	5.87

Based on Table 4.1, crude protein of raw mung bean seeds meal in this study is 28.20 % while crude protein of mung bean seeds meal that undergoes both soaking and de-hulling process from previous study by Mubarak (2005) is 27.60 %. Crude protein of mung bean seeds meal that undergoes boiling process is 26.14 % while previous study by Mubarak (2005), amount of crude protein is 26.80 %. There were slightly different amount of crude protein might be due to different sources of mung bean seeds and techniques.

According to previous study by Mubarak (2005), mung bean seeds meal that undergoes both soaking and de-hulling process had higher protein content at 27.60 % compared to mung bean seeds meal that only undergoes boiling process had protein content at 26.80 %. There were significantly differences ($p<0.05$) in protein content of mung bean seeds that undergoes de-hulling and boiling process might be due to diffusion of some proteins into water during processing.

According to Table 4.1, both of experimental diets were experienced soaking process that helps in facilitate de-hulling process in order to remove seed coat of mung bean seeds. This was supported by Shi et al. (2004) which stated, soaking technique helps in softening the texture and reduce toxic content of edible beans. Hence, increase soaking duration would cause greater reduction of saponin.

According to Mubarak (2005), percentage reduction of phytic acid in soaking and boiling processes were 26.70 and 25.86 mg/gm sample respectively. This was supported with Duhan et al. (1989) who noted that, phytic acid contents in chickpea (*Cicer arietinum*) and black gram (*Vigna mungo*) were reduced when soaking for 12

hours as anti-nutrients were leached out into soaking water. Greater loss of phytic acid in legumes happened when distilled water was used as soaking medium and supported by Vijayakumari et al. (1996).

Percentage reduction trypsin inhibitors and tannins of mung bean seeds experienced boiling process were 100 and 45.50 % respectively (Mubarak, 2015). This was full agreement with Saikia et al. (1999) and Elsheikh et al. (2000) stated that, cooking treatments were effective in deactivate tanning, phytic acid and trypsin inhibitors in faba bean seeds thus, improve protein digestibility and quality. According to Martín-Cabrejas et al. (2009), cooking after soaking in water improved protein digestibility from 7 to 12 %. Longer cooking duration would cause great decrease level of oligosaccharides in *Mucuna monosperma* compared to soaking technique (Vijayakumari et al., 1996).

Protein digestibility of uncooked legumes was range from 33.8 to 37.6 % while protein digestibility of cooked legumes undergoes ordinary boiling technique was improved by 86.0 to 93.3 %. Improvement of protein digestibility happened when increased accessibility of protein to enzymatic attack (Zia-ur-Rehman & Shah,2005).

4.2 Environmental Conditions

Water quality parameter throughout experimental period was presented in Table 4.2.

Table 4.2: Water quality parameter through experimental period

Water quality parameter	Experimental data
Temperature (°C)	26 – 28.2
pH	6.89 – 7.80
Dissolved oxygen (mg L^{-1})	5.45 – 6.86

Based on Table 4.2, water condition throughout experimental period was suitable for rearing Nile tilapia fingerlings. The experiment water temperature ranged from 26 to 28.2 °C. These temperature were in the preferred range of temperature recorded by El-Sherif and El-Feky (2009b) for Nile tilapia fingerlings. Other study by El-Shafai et al. (2004) stated that, temperature range of 26 to 34 °C was optimum temperature for growth of Nile tilapia.

During the experimental period, pH value was at minimum 6.89 and maximum 7.80. It was agreement with Lara-flores et al. (2003), suitable dissolved oxygen in rearing tilapia at 6.17±1.64 mg L^{-1}. This range was in the optimum values

of pH stated by El-Sherif and El-Feky (2009a) at range level 7 to 8 in order to obtain optimum growth performance and survival rate.

4.3 Growth Performance of Nile Tilapia

Initial and final weight of Nile tilapia fingerlings fed different experimental diets with and without raw and boiled mung bean seeds meal after six weeks of feeding was presented in Table 4.3.

Table 4.3: Initial, final weight and weight gain of Nile tilapia fingerlings fed different experimental diets

Treatment / Parameter	Initial weight (g)	Final weight (g)	Weight gain (g fish^{-1})
Diet 1	4.55±0.29^{a}	10.11±0.14^{a}	5.56±0.42^{a}
Diet 2	4.48±0.15^{a}	13.47±0.50^{b}	8.99±0.36^{b}
Diet 3	4.70±0.18^{a}	14.97±0.39^{c}	10.28±0.27^{c}

[1]Values are means±SE of three replicates. Values in the same column with different superscripts are significantly different ($p<0.05$).
[2]Diet 1: control without raw and boiled mung bean seeds meal; Diet 2: raw mung bean seeds meal; Diet 3: boiled mung bean seeds meal.

Initial body weights of fish recorded at the beginning of experiment were homogeneous. Mean individual final weight of Nile tilapia fingerlings reared for six weeks in Diet 1, Diet 2 and Diet 3 were 10.11 g, 13.47 g and 14.97 g respectively. Fingerlings in Diet 3 showed the highest final body weight (14.97 g) followed by Diet 2 (13.47 g). There were significant differences ($p<0.05$) in mean individual final weight of fish in different experimental diets.

From these result, it could be decided that mean individual final weight of Nile tilapia fingerlings was related with different experimental diets. This was in agreement with findings of Ganzon-naret (2014), reported that seabass, *Lates calcarifer* fingerlings fed on diets containing boiled mung bean seeds meal had best final weight at 8.33 g compared to control and raw mung bean seeds diet. Balogun and Ologhobo (1989) also reported that, final weight of *Clarias gariepinus* (Burchell) fingerlings fed cooked soybean meal were higher than fingerlings fed on raw soybean meal due to absence of some anti-nutrients in the cooked meal.

According to Table 4.3, average body weight gain in Diet 1, Diet 2 and Diet 3 were 1.01, 0.25 and 0.61 g $fish^{-1}$ respectively in the first seven days and then gradually increased and reaching 5.56, 8.99 and 10.28 g $fish^{-1}$ at the end of six weeks experimental period for Diet 1, Diet 2 and Diet 3 respectively. The differences among the mean body weight gain of tilapia in Diet 1, Diet 2 and Diet 3 were significant ($p<0.05$). For the first seven days of rearing Nile tilapia fingerlings, mean body weight gain in Diet 2 and Diet 3 were increased at slow rate compared to Diet 1. This slow growth rate of fish that consumed Diet 1 and Diet 2 because of fish required adaptation towards acceptance of mung bean seeds meal in form of microbound

diet.. According to Stickney (2009) stated that, MBD is not widely used in aquaculture diets as many species unable to recognize them as feed and might ignore them.

Specific growth rate of Nile tilapia fingerlings fed different experimental diets after six weeks of feeding trial was presented in Table 4.4.

Table 4.4: Specific growth rate of Nile tilapia fingerlings fed different experimental diets

Treatment / Parameter	Specific Growth Rate (% day^{-1})
Diet 1	1.91 ± 0.19^{a}
Diet 2	2.62 ± 0.12^{b}
Diet 3	2.76 ± 0.06^{b}

[1]Values are means±SE of three replicates. Values in the same column with different superscripts are significantly different ($p<0.05$).
[2]Diet 1: control without raw and boiled mung bean seeds meal; Diet 2: raw mung bean seeds meal; Diet 3: boiled mung bean seeds meal.

Specific growth rate at the end of experimental period were 1.91 % day^{-1}, 2.62 % day^{-1} and 2.76 % day^{-1} for Diet 1, Diet 2 and Diet 3 respectively. Diet 3 had highest value of specific growth rate (SGR) followed by Diet 2 and Diet 1. High value of SGR in Diet 3 indicates that, fish were growth at fast rate compared to fish in Diet 1. There were significant differences ($p<0.05$) in the mean specific growth rate at different experimental diets, but the difference was not significant ($p>0.05$) between Diet 2 and Diet 3.

Feed conversion ratio (FCR) of Nile tilapia fingerlings fed different experimental diets after six weeks of feeding trial was presented in Table 4.5.

Table 4.5: Feed conversion ratio of Nile tilapia fingerlings fed different experimental diets

Treatment / Parameter	Feed Conversion Ratio (FCR)
Diet 1	2.54 ± 0.19^{a}
Diet 2	2.08 ± 0.74^{a}
Diet 3	2.00 ± 0.03^{b}

[1]Values are means±SE of three replicates. Values in the same column with different superscripts are significantly different ($p<0.05$).
[2]Diet 1: control without raw and boiled mung bean seeds meal; Diet 2: raw mung bean seeds meal; Diet 3: boiled mung bean seeds meal.

Based on Table 4.5, feed conversion ratio (FCR) of Nile tilapia fingerlings at the end of experimental period were 2.54, 2.08 and 2.00. In this study, FCR of Nile tilapia fingerlings was higher for Diet 1, followed by Diet 2 and the lowest for Diet 3. This means less quantity of boiled mung bean seeds meal was required for a unit weight gain of fish, whereas raw mung bean seeds meal was required in great quantity. There was no significant difference ($p>0.05$) of feed conversion ratio between Diet 1 and Diet 2, even though significant differences ($p<0.05$) occurred between Diet 1, Diet 2 and Diet 3. These result supported by Inayat and Salim (2005), feed conversion ratio increased when decreased in dietary protein.

According to Goda et al. (2007), poor FCR was recorded in *Oreochromis niloticus* and *Sarotherodon galilaeus* fed with full-fat soybean (FFSB) at 2.0 and 2.1 respectively. Sea bass that fed with boiled mung bean seeds meal had the lowest value of FCR at 2.06 compared to diet contained raw mung bean seeds meal at 2.44 (Ganzon-naret, 2014). Good feed conversion ratio for most aquatic organism at ranged 1.50 to 2.00 (Stickney, 2009).

Feed conversion ratio values in all experimental diets were more than 2.00 might be due to improper feed and nutrient supply towards fish. In this study, Nile tilapia fingerlings were fed with microbound diets that coating the feedstuffs. Improper nutrient supply was due to loss of nutrients available in feeds when it was immersed in culture water. This was agreement with Genodepa et al. (2007) stated that, agar, alginate and carrageenan-bound MBD had highest rate of leaching in three different immersion duration compared to zein-bound MBD. Use of binder such as agar in this feeding trial were effect on nutrient release in water, acceptance by fish and digestion as different aquatic species had different digestion of production (Luzardo-Alvarez et al., 2010).

Nutrients available in feed were insufficiently assimilate by fish as previous study by Partridge and Southgate (1999) stated that, agar-bound MBD had low assimilation rate at 2.11 $\mu g\ mg^{-1}\ h^{-1}$ which means absorption of nutrients by fish was poor. Type and strength of binder used in fish diets influenced digestibility and attractiveness of fish towards feed (Genodepa et al., 2007).

Survival rate of Nile tilapia fingerlings fed different experimental diets after six weeks of feeding trial was presented in Table 4.6.

Table 4.6: Survival rate of Nile tilapia fingerlings fed different experimental diets after six weeks feeding

Treatment / Parameter	Survival rate (%)
Diet 1	94.44 ± 2.94^{b}
Diet 2	88.90 ± 4.01^{ab}
Diet 3	80.00 ± 1.92^{a}

[1]Values are means±SE of three replicates. Values in the same row with different superscripts are significantly different ($p<0.05$)
[2]Diet 1: control without raw and boiled mung bean seeds meal; Diet 2: raw mung bean seeds meal; Diet 3: boiled mung bean seeds meal

Based on Table 4.6, survival rate of Nile tilapia fingerlings during experimental period ranged at 80 to 94 % in all experimental diets. Mortality happened due to cannibalism. There were significantly differences ($p<0.05$) in survival rate among all experimental diets. Cannibalism happened due to age, size, stocking density, stress and improper feed supply (Abdel-Tawwab, El-Marakby, & Ahmad, 2006).

At early life stages of fish, they commonly show aggressive interactions and cannibalism (Gizaw, 2017). Cannibalistic rate increased as size differences increased where large fish highly cannibalistic compared with small fish (Abdel-Tawwab et al.,

2006). According to Bolivar et al. (2008), large fingerlings able to withstand varies environment conditions compared with small fingerlings that more vulnerable and death. High stocking density in tank caused competition for food and stress thus resulted in low survival rate (Yi, Lin & Diana, 1996; Gizaw, 2017).

Stress happened due to fluctuation of dissolved oxygen (DO) and water temperature during cold weather as fish were reared in open condition. Previous study by Charo-Karisa (2006) said, low temperature caused low survival of small fingerlings as their immune system is weak and more susceptible towards cold stress than large fingerlings. When water temperature increase or decrease beyond temperature 25 to 30 °C, fish in all age groups would eat less or stop from taking feed (Srijaya, Pradeep, Mithun, Shaharom & Chatterji, 2011).

In this study, agar was used as binder. Improper feed supply happened when agar had high leaching rate when immersed in water, thus cause loss of some water-soluble nutrients such as vitamin B and C that commonly found in legumes, cereal grains and fresh organ meats (Partridge & Southgate, 1999).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Combination of more than two methods in deactivate anti-nutrients were required as use of one method only had slightly decreased of some anti-nutritional factors. In this study, boiled mung bean seeds meal were undergoes three methods which soaking in distilled water for 12 hours, de-hulling mung bean coat seed and boiling in tap water until mung bean seeds were soft had low protein content but with improvement of protein digestibility.

Different methods of processing mung bean seeds showed effect on growth performance. This can be seen through growth performance of Nile tilapia fingerlings in Diet 3 had lowest feed conversion ratio (FCR) with best weight gain (10.28 g $fish^{-1}$) compared with other experimental diets within six weeks feeding trial. Thus, H_O was rejected as growth performance of Nile tilapia fingerlings in Diet 3 had significant differences ($p<0.05$) compared with Diet 1 and Diet 2.

5.2 Recommendations

In the future, different methods can be used in drying of mung bean seeds or other legumes such as solar drying. According to Aremu and Akintola (2004), oven-drying caused losses of quality and nutrients compared to sun- and solar- drying techniques. Legumes would expose to light, oxygen and heat either direct or indirect.

Losses of nutrients such as vitamin C from legumes can be controlled by proper drying duration and drying techniques. Percentage of vitamin C discharged from legumes that undergoes sun-drying was higher compared with solar-drying. Solar-drying also ensures safety of legumes from being direct exposed to UV rays as direct exposure cause high losses in β-carotene and vitamin C. Percentage of vitamin C of *Amaranthus hybridus* in sun- and solar-drying at 71.4 and 27.9 % respectively (Kiremire, Musinguzi, Kikafunda, & Lukwago, 2010).

This method also can be practiced by farmers. It is also suggested that mung bean seeds meal can be used as partial replacement of soybean meal in practical diets in forms of pelleted feed. Pellet feeds able to minimize nutrients from leaching off from diet.

REFERENCES

Anwar, F., Latif, S., Przybylski, R., Sultana, B., & Ashraf, M. (2007). Chemical composition and antioxidant activity of seeds of different cultivars of mungbean. *Journal of Food Science*, *72(7)*, 503–510.

Abdel-Tawwab, M., Ahmad, M. H., Khattab, Y. A. E., & Shalaby, A. M. E. (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, *298*, 267–274.

Abdel-Tawwab, M., El-Marakby, H. I., & Ahmad, M. H. (2006). Cannibalism in *Oreochromis niloticus* (Linnaeus): effect of stocking density, feed quality and submerged macrophytes. *Indian Journal of Fisheries*, *53(3)*, 245–251.

Abdel-Tawwab, M., Hagras, A. E., Elbaghdady, H. A. M., & Monier, M. N. (2015). Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.): growth performance, whole-body composition, and innate immunity. *Aquaculture International*, 1261–1274.

Adebayo, O. T., Fagbenro, O. A., & Jegede, T. (2004). Evaluation of *Cassia fistula* meal as a replacement for soybean meal in practical diets of *Oreochromis niloticus* fingerlings. *Aquaculture Nutrition*, *10*, 99–104.

Alonso, R., Orúe, E., & Marzo, F. (1998). Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. *Food Chemistry*, *63(4)*, 505–512.

Araba, M., & Dale, N. M. (1990). Protein solubility as an indicator of optimum processing of soybean meal. *Poultry Science*, *69*, 76–83.

Aremu, A. K., & Akintola, A. (2014). Effects of some Drying Methods on Nutritional Characteristics of Moringa (*Moringa Oleifera*) Seeds. *International Proceedings of Chemical, Biological and Environmental Engineering, 75(12)*, 66–72.

Azaza, M. S., Wassim, K., Mensi, F., Abdelmouleh, A., Brini, B., & Kraiem, M. M. (2009). Evaluation of faba beans (*Vicia faba* L. var. *minuta*) as a replacement for soybean meal in practical diets of juvenile Nile tilapia *Oreochromis niloticus*. *Aquaculture*, *287*, 174–179.

Balogun, A. M., & Ologhobo, A. D. (1989). Growth performance and nutrient utilization of fingerling *Clarias gariepinus* (Burchell) fed raw and cooked soybean diets. *Aquaculture*, *76*, 119–126.

Bolivar, R. . B., Bolivar, H. L., Sayco, R. M. V., Jimenez, E. B. T., Argueza, R. L. B., Dadag, L. B., Taduan, A. G., & Borski, R. J. (2008). Growth Evaluation, Sex Conversion Rate and Percent Survival of Nile Tilapia (*Oreochromis Niloticus* L.) Fingerlings in Earthen Ponds. *8th International Symposium on Tilapia in Aquaculture (ISTA).* Phillipines. pp. 413.

Bora, P. (2014). Anti-Nutritional Factors in Foods and their Effects. *Journal of Academia and Industrial Research (JAIR)*, *3(6)*, 285–290.

Charo-Karisa, H. (2006). Selection for Growth of Nile Tilapia (*Oreochromis niloticus* L.) in low-input environments. PhD Thesis. Wageningen University, Netherlands.

Chau, C. F., & Cheung, P. C. K. (1997). Effect of Various Processing Methods on Antinutrients and in Vitro Digestibility of Protein and Starch of Two Chinese Indigenous. *Journal Agricultural Food Chemistry*, *45(12)*, 4773–4776.

Chen, H., Qiao, L., Wang, L., Wang, S., Blair, M. W., & Cheng, X. (2015). Assessment of genetic diversity and population structure of mung bean (*Vigna radiata*) germplasm using EST-based and genomic SSR markers. *Gene*, *566*, 1–9.

Clemente, A., Śanchez-Vioque, R., Vioque, J., Bautista, J., & Millán, F. (1998). Effect of cooking on protein quality of chickpea (*Cicer arietinum*) seeds. *Food Chemistry*, *62*(1), 1–6.

Daudpota, A. M., Abbas, G., Kalhoro, H., Shah, S. A., Ferrando, S., Gallus, L., Kalhoro, I. B., Ghaffar, A., & Hafeez-ur-rehman, M. (2016). Comparison of Growth, Feed Conversion and Body Composition of Juvenile Hybrid Red Tilapia (*Oreochromis niloticus* x *O. mossambicus*) and Nile Tilapia (*O. niloticus*) Reared in Concrete Tanks. *Pakistan Journal Zoology*, *48(3)*, 809–816.

Duhan, A., Chauhan, B. M., Punia, D., & Kapoor, A. C. (1989). Phytic Acid Content of Chickpea (*Cicer arietinum*) and Black Gram (*Vigna mungo*): Varietal Differences and Effect of Domestic Processing and Cooking Methods. *Journal Science Food Agricultural*, *49*, 449–455.

Duhan, A., Khetarpaul, N., & Bishnoi, S. (2002). Content of phytic acid and HCl-extractability of calcium, phosphorus and iron as affected by various domestic processing and cooking methods. *Food Chemistry*, *78(1)*, 9–14.

El-Sayed, A.-F. M. (2004). Protein Nutrition of Farmed Tilapia: Searching for Unconventional Sources. *New Dimensions in Farmed Tilapia: Proceedings of the Sixth International Symposium on Tilapia Aquaculture*, 364–378.

El-Sayed, A.-F. M., & Kawanna, M. (2004). Effects of photoperiod on the performance of farmed Nile tilapia *Oreochromis niloticus*: I. Growth, feed utilization efficiency and survival of fry and fingerlings. *Aquaculture*, *231*, 393–402.

El-Shafai, S. A., El-Gohary, F. A., Nasr, F. A., Van Der Steen, N. P., & Gijzen, H. J. (2004). Chronic ammonia toxicity to duckweed-fed tilapia (*Oreochromis niloticus*). *Aquaculture*, *232*, 117–127.

El-Sherif, M. S., & El-Feky, A. M. I. (2009_a). Performance of Nile Tilapia (*Oreochromis niloticus*) Fingerlings. I. Effect of pH. *International Journal of Agriculture and Biology*, *11(3*), 297–300.

El-Sherif, M. S., & El-Feky, A. M. I. (2009_b). Performance of Nile Tilapia (*Oreochromis niloticus*) Fingerlings. II. Influence of Different Water Temperatures. *International Journal of Agriculture and Biology*, *11(3)*, 301–305.

Elsheikh, E. A. E., Fadul, I. A., & El Tinay, A. H. (2000). Effect of cooking on anti-nutritional factors and in vitro protein digestibility (IVPD) of faba bean grown with different nutritional regimes. *Food Chemistry*, *68(2)*, 211–212.

Eusebio, P. S., & Coloso, R. M. (2000). Nutritional evaluation of various plant protein sources in diets for Asian sea bass *Lates calcarifer. Journal of Applied Ichthyology*, *16*, 56–60.

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

Gallardo, P. P., Pedroza-Islas, R., García-Galano, T., Pascual, C., Rosal, C., Sánchez, A., & Gaxiola, G. (2002). Replacement of live food with a microbound diet in feeding *Litopenaeus setiferus* (Burkenroad) larvae. *Aquaculture Research*, *33*, 681–691.

Ganzon-naret, E. S. (2014). Use of raw and heat-treated mung bean seeds (*Phaseolus aureus*) as replacement for soybean meal protein in the diets for sea bass, *Lates calcarifer* fingerlings in tanks : effects on growth performance , nutrient utilization and survival rate. *Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society*, *7(6)*, 458–468.

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.

Gizaw, Y. D. (2017). Survival rate, feed utilization and growth performance of fingerlings of African catfish, *Clarias gariepinus* (Burchell, 1822), at different stocking densities under dark condition. M. Sc. Thesis. Addis Ababa University, Ethiopia.

Goda, A. M. A.-S., Wafa, M. E., El-Haroun, E. R., & Chowdhury, M. A. K. (2007). Growth performance and feed utilization of Nile tilapia Oreochromis niloticus (Linnaeus , 1758) and tilapia galilae Sarotherodon galilaeus (Linnaeus , 1758) fingerlings fed plant protein-based diets. *Aquaculture Research*, *38*, 827–837.

Gupta, A. & Sahu, R. K. (2017). *Agri-Fundamentals*. Retrieved November 1, 2017, from the Agri-Commodities Daily Report: http://app.investmentguruindia.com /mobile/researcharticles/2017/August/Daily_Agri_Report_Aug_23_2017.pdf

Hajos, G. & Osagie, A. (2004). Technical and biotechnological modifications of antinutritional factors in legume and oilseeds. In Muzquiz, M., Hill, G. D., Cuadrado, C., Pedrosa, M. M., & Burbano, C. *Recent Advances of Research in Antinutritional Factors in Legume Seeds and Oilseeds* (pp. 293-305). Spain: Wageningen Academic Publishers.

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.

Inayat, L., & Salim, M. (2005). Feed conversion ratio of major carp, *Cirrhinus mrigala* fingerlings fed on soybean meal, maize and maize gluten. *Pakistan Vet. Journal*, *25(1)*, 13–16.

Iqbal, K. J., Qureshi, N. A., Ashraf, M., Rehman, M. H. U., Khan, N., Javid, A., Abbas, F., Mushtaq, M. M. H., Rasool, F., & Majeed, H. (2012). Effect of different salinity levels on growth and survival of Nile tilapia (*Oreochromis niloticus*). *The Journal of Animal & Plant Science*, *22(4)*, 919–922.

Jahan, D. A., Hussain, L., & Islam, M. A. (2007). Partial replacement of fishmeal protein by soybean meal protein in the diet of mrigal, *Cirrhinus cirrhosus* (Ham.) fry. *Bangladesh J. Fish. Res.*, *11(2)*, 181–188.

Kataria, A., Chauhan, B. M., & Punia, D. (1989). Antinutrients and Protein Digestibility (in vitro) of mungbean as affected by domestic processing and cooking. *Food Chemistry*, *32*, 9–17.

Khattab, R. Y., & Arntfield, S. D. (2009). Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. *LWT - Food Science and Technology*, *42(6)*, 1113–1118.

Kiremire, B., Musinguzi, E., Kikafunda, J., & Lukwago, F. (2010). Effects of vegetable drying techniques on nutrient content: A Case Study of South-Western Uganda. *African Journal of Food, Agriculture, Nutrition and Development*, *10(5)*, 2587–2600.

Kolkovski, S., Curnow, J. & King, J. (2010). Development towards commercialization of marine fish larvae feeds – Microdiets. *Project No. 2004/258. Fisheries Research Report No. 198*. Department of Fisheries, Western Australia. pp. 180.

Kratzer, F. H., Bersch, S., Vohra, P., & Ernst, R. A. (1990). Chemical and biological evaluation of soya-bean flakes autoclaved for different durations. *Animal Feed Science and Technology*, *31*, 247–259.

Kruawan, K., Tongyonk, L., & Kangsadalampai, K. (2012). Antimutagenic and co-mutagenic activities of some legume seeds and their seed coats. *Journal of Medicinal Plants Research*, *6(22)*, 3845–3851.

Kumar, R. (1991). Anti-nutritional factors, the potential risks of toxicity and methods to alleviate them. *Proceedings of the FAO Expert Consultation held at the Malaysian Agricultural Research and Development Institute (MARDI).* Kuala Lumpur, Malaysia. pp. 160.

Kumar, V., Sinha, A. K., Makkar, H. P. S., & Becker, K. (2010). Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, *120*, 945–959.

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.

Langdon, C. (2003). Microparticle types for delivering nutrients to marine fish larvae. *Aquaculture*, *227*, 259–275.

Lara-flores, M., Olvera-Novoa, M. A., Guzmán-Méndez, B. E., & López-Madrid, W. (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast Saccharomyces cerevisiae as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *216*, 193–201.

Lee, S.-J., Lee, J. H., Lee, H.-H., Lee, S., Kim, S. H., Chun, T., & Imm, J. Y. (2011). Effect of mung bean ethanol extract on pro-inflammtory cytokines in LPS stimulated macrophages. *Food Science and Biotechnology*, *20(2*), 519–524.

Likongwe, J. S. (2002). Studies on Potential Use of Salinity To Increase Growth of Tilapia in Aquaculture in Malawi. *Nineteeth Annual Technical Report. Pond Dynamics/Aquaculture CRSP*, Corvallis, Oregon. pp. 167-174

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.

Luzardo-Alvarez, A., Otero-Espinar, F. J., & Blanco-Méndez, J. (2010). Microencapsulation of diets and vaccines for cultured fishes, crustaceans and bivalve mollusks. *Journal of Drug Delivery Science and Technology*, *20(4)*, 277–288.

Maga, J. A. (1982). Phytate: Its chemistry, occurence, food interactions, nutritional significance and method of analysis. *Journal of Agricultural and Food Chemistry*, *30(1)*, 1–9.

Martín-Cabrejas, M. A., Aguilera, Y., Pedrosa, M. M., Cuadrado, C., Hernández, T., Díaz, S., & Esteban, R. M. (2009). The impact of dehydration process on antinutrients and protein digestibility of some legume flours. *Food Chemistry*, *114(3)*, 1063–1068.

Mubarak, A. E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chemistry*, *89(4)*, 489–495.

Nair, R. M., Yang, R.-Y., Easdown, W. J., Thavarajah, D., Thavarajah, P., Hughes, J. d'A., & Keatinge, JDH (Dyno). (2013). Biofortification of mungbean (*Vigna radiata*) as a whole food to enhance human health. *Journal of the Science of Food and Agriculture*, *93*, 1805–1813.

Ng, W.-K., & Chen, M.-L. (2002). Replacement of Soybean meal with Plam Kernel Meal in Practical Diets for Hybrid Asian-African Catfish, *Clarias macrocephalus* x *C. gariepinus*. *Journal of Applied Aquaculture*, *12(4)*, 67–76.

Ogunji, J. O., & Wirth, M. (2000). Effect of dietary protein content and sources on growth, food conversion and body composition of tilapia Oreochromis niloticus fingerlings fed fish meal diet. *Journal of Aquaculture in theTropics*, *15*(4), 381–389.

Ojiako, O.A & Igwe, C.U. (2008). The Nutritive, Anti-Nutritive and Hepatotoxic Properties of *Trichosanthes anguina* (Snake Tomato) Fruits from Nigeria. *Pakistan Journal of Nutrition*, *7(1)*, 85–89.

Ologhobo, A. D. (1992). Nutritive Values of Some Tropical (West African) Legumes for Poultry. *Journal of Applied Animal Research*, *2*, 93–104.

Partridge, G. J., & Southgate, P. C. (1999). The effect of binder composition on ingestion and assimilation of microbound diets (MBD) by barramundi *Lates calcarifer* Bloch larvae. *Aquaculture Research*, *30*, 879–886.

Poel, A.F.B. Van Der, Gravendeel, S., & Boer, H. (1991). Effect of different processing methods on tannin content and in vitro protein digestibility of faba bean (*Vicia faba* L.). *Animal Feed Science and Technology*, *33*, 49–58.

Poel, A.F.B. Van Der, Mollee, P. W., Huisman, J., & Liener, I. E. (1990). Variations among species of animals in response to the feeding of heat-processed beans (*Phaseolus vulgaris* L.). 1. Bean processing and effects on growth, digestibility and organ weights in piglets. *Livestock Production Science*, *25*, 121–135.

Randhir, R., Lin, Y. T., & Shetty, K. (2004). Phenolics, their antioxidant and antimicrobial activity in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochemistry*, *39*, 637–646.

Reddy, N. R., & Sathe, S. K. (2002). Food Phytates. In *Phytase Expression In Transgenis Plants.* pp. 95.

Robinson, E. H., & Li, M. H. (1994). Use of Plant Proteins in Catfish Feeds : Replacement of Soybean Meal with Cottonseed Meal and Replacement of Fish Meal with Soybean Meal and Cottonseed Meal. *Journal of the World Aquaculture Society*, *25(2)*, 271–276.

Ruyet, J. P. Le, Alexandre, J. C., Thébaud, L., & Mugnier, C. (1993). Marine Fish Larvae Feeding: Formulated Diets or Live Prey?. *Journal of the World Aquaculture Society*, *24(2)*, 211–224.

Saikia, P., Sarkar, C. R., & Borua, I. (1999). Chemical composition, antinutritional factors and effect of cooking on nutritional quality of rice bean [*Vigna umbellata* (Thunb; Ohwi and Ohashi)]. *Food Chemistry*, *67(4)*, 347–352.

Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G., & Jiang, Y. (2004). Saponins from Edible Legumes : Chemistry, Processing, and Health Benefits. *Journal of Medicinal Food*, *7(1)*, 67–78.

Shimelis, E. A., & Rakshit, S. K. (2007). Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chemistry*, *103*, 161–172.

Stickney, R. R. (2009). *Aquaculture An Introductory Text.* USA: Cambridge University Press, Cambridge. 248-250

Soetan, K. O., & Oyewole, O. E. (2009). The need for adequate processing to reduce the anti- nutritional factors in plants used as human foods and animal feeds : A review. *African Journal of Food Science*, *3*(9), 223–232.

Soltan, M. A. (2005). Partial and Total Replacement of Soybean Meal By Raw and Heat Treated Linseed Meal in Tilapia Diets. *Egyptian J. Nutrition and Feeds*, *8(1091)*, 1091–1109.

Srijaya, T. C., Pradeep, P. J., Mithun, S., Shaharom, F., & Chatterji, A. (2011). General Biology of Tilapia. In: Shaharom, F., Pradeep, P. J., & Chatterji, A. Tilapia: Aquaculture Techniques & Potential. Selangor: Reka Cetak Sdn. Bhd.

Tacon, A. G. J., Hasan, M. R., & Metian, M. (2011). Demand and supply of feed ingredients for farmed fish and crustaceans : Trends and prospects. *FAO Fisheries and Aquaculture Technical Paper, 564*. 1-87

Talib, Z. (1993). Fish nutrition in Malaysia : status and problems. *Proceedings of the Aquaculture Workshop for SEAFDEC/AQD Training Alumni.* Phillipines. pp. 71–73.

Tang, D., Dong, Y., Ren, H., Li, L., & He, C. (2014). A review of phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts (*Vigna radiata*). *Chemistry Central Journal*, *8(4)*, 1–9.

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.

Tovar, J., Francisco, A. de, Björck, I., & Asp, N.-G. (1991). Relationship Between Microstructure and in Vitro Digestibility of Starch in Precooked Leguminous Seed Flours. *Food Structure*, *10(1)*, 19–26.

Tung, P.-H., & Shiau, S.-Y. (1991). Effects of meal frequency on growth performance of hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, fed different carbohydrate diets. *Aquaculture*, *92*, 343–350.

Udensi, E. A., Ekwu, F. C., & J, N, I. (2007). Antinutrient Factors of Vegetable Cowpea (*Sesquipedalis*) Seeds During Thermal Processing. *Pakistan Journal of Nutrition*, *6(2)*, 194–197.

Venter, C. S., & Eyssen, E. van. (2001). More legumes for better overall health. *South African Journal of Clinical Nutrition*, *14(3)*, 32–38.

Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1995). Effects of various water or hydrothermal treatments on certain antinutritional compounds in the seeds of the tribal pulse, *Dolichos lablab* var. *vulgaris* L. *Plant Foods for Human Nutrition*, *48(1)*, 17–29.

Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1996). Effect of soaking, cooking and autoclaving on phytic acid and oligosaccharide contents of the tribal pulse, *Mucuna monosperma* DC. ex. Wight. *Food Chemistry*, *55(2)*, 173–177.

Wei, L. S., & Wee, W. (2010). Ikan Tilapia Secara Umumnya. Kelantan: Universiti Malaysia Kelantan. 10-17.

Winfree, R. A., & Stickney, R. R. (2015). Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of Tilapia aurea. *Journal of Nutrition*, 1001–1012.

Wiryawan, K. G., & Dingle, J. G. (1999). Recent research on improving the quality of grain legumes for chicken growth. *Animal Feed Science and Technology*, *76*, 185–193.

Wu, W., Williams, W. P., Kunkel, M. E., Acton, J. C., Wardlaw, F. B., Huang, Y., & Grimes, L. W. (1994). Thermal Effects on in vitro Protein Quality of Red Kidney Bean (*Phaseolus vulgaris* L.). *Journal of Food Science*, *59(6)*, 1187–1191.

Yi, Y., Lin, C. K., & Diana, J. S. (1996). Influence of Nile tilapia (*Oreochromis niloticus*) stocking density in cages on their growth and yield in cages and in ponds containing the cages. *Aquaculture*, *146*, 205–215.

Yousif, O. M. (2002). The effects of stocking density,water exchange rate,feeding frequency and grading on size hierarchy development in juvenile Nile tilapia, *Oreochromis niloticus* L . *Emir. J. Agric. Sci.*, *14*, 45–53.

Yue, Y., & Zhou, Q. (2008). Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia , *Oreochromis niloticus* × *O. aureus*. *Aquaculture*, *284*, 185–189.

Zia-ur-Rehman, & Shah, W. H. (2005). Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chemistry*, *91(2)*, 327–331.

APPENDICES

A.1: One-way ANOVA

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
InitialWeight	Between Groups	.071	2	.036	.261	.779
	Within Groups	.820	6	.137		
	Total	.892	8			
FinalWeight	Between Groups	37.219	2	18.609	44.191	.000
	Within Groups	2.527	6	.421		
	Total	39.746	8			
WeightGain	Between Groups	35.736	2	17.868	47.439	.000
	Within Groups	2.260	6	.377		
	Total	37.996	8			
MeanWeightGain	Between Groups	9926.065	2	4963.032	47.428	.000
	Within Groups	627.867	6	104.644		
	Total	10553.931	8			
SpecificGrowthRate	Between Groups	1.253	2	.626	16.408	.004
	Within Groups	.229	6	.038		
	Total	1.482	8			
FeedConversionRatio	Between Groups	.517	2	.259	5.963	.037
	Within Groups	.260	6	.043		
	Total	.778	8			
SurvivalRate	Between Groups	318.481	2	159.241	5.607	.042
	Within Groups	170.407	6	28.401		
	Total	488.889	8			

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

Treatment	N	Subset for alpha = 0.05
		1
Diet 2	3	4.48133
Diet 1	3	4.55233
Diet 3	3	4.69533
Sig.		.518

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 Final Weight

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Diet 1	3	10.10867		
Diet 2	3		13.47333	
Diet 3	3			14.97200
Sig.		1.000	1.000	1.000

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 Weight Gain

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Diet 1	3	5.55633		
Diet 2	3		8.99200	
Diet 3	3			10.27667
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.5: Post Hoc Analysis of Duncan Multiple Test for Specific Growth Rate

Treatment	N	Subset for alpha = 0.05	
		1	2
Diet 1	3	1.9100	
Diet 2	3		2.6200
Diet 3	3		2.7633
Sig.		1.000	.404

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.6: Post Hoc Analysis of Duncan Multiple Test for Feed Conversion Ratio

Treatment	N	Subset for alpha = 0.05	
		1	2
Diet 3	3	2.00000	
Diet 2	3	2.07867	
Diet 1	3		2.54333
Sig.		.660	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Table A.7: Post Hoc Analysis of Duncan Multiple Test for Survival Rate

Treatment	N	Subset for alpha = 0.05	
		1	2
Diet 3	3	80.0000	
Diet 2	3	88.8900	88.8900
Diet 1	3		94.4433
Sig.		.087	.249

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Figure A.8: Nile tilapia (*Oreochromis niloticus*)

Figure A.9: Agar-agar powder as binder

Figure A.10: Analyse of moisture content

Figure A.11: Distillation process of crude protein

Figure A.12: Foss Soxtec 2055 System for crude fat analysis