The Effect of Mango Seed Extracts on the Nutritional Value of Fish Feed

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DECLARATION

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

Student

Name:

Date:

I certify that the report of this final year project entitled "The Effects of Mango Seed Extracts on the Nutritional Value of Fish Feed" by Nur Aqilah binti Mohamed Noor, matric number F15A0133 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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The Effects of Mango Seed Extracts on the Nutritional Value of Fish Feed

Mango fruit is a type of fruit that are widely being used by the people of the world not just as a food but also as an ingredient in cosmetics and medicine. However, there are several scientific researches that found the whole mango fruit are not fully utilised where the skin and seed parts of the mango which are 35 to 60 percents of the mango are being discarded as waste. The purpose of this research is to use the seed part of the mango by extracting it and making the mango seed extraction as a raw material for fish feed. Extraction of the mango seed used soxhlet extractor with water as solvent. Data of the nutrient content of the altered feed were taken from proximate analysis of the feed. Feeding trial was conducted for 6 weeks in which the weight of the fish were sampled every week. The findings of this research provide scientific information on the nutritional value of the fish feed when mango seed extraction was added. From the proximate analysis result of the altered feed, the crude protein, crude fat, crude fibre, moisture and ash content increased as the mango seed extract were added, while carbohydrate content decrease. The fish that were fed with the altered feed had gained more weight than the fish that were fed with controlled feed. This project also aim to utilise the mango parts that are usually being discarded as waste as a raw material for feed.

Keywords: mango seed, extraction, fish feed, nutritional value, proximate analysis

Kesan Ekstrak Biji Mangga Terhadap Nilai Nutrisi Makanan Ikan Berformulasi

Buah mangga adalah buah yang banyak digunakan oleh semua populasi dunia bukan sahaja sebagai makanan, tetapi juga untuk dijadikan bahan untuk kosmetik dan perubatan. Tetapi, beberapa kajian saitifik menunjukkan bahawa keseluruh buah manga tidak digunakan secara menyeluruh, dimana bahagian kulit dan biji manga iaitu yang terdiri daripada 35 hingga 60 peratus daripada buah mangga dibuang sebagai sampah. Tujuan kajian ini adalah untuk menggunakan biji mangga dengan cara mengekstraknya dan menjadikan ekstrak biji mangga sebagai bahan mentah untuk makanan ikan. Biji mangga tersebut diekstrak menggunakan pengekstrak soxhlet dan menggunakan air sebagai pelarut. Data kandungan nutrisi makanan yang diubah telah diambil dari anggaran analisa makanan ikan tersebut. Cubaan pemakanan telah dibuat selama 6 minggu, dimana berat ikan telah ditimbang setiap minggu. Hasil kajian ini adalah informasi saintifik untuk nilai nutrisi bagi makanan ikan apabila ekstrak biji manga ditambah. Hasil dari anggaran analisa makanan yang telah diubah tersebut, jumlah protein, lemak, fiber, kelembapan dan abu telah bertambah jika ekstrak biji mangga ditambah. Manakala, jumlah karbohidrat telah berkurang. Ikan-ikan yang telah diberi makanan yang diubah juga lebih berat dari makanan ikan biasa. Kajian ini bertujuan untuk menggunakan bahagian mangga yang selalunya dibuang sebagai sampah sebagai bahan mentah untuk dibuat makanan haiwan.

Kata kunci: biji mangga, ekstrak, makanan ikan, nilai nutrisi, anggaran analisa

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LIST OF ABBREVIATIONS

sp.	Species
ANOVA	Analysis of variance
SD	Standard deviation
DMRT	Duncan's Multiple Range Test

LIST OF SYMBOLS

ml	millilitre
ºC	degree Celsius
%	percentage
cm	centimetre
g	gram
m	meter
mg	milligram
µg	microgram
h	hour
min	minute

CHAPTER 1

INTRODUCTION

1.0 Focus of Study

Mango or *Mangifera indica* is a tropical fruit that is increasingly being consumed all around the world. Due to this, the mango production industry is also increasing. The country that by far is the largest in producing mango is India with 1,525,000 tons per year followed by China, Kenya, Thailand, Pakistan, and Mexico. According to statistics from FAOSTAT (2018), the production of mangoes, mangosteen and guavas are at a total of 102,046 tonnes in 2016. Although there are no specific data of mango production itself. Mango fruit contains a single seed surrounded by a fleshy mesocarp covered by a fibrous skin (Singh et al., 2013). The consumer usually only use 65 to 40 % of the fruit which is the flesh part while other parts like the seed and skin are discarded as waste and may lead to environmental issues if they are not disposed of properly.

Nutritional value is the most important keyword in this research as it defines as the content of a food or feed in term of nutrients and its' effect on the body. Mango seed kernel has a high nutritional value where they are high in protein and have other chemical components like fat, fibre, ash and tannins. 100 g of mango seed contains 32.24 g of Carbohydrate, 13 g of Total Fat, 6.36 g of Protein, 2.02 g of Total

Dietary Fibre, 0.19 mg of Vitamin B6, 1.3 mg of Vitamin E, 22.34 mg of Magnesium and 0.12 µg of Vitamin B-12 (S. Kittiphoon, 2012).

There are five major nutrients that is needed in a well formulated fish feed. The five major nutrients are Protein, Carbohydrates, Fats, Vitamins and Minerals. Most farmers use a diet that is formulated that contains an adequate amount of nutrients like 18 to 50 % of protein, 10 to 25% of fat, 15 to 20% of carbohydrate, less than 8.5% of ash, less than 1.5% of phosphorus, less than 10% of moisture and a trace amount of vitamins and minerals. However, the nutritional value will varies based on some factors like age, physical activity, size or weight, physiological processes like growth and reproduction.

By utilizing the mango seed as a raw material for making fish feed the percentage of waste that came from mango fruit will be reduced and mango seed can replace other raw materials that provide protein like soybean, barley and maize which are insufficient in Malaysia and needed to be imported from other countries. Addition of mango seed extraction into the formulated feed also altered the nutrient content like protein, carbohydrate, fibre, fat, moisture and ash of the formulated feed.

1.1 Problem statement

Mango is a tropical fruit that is considered as one of the most consumed fresh fruit in the world. Human consume the mango fruit in many ways like eating the pulp raw, process it by canning, frozen as concentrates, mashed, dehydrated, or processed as juice and jams (Torres-Leon C. et al., 2016). However, it is estimated that 35 to 60 % of the fruit is discarded as waste after processing or consuming (O'Shea et al., 2012) these discarded waste parts includes the mango seed. In spite of this, the mango seed can be utilized commercially because of their nutritional value.

Due to their nutritional value, mango seed extraction is added to formulated fish feed to increase the nutritional value of the formulated feed. By utilizing the mango seed as an additive in fish feed, the amount of mango fruit waste will decrease and at the same time improves the nutritional value of fish feed.

Protein is one of the important nutrient that is needed in making a good formulated feed in aquaculture. However, it is also one of the main reason to why the feed are expensive because the protein sources in Malaysia is limited and they needed imports from other countries.

Mango seed extraction also may be an alternative for protein source. In Malaysia, the feed usually uses protein source like soybean, wheat or other legumes. With mango seed extraction used in feed for the protein source, the cost may slightly be reduced as they replace or uses less amount of the protein source like soybean, wheat and other legumes.

1.2 Hypothesis

H_0: There is no effect on the nutritional value of formulated fish feed if mango seed extracts is added.

H_1: There are effects on the nutritional value of formulated fish feed if mango seed extracts is added.

1.3 Objectives

1) To determine the nutritional value of commercial fish feed by adding mango seed extraction of different concentration.
2) To observe and analyze the weight gain and survival rate of the fish during the feeding trial for 6 weeks.

1.4 Scope of study

The study was carried out in Universiti Malaysia Kelantan in Jeli Campus and the mango fruit were collected from the waste that were discarded by a student company that sells fruit juice. The objectives are to extract the mango seed using soxhlet extractor and adding it to the commercial fish feed to determine the alteration of the nutritional value of the feed. Next, a feed trial for 6 weeks that analyzed the weight gain and survival rate of red nile tilapia species when they were fed with the feed that were added with mango seed extraction and controlled feed.

1.5 Significance of study

The study aims to determine the effects on nutritional value of the formulated feed when mango seed extraction were added and at the same time, making the mango seed as one of the raw materials that can provide nutrients like protein, fibre, fat and carbohydrate for commercialize feed. Percentage of mango part that are discarded as waste also could be lessen when the mango seed are used as a raw material and help to give solution on the environmental issues.

CHAPTER 2

LITERATURE REVIEW

2.1 Mango

2.1.1 Taxonomy and Morphology

Mangifera indica L. or the common name mango is one of the popular, important and largely produced tropical fruits in the world. Mango is from the genus *Mangifera* that consists of 58 species in Tropical Asia and it is under the family of Anacardiaceae which is the flowering plant.

Mango fruit has many varieties in term of sizes that can weight up to 1 kilogram in some of the cultivars, shapes, colors and taste hence mango have more than 1000 cultivars all around the world. The mango fruit is usually green in colors when unripe and change into orange-reddish colors according to the cultivars. The fruit consists of endocarp the pit that surrounds the seed, an edible mesocarp or the flesh and an exocarp the leathery skin or peel. Each mango fruit also consists of a single seed that have two fleshy cotyledons that encased in a hard stony endocarp. The seeds varies in sizes and shapes. The seed constitute approximately up to 20% of the whole fruit weight depending on the cultivars.

The mango has many cultivars. In Peninsular Malaysia the most popular mango cultivars are Harumanis, Sala and Chok Anan. The most abundant cultivar in Malaysia especially in Kelantan is the Chok Anan. Chok Anan was originated from Thailand, India and Bangladesh. Due to its originality from Thailand, which is one of the closest countries to Malaysia, Malaysia had planted the Chok Anan commercially which about 9,739.7 ha. area has been planted with the Chok Anan cultivar (F. Abdullah, 2013).

2.1.2 Production and Consumption

In 2016, India is known to be the biggest producer of mango with a production that reaches over 18 million tonnes and followed by China with 4.77 million tonnes of mango and Thailand with 3.4 millions of tonnes of mango production (Pariona, 2018). In Malaysia, the mango production for the year 2016 was 102,046 tonnes but the 102,046 tonnes also includes mangosteen and guava production (FAOSTAT, 2018). Malaysia also received imported mangos from Thailand which was 2% of their production (Pariona, 2018).

Mango is consume mostly for their flesh because of the sweet taste and can be eaten fresh. Although most of mango were eaten fresh, mango can also be canned, frozen, concentrates, dehydrated or processed as juices or jams. According to O'Shea et al. (2012), the estimated fruit parts that were being discarded as a waste after processing are 35 to 60 % of the fruit. The seed particularly produced more than one million tonnes as waste annually and not utilized for any commercial purposes (Leanpolchareanchai et al., 2014).

2.1.3 Nutritional Value and Utilization of Mango Seed

Result from the proximate analysis of mango seed shows that mango seed kernel contain crude protein, fat, ash, crude fibre, and carbohydrate. Mango seed kernel also is high in potassium, magnesium, phosphorus, calcium and sodium. Potassium is important for synthesizing amino acids and proteins. Whereas, calcium and magnesium helps in photosynthesis process, carbohydrate metabolism, nucleic acids and binding of cell walls. Calcium also plays role in assisting teeth development, magnesium is an essential mineral for enzymatic activity and phosphorus is for bone growth, kidney functions and cell growth. There are also presence of antioxidant vitamins such as vitamin C, vitamin E and A. From the proximate analysis it can be shown that mango seed have many nutrition and can be utilize as food ingredient.

The article also discussed about the properties of the mango seed kernel oil. Mango seed oil have low free fatty acid which shows that the mango seed was almost free from hydrolytic rancidity brought almost by lipases. The oil has low level of peroxide value, the iodine number ranged from 39 to 53. The major saturated fatty acids in mango seed oil were stearic and palmitic acids and the main unsaturated fatty acids are oleic and linoleic acids. From the properties of mango seed oil, the article stated that mango seed kernel oil is more stable than some other vegetable oils rich in unsaturated fatty acids. The oil are suitable for blending with vegetable oils, stearin manufacturing, confectionery industry and soap industry. Cocoa butter is one of the expensive vegetable oil that is commonly used in confectionery products. It consist of palmitic acid, stearic acid and oleic acid and little amount of lauric acid and myristic acid. Because of the price, the industries tried to replace the cocoa butter in their manufacturing process. A research from Kaphueakngam et al., 2009. Shows that it is possible for mango seed kernel oil to replace the cocoa butter as an edible oil. However, the oil needed a certain amount of proportion and blend with palm oil can be an alternative to replace cocoa butter.

2.2 Formulated Fish Feed

A good formulated fish feed must consist all the nutrient that the fish requires. However, the nutrient content of the feed must be suitable to be consume by the fish according to their stages of life. Typically, a formulated fish feed should consist nutrients like protein, lipids, energy, vitamins and minerals that are required for the fish to grow, reproduce and for them to have their normal physiological functions. The amount of these nutrients differs according to the species, stages of life, sex, environment and reproductive state.

2.2.1 Nutritional Requirement of *Tilapia* sp.

In the early juvenile fish (0.02-10.0 g), they need a slightly higher amount of proteins, fats, vitamins and minerals. However less carbohydrate is needed. As for sub-adult fish (10-25 g) more energy are needed which comes from fats and carbohydrate and less proportion of protein for growth. Adult fish also need energy from fats and carbohydrates and less protein than juvenile stage.

2.2.1.1 Protein

Tilapia specifically Nile tilapia requires ten essential amino acids just like other finfishes. The protein requirement of tilapia vary from 45-50 % for first feeding larvae, 35-40 % for fry and fingerling (0.02-10 g), 30-35 % for juveniles (10.0-25.0 g) and 28-30 % for adult stage or on-growing stage of the tilapia. For brooding tilapia they require about 40-45 % of protein for their optimum reproduction, spawning efficiency, larval growth and survival.

Protein requirement in freshwater

Life stage	Weight (g)	Requirement (%)
First feeding larvae		45-50
Fry	0.02-1.0	40
Fingerlings	1.0-10.0	35-40
Juveniles	10.0-25.0	30-35
Adults	25-200	30-32
	>200	28-30
Broodstock		40-45

Table 2.2.1.1 Protein requirement for *Tilapia* sp.

Source: FAO, 2018

2.2.1.2 Lipid

Minimum requirement for lipid in tilapia diet is 5 % but to achieve a good growth and protein utilization efficiency, 10-15 % of lipids are needed. Lipid functions as transporter for fat-soluble vitamins and as energy provider to the fish as lipid are able to provide double the energy that carbohydrate provides (Craig S., 2017)

Crude lipid, essential fatty acid (EFA) and energy		
Crude lipid, % min		10 - 15
Essential fatty acids, % min		
	18:2n-6	0.5 - 1.0
	20:4n-6	1.0
	18:3n-3	
	20:5n-3	
	22:6n-3	
Carbohydrate, % max		40
Crude fibre, % max		8-10
Protein to energy ratio		110
(mg/kcal)		120

Table 2.2.1.2 Lipid requirement in *Tilapia* sp.

Source: FAO, 2018

2.2.1.3 Carbohydrate

The exact requirement for carbohydrate by tilapia species are not known. However, carbohydrates are included as one of the important nutrient in tilapia feed because they provide energy and to improve the pellet binding properties so that the pellet stays in shape even though when in water. Tilapia species can utilize 35-40 % of digestible carbohydrate. Larger hybrid of tilapia can utilize greater amount of digestible carbohydrate compare to smaller ones (FAO, 2018).

2.2.1.4 Vitamin

In semi-intensive farming of tilapia, vitamin supplement are not necessary. But in intensive farming vitamin are important for optimum growth and health of tilapia. The vitamin amount in a feed are sometimes influenced by the amount of dietary lipid level. Other than that, stability and bioavailability of the vitamin also influenced the amount of vitamins in a feed (FAO, 2018).

2.2.1.5 Mineral

Tilapia are able to absorb minerals from culture water just like any other aquatic animals. However, minerals supplement is still needed to ensure tilapia receive sufficient level of minerals to protect against mineral deficiency that may cause by reduced bioavailability (FAO, 2018).

2.3 Proximate Analysis

Proximate analysis is quantitative analysis of different macronutrients in feed. Proximate analysis can determine the carbohydrate, crude protein, crude fat, crude fibre, moisture and ash.

2.3.1 Carbohydrate

Sugars, starches and cellulose are what carbohydrate composed of, cellulose is actually classified as crude fibre which is the insoluble portion of carbohydrates. The amount of carbohydrate required in fish feed are not known, however fish specifically tilapia species can utilized as much as 35-40% digestible carbohydrate. Amount of carbohydrate in fish feed depends on the cultivated species. Carbohydrate function as a main energy source and as binder for the feed to stay in shape. Starches, pectins and hemicellulose have a pellet-binding properties which is very important function for the feed manufacturers.

To find the carbohydrate content in proximate analysis is to subtract the sum of the other component like crude protein, crude fat, crude fibre, ash and moisture in percentage from 100 percent.

2.3.2 Crude Protein

Crude protein means the amount of protein in animal feed or specific food. The crude protein depends on the nitrogen content of the feed or food. Protein is mainly required for the fish or other animals to obtain optimum growth.

Crude protein is measured by Kjeldahl method. Kjeldahl method or Kjeldahl digestion is a method that determine the nitrogen content of the feed or food quantitatively. This method is divided into three principles which are digestion, neutralization and titration. In digestion, any nitrogen in the feed or food is converted into ammonia and other organic matter to carbon dioxide and water. In neutralization, the digestion tube connects to a receiving flask by a tube where the solution in the digestion tube is then made alkaline by the addition of sodium hydroxide and converts the ammonium sulphate into ammonia gas. Ammonia gas will moves out of the digestion tube into the receiving flask where it is excess of boric acid. Low pH of the solution in receiving flask converts the ammonia gas into ammonium ion and converts boric acid into borate ion. Titration principle is where the nitrogen content is estimated. Titration of ammonium borate formed with standard sulfuric or hydrochloric acid. The nitrogen content can determine the crude protein.

2.3.3 Crude Fat

Crude fat is the amount of fat or lipid in the feed or food. Fats function as one of the energy source, supply essential fatty acids and as transporter for fat-soluble vitamins. However, excess of fats in diet of a fish may resulting a decrease in fish health, quality and shelf life of the final product (Craig S., 2017).

Fat content can be determine by extracting the samples with diethyl ether that will dissolve fat, oils, pigments and other fat-soluble substances. The ether will be evaporated from the fat solution. The residue is weighed and considered as crude fat.

2.3.4 Ash

Ash is the left-over substance after incineration of the feed. The ash contains the minerals and trace elements (Antonius, 2015). Minerals are inorganic elements that is divided into two groups which are macrominerals and microminerals (Craig, 2017). Although fish can absorb these minerals from the cultured water, supplementary minerals should be given in the feed to avoid any deficiency.

2.3.5 Crude Fibre

Fibres are the indigestible parts or compound of a plant. Fibre is mainly a carbohydrate. Crude fibre is a measurement of fibre content. It is the insoluble or indigestible residue of an acid hydrolysis followed by an alkaline one. This residue contain true cellulose and insoluble lignin. The residue is an end product or the ash of incineration of samples.

2.3.6 Moisture

Fish feed contain about 8-10% of moisture and the ingredients used usually contain 7-12% moisture. If the moisture content of feed is too high it is highly likely to be moulded and shorten the shelf life of the feed product. Moisture content is determine by drying the feed in a dryer or oven. From the drying process, dry matter is produced. Dry matter is the feed that is without the moisture.

2.4 Feeding Trial

Feeding trial are usually done to test the adaptation, growth and survival of an animal towards a newly formulated feed or a condition of the surrounding. Animals should be in their specific pens, aquariums, pond, farm or cage where the farmer or researcher can intervene or control the condition or environment of the place. Throughout the feeding trial, the animals must be well taken care of and abide the Good Animal Husbandry Practices (GAHP). There are five freedoms of animal welfare that the researcher or farmer need to pay attention to which are, the freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury or disease, freedom to express normal behaviour and freedom from fear and distress (FAO, 2018).

For aquatic animal like fish, condition of the cultured water must be same as their own habitat. Freshwater life and marine water have different environment for them to survive. Therefore, researcher must take into account of the water parameter of the aquarium, tank or pond where the feeding trial is made.

Sayed Ali et al. (2016) made a feeding trial for Nile tilapia fingerling that weighed around ± 2.00 grams. The capacity per fish tank that they used was 40 fishes per tank. The feeding trial was aimed to see the differences of weight of fish when they are given different feeding frequencies. The period for the feeding trial was 12 weeks and fish sampling or weighing were taken every week. The fish were given feed every 2 and 3 times a day according to their treatment frequencies.

In a feeding trial made by J. D. Ye, the fish tank that was used were completed with a recirculating systems connected to the circulation pump, biological and mechanical filters and supplemental aeration. Each tank were also provided with fresh water parameter as the fish species that were Nile tilapia species. The feeding trial were made for 44 days. For the sampling of the fishes, 3 random fish were selected to be weighed every week. The percent of weight gain, specific growth rate, feed efficiency, protein efficiency ratio, feeding rate, hepatosomatic index and condition factor were measured and calculated (J. D. Ye et al., 2016).

2.5 Nile Tilapia

Tilapia belong to a family of fish known as cichlids, among which most African are mouthbrooders. Nile tilapia or *Oreochromis* sp. is one of the economically important fish cultured in the South East Asia and have a high demand in market of Malaysia because of their tasty flesh and less fishy taste. Tilapia is one of the most cultured species because of their high tolerant towards extreme environment and easy adaptation. Tilapia has a rapid growth and resistance to poor quality environment, ability to grow under sub-optimal nutritional conditions and high fecundity made them an easy species to grow in aquaculture industry (Marina, 2013).

A male tilapia grow more rapidly and uniformly compared to a female tilapia. Nile tilapia and blue tilapia reach sexual maturity when they are about 5 to 6 months. The female tilapia can reproduce one every two months if they are under optimum condition by laying 300 to 500 eggs for each time.

CHAPTER 3

MATERIALS AND METHOD

3.1 Sample Preparation

The mango cultivar used in this research was Chok Anan cultivar. Chok Anan is a popular cultivar in Northern Peninsular Malaysia that are commercially planted in Malaysia other that Harumanis and Sala. Chok Anan cultivar originates from Thailand, hence it was highly imported to Kelantan because of the close proximity between Kelantan and Thailand (F. Abdullah, 2013).

The mango seeds of the mangoes were taken from a student company in University Malaysia Kelantan Jeli Campus that sells mango juice as one of their products. The mango seed were immediately collected on the day the mango flesh were used by the company to avoid any moulding. The mango seed were cleaned thoroughly so that no flesh were stuck to the seed. The seeds were then dried for 72 h in room temperature. After that, the seeds were grinded and packed in thimbles to be extracted.

3.2 Equipment and Chemical List

3.2.1 Equipment

Grinder, tray, Soxhlet extractor, flask, thimbles, precision balance, oven, desiccator, crucibles, grinder, furnace, digestion tube, 250 ml conical flask, retort stand, Soxhlet extractor, fish tanks, electronic balance, aerator.

3.2.2 Chemical list

Mango seed, water, tablet kjeldahl catalyst, concentrated Sulfuric acid, 4% Boric acid, Green Bromocresol, 40% Sodium Hydroxide, 0.1N Hydrochloric acid, Petroleum ether, Potassium hydroxide,

3.3 Soxhlet Extraction

Soxhlet extractor were used to extract the mango seed. Water acted as solvent and were boiled in a flask. The evaporated water would pass through the condenser and condensed at the top of the extractor. The condensed water would then runs into the thimble thus the mango seed in the thimble soaked and the constituents were extracted. After the chamber that held the thimble were full, the water will flow down to the flask. The process were repeated until the extraction were completed (Redfern et. al., 2014).

3.4 Feed Preparation

Formulated feed for juvenile fish were bought from a feed store in Bukit Bunga, Kelantan. Feed for juvenile fish were bought as the fish that were being used for feeding trial was red nile tilapia species that have the weight that ranged from 11 to 12 g which is classified as juvenile stage or sub-adult stage of a fish (FAO, 2018).

Three groups of feed were used in this research which are the control treatment which had no alteration of ingredients and nutritional values, Treatment 1 group that use a 1:20 ratio where 1 portion is the amount of mango seed extraction and 20 is the amount of feed used and the last group was Treatment 2 that used 1:10 ratio.

The mango seed extraction was sprayed on the formulated feed according to the ratio of the group.

3.5 Proximate Analysis of Fish Feed.

3.5.1 Moisture Content

The crucibles were dried in an oven for 30 min to remove moisture and cooled in a desiccator for 20 min to avoid any moisture absorption from the surrounding. The cooled crucibles were weighed. The samples which was the fish feed, was grinded and weighed approximately for 1.5 g and placed in each crucibles. The loaded crucibles were then dried at 105 ºC for 24 h. After 24 h, the crucibles were taken out and cooled in desiccator for 20 min. The cooled crucibles were weighed (M. A. Talpur et. al. 2011). The moisture content and dry matter were calculated by using this formula below:

Dry Matter (%) = (Weight of dry matter/Weight of sample) × 100% --- Equation 3.5.1.1

Moisture Content (%) = 100 – % of dry matter --- Equation 3.5.1.2

3.5.2 Ash Content

Crucibles were dried on the oven for 1 hour at 105 ºC and cooled in desiccator. Grinded samples were weighed approximately 1 g and loaded in each of the cooled crucibles. The crucibles were placed in a furnace at 660 ºC for at least 4 h and cooled in desiccator for 20 min. The final weight of the crucibles were taken (Thiex, 2012). The ash content were calculated using the formula below:

Ash Content (%) = [(A/B) × 100%] --- Equation 3.5.2.1

Where,

A= Weight of ash (g)

B= Initial weight of sample (g)

3.5.3 Crude Fat

The Soxtec Method was used to get the crude fat content. Firstly, the aluminium cups were heated in oven at 105 ºC for 30 min and cooled in desiccator for 20 min. The cooled aluminium cups were weighed. ±1.5 g of grinded samples were weighed and placed in each of the extraction thimbles. A layer of cotton were inserted on the surface of the thimbles to avoid the samples from getting out when they were soaked. The thimbles were then fixed into the extraction unit. Next, 80 ml of petroleum ether were filled in the aluminium cups and the aluminium cups were fixed into the extraction unit. When the Soxtec machine started to operate, the thimbles were submerged and boiled for 10 min. After that, the thimbles were in rinsing position for 20 min and followed by solvent evaporation for 5 min. As evaporation process ended, the aluminium cups were removed and heated in the oven for 30 min and then the aluminium cups were weighed (Luthria, D. L.,2004). Crude fat content were calculated using the formula:

$$\text{Crude Fat (\%)} = [(A - B) / C] \times 100\% \qquad \text{--- Equation 3.5.3}$$

Where,

A= Final weight of aluminium cup (g)

B= Initial weight of aluminium cup (g)

C= Sample weight (g)

3.5.4 Crude Protein

3.5.4.1 Heating up

The control unit were turned on and let it reached 400 ºC. ± 1 g of grinded samples, 2 pieces of Kjeldahl catalyst and 12 ml of concentrated sulphuric acid solution were inserted into each of the digestion tubes.

3.5.4.2 Digestion

The digestion tubes were put in a rack and was put in the hot digestion block. The insert rack were covered by placing the exhaust system on top of rack. The scrubber unit were turned on at the right control unit. Then, leave it for 1 h 30 min.

3.5.4.3 Cooling Down

After that, the control unit and the scrubber unit were turned off. The exhaust system were lifted up and the rack was moved into the fume hood and leave for 1 h.

3.5.4.4 Distillation

To prepare 30ml of receiving solution, 4 g Boric acid and 1ml of Green Bromocresol indicator and 0.7 ml of methyl were filled into 250 ml conical flask. Then place the receiving solution in distillation unit. 50 ml of 40 % of Sodium Hydroxide automatically flows into the tubes. Distillation process was operated for 4 min.

3.5.4.5 Titration

The product from distillation process or receiver were titrated with 0.1 N HCl until the color turns to pinkish grey. Record the titration volume (AOAC, 2000). The crude protein were calculated using these formula:

Nitrogen (%) = $[(T - B) \times N \times 14.007] / W$ --- Equation 3.5.4.5.1

Crude protein (%) = Nitrogen (%) x 6.25 --- Equation 3.5.4.5.2

Where,

T= Sample titration (ml)

B= Blank titration

N= Normality of HCl

W= Weight of sample (mg)

3.5.5 Crude Fibre

The fibre bags were dried for 1 h at 105 ºC and cooled in dessicator for 30 min.To get M1, the fibre bags were weighed. Approximately 1 g of sample were prepared for M2. After that, put the samples in the fibre bags. Fibre bags with glass spacers were inserted into the carousel. The fibre bags were washed or submerged 3 times in petroleum ether for defattening. The fibre bags were dried carefully. For washing phase 1, the fibre bags were boiled in 300 ml of sulphuric acid for 30 min. Then, the fibre bags were washed in hot water for 3 times to remove the acid. For washing phase 2, the fibre bags were boiled in 300ml sodium hydroxide for 30 min. Then the alkali were removed by washing the fibre bag for 3 times. The fibre bags were removed from the carousel and dried for 4 h at 105 ºC and cooled in dessicator for 30 min. The crucibles for incineration were prepared by heating it in furnace for 30 min at 600 ºC and cooled off in drying chamber at 105 ºC for 30 min. Then they were cooled off in dessicator for 30 min. The crucibles with fibre bags were weighed to get M3. For incineration of fibre bags, the crucibles were heated for 4 h in furnace at 600 ºC, cooled off in drying chamber for 30 min at 105 ºC and cooled in dessicator for 30 min. The crucibles with ashes were weighed to get M4 (FibreBag, 2006). The percentage of crude fibre were calculated using the formula:

$$\text{Crude Fibre (\%)} = [(M3 - M1 - M4) - (B3 - B1 - B4) \times 100)] / M2 \quad \text{--- Equation 3.5.5.1}$$

Where,

M1= Weight of dried fibre bag (g)

M2= Weight of samples (g)

M3= Weight of incinerating crucible + dried fibre bag after digestion (g)

M4= Weight of incinerating crucible + ash (g)

B1= Blank value of dried fibre bag (g)

B3= Weight of incinerating crucible + dried fibre bag after digestion (g)

B4= Weight of incinerating crucible + ash blank value(g)

3.5.6 Carbohydrate Content

The carbohydrate content of the sample can be calcualted by substrating the sum of moisture content, ash content, crude fat content, crude protein content and crude fibre content from 100.

3.6 Feeding Trial

Red Nile Tilapia or *Oreochromis Niloticus* were used for the feeding trial. The fish were bought from Mr. Ibrahim in Gual Ipoh, Kelantan. The feeding trial were made in Jeli Campus, Universiti Malaysia Kelantan. The fish were divided into 3 groups and each group have 2 replications. Each aquarium were filled with 20 fish. The fish were fed two times a day every day for 6 weeks. The amount of feed that were given to the fish were constant which was about 40 g for each time. The fish were weighed 3 times randomly for each week to get the average weight of fish. The mortality of fish were also recorded.

3.7 Statistical Analysis

The result analysis of the proximate analysis of carbohydrate, crude fat, crude protein, crude fibre, ash and moisture were made using one-way ANOVA and Duncan's Multiple Range Test (DMRT). One-way ANOVA were used to determine whether there are statistically significant differences between the means of two or more independent groups. Whereas, Duncan Multiple Range Test was used to compare the sets of means. Result of DMRT is a set of subset means, where the each of the subset means have been found not to be significantly different from each other.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Proximate Analysis

The analyzed result of the proximate analysis of carbohydrate, crude fat, crude protein, crude fibre, ash and moisture were presented in percentage unit and were summarized into figures in Figure 4.1.1, 4.1.2, 4.1.3, 4.1.4, 4.1.5 and 4.1.6 respectively.

4.1.1 Crude Fat

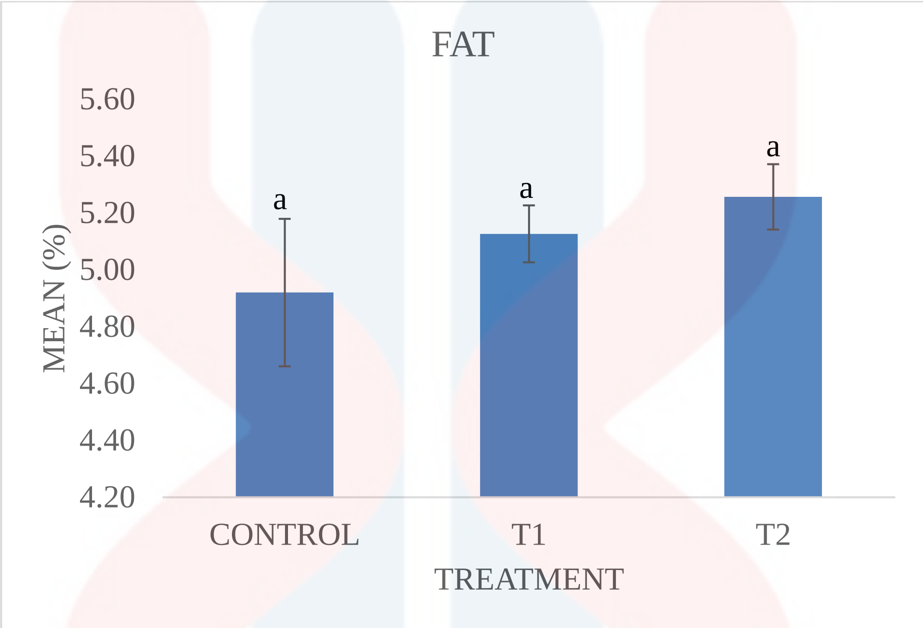

Figure 4.1.1 Fat content in different feed treatment. Vertical bars represent Standard Deviation (SD) of the mean.

Crude fat of different feed treatments were ranged from 4.92% to 5.26%. Treatment 2 has the highest crude fat which is 5.26%, followed by Treatment 1 with 5.13% and Control treatment with the lowest percentage of 4.92%. There were no significant differences (P>0.05) that were detected between Control treatment, Treatment 1 and Treatment 2.

It can be observed that the crude fat of the treatment increase as the ratio of mango seed extraction to formulated feed increase where treatment 2 used the ratio of 1:10 while Treatment 1 used ratio of 1:20. This shows that mango seed extraction can elevate the fat content of the fish feed.

A study by Torres-Leon et al. (2016), states that mango seed has a high fat content that ranged from 8.15% to 13.16%. However, in this research the fat content only increase for about 0.21% to 0.34% which differs a lot from the expected fat content of the mango seed. This may due to the concentration or ratio of the mango seed extraction to fish feed used, error in the soxhlet extraction method like unsuitable solvent to extract crude fat. Analysis by Ramluckan et al. (2014) showed that ethanol, chloroform and hexane work more efficiently in the extraction of fats.

Fat content in fish feed function to give energy to the fish when the fish consume the feed and supply the essential fatty acids and as transport for fat-soluble vitamins (However, when higher level of fat was used in a feed, there would be problem like excessive fat deposition in the liver of fish which makes the health, quality and shelf life of end product of fish decrease (Craig S., 2017)..

4.1.2 Crude Protein

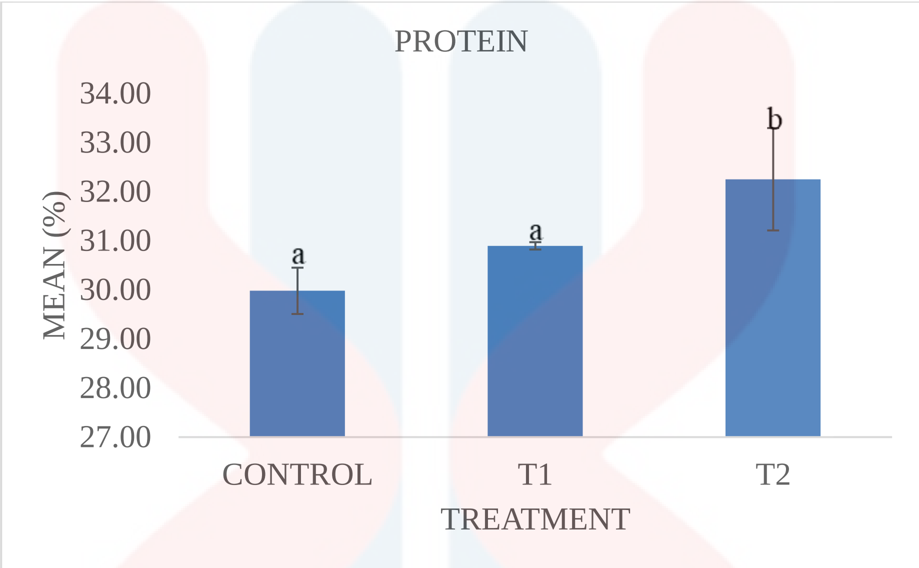

Figure 4.1.2 Crude Protein in different feed treatment. Vertical bars represent Standard Deviation (SD) of the mean.

The crude protein of the different feed treatment is as shown in figure 4.1.2 ranged from 29.98% to 32.25%. Treatment 2 has the highest crude protein with 32.25%, followed by Treatment 1 at 30.90% and control treatment at 29.98%. The P-value between control treatment with treatment 1 is P>0.05 where there was no significance difference between the two treatment group. However, the p-value between treatment 1 and control treatment from treatment 2 is P<0.05 which showed that there is significance difference between the treatments. The crude protein of the formulated fish feed increased as the concentration of mango seed extraction increased.

From the results, it can be observed that by adding mango seed extraction into the formulated fish feed, the protein content increased for about 0.92% to 2.27%. Torres-

Leon et al. (2016), claimed that mango seed contain 6% to 7.76% of protein by dry weight.

Protein function in the growth of fish if the fish receive adequate level of fats and carbohydrates. The protein level of the feed usually differs according to the stages of lifes of fish, environment and other nutrient content.

In spite of that, the crude protein content that was analysed using kjeldhal method may not be accurate as it does not give a true protein measure, since all nitrogen in food or feed was not in protein form (McClement, n.d.).

4.1.3 Crude Fibre

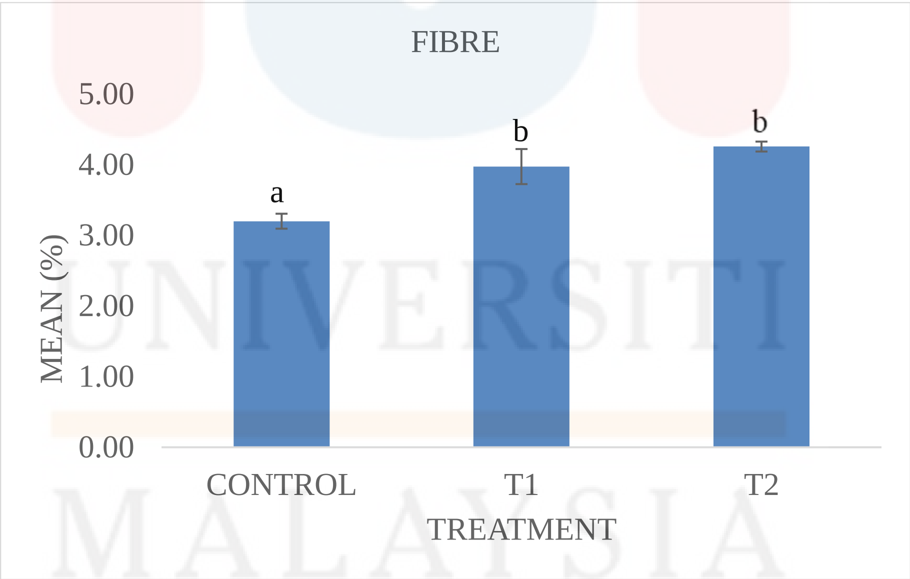

Figure 4.1.3 Crude fibre in different feed treatment. Vertical bars represent Standard Deviation (SD) of the mean.

As shown in the figure 4.1.3, the crude fibre in the control treatment, treatment 1 and treatment 2 varied from 3.20%, 3.97% to 4.26% respectively. The control treatment had the lowest crude fibre because there were no alteration of the ingredients, while treatment 1 and treatment 2 increase in ascending order because of the addition of mango seed extraction. There was a significant difference (P<0.05) between control treatment and treatment 1. Whereas there was no significant difference (P>0.05) between the crude fibre in treatment 1 and treatment 2.

The fibre content increased up to 1.06 % when mango seed extraction was added. According to Nzikou et al. (2010), the mango seed contains 2.02% crude fibre. From the amount of increase fibre content when mango seed extraction was added, it can be seen that almost 50% of the crude fibre in mango seed was extracted and been added to the formulated feed to add the nutritional value.

4.1.4 Ash Content

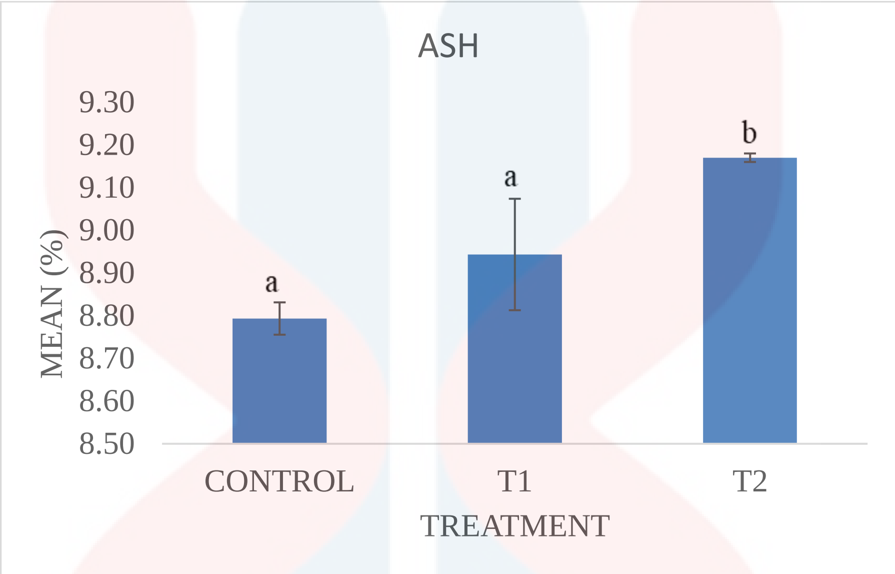

Figure 4.1.4 Ash content in different feed treatment. Vertical bars represent Standard Deviation (SD) of the mean.

The ash content of the three treatment range from 8.79% to 9.17% as shown in the figure 4.1.4 above. The highest ash content was in treatment 2 with 9.17%, followed by treatment 1 with 8.94% and 8.79% ash content for control treatment. The ash content of the formulated fish feed only increase slightly according to the concentration of mango seed extraction added. According to Nzikou (2010), the ash content of mango seed was about 3.20%.

Control treatment and treatment 1 had no significant difference between them. While there was a significant difference between treatment 1 and treatment 2.

Ash content of the fish feed usually contain the minerals and vitamins of the feed.

4.1.5 Moisture Content

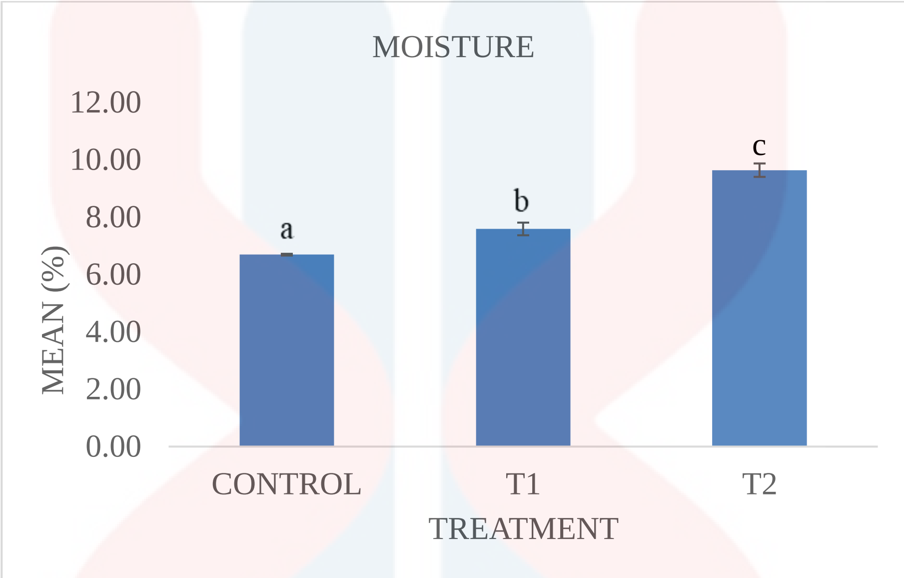

Figure 4.1.5 Moisture content in different feed treatment. Vertical bars represent Standard Deviation (SD) of the mean.

Moisture content were shown in figure 4.1.5. It can be observed that the moisture content of the treatments varies from 6.69% to 9.64%. The highest moisture content was in treatment 2 with 9.64%, while treatment 1 contained 7.59% and control treatment contain 6.69%. There was a significant difference between the control treatment, treatment 1 and treatment 2.

However, the result contradict to the research from Miles et al (2007), where it stated that the moisture content of the fish feed influence the decreasing of other nutrient as water only added weight but no nutrients or calories to the feed.

The result of increasing moisture content may due to the moisture from the environment or the moisture that came from the water solvent that were used in extracting the mango seed.

4.1.6 Carbohydrate Content

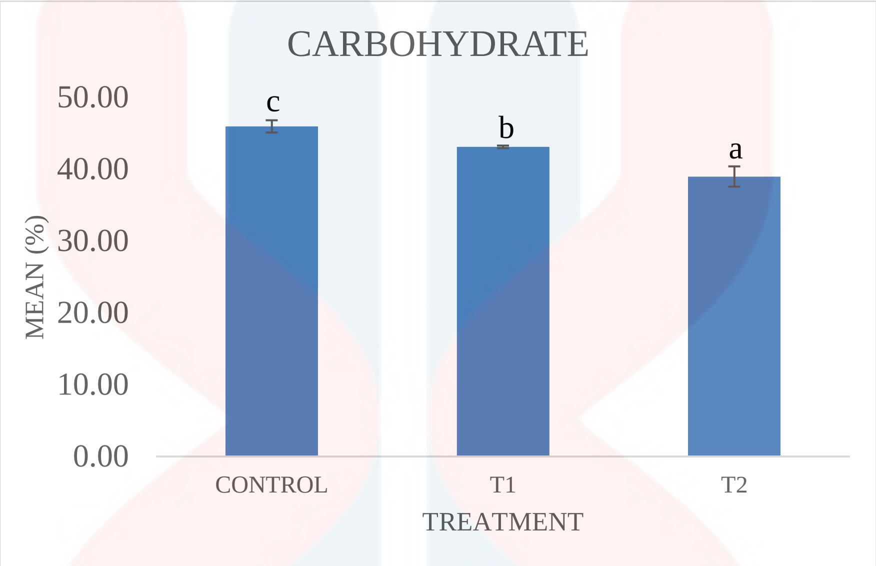

Figure 4.1.6 Carbohydrate content in different feed treatment. Vertical bars represent Standard Deviation (SD) of the mean.

Based on figure 4.1.6, the carbohydrate content of the treatments vary from 38.98% to 45.95%, where the highest amount of carbohydrate is in the control treatment with 45.95%, treatment 1 at 43.11% and lowest carbohydrate content is 38.98% in treatment 2. It was found that there were significant differences (P<0.05) between the treatment control, treatment 1 and treatment 2.

According to Miles (2007), in order to add or increase nutrient and energy density in a feed, another nutrient must be taken out to provide room for the selected nutrients. Usually, the nutrient that would decrease in amount is carbohydrate. Most species of fish get their energy from proteins and fats more efficiently than from carbohydrate.

4.2 Feeding Trial

The feeding trial of the treatments were made for six weeks and the results and data were taken and analysed by using one-way ANOVA method and Duncan's Multiple Range Test (DMRT). Data that were taken from the feeding trial were the weight gain and the survival rate of the tilapia. The analysed data of both weight gain and survival rate were summarised into figures which are Figure 4.2.1 for weight gain and Figure 4.2.2 for survival rate of tilapia. The weight gain of tilapia used the unit of gram (g) while for survival rate was presented by percent (%) unit.

4.2.1 Weight Gain

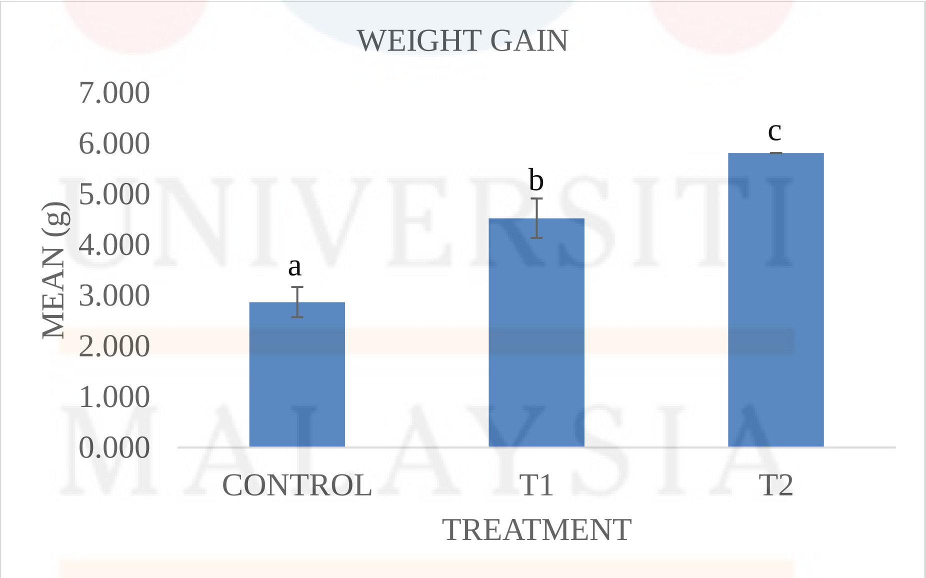

Figure 4.2.1 Weight gain of fish in different treatment. Vertical bars represent Standard Deviation (SD) of the mean.

From the figure 4.2.1, it can be shown that the weight gain varies according to their treatments. The highest mean weight gain was 5.81 g in treatment 2, followed by treatment 1 with 4.53 g and the lowest mean weight gain was in control treatment with a weight gain of 2.87 g. There was a significant difference between all three of the treatments.

The weight gain of the treatment 2 was the highest because of the higher nutritional value that the tilapia fish consumed from the altered feed. Treatment 1 also had higher weight gain than in control treatment. The weight gain in treatment 2 was more because of the higher concentration of mango seed extract used in the ingredients followed by concentration of mango seed in treatment 1 which is 10% less concentration than treatment 2.

Although theoretically, the weight gain of the treatment groups correlate with the nutritional content of the feed given, there were many factors of why the tilapia does not gain weight as much as they are expected. Some of them are the stress condition because of the weather, loss of nutrient of feed, competition between the fish and improper water parameters.

4.2.2 Survival Rate

Survival rate is the percentage of fish that survive the feeding trial that were made for 6 weeks. The survival rate of the fish was taken into account to determine whether the difference of feed nutrition between treatments affect the mortality of the fish or not.

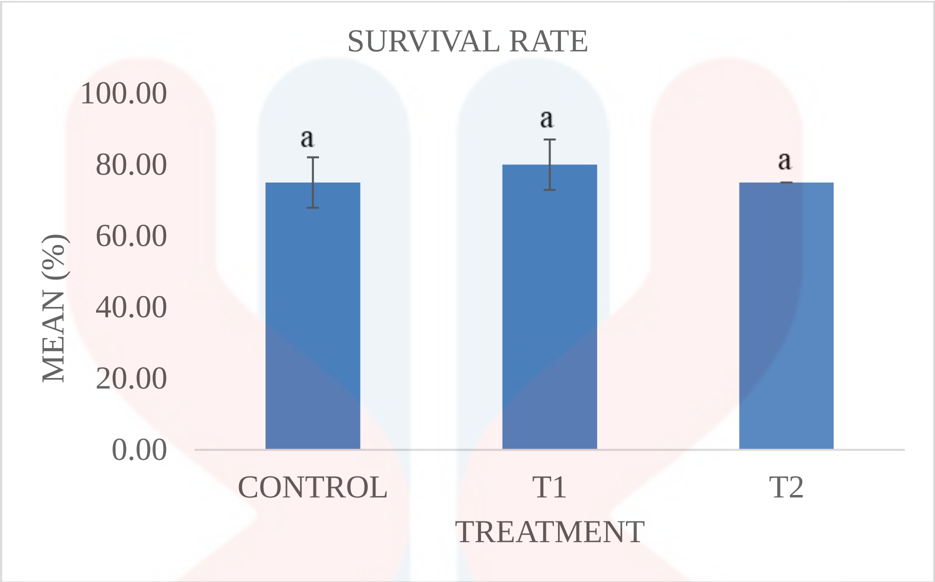

Figure 4.2.2 Survival rate of fish in different treatment. Vertical bars represent Standard Deviation (SD) of the mean.

The survival rate of tilapia fish for all three treatment are at a quite high percentage which range from 75% to 80%. The highest survival rate was in treatment 1 at 80% and both treatment 2 and control treatment have the same survival rate which was 75%. There are no significant difference between all three treatment groups.

Throughout the whole six weeks, the survival rate of all three groups are slightly higher in the fourth week, which means that many fish died in that week. This condition was mainly because of the extreme weather which is raining season and low temperature which caused the fish to be stressed (FAO, 2018).

The survival rate also could be affected by the water quality or cleanliness of the aquarium. Other than that, the fish also died of stress and not being able to adapt for the first week. These are the main factors of the death of the fish, it can be observed that the difference in feed treatment does not affect the survival rate.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In the study, the nutritional value of the formulated fish feed in Treatment 2 showed the highest overall nutritional value. Treatment 2 has the highest value in term of crude fat (5.26±0.12%), crude protein (32.25±1.04%), crude fibre (4.26±0.07%), ash content (9.17±0.01%) and moisture content (9.64±0.23%) compared to other treatments. However, the highest carbohydrate content was in Control Treatment with (45.95±0.85%) compared to other treatments. The weight gain of the red nile tilapia was also the highest in the aquariums that were fed with Treatment 2 with an increase of 5.81g in average. However, the survival rate of all three groups were slightly the same within the range of 75 to 80 % of survival rate. It can be concluded that Treatment 2 had the highest nutritional value and the mango seed extraction does affect the nutritional value of the feed by increasing the values of crude protein, crude fat, crude fibre, moisture and ash. Treatment 2 also had the highest weight gain which shows that the red nile tilapia that were fed with Treatment 2 get more nutrition than in Treatment 1 and Control Treatment. Thus, making the mango seed as a potential raw material that can be used to provide nutrition for aquatic feed making industry. The proper usage of mango seed as additives in feed making industry could gain economic benefits, promote a better heathy fish, provide extra nutrition and help to reduce environmental problem of too many food waste.

5.2 Recommendation

Future research and investigation on nutritional composition, benefits and potential usage of mango seed or any mango parts that were usually discarded as waste should be conducted to lessen the food waste and provide aquatic feed industry a raw material that could provide nutrition like protein, fat, fibre, minerals and vitamins. In addition, researches on improving the method of extraction of the mango seed to be used in fish feed and extracting only selected nutrient from the mango seed. This is because, not all nutrients in the mango seed are suitable to be used in feed making. Next, to improve the research in vitro digestibility test could also be done to test or determine the exact amount of nutrients that the fish consumed from the treatment given. In a nutshell, there are many researches and scientific information that is necessary to make the mango seed as one of the raw materials to be used in feed making industry.

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APPENDIX A

Photograph A.1: The aquariums used during feeding trial.

Photograph A.2: Extraction of mango seed using soxhlet extractor.

APPENDIX B

TREATMENT	N	Subset	
		1	2
CONTROL	3	8.7933	
T1	3	8.9433	
T2	3		9.1700
Sig.		.058	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .006.
a. Uses Harmonic Mean Sample Size = 3.000.
b. Alpha = .05.

Table B.1: Effect of different treatment towards ash content (%).

TREATMENT	N	Subset
		1
CONTROL	3	4.9200
T1	3	5.1267
T2	3	5.2567
Sig.		.062

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .030.
a. Uses Harmonic Mean Sample Size = 3.000.
b. Alpha = .05.

Table B.2: Effect of different treatment towards fat content (%).

TREATMENT	N	Subset	
		1	2
CONTROL	3	3.2000	
T1	3		3.9733
T2	3		4.2567
Sig.		1.000	.075

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .026.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Table B.3: Effect of different treatment towards crude fibre (%).

TREATMENT	N	Subset		
		1	2	3
CONTROL	3	6.6933		
T1	3		7.5900	
T2	3			9.6400
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .035.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Table B.4: Effect of different treatment towards moisture content (%).

TREATMENT	N	Subset	
		1	2
CONTROL	3	29.9800	
T1	3	30.8967	
T2	3		32.2533
Sig.		.141	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .438.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Table B.5: Effect of different treatment towards the crude protein (%)

TREATMENT	N	Subset		
		1	2	3
T2	3	38.9800		
T1	3		43.1067	
CONTROL	3			45.9533
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .918.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Table B.6: Effect of different treatment towards the carbohydrate content (%).

TREATMENT	N	Subset		
		1	2	3
CONTROL	2	2.8700		
T1	2		4.5250	
T2	2			5.8100
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square

(Error) = .080.

a. Uses Harmonic Mean Sample Size = 2.000.

b. Alpha = .05.

Table B.7: Effect of different treatment to the weight gain of *Tilapia* sp.

TREATMENT	N	Subset
		1
CONTROL	2	75.0000
T2	2	75.0000
T1	2	80.0000
Sig.		.447

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 33.333.

a. Uses Harmonic Mean Sample Size = 2.000.

b. Alpha = .05.

Table B.8: Effect of different treatment towards survival rates *Tilapia* sp.