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Mixture of *Hydrilla verticillata* (Order: Hydrocharitales) and *Blaptica dubia* (Order: Blattodea) as Fish Feed

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

Faculty of Agro Based Industry

Universiti Malaysia Kelantan

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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: Nur Hazwani Binti Abdull Hadi

Date:

I certify that the report of this final year project entitled Mixture of *Hydrilla verticillata* (Order: Hydrocharitales) and *Blaptica dubia* (Order: Blattodea) as Fish Feed by Nur Hazwani Binti Abdull Hadi, matric number F15A0149 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agrotechnology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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Mixture of *Hydrilla verticillata* (Order: Hydrocharitales) and *Blaptica dubia* (Order: Blattodea) as Fish Feed

ABSTRACT

In Malaysia, there is an issue of increasing demand and unstable production of fish meal which led to an increasing cost of aquaculture production. Despite the nutritive value of fish meal, the escalated cost of fish meal has urged the industry to seek for alternative protein source to replace the costly fish meal. In this research, different proportion of *Hydrilla verticillata* and *Blaptica dubia* were used to produce fish meal. The proportion were named as T1(60% *Hydrilla verticillata* and 40% *Blaptica dubia*), T2(70% *Hydrilla verticillata* and 30% *Blaptica dubia)*, T3(80% *Hydrilla verticillata* and 20% *Blaptica dubia), T4(90% Hydrilla verticillata* and *10% Blaptica dubia)* and *T5(100%Hydrilla verticillata*. T5 had the highest floatation compared to other diets. Thus, T5 can be indicate as pellet that has good water stability. From the result obtained, T3 is the most suitable diet to be use as commercial pellet in the future. However, others diet can be suggested to be consume according to life stages of fish and based on their species as differ species require different amount of protein content. The result showed that, these treatments can be locally developed to substitute commercial pellet in the future as both raw material contain high protein content and easily available.

Keywords: fish meal, *Hydrilla verticillata, Blaptica dubia,* floatation, crude protein

FYP FIAT

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Campuran *Hydrilla verticillata* (Order: Hydrocharitales) dan *Blaptica dubia* (Order: Blattodea) Sebagai Makanan Ikan

ABSTRAK

Di Malaysia, terdapat isu peningkatan dalam permintaan dan penghasilan makanan ikan juga tidak stabil yang telah mengakibatkan peningkatan kos dalam pengeluaran akuakultur. Selain daripada isu nutrisi makanan ikan, peningkatan kos penghasilan makanan ikan telah menyebabkan industri akuakultur memerlukan alternatif dalam pencarian sumber protein baharu untuk menggantikan pembuatan makanan ikan sedia ada. Dalam kajian ini, *Blaptica dubia* dan *Hydrilla verticillata* telah digunakan dalam campuran yang berbeza dalam menghasilkan makanan ikan. Bahagian campuran yang berbeza ini dilabelkan sebagai T1(60% *Hydrilla verticillata* and 40% *Blaptica dubia*), T2(70% *Hydrilla verticillata* and 30% *Blaptica dubia),* T3(80% *Hydrilla verticillata* and 20% *Blaptica dubia), T4(90% Hydrilla verticillata* and *10% Blaptica dubia)* dan *T5(100%Hydrilla verticillata.* T5 mempunyai daya apungan yang tertinggi berbanding dengan kesemua campuran. Oleh itu, T5 boleh dikatakan mempunyai daya apungan yang baik dan stabil. Daripada hasil kajian, T3 adalah campuran yang paling sesuai untuk dijadikan makanan ikan komersial di masa hadapan. Walau bagaimanapun, campuran yang lain boleh dijadikan makanan ikan komersial berdasarkan pembesaran ikan dan jenis spesies yang berbeza. Spesies ikan yang berbeza memerlukan kandungan protein yang berbeza. Hasil kajian menunjukkan campuran ini boleh dijadikan pengganti sumber makanan ikan sedia ada di masa hadapan kerana bahan pembuatannya mempunyai kandungan protein yang tinggi dan ianya mudah didapati

Kata kunci: makanan ikan, *Hydrilla verticillata*, *Blaptica dubia*, keupayaan terapung, kandungan protein

FYP FIAT

UNIVERSITI MALAYSIA KELANTAN

TABLE OF CONTENT

CONTENTS **PAGE**

FYP FIAT

UNIVERSITI MALAYSIA KELANTAN

CHAPTER 2 LITERATURE REVIEW

CHAPTER 3 METHODOLOGY

FYP FIAT

UNIVERSITI MALAYSIA KELANTAN

FYP FIAT

UNIVERSITI MALAYSIA KELANTAN

LIST OF TABLE

FYP FIAT

UNIVERSITI MALAYSIA KELANTAN

LIST OF FIGURES

FYP FIAT
UNIVERSITI
MALAYSIA
KELANTAN

LIST OF ABBREVIATION AND SYMBOLS

g	Gram
kg	Kilogram
DM	Dry matter
%	Percentage
SD	Standard deviation

FYP FIAT
UNIVERSITI MALAYSIA KELANTAN

FYP FIAT

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In modern times, aquaculture has sustained a global growth, continue to grow and is expected to increasingly fill the shortfall in aquatic food products resulting from static or declining capture fisheries and population increase well into the year 2025 (De Silva, 2000). Fish contributes a high proportion of animal protein in human diets in most Asian countries and most countries are trying to increase fish production through aquaculture. In Malaysia, production of aquaculture has increased drastically from 15,000 tonnes in 1990 to 90,000 in 2003. Aquaculture production in Malaysia is targeted to reach 600,000 tonnes annually by the year 2010 (Musa and Nuruddin, 2005).

Currently, production of fish in Malaysia mainly depends on trash feed, formulated feed, waste from poultry industry and other agriculture by-product. However, production of marine-cultured fish is totally relying on trash fish, as the supply is readily available.

Direct use of trash fish will exploit marine fish resources. Several ways to improve feeds for aquaculture include invest in feed research for inland/marine species and promote adoption and change over to pellet feeds. However, nutritious feed is the most expensive component in incentive aquaculture industry. Fish feed represents about 50% of the production cost in the Atlantic salmon cage-culture industry (Vielma *et al*., 2000

Fish diet production begins with ingredient selection and feed formulation (Adeparusi and Famurewa, 2011). To formulate a diet that meets a target fish requirement, information on its nutrient requirement, the nutrient composition of individual ingredients as well as their anti-nutrient compositions are required (Craig and Helfrich, 2009). Selection of ingredients can be done from a wide range of choices, therefore it is often necessary to consider the locally availability and cost of the ingredients.

In this research, the fish pellet will be produced by using insects which is Dubia roaches, (*Blaptica dubia*) and water thyme, (*Hydrilla verticillata*). Several publications report insect meal to be easily digestible (Bosch *et al*., 2014; Jabir *et al*., 2012; Marco *et.al*., 2015), having a high protein efficiency ratio (Razak *et al*., 2012) and being potentially cheaper than conventional protein sources such as fish meal. Insects are generally rich in both macro (protein and energy) and micro (vitamins and minerals) nutrients. While insect species vary in composition, they generally do not differ significantly from the widely used conventional feed stuffs (Makkar *et al*., 2014). Water thyme, *(Hydrilla verticillata)* has been used in India as fish food, storage and packing material for live crabs, and an organic amendment for crop production (Misra *et al*., 2012).

1.2 Problem Statement

Fish meal is a major component in aqua feed due to its highly digestible protein, amino acids, as well as good palatability. However, an increasing demand and unstable production of fish meal led to an increasing cost of aquaculture production (Jabir *et al*., 2012). Fish feed constitutes 80% of operating costs in aquaculture where protein is the limiting factor that influences the market price of fish feed (Shepherd and Jackson, 2013). The availability and cost of feed are of critical importance to aquaculture and livestock

FYP FIAT
UNIVERSITI
MALAYSIA
KELANTAN

development (FAO,2007). Livestock feed contributes 60-80% of the total production cost with the protein ingredient accounting for about 70% of the total feed cost (Abu et. al., 2015); Hasan, 2007); Lucas and Southgate, 2012); Swanepoel *et. al*., 2010). Livestock production currently depends on fish and soy meal as the main protein ingredients, utilizing 10% (Van Huis *et. al*., 2013) and over 85% (Riaz, 2005) of the total world's fish and soy meal, respectively. These conventional protein sources have become scarce and expensive (Davis, 2005) thus affecting fish and poultry production.

In Malaysia, over 80% of fish meal supply is imported while the rest is locally sourced. The quality of local fish meal in several aspects such as lower crude protein(CP) (50-55%), higher ash (26-28%), higher salt (>3.5%) and fats (>10%). Moreover, inconsistency in the supply of thrash fish or fishery wastes has also been a problem faced by the local suppliers in quality assurance of the fish meal.

Despite the nutritive value of fish meal, the escalated cost of fish meal has urged the industry to seek for alternative protein source to replace the costly fish meal (FAO, 2013; Barroso *et al*., 2014). The Food and Agriculture Organization of the United Nations(FAO) recommended insects as an alternative protein source in livestock feed (Van Huis *et al*., 2013). However, adapting the recommendation requires adequate scientific data on the technical and economic viability as well as the social acceptability of insect-based feeds, if their benefits are to be realised.

1.3 Objectives

1. To observe the floatation of the fish pellet that made up by the mixture of *Hydrilla verticillata* and *Blaptica dubia.*

2. To analyze protein content in the fish pellet mixture through crude protein analysis by

FYP FIAT

UNIVERSITI MALAYSIA KELANTAN

using Kjeldahl method.

1.4 Hypothesis

The fish feed that made up from the mixture of *Hydrilla verticillata* and *Blaptica dubia* might have a better floating ability and contain high protein content.

1.5 Scope of Study

The research covers the physical quality of the fish pellet made from a different proportion mixture *Hydrilla verticillata)* and *Blaptica dubia* in the terms of floating ability and edible

1.6 Significant of the Study

This study should be significant to promote the utilization of the waste by product which is *Hydrilla verticillata* to its maximum use. *Hydrilla verticillata* can be used to produce fish feed due to its nutrients. The use of this fish pellet made from the mixture of *H. verticillata* and *B. dubia* can be eco-friendly which could be better compared to the commercial pellet in the industry. Apart from that, the significant of this study also could be significant to ensure a proper waste management for the nature importance in the future. The use of these natural components to produce a better product of fish feed can be a better way to conserve the nature. Instead of that, these two components also can be used as an alternative way in order to produce fish feed with the same quality as other commercial fish feed. Apart from that, the production of aquaculture also can be improved and the cost for the fish feed production can be lowered by using these alternative ways.

1.7 Study Limitation

Hydrilla verticillata might be limited in University Malaysia Kelantan(UMK) and quite difficult to be found in other places. Without a proper management of the *H.*

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verticillata and *B. dubia,* the contamination could cause the treatments to be effected in the results of this experiment. Apart from that, the mixture also need to be suitable in its proportion and need to be handle properly to ensure any contamination in the fish pellet can be avoided. Instead of that, the proper process also will lead to the production of a better quality of the fish feed and could be more safe for the fish to consume the fish pellet.

FYP FIAT

UNIVERSITI MALAYSIA KELANTAN

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CHAPTER 2

LITERATURE REVIEW

2.1 Fish Formulation

Over the last decades, improvement in aquaculture production have been depending on the application of aquaculture feed. About 60-70% of investment in aquaculture is due to feeds and feeding management and with annual growth rate of roughly 5% and 75% of production being due to feed taking species. Apart from that, feed demand also exceeds 20 million metric tons annually. Next, average percentage of crude protein for the aquafeed are about 30%, which lead to the protein requirement could exceeds 6 million metric tons per year. At these rate, aquafeed cannot continue its dependence on fish meal and need to find new alternative sources of protein (Coloso, 2015).

Feed formulation is the process of combining different feed ingredients in proportions necessary to provide the fish with proper amounts of nutrients needed at a

particular stage of production at a reasonable cost (Coloso, 2015). As aquaculture production of tropical fish and crustacean species becomes more productive, feed formulations need to be formulated to be more cost effective and practice of environment-

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friendly (Coloso, 2015). Ahmad *et al*. (2012) reported that main aim of feed formulation is to provide fish species under culture with an acceptable diet that could fulfil its nutritional requirements at different stages of life and obtained maximum production at minimum costs. Feed formulation for sustainable aquaculture should aiming at increasing aquaculture system performance and profitability. Apart from that, fish meal also should focus on enhancing the animal disease resistance, increasing attract ability, palatability and digestibility of practical diets. It is also crucial to maintaining environmental quality through sound feeding management and good aquaculture practices (Coloso, 2015). Nowadays, few feed stuffs being used in rations for both terrestrial animals and fish (Udo and Umoren, 2011).

Fish diet production begins with ingredient selection and feed formulation (Adeparusi and Famurewa, 2011). According to Coloso (2015), in order to produce a good fish formulation, it required knowledge about nutrients and feedstuffs, nutrient requirements, palatability, acceptability, digestibility, toxicity and costs. In order to satisfy the nutrient requirements of the animal, promote optimal fish growth and boost the income of small-scale farmers and commercial producers with minimal impacts to environment thus alternative ingredients need to be included (R. M. Coloso, 2015). According to Bolorunduro (2002), animal protein was necessary compared to plant protein that can be added into fish formulation as its could supply sufficient amino acid. A complete diet should be nutritionally balanced, palatable, water stable and need to have proper size and texture (Ahmad and Ibrahim, 2016). A report by Heuze *et al.* (2015) said that effective feed formulation can be achieved by knowing its protein of the culturable fish species. A study by Ahmad and Ibrahim (2016) said that wide range of feed stuffs normally produced as by-products from animal processing industries. Selection of meal ingredients to be used to produce fish meal would play a major role in order to have

FYP FIAT
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MALAYSIA
KELANTAN

ultimate nutritional and economic success (Ovie and Eze, 2013). A study by Julius (2015) reported that selection of protein ingredient is not limited to only assessing crude protein levels of ingredients, yet also include in depth knowledge of amino acid profile and bioavailability. Most important factors need to be consider in formulating a feed for aquaculture are nutrient requirements and feeding behaviour of fish or shrimp. Adequate nutrients need to be given to fish for faster growth and survival and feeds that suited to the feeding behaviour of animals should be offered. Thus, Coloso *et al*. (2015) stated that culturist need to know what feed to be given to the fish as its nutrient requirements could be vary due to life stages. Balanced nutrition leads to good growth, low feed conversion ratios, lower production costs, higher profit and could sustained production of aquaculture (Coloso, 2015).

Based on study by Julius (2016), the nutritional requirements for protein, lipids and energy for optimum growth of specific fish species are vital for formulating a balanced diet. Several studies have been conducted on the Amino Acid Nutrition requirements, composition, supplementation and balanced ratios of protein in aqua feeds (Ketola 1982, NRC 1993, Ahmad 2008, NRC 2011, Munguti *et al.* 2012). Several studies (Hasan *et al.* 2007, Tacon *et al.* 2009, 2011) have been done to profile nutritional value of different feed ingredients from differ agro-ecological and geographical locations in the world. Fish formulations can be improved by inclusion of essential amino acids (EAAs) and EAA supplements, whenever they become limiting for fish growth. It is vital to compose and process a balanced and biologically available levels of EAAs that would fulfil target of species nutrient requirements (Nunes *et al.*, 2014). Proper formulation of fish feeds with sufficient quantity of lysine needed by fish fingerlings will cause in maximum growth (Hamid, 2016). Rawles (2013) had stated if lysine needed by fish diet

FYP FIAT

is met, the other amino acids are present in amounts that meet or exceed their requirements.

Purpose of formulating local fish meal is to ensure the fish grow fast and economically such as cheaply as possible (Ahmad and Ibrahim, 2016). Apart from that, by using local ingredients, it could have impact on the innovations and lead to grow of micro-technologies which farmers already trying different trials on farm that could increase output (Ahmad and Ibrahim, 2016). A study by Spinelli (2014) reported that some farmers used boiled chicken egg yolk to feed catfish which causing other researchers to conduct research on the same. Julius *et al*. 2016 demonstrated that plant proteins are cheap and locally available which mainly being used to produce supplement animal protein at lower costs.

Nowadays, feed formulators are adopting modern and environmentally sound formulation techniques based on nutrient value, supplementation with crystalline EAAs and on animal nutrient requirements. Commercial feed formulation is focused on producing cheaper fish meal depend on type of fish species grown while improving quality products in nutritional requirement. Based on research by Julius *et al*. (2016), commercial aquaculture production feed costs can be reduced by producing proper feed management and husbandry strategies to improve fish growth.

Based on research by Ahmad and Ibrahim (2016), they stated that there are two main types of fish feeds being produced by industry which are herbivorous fish (Tilapia) feeds that contain 30-35% crude protein and carnivorous fish (catfish) feeds which contain 45-50% crude protein. Most of the fish meal is made up from crop residues, mill by-products, food processing wastes or agro-industrial by-products (Ahmad and Ibrahim, 2016). Feeds are formulated to be dry, with a final moisture content of 6-10%, semi-moist

FYP FIAT
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with 35-40% water or wet with 50-70% water content. Most feeds produced are normally being used in intensive production system or home aquaria that commercially produced as dry feeds (Ahmad and Ibrahim, 2016). According to Lovell (2013), most farmers would use combination of both types, about 15% floating feeds and 85% sinking feed.

2.2 *Hydrilla verticillata*

2.2.1 Biology of *Hydrilla verticillata*

Hydrilla verticillata is a submerged aquatic plant in lentic freshwater ecosystems of the world, and is a highly controversial weed. *H. verticillata* is popularly known as water thyme, or the Indian star vine or Florida elodea (Sumithran, 2013). *Hydrilla verticillata* is a water plant that grown rapidly which their existence could be abundant and causing blooming problem on the water (Dhamayanti, Nursyam, & Hariati, 2016). *Hydrilla verticillata* is the only species for the genus Hydrilla, which belong to family of Hydrocharitaceae (Sumithran, 2013). *Hydrilla. verticillata* might differ in terms of its associated organisms (e.g., microalgae and ostracods; Theel *et al*. 2008; Mormul *et al*. 2010), which may serve as food resources for small sized-fish (Casatti *et al*. 2003; Pelicice & Agostinho, 2006).

According to Sumithran *et al.* (2013), *H. verticillata* was produced through vegetative propagation which mean by process of broken fragments that float and get carried by currents and flood or through trade in of ornamental fish. *Hydrilla verticillata* can survive even with one node or bits of 2-3cm long and its can regenerate, growing at rate of 2cm per day, with stems as long as 750cm and its biomass can achieve about 130 acres per year. Next, *Hydrilla verticillata* could resists herbicides, can tolerate oxygen depletion, low salinity of water and resistance towards chemical pollution. Apart from that, it is also resistance to drought due to its root-tubers or rhizomes can bear in bottom

FYP FIAT

sediment (Sumithran *et al*., 2013). *Hydrilla verticilllata* can survive without roots as floating vegetation because it is independent of the depth of water its inhabits (Sumithran *et al*., 2013). A report by Sanjeeva Raj (2008) stated that *H. verticillata* is a producer and primary keystone species. Next, *H. verticillata* also tolerant to a wide range of temperature and it is adaptable to waters of all continents (Sanjeeva Raj, 2008).

According to the research by Stephen Sumithran, *H. verticillata* is being stated as the native of Sri Lanka, South India, Africa, Southeast Asia and Australia. *Hydrilla verticillata* once being labelled as invasive weed, nuisance plant, pernicious aquatic weed and as naturalists' curse due to its canopies in water interfere with game-fishing, boating and swimming (Stephen, 2013). However, in some native countries, *Hydrilla verticillata* does performs multiple ecosystem functions such as becoming keystone niches in order to attract an enormous biodiversity and it is highly being used to clean waters of heavy metal contamination, through process of bioremediation (Stephen, 2013).

In another article, *H. verticillata* have been proved can affect fish feeding activity and diet composition of the fish itself. Researcher also said that *H. verticillata* could provide a suitable amount of food resources to the small-sized fish that inhabit its patches (C. Natalia *et. al*., 2014).

2.2.2 Nutrient of *Hydrilla verticillata*

A study by Dhamayanti *et al*. (2016) demonstrated *Hydrilla verticillata* can be used as main raw material to produce fish meal due to their high nutrients content. *Hydrilla verticillata* was stated to have about 20.15% of carbohydrate, 32.12% of crude fiber, 2.92% fat, 17.82% protein and 28.82% ash (Uktolseja, 2013). Due to this nutrient content, *H. verticillata* are actively be used to produce feed formulation which lower in cost and commercially available.

FYP FIAT

Next, *H. verticillata* also reported contained a greater source of nutrients and chemical constituents such as saponins, vitamins, minerals, antioxidants, amino acids, detoxifying agents, etc (Prabha & Rajkumar, 2015). Apart from that, *H. verticillata* also being stated to have a low fat source of protein yet they contained hundreds of enzyme, which are unique to this aquatic 'nutrient powerhouse' (Pal & Nimse, 2006). According to Pal and Nimse (2006), *Hydrilla verticillata* contained more calcium than any other whole food source on Earth. *Hydrilla verticillata* also do have high values of calcium, vitamin B-12, polysaccharides, amino acids, micro and macro nutrients. Due to these nutrients content, it is considered to have a great value in terms of food to everyone (Pal & Nimse, 2006).

2.3 *Blaptica dubia (*Dubia Roach)

2.3.1 Biology of *Blaptica dubia*

Today, the usage of insects has been an alternative source of nutrients in farm animal diets (Kulma *et al*., 2016). Based on report by Mitsuhashi (2010), insects do have a great potential as feed due to their nutritional value, low space requirement, and the higher demand of poultry, fish and reptiles since these insects belong to their diet in their natural habitat. A study by Rumpold and Schulter (2012) stated that edible insects would contain high fat, protein and mineral contents thus makes them been used as alternative food and feed source and could be a potential substitute such as fishmeal in feed formulae.

Blaptica dubia adults can reach 40mm in length and they are differ based on their sex. Adult males have long wings that cover the abdomen entirely, whereas ovoviviparous females are lack of wings compared to the males (Kulma *et al*., 2016). Based on study by Kulma *et al.* (2016), he stated that *B. dubia* are considered as suitable feed for animals and they are commercially available. *Blaptica dubia* are easy to be handle as they are

FYP FIAT

unable to fly or climb on smooth surfaces. This feature is significantly easier to manipulate and could prevents contamination and their accidental escape (Kulma *et al.*, 2016). According to Adamo *et al*., (2015) *B. dubia* lack in muscles which causing them unable to fly.

Adult coloration of *B. dubia* ranges from dark brown to black with lighter orange spot/stripe which sometimes are visible only in bright light (Adamo *et al*., 2015). Apart from that, coloration of *B. dubia* also might be differ from one colony to another due to relation of environment and diet conditions. *Blaptica dubia* is ovoviviparous which producing live young ranging from 20 to 40 nymphs per month under suitable condition (Adamo *et al*., 2015). Based on research by Adamo *et al*., (2015), the ideal temperature for the *B. dubia* to breed is around 24-35°C and they cannot breed below 20°C and do not moult properly when the humidity values are too low. *Blaptica dubia* can cause public health problems such as allergies, asthma and sometimes can transmit infective diseases (Adamo *et al*., 2015).

2.3.2 Nutrient of *Blaptica Dubia*

Blattodea species generally known to have sufficient source of protein and lipids (Kulma *et al*., 2015). Based on research by Yi *et al*. (2013), amino acid profile is the decisive characteristic of protein quality and Blattodea species are being stated to have higher protein quality compared to soya bean and lower than casein proteins. A report by Bosch *et al*. (2014) said that *in vitro* digestibility of insect protein is lower than commercially available protein feedstuffs. The quality of lipids would depend on their fatty acids profiles thus, the determination of lipids composition in insects is vital (Kulma *et al*., 2015). Several studies have been done by (Finke, (2002): Barroso *et al*. (2014):

FYP FIAT
UNIVERSITI
MALAYSIA
KELANTAN

Oonincx *et al. (*2015) which analysed the fatty acid composition of the *Blattodea* species. A report by Sanchez-Muros *et al*., (2014) shows that fatty acid profile could be vary significantly due to its reflect of the fatty acid composition of animal diet.

Carbohydrate in Blattodea species are present in the form of chitin which normally are indigestible for monogastric animals and it is available in low quantities (Kulma *et al*., 2015). Next, *Blattodea* species also have long chain polymer of N-acetyl glucosamine which could provide positive effect on the immune system (Van Huis *et al*., 2013). Apart from that, N-acetyl glucosamine also do have effect in antioxidative, hypocholesterolaemic and prebiotic (Hader *et al*., 2013). Sanchez-Muros *et al*., (2014) stated that currently there are more than 150 commercially available species which their nutrient content is species-dependent. A study by Kulma *et al.* (2016) demonstrated that Blattodea species have different developmental stages which can be used as feed based on individual requirements. A study by Kulma *et al*. (2016) proved that *B. dubia* contained about 630 $g \cdot kg^{-1}$ DM of crude protein in adult phase while in sub-adult there would be about 525 $g \cdot kg^{-1}$ DM of crude protein. Apart from that, Kulma *et al*. (2016) also studied amount of energy content in adult and sub-adult phase of *B. dubia*. The amount of energy in adult phase of *B. dubia* was 19 $MJ \cdot kg^{-1}$ DM while sub-adult contained 22 $MJ \cdot kg^{-1}$ DM (Kulma *et al*., 2016).

Several studies by (Yi *et al*. 2013: Bosch *et al*. 2014) stated that proximal composition only been investigated for the adults of *B. dubia* only. A study by Bosch *et al*. (2014) said that Blattodea species have a similar source of proteins and lipids. For adult females of *B. dubia*, they could contain high contents of valine, leucine, histidine, lysine and arginine (Bosch *et al*., 2014). Next, in the terms of fatty acid profile, a study by Finke (2002) and Oonincx *et al.* (2015) analysed the fatty acid profile of *B. dubia* and found the same major fatty acids which is oleic acid. Based on the research by Barroso *et*

FYP FIAT

al. (2014), the fat found in *B. dubia* contained high proportion of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Next, proportion of polyunsaturated fatty acids (PUFA) found in this species are comparable to commonly used oils such as olive, sunflower or rapeseed oil (Valisek, 2014). All of these elements can be improved during rearing process by adding suitable supplements to the feeding mixtures for insects (Kulma *et al*., 2016). Several studies by (Yi *et al*. 2013: Tzompa-Sosa *et al*. 2014) reported 7.6 g of total fat found from 100 g of fresh samples of *B. dubia* and about 214 g of total fat found per kg of DM.

For the conclusion, a report by Kulma *et al*. (2016) proved that adults *B. dubia* have a better source of crude protein, ash and calcium yet contained less fat than sub-adults.

2.4 Physical Test for Fish Pellet

2.4.1 Floating Test

Pellet water stability is a crucial quality parameter in the manufacture of aquaculture diets. High pellet water stability is defined as the retention of pellet physical integrity with minimal disintegration and nutrient leaching while in the water until pellet being consumed by the fish (Keri *et al*., 2013). Based on study by (Momoh: Abubakar: Ipinjolu, 2016), floating extruded pellets are in greater demand compared to sinking pellets. This way could ease farmer to observe the feeding activity of fish and also could lowered the possibility of wastage of feed itself. Due to this character, Almaraj (2010) said that pellet could have greater water stability, digestibility, water protection, zero water pollution and zero wastage of raw materials. A study by Chen and Jenn (2013)

FYP FIAT

showed that water stability, composition density and supplementation are the most crucial physical characteristics of aquatic animal feeds.

In order to achieve pellets with relatively low bulk density and high buoyancy, process of combining ingredients need to be done carefully (Obi *et al*., 2011). Pellets need to be stable in the water for only a few minutes for fish, while for shrimps needs a few hours (Keri, 2013). Based on study by Zakaria (2013), a binder agent is suggested to be apply into feed formulation in order to increase the stability of pellets in water. Yogendra *et al*. (2011) said that binder agent need to be apply steaming process to help in gelatinization and leads performing strong bonding among ingredient particles. Optimization of temperature and time is vital to help in maximization of gelatinization process and hence could increase stability of pellets in water, commonly refer as water stability (Zakaria, 2013). Carbohydrates could improve the pellet ability and water stability of feeds (Keri *et al*., 2013). Several studies (Solomon *et al.* 2011: Falayi and Sadiku. 2013: Obi *et al*., 2011) suggested cassava tuber starch, maize flour starch, wheat flour starch and addition of other ingredients such as yeast, duckweed, honeycomb and melon shell as a good quality binder for feed formulation.

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CHAPTER 3

METHODOLOGY

Materials and Method

3.1 Experimental Design

Treatments for this experiment consists of five different combinations of *Hydrilla verticillata* and *Blaptica dubia,* namely T1(60% *Hydrilla verticillata and* 40% *Blaptica dubia*), T2(70% *Hydrilla verticillata and* 30% *Blaptica dubia),* T3(80% *Hydrilla verticillata and* 20% *Blaptica dubia), T4(90% Hydrilla verticillata and 10% Blaptica dubia) and T5(100%Hydrilla verticillata).* Meanwhile, the control used in this experiment was commercial pellet (Cargill, Malaysia). All feed samples were subject to crude protein analysis.

3.2 Water Thyme, *Hydrilla verticillata* Preparation

Water thyme, *Hydrilla verticillata* was freshly collected from the area of Universiti Malaysia Kelantan Jeli Campus. Sample of *Hydrilla verticillata* was washed by using distilled water, air dried for 48 hours and were fine-mill by using a laboratory blender. Powder obtained were sieved to get the powder with size about 425µm. Powder been stored in a cold room at 4ºC to avoid any fungal infestation.

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3.3 Dubia Cockroach, *Blaptica Dubia* Preparation

Blaptica dubia were reared in a storage box and being feed with fruits and vegetables throughout their life cycle. Then, they were transported to the laboratory and refrigerated at −20ºC before being dried in an oven at 60ºC for 48hours. The dried cockroach was grinded with a dry feed grinder. Then, the powder was sieved through 425µm to obtain the fine powder and the powder will be keep in a cold room (4ºC).

3.4 Diet formulations

Powder of the *Hydrilla verticillata* and *Blaptica dubia* were manually mix. Then, the mixture was slowly mixed with hot water (80°C) in proportion 1:2 (v/w) mixing to accomplish agglutination. The pellets were passed through piping bag to obtained 2mm diameter pellet. The pellets were cut around 2 to 3 mm length. The pellets were dried at 70ºC for 24h in an oven. Then, the sample will be stored in the cold room at 4ºC and prior to proximate analysis of crude protein analysis.

3.5 Crude Protein Analysis

Crude protein analysis of samples in the present study according to Hanan *et al*. (2011) and Jabir *et al.* (2012). Samples were digested using Tecator Digester (FOSS,USA) with eight digestion tubes. The samples were then subjected to Kjeldahl test. Total nitrogen will be calculated using formula as below:

$$\text{Percentage of nitrogen} = \frac{(Vs - Vb) \times N \times 14.007}{W \times 10} \quad (3.1)$$

Where

Vs=volume of 0.1M HCl used to titrate sample

Vb=volume of 0.1M HCl used to titrate blank

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N=normality of HCl

14.007 is the atomic weight of nitrogen

W=weight of sample, in g

Crude protein (%)= nitrogen in sample×F

F=6.25, factor to convert nitrogen to protein

(Jabir *et al*., 2012)

3.6 Floating and Edible Test

Floating Test

Floating test been done to determine the time of pellet retain on water surface before sinking. Samples containing 10 pellets (3 times replication) were placed in the bowl contains 75% of water. Number of pellet from each sample that remained afloat after every 60s interval were recorded for period of 30 minutes.

$$\% \, flotation = \frac{a}{b} x100\% \qquad (3.2)$$

a= number of pellet afloat

b=number of pellet in sample

(Misra *et al*.,2012)

Edible Test

Fish pellet from each sample were fed to the 2-month olds Siamese Fighting Fish *(Betta splendens).* 10 pellets from each sample were given to 5 fishes and observation were been done in 2hrs to observe whether the Siamese Fighting Fish eat the pellet or not. The feeding was done once per day. The eating behaviour of the fish also been observed.

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3.7 Statistical Analysis

All the data were analyzed by using Software Program (SPSS) for Windows, Version 24.0. One-way analysis of variance (ANOVA) was tested followed with Post Hoc Tukey to test multiple range tests with significance ($P < 0.05$

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CHAPTER 4

RESULT AND DISCUSSION

4.1 Crude Protein Analysis

Protein is the major growth promoting factor in feed. However, protein requirement of the fish was influenced by several factors such as fish size, water temperature, feeding rate, availability, quality of natural foods and overall digestible energy content of diet (Nasim *et al*., 2012). A study by Rahim *et al.* (2017) stated that protein being considered as vital component of fish feed. Generally, fish meal contains about 60% to 72% protein (Shepherd & Jacksona, 2013). In order to obtain fish with protein content of 60% to 72% protein, fish need to be feed by fish pellet that contain about 30% to 45% of protein content (Rahim *et al*., 2017). Thus, fish meal must be included in fish diets so as to match the protein requirements of fish (Rahim *et al*., 2017).

In this study, crude protein analysis had been done to observe the protein content in the fish formulations. The mean and standard deviation analysis had been summarized in the Table 4.1.

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Table 4.1: Result of mean and standard deviation of crude protein analysis

Treatment	Protein (Mean ± SD)
Control	24.27 ± 0.88^{b}
T1	45.97 ± 0.12^{f}
T2	41.79 ± 1.35^{e}
T3	35.67 ± 0.53^{d}
T4	27.36 ± 0.60^{c}
T5	21.33 ± 1.14^{a}

^{abcdef} Groups with different superscripts differ significantly at $p<0.05$.

T1 (60% *H. verticillata* + 40% *B. dubia*), T2 (70% *H. verticillata* + 30% *B. dubia*), T3 (80% *H. verticillata* + 20% *B. dubia*), T4 (90% *H. verticillata* + 10% *B. dubia*), T5 (100% *H. verticillata*)

In this study, Table 4.1 demonstrated that T1 had the highest amount of crude protein content which is 45.97%. This value is much higher compared to the amount of protein content in the control pellet. Result obtained also demonstrated that as amount of *Blaptica dubia* increases, the amount of crude protein also increases. This statement can be related to the previous study by Yi *et al*. (2013) that stated *Blaptica dubia* did contain higher amount of protein compared to soy bean meal which mainly used as raw material to produce fish feed. The feeds also showed significant differences (P<0.05) in this crude protein analysis.

Based on study by Nasim *et al.* (2012), the analysed crude protein contents in fish meal varied between 51.32% to 65.34%. The highest crude protein content was found by Nasim *et al*. (2012) in Lakshmipur was about 65.34%. Apart from that, a study by Lee *et*

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al. (2017) found that about 30% of protein content in commercial pellet. Next, a study by Hamid *et al*. (2016) also found that fish meal contained about 46% crude protein. This finding can be more or less similar with the result of T1 which is 45.97% of crude protein content. According to Steven *et al*. (2017), artificial feeds typically made up from protein content that range of 18% to 50%. However, nutritional content of the feed depends on what species of fish being cultured and also depends on their life stages (Steven *et al.*, 2017). Fish that been reared in high-density indoor systems such as cages and cannot feed on their own need to have a complete diet. Yet, fish in ponds or outdoor normally being feed by partial diets only as they can feed freely on natural food (Steven *et al.*, 2017).

Based on the result obtained, T1, T2 and T3 can be considered to be formulate as commercial feeds as their protein content are higher compared to T4 and T5. However, all of the formulations does have their own advantages as they can be consumed according to amount of protein needed by the type of species of the fish, feeding rate and their life stages. Based on study by Steven *et al*. (2017), a smaller fish need more amount of protein compared to adult fish. Thus, T1, T2 and T3 can be considered to be produce as fish formulations for fingerling while T4 and T5 can be considered to be use as adult feeds due to their lower amount of protein content. Small larval fish and fry need to be fed at high-protein diet frequently and normally in excess. Small fish have a high energy demand and must eat nearly continuously and be fed almost hourly.

In general, protein requirements are lower for herbivorous fish (plant-eating) and omnivorous (plant and animal eaters) while carnivorous (flesh-eating) required higher amount of protein (Steven *et al*., 2017). Apart from that, protein levels needed by fish also varied among different species. According to Steven *et al.* (2017), catfish would require about 28% to 32% of protein, while tilapia would require about 35% to 40%

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protein content. Thus, by referring to this result of crude protein analysis, T3 and T4 can be consumed by catfish while tilapia can consume the formulations of T1, T2 and T3.

4.2 Floatation Ability

Commercial fish diets are manufactured as either extruded (floating or buoyant) or pressure-pelleted (sinking) feeds. Both floating and sinking feeds could produce satisfactory growth, however some species prefer floating compared to sinking (Steven *et al*., 2017). For example, shrimp cannot consume on floating feed, but most of fish species can be trained to accept a floating pellet (Steven *et a*l., 2017). Next, it is advantageous to feed a floating feed because farmer can directly observe the feeding intensity and can ease them to adjust feeding rates accordingly.

In this experiment, Table 4.2 shows the mean and standard analysis done to observe the floatation ability of the fish formulations.

Table 4.2: Result of mean and standard deviation analysis of floatation

Treatment	Floating (Mean ± SD)
Control	91.75 ± 0.99^{e}
T1	45.81 ± 0.37^{a}
T2	51.47 ± 1.25^{b}
T3	53.88 ± 0.59^{c}
T4	87.19 ± 0.82^{d}
T5	89.65 ± 20.35^{e}

^{abcde} Groups with different superscripts differ significantly at $p<0.05$.

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T1 (60% *H. verticillata* + 40% *B. dubia*), T2 (70% *H. verticillata* + 30% *B. dubia*), T3 (80% *H. verticillata* + 20% *B. dubia*), T4 (90% *H. verticillata* + 10% *B. dubia*), T5 (100% *H. verticillata*)

The results indicated that the mean floatability of the fish feed pellets increase as the amount of *Blaptica dubia* decreases. Based on research by Orire and Sadiku (2015), the choice of ingredients played a significant role as it was evident from the results which indicated that, feeds containing ingredients with high molecular weight did not float as in the case of soy bean based feeds despite the presence of catalysts. Right selection of feedstuffs and appropriate inclusion level of locally available floating catalysts will achieve positive buoyancy in feeds (Orire & Sadiku, 2015).

Table 4.3: Assessment of pellets buoyancy

Treatment	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes
Control	10	10	10	10	9	9
T1	8	7	5	3	2	0
T2	8	7	6	5	3	1
T3	8	7	6	5	4	3
T4	10	9	9	9	8	8
T5	10	9	9	9	9	8

T1 (60% *H. verticillata* + 40% *B. dubia*), T2 (70% *H. verticillata* + 30% *B. dubia*), T3 (80% *H. verticillata* + 20% *B. dubia*), T4 (90% *H. verticillata* + 10% *B. dubia*), T5 (100% *H. verticillata*)

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In the first 10 minutes of the test, all the diets floated well with over 70% floatation. This could be as a result of the uniform particle size of ingredients used in this experiment. Based on study by Khater *et al*. (2014), they found that as the protein ratio decrease, the floatability of pellet also decreases. The finding is different from the result of this experiment as the protein ratio decreases, the floatability of the pellet increases.

Based on the result obtained, T5 had the highest floatation which is 89.65%. The result of mean in floatation test ranged between 45.81% 91.75%. T5 can be considered to have a good water stability compared to other diet formulations. The subsequent difference in floatation could be attributed to the difference of proportion used within the formulation diet (Adeparusi & Famurewa, 2011).

A study by Saalah *et al.* (2010) analysed that floatation of fish pellet depends on mash particle size, binding agent and temperature during feed processed. A good fish pellet need to have a good water stability and floatation ability (Khater *et al.*, 2014). Thus, based on the result obtained above, T5 can be considered to be produce as commercial in the future research as it had floatation ability almost the same with available commercial pellet. However, in terms of protein, T5 might lack as it contains lower protein compared to other treatments.

According to Steven *et al.* (2017), most of the farmers would prefer floating pellet compared to sinking pellet. Thus, it is suggested that T4 and T5 can be considered to be produce in the future as commercial pellet as both of them do have a good floatation. A future research can be done to improve their floatation by adding binding catalyst in good proportion. T1, T2 and T3 are lack in floatation yet, it can be substitute shrimp feed as shrimp prefer sinking pellet compared to floating pellet. Thus, it is suggested to choose T5 as the best pellet that have good floatation ability.

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4.3 Edibility

All of the samples being eaten by the Siamese fighting fish. Most of the samples ate by the fish in the first 30 minutes. Eating behaviour of the fish been observed. Some fish did spit out the pellet. However, it can be concluded that these pellet are edible for the fish to consume protein in their growth. Somehow, it is suggested to add few more ingredients in order to ensure the fish ate the pellet well.

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CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

To conclude, in terms of crude protein analysis, all the pellets can be considered to be produce as commercial pellet. However, the pellets need to be consider to be feed for differ species of fish and development stages of fish itself. T1 (60% *H. verticillata* + 40% *B. dubia*) are suitable to be consume by fingerlings instead of adult fish as its contain higher protein content. While T4 (90% *H. verticillata* + 10% *B. dubia*) and T5 (100% *H. verticillata*) can be consumed by adult fish as it contains lower protein content. In terms of floatation, T5 (100% *H. verticillata*) had the highest floatation ability and can be suggested to be produce as commercial pellet in the future. Thus, it can be concluded that these two main ingredients can be used to replace the costly protein source of fish meal such as soy bean meal as both of these ingredient do have advantages in terms of crude protein content and floatation abili

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5.2 Recommendation

In this experiment, there could be error in the process of mixing the feed ingredients as all of the steps have been done manually. Thus, in the future research it is recommended to use machine when mixing the ingredients. As for the floatation test, the composition of carboxymethylcellulose as a binder agent does have effect on the pellet floatation ability. The error during mixing the ingredients with the binder can cause the differences in the data collected.

During the process of this experiment, there could be error such as inaccurate data taken. Thus, in the future research it is recommended to ensure the process of collection data need to be taken properly. Wrong data taken can cause the result be variety and differences of the result itself.

In the terms of crude protein analysis, the process need to be done properly. Raw material used need to be process properly such as by using a right temperature during drying process. A high temperature used can cause the protein content in the raw material to denature. Thus, it is crucial to know the right temperature before starting the drying process.

The formulation diets of fish meal in this experiment could be a benchmark for future researcher. Raw material used in this experiment do have positive effect in terms of protein content and floating ability which might be further study in the future.

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APPENDIX A

Table A1: Homogenous Subsets of Crude Protein Analysis

Protein

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
T6	3	21.3266					
Control	3		24.2720				
T5	3			27.3584			
T4	3				35.6708		
T2	3					41.7918	
T1	3						45.9661
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.2: Homogenous Subsets of Floating Test

Floating

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
T1	3	45.8067				
T2	3		51.4700			
T4	3			53.8767		
T5	3				87.1867	
T6	3					89.6467
Control	3					91.7533
Sig.		1.000	1.000	1.000	1.000	.063

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

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Table A.3: Test of Homogeneity of Variances

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Protein	3.478	5	12	.036
Floating	1.226	5	12	.355

Table A.4: One-way ANOVA test

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Protein	Between Groups	1489.169	5	297.834	390.653	.000
	Within Groups	9.149	12	.762		
	Total	1498.318	17			
Floating	Between Groups	7029.628	5	1405.926	2194.549	.000
	Within Groups	7.688	12	.641		
	Total	7037.316	17			

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APPENDIX B

Figure B.1: Weight the *Hydrilla verticillata* to be use as main raw material

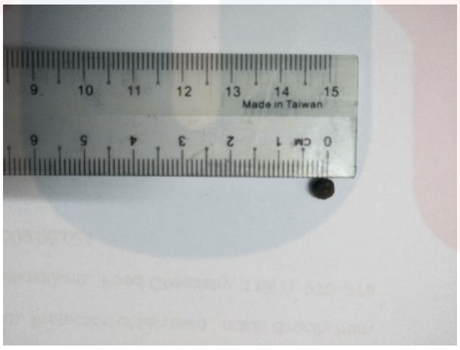

Figure B.2: Size of the pellet approximately about 2mm

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Figure B.3: Ready-made pellet

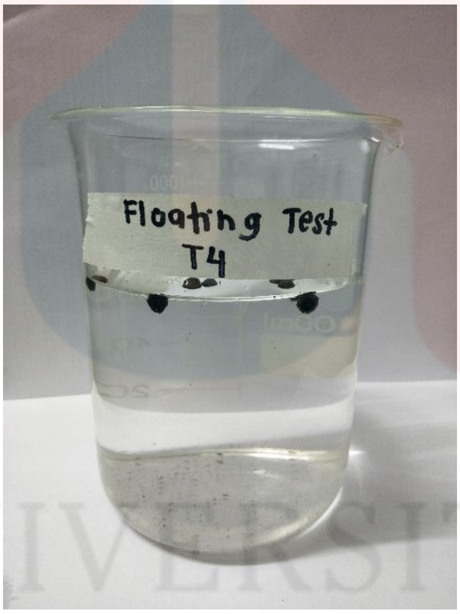

Figure B.4: Floating test of fish pellet

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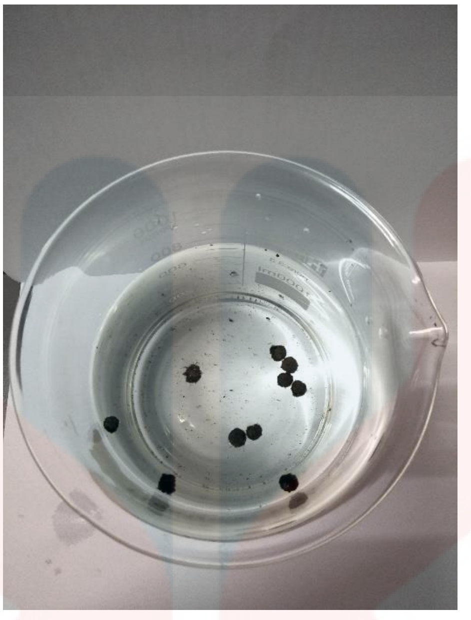

Figure B.5: Floating test by using 10 pellets

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