Formulation of Poultry Feed with Supplementation Protein Content from *Trichanthera gigantea* and *Morus alba* Leaves Meal for Japanese Quail (*Coturnix japonica)*

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DECLARATION

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

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ABSTRACT

The poultry industry in Malaysia is a major source of meat protein. Feed, particularly the protein source ingredient, is the most critical single cost associated with poultry production. Because feed contribute for 60% to 70% of total production costs, any effort to reduce feed costs will result in a significant reduction in total feed production costs. This study aims to identify the best protein source ingredient and formulation for Japanese quail's diet using Design Expert Software Version 7.1 through Central Composite Design (CCD) as a statistical tool. This quail formulation diet included four main ingredients: Madre de agua leaves meal (MDALM), Mulberry leaves meal (MLM), rice bran (RB), and fish meal (FM) as a protein source. The recommended optimum value was 29.5 %, 27.5 %, and 19.5 % for leaves meal (MDALM & MLM), RB, and FM, respectively, resulting in an optimum protein content of 27.0116 % for MLM inclusion. In the future, the formulation of quail feed from leaves meal can be an option in the poultry industry. Together with cost-effectiveness, leaves meals have a high potential to replace pricey and imported ingredients in the feed industry.

Keywords: Protein source, Madre de agua leaves meal, Mulberry leaves meal, Central Composite Design (CCD), Feed industry.

Formulasi Makanan Unggas dengan Tambahan Kandungan Protein daripada Serbuk Daun *Trichanthera gigantea* dan *Morus alba* untuk Puyuh Jepun *(Coturnix japonica)*

ABSTRAK

Industri unggas di Malaysia merupakan sumber utama protein daging. Makanan unggas, terutamanya sumber bahan protein adalah kos paling tinggi dalam pengeluaran unggas. Oleh kerana makanan unggas menyumbang 60% hingga 70% daripada jumlah kos pengeluaran, maka sebarang usaha untuk mengurangkan kos makanan akan menggalakkan pengurangan ketara dalam jumlah kos pengeluaran makanan. Kajian ini bertujuan untuk mengenal pasti bahan terutamanya serbuk daun dan formulasi sumber protein terbaik untuk pemakanan puyuh Jepun menggunakan 'Design Expert Software' Versi 7.1 melalui 'Central Composite Design' (CCD) sebagai alat statistik. Formulasi diet puyuh ini merangkumi empat bahan utama: Serbuk daun ketum ayam (MDALM), serbuk daun mulberi (MLM), RB, dan FM sebagai sumber protein. Nilai pemakanan optimum yang disyorkan ialah 29.5 %, 27.5 %, dan 19.5 % untuk serbuk daun (MDALM & MLM), RB, dan FM, masing-masing, menghasilkan kandungan protein optimum sebanyak 27.0116 % untuk kemasukan MLM. Pada masa hadapan, formulasi makanan puyuh daripada serbuk daun boleh menjadi pilihan utama dalam industri unggas. Bersama-sama dengan keberkesanan kos, serbuk daun mempunyai potensi tinggi untuk menggantikan bahan yang mahal dan perlu diimport dalam industri makanan haiwan.

Kata kunci: Sumber bahan protein, Serbuk daun ketum ayam, Serbuk daun mulberi, Central Composite Design (CCD), Industri makanan haiwan.

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LIST OF SYMBOL/ABBREVIATION

SYMBOL/ABBREVIATION	MEANING
%	Percent
-	Dash
℃	Degree Celsius
NH4=	Ammonium ion
NH3	Ammonia
m	Meter
cm	Centimeter
g	Gram
kcal	Kilocalories
mm	Millimeter
hr	Hour
min	Minute
MDALM	Madre de agua leaves meal
MLM	Mulberry leaves meal
RB	Rice bran
FM	Fish meal
CCD	Central Composite Design
CP	Crude protein
CF	Crude fiber
EE	Ether extract
V	Volume
N	Concentration of HCl
W	Weight
C	Blank
WBA	Weight before ashing
WAA	Weight after ashing

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CHAPTER 1

INTRODUCTION

1.1 Research background

In Malaysia, nutritionists should focus on investigating and testing inexpensive and new feedstuff as a protein source for livestock, poultry, and another farm animal. Nutrition is described as the process of supplying and obtaining food required for an animal's health and growth (Arai, 2014). Nutrients are available in all feeds. Animals, like humans, require specific nutrients in their daily feed to stay healthy and grow. Some feeds are grown specifically for animals, such as pasture grasses, hay and silage crops, and certain cereal grains. Other feeds, such as sugar beet pulp, brewers' grains, and pineapple bran, are byproducts of food crops that have been processed for human consumption.

Carbohydrates, protein, fat, minerals, vitamins, and water are the basic nutrients that animals require for maintenance, growth, reproduction, and good health. Carbohydrates and fats provide most of the energy required for growth and activity.

Protein will also provide energy, especially if carbohydrate and fat intake are insufficient or protein intake exceeds the body's needs. Animals require an energy source to sustain life processes within the body as well as muscular activity. When an animal's energy intake exceeds its requirements, the excess is stored as body fat, which can be used as a source of energy later if less food becomes available (Holden & Loosli, 2018).

Malaysia's poultry industry is a significant source of meat protein. The most critical single cost associated with poultry production is feed (Ravindran, 2013). The animal feed includes feedstuffs, ingredients, additives, supplements, and any single or multiple products, whether processed, semi-processed, or raw, that are intended to be fed directly to the food-producing animals. Poultry feeds are a form of feed used by far poultry such as chicken, geese, duck including quail. It is critical to provide the proper nutrition for poultry development, production, and health. Depending on factors such as the age of birds and production status, different amounts of energy are needed.

The second most important aspect of functional poultry diets is protein sources. The conventional commercial poultry industry uses a few ingredients to supply protein, which is limited in supply and usually more expensive than energy sources (Iji et al., 2017). The poultry industry's primary protein source is soybean seed and cornmeal. Malaysia lacks soybean and corn meal sources. A large portion of Malaysia's corn and soybean meal is imported for poultry feed. Malaysia cannot stop importing corn and soybeans because the yields are not strong enough to grow them locally. Palm oil and rice bran are the only feed ingredients provided locally, but they only make up around 6% of the total feed, and it is suitable for cow instead of poultry (Lim, 2015).

Malaysia is still struggling on planning to cut back on imported poultry feed. To remain self-sufficient in the livestock industry, Malaysia must grow its feed ingredients, reducing reliance on imported agricultural products (Byrne, 2018). Alternative protein sources for poultry diets are needed to reduce farmers' dependence on conventional protein sources. Supply, affordability, and nutritional value are critical factors to consider when looking for suitable alternatives. Due mainly to shortages and cost issues, it is necessary that more attention be given to alternative protein supplements for use in poultry feeds in the coming years.

Leaf meal is ground, and dried product made from young leafy alfalfa plants to supplement poultry and livestock feed. Leaf meals with high protein content have been used in broiler diets as an alternative protein-rich feedstuff. In addition to being high in protein, Leaf meals contain several biologically active components that can help broiler chickens develop and improve their health (Sugiharto et al., 2019). In the broiler diet, leaf meals are now encouraged in Malaysia due to increased feed prices, especially for protein-rich feed ingredients.

Trichanthera gigantea, a new commercialized product, is one of the alternative protein sources for animal feed. It is also known as Madre de Agua and has many more familiar names. Madre de Agua is a high-protein source that can be used to feed chickens, pigs, goats, cows, buffalo, and even fish. Chemically, it is composed of 18.21% crude protein, 88.44% dry matter, 12.5% crude fiber, 2.66% crude fat, 21.80% ash, 11.56% moisture, 5% calcium, and 41% phosphorus (Jaya et al., 2009). Madre de

Agua is a type of leaf species in this world that is categorized into the Lamiales order, *Trichanthera* genus, and *T. gigantea* species.

Besides, *Morus alba*, or its common name is Mulberry, is a plant that grows well in the tropic and subtropic and seems to have a high nutritional value. Moreover, its chemical composition of 4 weeks of age is a crude protein (29.8%), dry matter (89.25%), ether extract (5.57%), ash (11.81%), crude fiber (13.11%), calcium (2.73%), and phosphorus (0.28%) (Riyadh et al., 2013). Mulberry leaves have provided excellent results in the previous study as a poultry feed, especially broiler (Ustundag & Ozdogan, 2015). Due to arising problems, nowadays many farmers also try to use leaves since the soybean and corn meals have been so pricy in feed production.

Japanese quail (*Coturnix japonica*) is an agricultural species that produces both egg and meat in Asian countries and, Malaysia. Also known as a species of Old-World quail, the Japanese quail belongs to the Phasianidae family. They are small, round birds that only reach a height of six to eight inches, making them suitable for raising in small spaces (Torie, 2014). This animal is a vitamin-rich source of low-fat alternative animal protein and eggs that contain 2.47% less fat than chicken eggs, all of which help improve the human immune system. Quail’s high levels of phosphorus, iron, and vitamin A, B1, and B2 all help it fit into society’s concept of a balanced diet and lifestyle (Bruce, 2019). This study aims to aim at the effect of protein content in Madre de agua leaves meal (MDALM) and Mulberry leaves meal (MLM) and identify the best protein source ingredient in the Japanese quails' diet.

1.2 Problem Statement

Feed formulation in animal feed is important because it is one of the best ways to maintain the quality. Poultry farmers worldwide are still dealing with the high production cost of poultry feed. Producers must find a way to formulate a suitable formulation of feed and minimum input cost in poultry feed to prevent loss. Besides, protein ingredient in Malaysia remains the priciest feedstuff in animal feed. One of the reasons is that farmers in Malaysia need to import the ingredient from others country. In poultry feeding, leaf meal may be used as an alternative protein feed.

1.3 Hypothesis

H_0: No effect of protein content in *Trichanthera gigantea* leaves meal and *Morus alba* leaves meal used on quail's formulation diet.

H_1: Have effect of protein content in *Trichanthera gigantea* leaves meal and *Morus alba* leaves meal used on quail's formulation diet.

1.4 Research Objective

The research has been conducted to determine the best protein source ingredient and formulation for Japanese quail's diet using Design Expert Software Version 7.1 through Central Composite Design (CCD).

1.5 Scope of Study

The scope of this study is to investigate the best protein content of two types of leaves meal which is Madre de agua and mulberry, rice bran, and fish meal, in the Japanese quails' diet. The feed has been formulated according to their standard nutrient requirement by only replacing the protein source, which is soybean meal, in their commercial feed. Moreover, proximate analyses were used to determine the chemical composition of leaves, rice bran, broken rice, and fish meal for feed formulation. The element that has been analyzed in the proximate analysis is a crude protein (CP), crude fiber (CF), ether extract (EE), ash, and moisture content.

1.6 Significant od Study

Leaves meal as an alternative source of protein ingredient in poultry feed can reduce the imported protein ingredients such as soybean and cornmeal. The study can also provide information on the positive effect of leaf meal as a protein source in the quail diet. This study can also benefit many farmers, especially small-scale farmers, by using a local ingredient that can replace a cheaper protein source from the market and as a guideline for the formulation of feed for their farm animals. So, farmers can minimize the dependency on imported soybean and cornmeal in their farm animal feed.

CHAPTER 2

LITERITURE REVIEW

1.1 *Trichanthera gigantea* (Madre de agua)

Figure 2.1: *Trichanthera gigantea* leaves

Madre de agua, or its scientific name *Trichanthera gigantea*, belongs to the order Lamiales, family Acanthaceae, genus Trichanthera was a forage species in Malaysia. It is a multipurpose, adaptable South American tree that grows in various tropical ecosystems. Pigs, rabbits, and ruminants eat it as fodder. *Trichanthera gigantea* is a small and medium-sized shrub that grows to a height of 12-15 m in the wild. The tree's crown has a diameter of 6 meters and is densely branched. Quadrangular branches with rounded nodes and finely haired ends. On 1-5 cm long petioles, the leaves are borne in opposite directions (Heuze et al., 2017). Madre de agua can be grown in multi-strata agroforestry systems alongside forage trees. Pigs, ruminants, and rabbits have historically been fed Madre de agua foliage. It is used in fodder bank systems as a source of forage.

Madre de Agua is a high-protein source that can be used to feed chickens, pigs, goats, cows, and even fish. Chemically, it is composed of 88.44% dry matter, 18.21% crude protein, 12.5% crude fiber, 2.66% crude fat, 21.80% ash, 11.56% moisture, 5% calcium, and 41% phosphorus. Replacement of up to 30% soybean meal in the diet of pregnant pigs resulted in cost savings without impacting resale value. Replacement of up to 30% soybean meal in the diet of pregnant pigs resulted in an economic gain without affecting reproductive efficiency. Fresh young leaves can be given to chickens. It can also be made into leaf meals and used as a mash ingredient. It can be added to the diets of ducks, hens, quails, rabbits, and large such as cattle and buffaloes and small ruminants like pigs, sheep, and goats. When commercial feeds are partially replaced with Madre de agua, the weight gain, meat quality, and income are largely like those chickens fed only commercial feeds.

The research from Charlie (2015) has study on effect of different level of leaf as a partial replacement of feed on poultry. In this study, an experiment was set in simple completely randomized design and replicated 6 times. In poultry diets, supplemental protein sources are important. Protein, as well as vitamins and minerals, are likely to be the first limiting factors. There is a need for further research into the possibility of using locally accessible plant-based protein sources. The leaves and twigs of the multi-purpose tree Madre de agua contain a high crude protein content. The leaves and thin stems of the multi-purpose tree Madre de agua contain a high crude protein content, which is also eaten by animals, and apparently most of it is true protein with a strong amino acid balance.

Next, Nguyen et al. (1997) have been done research about the use of Madre de agua leaf meal and fresh leaves as livestock feed. Studies were conducted to determine the optimal inclusion rates of meals made from sun dried *T. gigantea* leaves in poultry diets, as well as fresh leaves in duck diets, as locally available sources of carotene and plant protein. To replace current protein-rich concentrate meals, more research is needed to investigate the possibility of using locally available protein resources, especially the leaves of multipurpose trees and water plants. Small quantities of green feed are commonly used by farmers in tropical countries to protect against vitamin deficiencies and provide unidentified growth factors.

Ly et al. (2001) was studied about nutritive value of two type of leaves meal on Mong Cai pig. Two experiments were conducted to determine the nutritional value of sun-dried leaves from Madre de agua (*Trichanthera gigantea*) and mulberry (*Morus alba*) for pigs. In the view of protein supply from these materials, the addition of significant quantities of forages to the feed has largely been ignored. The different between both leaves were investigate. The high nutritional value of the mulberry leaves used in these experiments suggests that they could be used as a protein source in pig feed.

1.2 *Morus alba* (Mulberry)

Figure 2.2: *Morus alba* leaves

Mulberry or its scientific name Morus alba belongs to the order Rosales, family Moraceae and genus *Morus* was one of the forage species available in Malaysia. It is a medium-sized pantropical and subtropical tree with a high yield. Mulberry offers a highly palatable forage suitable for most farm animals, including its traditional use as silkworm fodder (Martin et al., 2017). *Morus alba* is a medium-sized deciduous tree that grows to a height of 25-35 m. It has a thick, spreading crown that is usually wider than the tree's height. A common mulberry can grow in a pyramidal or drooping form. It has a straight, cylindrical bole with no buttresses and a circumference of up to 1.8 m. Its foliage can be fed to livestock as a source of fodder. The leaves and stems can be eaten raw or baked. The fruits are edible and can be eaten raw or dried and can be substituted for raisins. Juice and drinks can be made from fruits.

Mulberry trees are part of a millennial circular economy system in China, which includes mulberry trees, silk processing, fish farming, agriculture, and livestock farming: silkworms eat the leaves, and the pupae of silkworms are fed to fish. Fishponds are fertilized with silkworm feces and wastewater from silk processing, whereas pond silt is a suitable fertilizer for fodder crops. Silkworm feces and wastewater from silk production are used to fertilize fishponds, while pond silt is used to fertilize fodder crops, which are then fed to livestock (Cook et al., 2005).

Mulberry leaves have been tested on pigs and chickens and have a high protein content, making them a good candidate for feed supplementation. A report from Omar et al. (1999) and Islam et al. (2015) state that mulberry leaves are high in protein (15-35%), minerals (2.42-4.71%) calcium, (0.23-0.97%) phosphorus and other nutrients. with metabolizable energy (1130-2240 kcal/kg) and no or minor anti-nutritional factors. Wang et al., (2016) reported that the composition on a dry matter basis of mulberry leaf meal is 4.94 mcal gross energy, 18.81% crude protein, 11.65% ether extract, 12.45% crude fiber, 2.46% calcium, 0.24% phosphorus and 0.26-1.92% amino acids.

Besides, Riyadh et al. (2013) was study about nutrient digestibility of mulberry leaves. The aim of this study was to figure out the chemical composition of mulberry (*Morus alba*) leaf meal (MLM) and its nutritional value as a feed ingredient. In the digestibility test, fifteen layer and fifteen broiler chickens were used. Nutritionists should focus on investigating and testing fresh and inexpensive protein sources for livestock and other farm animals. The researchers used layer and broiler chickens to evaluate the nutrient digestibility and metabolizable energy content of mulberry leaf meal. Resulting in a high fiber content, incorporating MLM into the chickens' diet may be a good source of protein.

A study about the effect of nutritive value of mulberry leaves meal on the performance of geese have been done by Wang et al. (2017). This study was determined the nutritional value of mulberry leaf meal (MLM), as well as the energy and amino acid digestibility of MLM in male Sichuan white geese, and to assess the efficiency of these geese fed an MLM-supplemented diet. Mulberry leaves are highly palatable and readily digestible (70-90%) for herbivores, and monogastric can be fed them. Mulberry leaves have previously been evaluated as a protein source for beef cattle, sheep, pigs, laying hens, and broilers.

Performance of broiler fed with diets substituted with mulberry leaf powder was studied by Carlina et al. (2012). The need for a low-cost, easily accessible, and similarly nutritive feed to partially replace commercial poultry diets has never been pressing. This experiment was carried out to see how adding up to 50% mulberry leaf powder (MLP) to a commercial broiler diet affected feed consumption, growth rate, feed conversion ratio, and mortality in broiler chickens. The benefits of including up to 30% MLP in broiler diets include a significant reduction in feed costs and, as a result, lower production costs.

1.3 Rice Bran

Rice is the world's second-largest cereal crop, and the by-products of rice processing for human consumption could be used to supplement poultry diets. One of the byproducts from rice industry is rice bran. The hard outer layer of rice grains is called the bran where it will be removed when the process of brow rice into white. Rice bran is a powdery finely, fluffy substance made up of seeds or kernels, as well as pericarp, seed coat, aleurone, germ, and fine starchy endosperm particles. It is rich in protein and fat as well as containing high level of vitamins and some mineral (Samli et al., 2006). Both the protein and fat in rice bran have a high biological value (Khan, 2004).

The Feedstuffs Ingredient Analysis Table is designed to give nutritionists a good idea of the nutritional values of common feed ingredients. A study from Batal and Dale (2010) state that rice bran containing 91% dry matter, 13.5% crude protein, 5.9% crude fat, 13% crude fiber, 11% ash and 0.1% calcium. In another study of proximate composition of rice bran, it states that there are 87.9% of dry matter, 16.98% of ether extract, 14.96% of crude protein, 11.42% of crude fiber and 8.64% of ash contain in rice bran (Samli et al,2006).

1.4 Fish Meal

Processed whole or sections of fish produce a fish product or a fishery byproduct. Fish meal is a commercial product made primarily from fish that are not consumed by humans. It is an internationally traded commodity that is widely used as a feed for aquaculture, poultry, and other livestock. Small pelagic oceanic fish such as menhaden, herring, anchovies, and sardines are used to make fish meal. The oil and water are pressed out of the small fish after it have been pulverized. The solids that remain are cooked and ground into a meal. Fish oil is produced as a byproduct of the fish meal manufacturing process by separating water from the remaining liquid (Boyd, 2015).

As state by Boyd (2015), fish meal is a good source of calcium, phosphorus, and other minerals, and it is used in animal feeds because of its high protein content. The proximate composition containing in fish meal are 91-92% dry matter, 60-72% crude protein, 6-10% crude fat, 0.8-3% crude fiber, and 7-16% ash (Cho & Kim, 2010). Research from Karimi (2006) evaluated the effect of different inclusion of fishmeal in broiler diet. The findings of this study revealed that the beneficial effects of fishmeal on broiler outputs are most noticeable at higher usage levels and during later growth periods, owing to feed intake stimulation

A study about fish meal on understanding why this feed ingredient are so valuable in poultry diet have been done by Richard and Jacqueline (2008). According to the findings of this report, fishmeal is an excellent source of protein for poultry because it contains enough of all the essential amino acids needed by chickens, with a focus on lysine and methionine. The consistency of fishmeal varies greatly depending on the processing conditions used in its production.

1.5 Overview of Japanese Quail (*Cortunix japonica*)

1.5.1 Origin, Taxonomy and Distribution

Belonging to the Phasianidae family, Japanese quail, or its scientific name *Coturnix japonica* is a highly diverse genus of animals. The pheasant family contains species that have a great economy importance to human. Grouse, quail, partridges, pheasants, and turkeys are all valuable game birds that are hunted daily in the world. They are mainstay of global food supplies because most of them are reared for human's consumption. In several parts of the world, the Japanese quails have gained a lot of economic importance as an agricultural species for egg and meat production.

The Japanese quail is the most common domesticated quail species in Malaysia. East Asia is the origin of the Japanese quail. Wild Japanese quail can be found in Manchuria, South-Eastern Siberia, Northern Japan, and the Korean Peninsula due to their migratory nature. It can also be found in Sikkim, Bihar, and parts of Assam in India's North-Eastern states. Grasslands and cultivated fields are their preferred habitats. Because of their small body size, short sexual maturity, high rate of reproduction, ability to produce 3 to 4 generations per year, and relative ease of colony maintenance, they have become a significant laboratory animal (Shim & Vohra, 1984; Baer et al., 2015).

Table 2.1: Taxonomy of Japanese quail

Kingdom	Animalia
Phylum	Chordata
Class	Aves
Order	Galliformes
Family	Phasianidae
Genus	Cortunix
Species	*Coturnix japonica*

Source: (Myers, 2021)

2.5.2 Morphological Characteristic

The Japanese quail's morphology varies depending on its life stage. Both females and males' individuals have the same plumage and coloring as chicks. Small black patches litter the area above the beak, giving them a tawny appearance. The chick's wings and back are a pale brown color, with four brown stripes running the length of the back. A pale yellow-brown line runs down the top of the head, surrounded by smaller black lines. The Japanese quail's plumage is sexually dimorphic, allowing the sexes to be separated from one another. Adults, both male and female, mostly have brown plumage. However, markings on the throat and breast, as well as the appearance of the plumage, may differ significantly.

Females' breast feathers are littered with dark spots between their otherwise pale feathers. Male breast feathers, on the other hand, are a uniform dark reddish-brown color with no dark spots. Male cheek feathers are also reddish-brown in color, while female cheek feathers are cream in color. The development of a white collar is also seen in some males, but not in any female members of the species.

Males have a smaller size than females. The production of quails in Malaysia usually takes only 6 to 7 weeks to reach marketable size and 7 to 8 weeks for egg production. A study from Mizutani, (2003) stated that the meat type quail have 2 to 3 times higher than the egg production type. However, the weight of domesticated lines varies greatly, with commercial meat-producing strains weighing up to 300 g.

Table 2.2: Normal data for Japanese quails

Table 1. Normal data on Japanese quail

Trait	Range
Body weight at one-day-old	6 ~ 8 g
Adult male	100 ~ 130 g
Adult female	120 ~ 160 g
Egg weight	9 ~ 10 g
Egg number/100 days	80 ~ 90
Age at sexual maturity	38 ~ 42 days of age
Life span	Max: 7 years in male Mean: 3 ~ 4 years

Source: (Mizutani, 2003)

2.5.3 Production and Economic Important

Japanese quail has grown in economic importance as an agricultural species that produces eggs and meat with a distinct flavor. In Japan and Southeast Asia, egg production is essential, whereas in Europe, meat is the main commodity. Second, due to its small body size (80-300 g) and short generation period (3-4 generation per year), it has a low maintenance cost and is resistant to disease. Besides, its small body size (80-

300 g) combined with its short generation period (3-4 generation per year), disease tolerance, and high egg production made it an ideal laboratory animal (Nasrollah,2008). Finally, the Japanese quail is the smallest avian species farmed for meat and eggs. As a result, it has been used in a lot of research. The Japanese quail is bred to produce eggs and meat. As state by Department of Veterinary, in 2017, there are 3,123,811 numbers of quails that are reared by farmers in Malaysia, and it were estimated to increase to 3,431,488 in 2018 as shown in table 2.3 and 2.4.

Table 2.3: Japanese quail population in Malaysia (2017)

MALAYSIA : BILANGAN TERNAKAN MENGIKUT NEGERI, 2017
Malaysia : Livestock Population By States, 2017

NEGERI *State*	Kerbau *Buffalo*	Lembu *Cattle*	Kambing *Goat*	Bebiri *Sheep*	Babi *Swine*	Ayam *Poultry*	Itik *Duck*	Burung Unta *Ostrich*	Puyuh *Quail*	Rusa *Deer*
Perlis	224	6,713	3,178	1,653	0	1,570,560	7,790	6	10,900	284
Kedah	5,437	56,118	46,982	11,428	1,755	50,712,440	515,166	14	222,143	1,119
Pulau Pinang	574	11,642	10,215	2,757	317,897	10,869,583	335,043	2	147,450	86
Perak	9,199	44,749	26,741	2,948	540,816	37,107,300	6,837,100		47,545	4,835
Selangor	2,812	24,831	19,997	3,090	267,317	17,654,459	5,305	190	106,800	44
N. Sembilan	1,992	39,614	32,488	16,128	349	20,042,199	32,303	91	389,300	1,124
Melaka	4,404	28,721	35,804	6,906	47,250	24,883,996	79,091		343,500	394
Johor	3,710	101,967	40,462	21,756	232,391	69,594,084	1,030,715	297	1,380,569	1,992
Pahang	11,680	123,299	31,621	18,605	4,282	11,301,623	11,240	2	76,530	1,832
Terengganu	8,972	84,427	28,401	6,860	0	2,674,040	27,176		84,597	655
Kelantan	5,628	98,237	42,037	34,030	680	3,657,188	64,142		9,544	219
W. Persekutuan		203	106							48
Jumlah S. M'sia *Total For P. M'sia*	**54,632**	**620,521**	**318,032**	**126,161**	**1,412,737**	**250,067,472**	**8,945,071**	**602**	**2,818,878**	**12,632**
Sabah	53,850	73,200	54,525	2,565	103,095	9,604,845	53,725	59	16,762	
Sarawak [P]	5,531	10,111	12,747	1,932	333,519	33,629,241	285,104		288,171	1,061
JUMLAH BESAR *Grand Total*	**114,013**	**703,832**	**385,304**	**130,658**	**1,849,351**	**293,301,558**	**9,283,900**	**661**	**3,123,811**	**13,693**

P : Sementara (Provisional) n.a : Tiada maklumat *(Not available)*

Source: (Department of Veterinary Science Malaysia, 2017)

Table 2.4: Estimated Japanese quail population in Malaysia (2018)

MALAYSIA : BILANGAN TERNAKAN MENGIKUT NEGERI, 2018 [E]

Malaysia : Livestock Population By States, 2018 [E]

NEGERI *State*	Kerbau *Buffalo*	Lembu *Cattle*	Kambing *Goat*	Bebiri *Sheep*	Babi *Swine*	Ayam *Poultry*	Itik *Duck*	Burung Unta *Ostrich*	Puyuh *Quail*	Rusa *Deer*
Perlis	249	6,718	3,176	1,644		1,784,417	7,838	5	10,203	287
Kedah	5,219	54,297	48,459	10,833	1,588	56,529,903	561,819	11	237,883	1,205
Pulau Pinang	608	11,934	11,008	2,811	320,165	11,342,444	343,397	1	142,930	78
Perak	9,174	45,516	27,167	3,008	545,258	39,897,038	7,463,087		43,197	5,251
Selangor	2,896	25,143	21,515	3,117	262,060	18,064,933	5,236	213	127,110	48
N. Sembilan	1,899	39,667	33,986	16,084	241	21,187,714	32,068	101	384,402	1,108
Melaka	4,408	29,272	38,121	6,902	44,025	27,000,465	84,314		381,794	422
Johor	3,788	105,712	42,463	21,870	228,639	72,686,534	1,057,353	297	1,570,996	1,749
Pahang	11,604	124,530	32,433	18,826	3,893	12,915,104	11,284	1	87,029	2,061
Terengganu	8,935	85,155	29,798	6,892		2,749,331	26,453		78,916	745
Kelantan	5,425	96,109	41,501	33,589	645	3,727,520	62,144		9,349	230
W. Persekutuan		210	108							47
Jumlah S. M'sia *Total For P. M'sia*	**54,205**	**624,263**	**329,735**	**125,576**	**1,406,514**	**267,885,403**	**9,654,993**	**629**	**3,073,809**	**13,231**
Sabah	54,680	75,766	56,346	2,597	101,840	9,814,509	54,861	59	16,762	
Sarawak	5,436	10,452	12,964	1,821	334,074	34,278,682	298,400		340,917	1,121
JUMLAH BESAR *Grand Total*	**114,321**	**710,481**	**399,045**	**129,994**	**1,842,428**	**311,978,594**	**10,008,254**	**688**	**3,431,488**	**14,352**

E : Anggaran (Estimate) n.a : Tiada maklumat *(Not available)*

Source: (Department of Veterinary Science Malaysia, 2017)

Based on Thirumalaisamy et al. (2016), a study about cost effective feeding of poultry have been done. This experiment focuses on lowering feed costs by using low-cost feed ingredients, with the aim of improving feed quality and increasing production output. To minimize feed costs, scientific information about using low-cost locally available agro-industrial by-products in poultry feed must be improved. Since feed accounts for 60 to 70% of total production costs, any effort to reduce feed costs may result in a substantial reduction in total costs.

2.6 Feed Formulation and Nutrient Requirement

The purpose of feed formulation is to determine the volumes of ingredient and additives to blend to produce formulated feed that meet the nutrient requirement by animal as well as achieve the production goal at optimized cost (Lall & Dumas, 2015).

To formulate a good feed, there are some aspects that need to be concerned on. For instance, nutrient requirement for type of animals. Similar nutrient that contains in local ingredient can used as alternative ingredient to replace the imported ingredient. It is important to focus on proper formulation of feed to sustain the development of poultry industry.

Early in life, young quail have a high demand for dietary protein and amino acids, but as the bird grows older, these needs decrease. The recommended nutrient levels for breeding and growing birds vary from those for young birds. At all times, clean drinking water must be available. Water intake rises with age and is higher in lines bred to produce body mass (Cheng et al., 2010). Water, protein, carbohydrate, fat, minerals, and vitamins are the nutrients that make up a quail diet. Even though they are all essential, adequate water may be the most critical nutrient. Fresh, clean water should be always available to all birds, particularly in a tropical climate. Quails need at least twice the amount of water in weight as they do dry feed (Olawale, 2019). If there are too many salts in the feed or it is a humid, dry season, they may need more water. Table 2.5 shows the nutrient requirement of domestic quails under tropical conditions (Prabakaran, 2003; Altine et al., 2016).

Table 2.5: Nutrient requirement of domestic quails under tropical conditions.

	Quail broiler		Quail layer		
	0-2	3-5	0-2	3-5	
Nutrient	**Weeks**	**Weeks**	**Weeks**	**Weeks**	**> 6 weeks**
Metabolizable Energy (Kcal/kg)	2800	2900	2750	2700	2650
Protein (%)	27	24	24	20	19
Minerals					
Calcium (%)	0.8	0.6	0.8	0.6	3.0
Phosphorus %)	0.3	0.3	0.3	0.3	0.45
Vitamins					
Vitamins A (IU)	8000	8000	8000	8000	8000
Vitamin D3(ICU)	1200	1200	1200	1200	1200
Riboflavin, (mg)	6	6	6	6	6
Amino acids					
Lysine (%)	1.30	1.20	1.20	1.10	0.80
Methionine (%) Methionine + Cystine %	0.48 0.75	0.45 0.70	0.45 0.70	0.40 0.65	0.33 0.60

Source: (Prabakaran, 2003; Altine et al., 2016).

2.6.1 Protein Requirement

Proteins are large, complex molecules that play several important roles in the body. Proteins are made up of hundreds or thousands of smaller units known as amino acids that are linked in long chains. A protein is made up of 20 different types of amino acids that can be mixed in various ways. One of the most essential nutrients for quails is high-quality protein with a sufficient amino acid balance. It is also one of the costliest nutrients. Increased nitrogen excretion and lower feed performance for egg production result from high protein intake. The type of protein fed to quails must come from a high-quality source.

The amino acid composition of the feedstuff and the availability of these amino acids from the feedstuff following digestion in the gut are used to determine protein content. Hyankova et al. (1997) and Altine et al. (2016) recommended protein level of 26% for Japanese quail in the age of 1 to 21 day had a good performance and 21.6% of protein in the age of 22 to 35 days. It means that the requirement of protein was reduce with age. Babatunde et al. (2016) reported that Japanese quails fed 26% crude protein can increase the weight gain and it was suitable for optimum performance of quail. For first few weeks of growth, Reda et al. (2015) reports that 22% of crude protein are adequate while Jahanian and Edriss (2015) reported 26% of crude protein for the same period. During laying period, Soares et al. (2003) recommended 20% of crude protein higher than the 18% crude protein that recommended by Murakami et al. (1993) and Altine et al. (2016).

Several researched have been done about Japanese quail, mulberry leaves, and Madre de agua. Frempong et al. (2019) did research about the effect of replacing protein ingredient in poultry diet and total cost of gain. The aim of this research was to see how replacing fish meal with soybean meal or poultry by-product meal in broiler diets affected growth efficiency and total feed cost per kilogram of gain. In developing country, imported protein feed ingredient like soybean meal and corn meal have the highest cost among all the protein sources. The demand of these both ingredients are high due to their protein content. The experiment was conducted to determine the effect of replacing protein ingredient in broiler diet can help in gaining body weight and reducing the cost.

Crude protein and energy requirement of Japanese quail is one of research from Babatunde et al. (2016). The aim of this study was to examine into the energy and crude protein requirements of increasing Japanese quail in a tropical environment during the rearing period by feeding them different dietary levels of protein and energy. To make effective use of dietary protein, the diet must provide enough energy. It has been discovered that the energy to protein ratio, rather than the volume of protein, determines the production performance. It is important to know the percentage of protein needed by Japanese quail

2.6.2 Energy Requirement

Metabolizable Energy (ME) is the net energy left after fecal and urinary energy loss, and it reflects the energy required for growth and reproduction, as well as metabolic processes like work and respiration. For poultry, true metabolizable energy (TME) is the gross energy of the feed consumed minus the gross energy of the feed's excreta. Adequate amount of energy in poultry diet help in bird's maintenance, optimum growth, reproduction, and egg production. There is different amount of energy needed by Japanese quails depend on their age. A report from Reda et al. (2015) state that adequate energy level during the first few weeks of growth in Japanese quail is 2900 kcal while 3000 kcal were reported by Jahanian and Edriss (2015) for the same age. Besides, a result from Babatunde et al. (2016) state that the suitable metabolizable energy for optimum performance in term of weight gain in Japanese quail is 3200 kcal. Next, in temperate region, Olawale (2019) recommended that energy requirement for growing quail is 2600 kcal to 3000 kcal. It is crucial to analyze the effects of alternative dietary feed ingredients on poultry performance to incorporate them into current poultry diets properly. According to Muhammaed et al. (2015), increased fiber in feed ingredients has been shown to harm poultry digestion and nutrient absorption. However, the inclusion of high fiber ingredients at moderate levels, 60 – 120 g/kg of diet in broiler and layer diets, has no adverse effect on performance.

2.6.3 Fiber Utilization in Poultry

The use of fiber as a supplement in poultry diets is essential for poultry health and poultry production. Natural plant fiber sources could be used as a nutritional and economical alternative source to supplement the scavenging intake of free-range chickens (Muscat et al., 2019). Dietary fiber is a heterogeneous class of components not hydrolyzed by nonruminant animal digestive enzymes. As a result, they are the primary substrates for bacterial fermentation in the distal part of the gut. The most recent studies precisely identify the percentage of crude fiber for poultry; crude fiber could be in the range of 3 to 4 percent for a more extended period, while it could be applied by 5 percent for layers (Varastegani, 2018). In general, poultry-feed manufacturers and poultry producers believe that fiber content in poultry feed should be kept below 7%. Fiber is viewed negatively because it reduces production and chicken growth. It seeks to reduce the efficiency with which feed is utilized.

2.6.4 Vitamin and Mineral Requirement

There are several vitamins in animal diet. It can be categorized in two type which are fat and water soluble. Fat soluble consist of vitamin A, D, E and K while water soluble consist of B complex vitamin. Vitamin deficiencies in poultry can lead to poor feeding management. Minerals, including vitamins, must be given in sufficient quantities to all quails. Minerals are particularly important in breeder feeds. For eggshell formation, laying quail need a higher level of minerals. For proper bone formation and growth, young quails need a lot of minerals. Only laying quails are fed breeder feeds. If young quails are fed a breeder feed, they may develop slower and experience more tension. When young quails are fed a breeder feed, their development is stunted, and they are subjected to undue stress. Mineral also can be divided into micro and macro minerals. Large amount of mineral is call as macro mineral which are calcium, phosphorus, potassium, magnesium, sulphur, sodium, and chloride.

Calcium and phosphorus play a main role in quail feed since both elements help in makeup of bones in the body and essential for eggshell. A study from Shrivastav (2000) and Altine et al. (2016) state that a minimum of 0.8% of calcium needed in young quail diet and 0.48% as available phosphorus while 2.5% to 3% of calcium are needed for laying quails to help in eggshell development. In growing quail, 0.5% calcium was adequate in their feed (Altine et al., 2016). Some research reported that the best amount of calcium and phosphorus needed in layer quail are 3.2% and 0.6%. A high amount of calcium in the quail feed can lead to retarded growth and reduce its hatchability.

2.7 Central Composite Design (CCD)

The most common response surface designed experiment is a central composite design. A factorial or fractional factorial design with center points, augmented with a group of axial points that enable curvature estimation, is known as a central composite design. CCD can used to estimate first- and second-order words efficiently and add center and axial points to a previously completed factorial template to model a response variable with curvature. It can also be used to investigate the interactions between the different parameters that affect the process. To optimize the protein content in formulated feed, Response Surface Methodology (RSM) via CCD can be used. It can also be used to find the best protein content for a given parameter composition. CCD was used to find the best combination of selected parameters in poultry feed formulation using a regression model.

A previous study from Hamid et al. (2016) were to formulate fish feed with optimum lysine for American catfish. The aim of this research is to maximize lysine content in fish feeds by varying the percentages of various feed ingredients. The statistical method used in the optimization analysis was central composite design (CCD). RSM is used in this experiment to construct a prediction model for determining the best lysine content by varying the composition of the fish feed and ANOVA is used to determine the model's adequacy statistically. The experimental data collected using 30 different experiment or formulas. Six formulations were the repetition of the central point among the 30 formulations. From the result of this study, fish feed composition can be used as a guideline for the feed formulation.

Besides, there are also research about optimization of protein content in earthworm powder for catfish (Zakaria et al., 2012). This study was also conducted through CCD to know the optimum mixture of the parameter in the formulated feed according to regression model. In this study, there are 17 run that have been shown from CCD for the percentage of protein concentration of the ingredient for fish feed. As a result, CCD's optimization process revealed the main effect and interactions of the parameters that contributed to the highest crude protein in the fish feed formulation.

The function of CCD in a research study has been well used. The goal of the study from Zhang et al. (2021) was to enhance value of soybean meal (SBM) through solid-state fermentation (SSF) with *Bacillus subtilis natto (B. s. natto*) to overcome the limitation of SBM utilization in aquaculture feed. The response surface methodology (RSM) was used to examine the effect of fermentation process on the degree of protein hydrolysis (DH) and crude protein (CP) content. There are 30 runs that have been provided by CCD. Thus, CCD was used to determine the true optimal culture temperature, fermentation period, water–material fraction, and SBM layer thickness for improved DH and CP.

A study from Hassan et al. (2021) was conducted using CCD to investigate the effect of materials on the physicochemical properties of solid lipid nanoparticles. The topic of the paper is formulation and optimization of solid lipid nanoparticles to improve acyclovir oral bioavailability. This study necessitates many experiments, and the interaction of factors is complicated. The results of the experiments could also be interpreted incorrectly. To address this issue, during the design and development process, the use of the design of experiment methods such as Central Composite Design (CCD) of Response Surface Methodology (RSM) could simultaneously identify the interactive effect of various variables that influence the study's results. CCD has been used successfully in several studies for formulation development and optimization, as the data obtained with CCD showed strong and reliable estimations.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Materials and Method

The Madre de agua and mulberry leaves have been obtained from the local farm of individual persons in Pulau Pinang. Rice bran, broken rice, and fish meal were purchased from a local store in Kelantan. The other ingredients used in this experiment were palm oil, vitamin, mineral premix, limestone, salt, and methionine.

The equipment used in this study are weight scale, oven 105, Kjeldahl machine, Soxtec machine, Fibertex machine, muffle furnace, moisture balance machine, the crucible, and the others laboratory apparatus in FIAT laboratory, UMK Jeli. Microsoft Excel was used to record the data, and Win Feed software has been used to formulate the feed and CCD software.

3.2 Preparation Sample

Fresh leaves of Madre de agua and mulberry have been separated from the stem and dried at 60℃ for 24 h using an oven. The dried leaves then were crushed and ground to the size needed to pass a 1mm screen using a grinder. The leaves meal was stored in airtight plastic bags. All dried ingredients were grounded and sieved to a particle size of less than 500 mm. All four main ingredients, Madre de agua leaves meal (MDALM), mulberry leaves meal (MLM), rice bran (RB), and fish meal (FM), have been mixed with other ingredients such as broken rice, vitamin and mineral premix, palm oil, limestone salt, and methionine. All the ingredients were mixed by using a feed mixer. Then, the effect of different weight percentages of raw materials on the protein content was investigated in the optimization study.

3.3 Proximate Analysis

Typically, proximate analysis was used for determining the composition in the feed. It is expressed as a percentage (%) feed. Proximate analysis methods analyzed all the samples. The parameter that has been taken in this method was crude protein (CP), crude fiber (CF), ether extract (EE), ash, and moisture analysis. This process was conducted at Animal laboratory, UMK Jeli Campus.

Preparation samples of feed have been done first before undergoing the proximate analysis. The feed sample needs to be small in particles or in homogeneous materials to ease the proximate analysis and get an accurate result. The drying and grinding processes have been used to prepare the feed sample. The drying process was used to remove any water or moisture in the sample, while the grinding process made the sample become small particles.

The equipment that has been used to run this project were weighing scale, aluminum oil, air drying oven, Kjeldahl machine, Soxtec machine, Fibertec machine, muffle furnace, and moisture analyzer machine. The proximate composition of the feed sample was determined using methods developed by the Association of Official Analytical Chemists (AOAC, 2000).

3.3.1 Determination of Crude Protein

The crude protein content of MDALM, MLM, RB, FM and broken rice have been analyzed using the standard method of Kjeldahl nitrogen assay for their crude protein content. Kjeldahl developed a process involving the conversion of organic nitrogen into ammonium by sulphuric acid boiling and alkali distillation to release ammonia by titration process (Mohammad & Flowers, 2014).

The total amount of protein used in the feed or sample is known as crude protein. The Kjeldahl method was used in this process to know the crude protein. It involves three steps which are digestion, distillation, and titration. The nitrogen in feed samples was decomposed using a concentrated acid solution during the digestion process. A homogeneous sample is boiled in concentrated sulfuric acid to achieve this. As a result, an ammonium sulphate solution is formed. Following that, distillation is how NH4+ is converted to NH3. The excess base solution was added to the acid digestion mixture, and the ammonia NH3 gas was boiled and condensed in a receiving solution. Finally, titration is a technique for determining the amount of ammonia in a solution. The amount of nitrogen in a sample can be calculated using the quantified amount of ammonia ions in the obtaining solution.

Calculation,

$$CP\ (\%) = \frac{(Vsample - Vblank)\ \times 0.014\ \times N\ \times 6.25}{W} \times 100 \tag{3.1}$$

Where,

V = Volume of acid neutralized sample (ml)

N = Concentration of HCl

W = Weight of sample (g)

3.3.2 Determination of Crude Fiber (CF)

The crude fiber contained in sample can be determined through organic residue left after sequential extraction of sample with ether. This process has been run using Fibertec 8000 machine brand Foss. At first, 1 g of celite was added to the glass crucible. Then, 1 g of feed samples from the fat analysis was put in a glass crucible. Secondly, the samples were placed into the Fibertec 8000 machine for two and a half hours. After that, the samples were heated in the oven at 130 °C for 2 hr and then cooled for 20 min in a desiccator until it reached room temperature. Next, the samples were weighed and then put in the furnace Carbolite at 550 °C for 3 hr. Lastly, cooled the samples in a desiccator for 20 min and weighed again.

Calculation,

$$CF\ (\%) = \frac{W2 - W3 - C}{W1} \times 100 \tag{3.2}$$

Where,

W_1 = Weight of the sample

W_2 = Weight of Crucible and residue

W_3 = Weight of crucible and ash residue

C = Blank

3.3.3 Determination of Ether Extract (EE)

The ether extract is the amount of fat and oil in the feed sample. This analysis has been run using Soxtec 2055 machine brand Foss in Animal Laboratory, UMK Jeli. This technique involves four processes: boiling, rinsing, recovery, and pre-drying. 1 g of feed samples was placed in the thimbles fitted with metal adaptors, then loaded into a fully automated Soxtec fat extraction system. In brief, beakers that had been dried at 105 °C for 30 min in an oven and weighed were put in the extraction unit under each extraction thimble, and 80 ml petroleum ether was applied to each of the six extraction chambers. The extract was dried in an oven at 105 °C for 30 min after extraction and then cooled in a desiccator for 20 min until it reached room temperature before gravimetric fat determination. Lastly, the beakers were weighed, and the percent crude fat was calculated. There are several benefits of Automatic

Soxhlet extractors, including shorter extraction time, reduced extractant volume and simultaneous extraction of several samples (Shin & Park, 2015).

Calculation,

$$EE\ \% = \frac{Final\ cup\ weight\ (g) - Innitial\ cup\ weight\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

(3.3)

3.3.4 Determination of Ash

The total amount of minerals present in the feed sample is referred to as the ash content. The ash portion of the feed represents the inorganic content of the feed, which is mostly minerals. These are essential nutrients needed for stronger tissue, blood clotting, enzyme activation, muscle contraction and eggshell forming quantities in poultry diets (Ofori et al., 2019). The crucible was heated for 30 min at 105 °C and then cooled for 20 min to determine the ash content. The sample was then weighed and placed in the crucible. To determine the ash content, the crucible was placed in a muffle furnace at 550 °C for 6 hr. The crucible was then removed and placed in a desiccator to cool until it reached room temperature. Finally, the crucible was removed, and the ash was quickly weighed.

Calculation,

$$Ash\ \% = \frac{WBA - WAA}{WBA} \times 100$$

(3.4)

Where,

WBA = Weight before ashing

WAA – Weight after ashing

3.3.5 Determination of Moisture Content

Moisture analysis has been conducted because moisture is an essential nutrient in feed formulation. The moisture content is important because the physicochemical and pellet stability can be affected (Zainuddin et al., 2014). The high moisture content will cause the ingredient to spoil quickly due to fungal contamination. This causes the feed to become toxic to the animals, causing illness or even death. The sample's moisture content has been determined by using Moisture Balance MX 50 machine in Animal Laboratory, UMK Jeli.

3.4 Optimization Study and Ration Formulation

One of the most critical aspects of animal feed production is feed formulation. Balancing feedstuffs is a significant challenge for industries as customer demand and supply grow. The animal feed industry's development depends on the quality of feed, and the quality of feed is determined by the raw materials used to formulate feed. The ratio is the total amount of feed given to the animal daily. In contrast, ration formulation is the process by which different ingredients are combined in the proportion required to provide the animal with the proper amount of nutrients needed for a specific stage of production (Alfoyan et al., 2008).

The feed formulation in this study was involved commercial feed as control, three types of formulated feed with the inclusion of MDALM, and three types of formulated feed with the inclusion of MLM. The commercial feed was directly bought from a local seller, and the ration formulation for formulated feed has been calculated using Design Expert Software Version 7.1 through CCD.

Win Feed software was used to know the low level and high level of the parameter before formulating the feed using Design Expert software by fixing the minimum and maximum limit for nutrients needed. Then, the minimum and maximum level obtained from Win Feed has been inserted in Design Expert software as low and high levels of four-parameter used (Table 3.1). Design-Expert Software Version 7.1 through CCD has been used in this study to perform the optimization analysis in determining the optimum protein content (%) in parameter composition that was suggested. From the low and high levels of four-parameter, a total of 30 formulations have been given to be tested (Table 3.2). The three best formulations have been chosen to formulate feed and undergo proximate analysis to know the nutrient content in the feed formulated for Japanese quails (Table 3.3).

A validation process was carried out to ensure that the best combination of MDALM, MLM, RB and FM were used to provide the most protein. The target for each parameter has been set to be in the recommended range, while the responses, which were the protein content, have been set to the maximum.

Table 3.1: Parameter level for Design Expert Software.

Parameter	Unit	Low level (-)	High level (+)
MDALM *(x_1)*	% w/w	20	25
MLM *(x_2)*	% w/w	20	25
RB *(x_3)*	% w/w	5	10
FM *(x_4)*	% w/w	10	15

Table 3.2: 30 formulation of Japanese quail feed based on Design Expert Software

Run	**x_1 & x_2**	**x_3**	**x_4**	**BR**
1	29	30	19	19
2	29.5	27.5	19.5	19.5
3	29	30	20	20
4	29.5	27.5	19.5	19.5
5	29.5	22.5	19.5	19.5
6	29	30	20	19
7	29.5	27.5	19.5	19.5
8	30	25	20	20
9	29.5	27.5	19.5	19.5
10	30.5	27.5	19.5	19.5
11	30	30	20	19
12	29	25	19	19
13	29.5	27.5	19.5	20.5
14	29	25	20	20
15	30	25	20	19
16	29	30	19	20
17	29.5	27.5	20.5	19.5
18	30	30	19	20
19	29	25	19	20
20	29.5	27.5	19.5	18.5
21	29.5	27.5	19.5	19.5
22	29	25	20	19
23	30	30	19	19
24	28.5	27.5	19.5	19.5
25	30	25	19	20
26	30	25	19	19
27	29.5	27.5	19.5	19.5
28	29.5	32.5	19.5	19.5
29	30	30	20	20
30	29.5	27.5	18.5	19.5

Table 3.3: Three best formulation from Design Expert Software

Formulation	Leaves meal (x_1 & x_2)	Rice bran (x_3)	Fish meal (x_4)	Broken rice (BR)
1	28.5	27.5	19.5	19.5
2	29.5	27.5	19.5	19.5
3	30	25	20	20

The inclusion of two types of leaves meal in Japanese quail feed based on the three best formulations has been formulated. Table 3.4 shows three formulations with the inclusion of MDALM, and table 3.5 shows three formulations with the inclusion of MLM.

Table 3.4: Three formulation inclusion of MDALM

INGREDIENTS	F1		F2		F3	
	(%)	(g)	(%)	(g)	(%)	(g)
MDALM	27.8023	8.4285	29.0808	8.6334	29.5737	8.8721
RB	26.8268	8.1327	27.1092	8.048	24.6447	7.3935
Broken Rice	19.0227	5.7669	19.2229	5.7068	19.7158	5.9147
FM	19.0227	5.7669	19.2229	5.7068	19.7158	5.9147
Palm Oil	2	0.6	2	0.6	2	0.6
Mineral Premix	1	0.3	1	0.3	1	0.3
Vitamin	1	0.3	1	0.3	1	0.3
Limestone	1	0.3	1	0.3	1	0.3
Salt	0.35	0.105	0.35	0.105	0.35	0.105
Methionine	1	0.3	1	0.3	1	0.3
TOTAL	**100**	**30**	**100**	**30**	**100**	**30**

Table 3.5: Three formulation inclusion of MLM

INGREDIENTS	F1		F2		F3	
	(%)	(g)	(%)	(g)	(%)	(g)
MLM	27.8023	8.4285	29.0808	8.6334	29.5737	8.8721
RB	26.8268	8.1327	27.1092	8.048	24.6447	7.3935
Broken Rice	19.0227	5.7669	19.2229	5.7068	19.7158	5.9147
FM	19.0227	5.7669	19.2229	5.7068	19.7158	5.9147
Palm Oil	2	0.6	2	0.6	2	0.6
Mineral Premix	1	0.3	1	0.3	1	0.3
Vitamin	1	0.3	1	0.3	1	0.3
Limestone	1	0.3	1	0.3	1	0.3
Salt	0.35	0.105	0.35	0.105	0.35	0.105
Methionine	1	0.3	1	0.3	1	0.3
TOTAL	**100**	**30**	**100**	**30**	**100**	**30**

CHAPTER 4

RESULT AND DISCUSSION

4.1 Parameter used and the Nutrient Content in the Parameter

As shown in Table 4, the parameter used in this research was undergo proximate analysis to know the nutrient content inside the ingredient. In this study, the main ingredient (parameter) used to make the Japanese quails feed was MDALM, MLM, RB and FM while the others ingredient is broken rice, palm oil, vitamin, mineral premix, limestone, salt, and methionine. The proximate analysis procedure was analyzed the crude protein (CP), crude fiber (CF), ether extract (EE), ash, and moisture content of the ingredient.

Table 4.1: Nutritional value of ingredients based on proximate analysis

Ingredient	Crude Protein (%)	Crude Fiber (%)	Ether Extract (%)	Ash (%)	Moisture (%)
MDALM	19.9945	10.2758	3.8919	6.0666	10.57
MLM	25.2829	11.0597	5.8127	4.8651	8.81
RB	16.5715	6.7176	5.8993	7.4377	12.84
FM	55.3777	1.9552	9.8881	6.436	10.02
Broken Rice	8.87	1.1575	4.8742	4.0515	13.61

The inclusion of leaves meal in the quail diet aims to replace the imported and expensive protein ingredient since leaves meals are types of forage that easy to get. Both Madre de agua and Mulberry leaves are available in Asia and effortlessly grow. Besides, fishmeal is used as a source of highly digestible and high-quality animal protein ingredient in quail diets. Quail diets are supplemented with protein to provide the amino acids needed for maintenance, growth, and egg production. Crude protein is a reliable measure of how much protein is in feed results of laboratory tests which examine the chemical composition of the feed. As claimed by Soares et al. (2003), one of the most important nutrients for quails is a high-quality protein with an adequate amino acid balance. It is also one of the costliest nutrients.

From the proximate analysis result, FM has the highest in the crude protein followed by MLM, MDALM, RC and the lowest is broken rice. It is usual for FM to have a higher crude protein content because it is a superior animal feed ingredient due to its high protein content (Ween et al., 2017). Typically, protein content in high-quality FM is between 60% to 72% (Cho & Kim, 2010), but in this experiment, protein content in FM is 55.38%. There are some reasons why the FM used in this experiment has lower crude protein than the past studies. The losses of crude protein in the FM can occur during the storage because of contamination that can affect the quality. The usage of low-quality fish also affected the quality of the FM.

Between the two types of leaf meal, MLM contains higher crude protein content than MDALM. The protein content in the MDALM has almost the same value as the studies from Jaya et al. (2008), 18.21%, and 18%-20% from Nguyen et al. (1997). According to Riyadh et al. (2013), MLM content 29.8% of crude protein is a bit higher than the result obtained in this experiment. Hence, these two leaf meals are suitable for the main protein content that can contribute to feeding formulation and replace the imported protein ingredient in Malaysia.

As we can see from the table, protein content of rice bran is 16.57% which almost the same to research from Faria et al. (2012) that indicate the protein content of rice bran ranges from 16.61% - 19.38%. Besides, broken rice has the lowest crude protein content (8.87%), making it more of a filler than a protein source. Paper from Zhang et al. (2021) also state that the protein content in broken rice is 10.24% which are close from the result in this experiment. Hence, in this study only MDALM, MLM, RB, and FM were selected as parameters, the main protein content for feed formulation.

4.2 Nutrient Content in the Formulated Feed with the Inclusion of Madre De Agua Leaves Meal

Table 4.2 shows three best formulations of MDALM inclusion from Design Expert software and nutrient content of each formulation. These three types of feed prepared used in this research have been undergoing proximate analysis to know the nutritional value inside the feed. The formulation used to formulate this feed is based on the Design Expert software that has been run.

Table 4.2: Three best formulation and nutrient content of MDALM inclusion from CCD

FORMULATION	F1	F2	F3
MDALM (%)	28.5	29.5	30
RB (%)	27.5	27.5	25
FM (%)	19.5	19.5	20
Broken Rice (%)	19.5	19.5	20
PROXIMATE ANALYSIS			
CP (%)	25.6949	26.2739	25.6856
CF (%)	8.6444	8.4099	9.9199
EE (%)	8.6746	8.6649	10.905
Ash (%)	3.4228	3.2626	3.2537
Moisture (%)	12.74	11.6	11.71

From the proximate analysis, F2 has the highest result of CP, which is 26.2739%, while F1 and F3 were almost the same, which are 25.6949% and 25.6856%, even though F3 has the highest content of MDALM as shown in table 4.2. The high content of CP in F2 is because of the content of MDALM and the support from CP in rice bran. In this experiment, CP in rice bran was 16.57% which is slightly similar to the study from Bhosale (2015), 17.5 %. The content of rice bran in F2 helps to gain high protein content in F2. F2 is the best formulation with the inclusion of MDALM since it has the highest result of CP, and we know that CP is the most important nutrient for quail (Soares et. al., 2003). According to the formulation, the amount of MDALM in F2 is in the middle between F1 and F3. The amount of MDALM needed for F2 is not much as F3 so we can save the use of MDALM.

As we can see from the result obtained, MDALM can be used by the animal feed industry as a substitute for soybean meal where it can help reduce the feed cost and increase the farmer's income. MDALM can be one of the raw and primary materials in animal feed since it is easily found in Asia, and this tree can reproduce quickly with low onset disease. It can be an alternative food to animal feed, particularly in Japanese quail feed, because Malaysia is currently facing a significant problem of insufficient supply of essential raw materials as well as unreasonably high import fees that are affecting our country's economic crisis due to the animal feed industry's increased reliance on feed importation. Furthermore, a previous study from Libatique (2021) stated that inclusion of MDALM in the poultry diet has no negative effects on health conditions. Instead, it contributes significantly to the excellent health and wellness of poultry.

Meanwhile, a study on MDALM by Sarwatt et al. (2003) shows that feed intake, growth performance, and dressing percentage in rabbit diets increase with the inclusion of MDALM in their feed. Rosales (1997) reported that the protein in MDALM is balanced in essential amino acids, which are found to improve voluntary rabbits' feed intake. It was determined that MDALM could replace up to 270 g/kg of compounded rabbit feeds.

Jaya et al., (2008) discover the supplemented of MDALM in growing-finishing pig's feed. There is an effect on the feed consumption and feed cost with the MDALM inclusion in growing-finishing pigs diet while there is no effect on other parameters studied such as final weight, daily gain, and weight gain. Referring to Manaig (2017) study about the effect of MDALM and fermented leaves of Madre de agua (MDAFL) on the growth of grower-finisher pigs shows that the total weight gain, average daily gain, and feed conversion of pigs fed MDAFL performed significantly better than pigs fed the basal diet alone and pigs fed MDALM. Using MDAFL provided the greatest benefit in terms of costs and returns, followed by using MDALM. As a result, Madre de agua leaves in leaf meal and fermented forms can be used effectively with growing finishing pigs,

4.3 Nutrient Content in the Formulated Feed with the Inclusion of Mulberry Leaves Meal

Three best formulations of MLM inclusion from CCD and nutrient content of each formulation have been shown in table 4.3. From the table, the highest result of CP is from F2, which is 27.0116%, followed by F1 (26.9926%), and F3 is the lowest CP (26.1704%). The amount of CP in rice bran helps increase the amount of CP in F2 since we can see in the table that F3 has the highest content of MLM.

Table 4.3: Three best formulation and nutrient content of MLM inclusion from CCD

FORMULATION	F1	F2	F3
MLM (%)	28.5	29.5	30
RB (%)	27.5	27.5	25
FM (%)	19.5	19.5	20
Broken Rice (%)	19.5	19.5	20
PROXIMATE ANALYSIS			
CP (%)	26.9926	27.0116	26.1704
CF (%)	8.7726	7.4712	9.471
EE (%)	8.6609	9.1894	10.1279
Ash (%)	3.3042	3.0222	3.0016
Moisture (%)	11.12	10.33	10.81

Among three formulations with MLM inclusion, F2 is the best since it has the highest content of CP even though F3 contains a high amount of MLM and fish meal. The rice bran contains in F2 helps raise the CP content in F2 since it is higher than F3. Rice bran is one of the most used byproducts in animal feed. The price of rice bran has recently increased, though not as much as the price of fish meal. From the result, we can see that F2 can help save the cost of farmers in animal feed even though it uses a high amount of rice bran compared to F3 and can save the usage of MLM.

It is uncommon to feed mulberry leaves to poultry. One of the most severe issues in animal feed production is the high cost of feed raw materials, which raises costs and reduces profitability. This increases agricultural waste and waste from the feed processing industry in animal nutrition. Mulberry leaves are one type of leaf that can be used in animal breeding. It can be used to feed animals (Sengul et al., 2021). Hence, we know that MLM can be used in Japanese quails feed as a substitute for another pricy imported feed ingredient and can help reduce the cost. As a result, using leaf meal as a source of feed ingredients may not only reduce production costs but also improve the health of almost all animals (Libatique, 2021).

As state by Wang et al. (2017), mulberry leaves have previously been evaluated as a protein source for laying hens, and broilers (Al-Kirshi et al., 2009), sheep (Kandylis et al., 2009), beef cattle (Huyen et al., 2012) and pigs (Ly et al., 2001). So, it is a prove that MLM can be used as a potential feed staff for farm animals since it contains high CP and amino acid.

4.4 Best Formulation with Inclusion of MDALM and MLM

The best formulation of MDALM and MLM inclusion shown in table 4.4 was F2. F2 from both formulations was chosen as the best formulation based on the protein content obtained from the proximate analysis. As shown in the table, CP from MLM inclusion was higher than MDALM inclusion which is 27.0116%. This is because the protein content in MLM was higher than MDALM. So, the CP in MLM was affecting the CP in the formulation with the inclusion of MLM compared to MDALM. Both leaves are among farm animals' favorite and most essential leaf meals for healthy and optimum growth, but MLM has more protein content.

Table 4.4: Best formulation and nutrient content of feed with MDALM and MLM inclusion

FORMULATION	MDALM	MLM
Leaves Meal (%)	29.5	29.5
RB (%)	27.5	27.5
FM (%)	19.5	19.5
Broken Rice (%)	19.5	19.5
PROXIMATE ANALYSIS		
CP (%)	26.2739	27.0116
CF (%)	8.4099	7.4712
EE (%)	9.9199	9.1894
Ash (%)	3.2626	3.0222
Moisture (%)	11.6	10.33

Besides, MLM formulation is recommended since the CP obtained from the formulated feed with MLM inclusion are matched the nutrient requirement of zero to two weeks Japanese quail under tropical conditions based on a previous study from Prabakaran (2003), which are 27.0116% and 27%. Because Japanese quail grow so quickly, they require more protein and amino acids.

Based on commercial quail feed 2230 from 'Perternakan Hong Lee Sdn. Bhd.', the specification of nutrient content in the feed is minimum of 20% for protein, minimum 2.5% for far, maximum 6% for fiber, and maximum of 13.5% for moisture. So, we can see that the recommended formulation in this study has a greater amount of protein content. Typically, the commercial feed contains maize and soybean meal, which is known as the imported and expensive ingredient, same to this commercial feed from 'Perternakan Hong Lee Sdn. Bhd.'. Thus, farmers can use the recommended formulation from this study to reduce the feed cost. Besides, it also can increase Malaysian's economy by using local ingredients and stop depending on the imported ingredients. Farmer can replace the soybean and cornmeal with leaves meal. Besides, animal feed nutritionists can reformulate the formulation to obtain the required value of protein needed by the quail.

Prabakaran (2003) stated that protein contributes to approximately 90% of the total cost of the diet, and any attempts to save cash on the diet mean lowering the level of protein. So, leaf meals are the best alternative feed ingredient in quail ration to replace soybean meal and cornmeal which is often the expensive and major dietary plant poultry source. The usage of MDALM and MLM in quail feed can help reduce the feed cost and can help in increasing the profit. This statement can be supported by the study from Chowdary et al. (2009), which mentions that MLM supplementation at 10% would reduce the cost of poultry feed. MDALM and MLM are also easy to find in Asia since both plants adapt well to local tropical conditions. Leaf meal, on the other hand, has a higher crude protein content than the agro-industrial by-product (Tesfaye, et al, 2013).

Crude protein is very important in quail's diet since it is contributing to the quail performance. Generally, performance of quails improves as dietary crude protein level increase. Instead that, it will reduce performance and waste nitrogen by consuming an excessive amount of crude protein. It is not only a loss of benefit, but also a polluter of the environment (Dumont et al., 2017). So, farmer need to know the right amount of crude protein in quail's diet since it is very important.

The effect and function of crude fiber in poultry feed have been a source of debate. Feeders believe that the percentage of fiber should be kept low. In this study, the CF level for the best formulation of MLM inclusion is 7.47% while MDALM inclusion is 8.41%. The commonly recommended CF for quail's diet was only 3 to 5%. According to a study from Salami and Odunsi (2019) shows that broiler chickens can tolerate up to 8% of CF at optimal ME levels. Although most poultry producers and feed manufacturers believe that the fiber content of rations should be kept below 7%, an increase to 8-10% does not appear to have a significant impact on production (Salah, 2012).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study provides novel, helpful information on the use of MDALM and MLM as a supplemental source of protein in Japanese quail diets at different inclusion levels based on Design-Expert software. In this study, proximate analysis has figured out all chemical composition of ingredients and formulated feeds. All formulated feed has followed the formulation suggested by CCD in Design-Expert software.

Overall, the best formulation feed recommendation is F2 with MLM inclusion (29.5% MLM, 27.5% RB, 19.5%FM) as it gives the best result which the highest CP. Compared to commercial feed and other formulations with leaves meal inclusion, the CP content of F2 with MLM inclusion is relatively high. Unfortunately, the CF contain in the formulation are a bit high, so suggestion for minimum and maximum value of ingredient for CCD need to improve to get a better formulation that follow exactly the quail's requirement. As a result, the inclusion of MLM in F2, which can replace soybean and cornmeal in commercial feed formulation, is highly recommended for animal farm farmers looking to reduce production costs and increase profit.

5.2 Recommendation

In a nutshell, the more research is needed to confirm this study's findings and improve the formulation that followed the recommended nutrient for Japanese quail's diet. Furthermore, the feeding trial also needs to be carried by the next researcher so that the result will come out with the prove of quail performance. Aside from that, the feed formulation should be as close to the recommendation as possible.

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APPENDIX

APPENDIX A

Table A1: Crude Protein Analysis for Ingredient

SAMPLE		SAMPLE WEIGHT (g)	VOLUME HCl (ml)	% N	% CP	AVERAGE % CP
MDALM	1	1.124	25.3	3.141	19.6315	19.99446667
	2	1.156	26.9	3.248	20.3	
	3	1.131	26	3.2083	20.0519	
MLM	1	1.145	35.5	4.3315	27.0719	25.28293333
	2	1.201	33.4	3.8845	24.2781	
	3	1.301	36.5	3.9198	24.4988	
RB	1	1.163	23.4	2.8068	17.5425	16.57146667
	2	1.146	21.6	2.6284	16.4275	
	3	1.129	20.4	2.5191	15.7444	
BR	1	1.281	12.4	1.3452	8.4075	8.870033333
	2	1.292	13.4	1.4422	9.0138	
	3	1.296	13.7	1.4702	9.1888	
FM	1	1.276	79.4	8.7068	54.4175	55.37773333
	2	1.287	82.1	8.9263	55.7894	
	3	1.323	84.6	8.9482	55.9263	

Table A2: Crude Fiber Analysis for Ingredient

SAMPLE		SAMPLE WEIGHT	CRUCIBLE & RESIDUE	CRUCIBLE & ASH RESIDUE	% CRUDE FIBER	AVERAGE % CF
MDALM	1	1.147	30.8146	30.7135	8.814298	10.275753
	2	1.1099	29.985	29.8643	10.87485	
	3	1.0289	30.5617	30.4471	11.13811	
MLM	1	1.0872	30.3834	30.2633	11.04673	11.059676
	2	1.0298	29.851	29.7391	10.86619	
	3	1.0394	30.537	30.4199	11.26612	
RB	1	1.03	31.3039	31.2351	6.679612	6.7175994
	2	1.0358	30.9543	30.8863	6.564974	
	3	1.035	31.2834	31.2119	6.908213	
BR	1	1.0224	30.7369	30.7278	0.890063	1.1575406
	2	1.0528	30.5046	30.4916	1.234802	
	3	1.1278	30.5158	30.5006	1.347757	
FM	1	1.0355	30.4951	30.4804	1.419604	1.9552102
	2	1.17	30.3779	30.3515	2.25641	
	3	1.329	30.7089	30.6798	2.189616	

Table A3: Ether Extract Analysis for Ingredient

SAMPLE		SAMPLE WEIGHT	INITIAL CUP WEIGHT	FINAL CUP WEIGHT	% ETHER EXTRACT	AVERAGE % EE
MDALM	1	1.301	40.0009	40.0512	3.86625673	3.891923
	2	1.317	40.0111	40.0588	3.62186788	
	3	1.311	41.4259	41.4808	4.18764302	
MLM	1	1.323	40.2412	40.3143	5.52532124	5.812681
	2	1.293	41.4772	41.5501	5.63805104	
	3	1.216	39.0702	39.1465	6.27467105	
RB	1	1.367	40.8856	40.9689	6.0936357	5.899349
	2	1.361	40.6471	40.7312	6.17927994	
	3	1.329	40.8061	40.8782	5.42513168	
BR	1	1.349	41.4359	41.515	5.86360267	4.874185
	2	1.304	40.6744	40.7186	3.38957055	
	3	1.313	40.9251	40.9956	5.36938309	
FM	1	1.38	40.798	40.9471	10.8043478	9.888106
	2	1.38	40.7918	40.9318	10.1449275	
	3	1.323	40.9981	41.1134	8.71504157	
PO	1	1	41.345	42.1862	84.12	84.7
	2	1	40.3872	41.224	83.68	
	3	1	40.689	41.552	86.3	

Table A4: Ash Analysis for Ingredient

SAMPLE		SAMPLE WEIGHT	WEIGHT BEFORE ASHING	WEIGHT AFTER ASHING	% ASH	AVERAGE % ASH
MDALM	1	1.147	38.5648	36.2194	6.0817118	6.0665676
	2	1.1099	36.6274	34.5001	5.8079471	
	3	1.0289	36.4942	34.1914	6.3100438	
MLM	1	1.0872	34.8242	33.1168	4.9029123	4.8651061
	2	1.0298	35.2824	33.5874	4.804095	
	3	1.0394	36.2211	34.4505	4.888311	
RB	1	1.03	35.8846	33.3142	7.1629613	7.4376817
	2	1.0358	37.7729	34.9011	7.6028052	
	3	1.035	39.923	36.9099	7.5472785	
BR	1	1.0224	34.7301	33.3498	3.9743623	4.0514948
	2	1.0528	34.5276	33.1349	4.0335847	
	3	1.1278	34.0091	32.5989	4.1465373	
FM	1	1.0355	34.5386	32.2362	6.6661648	6.4359826
	2	1.17	36.1364	33.6601	6.8526472	
	3	1.0329	36.1522	34.0593	5.7891359	

Table A5: Moisture Content Analysis for Ingredient

SAMPLE		SAMPLE WEIGHT	% MOISTURE	AVERAGE % MOSITURE
MDALM	1	5.014	10.54%	10.57%
	2	5.066	10.66%	
	3	5.087	10.50%	
MLM	1	5.13	9.38%	8.81%
	2	5.096	8.51%	
	3	5.01	8.55%	
RB	1	5.032	13.78%	12.84%
	2	5.087	12.84%	
	3	5.028	11.90%	
BR	1	5.012	14.06%	13.61%
	2	5.009	13.17%	
	3	5.036	13.60%	
FM	1	5.02	10.56%	10.02%
	2	5.018	10.42%	
	3	5.052	9.09%	

APPENDIX B

Table B1: Crude Protein Analysis for Feed Formulation

SAMPLE	SAMPLE WEIGHT (g)	VOLUME TITRATION (ml)	%N	%CP
F1 (MDALM)	1.1382	33.5	4.111176	25.69485
F1 (MLM)	1.0503	32.5	4.321851	27.01157
F2 (MDALM)	1.0298	31	4.203816	26.27385
F2 (MLM)	1.1516	35.6	4.318817	26.99261
F3 (MDALM)	1.0977	32.3	4.109702	25.68564
F3 (MKM)	1.1309	33.9	4.187267	26.17042

Table B2: Crude Fiber Analysis for Feed Formulation

SAMPLE	SAMPLE WEIGHT (g)	CRUCIBLE & RESIDUE (g)	CRUCUBLE & ASH RESIDUE (g)	% CF
F1 (MDALM)	1.0342	29.5254	29.436	8.644363
F1 (MLM)	1.0111	29.8094	29.7207	8.772624
F2 (MDALM)	1.0226	30.2546	30.1686	8.409935
F2 (MLM)	1.0052	29.2437	29.1686	7.47115
F3 (MDALM)	1.0179	30.2886	30.2004	8.664898
F3 (MLM)	1.0284	29.7632	29.6658	9.471023

Table B3: Ether Extract Analysis for Feed Formulation

SAMPLE	SAMPLE WEIGHT (g)	INNITIAL CUP WEIGHT (g)	FINAL CUP WEIGHT (g)	% EE
F1 (MDALM)	1.1159	40.9956	41.0924	8.674612
F1 (MLM)	1.1142	40.8706	40.9671	8.660923
F2 (MDALM)	1.1734	41.0017	41.1181	9.919891
F2 (MLM)	1.1165	41.4034	41.506	9.189431
F3 (MDALM)	1.116	41.0559	41.1776	10.90502
F3 (MLM)	1.1177	39.278	39.3912	10.12794

Table B4: Ash Analysis for Feed Formulation

SAMPLE	SAMPLE WEIGHT (g)	WEIGHT BEFORE ASHING (g)	WEIGHT AFTER ASHING (g)	% ASH
F1 (MDALM)	1.1209	25.073	24.2148	3.422805
F1 (MLM)	1.1279	25.3104	24.4741	3.304175
F2 (MDALM)	1.1256	25.4058	24.5769	3.262641
F2 (MLM)	1.1241	28.9688	28.0933	3.022217
F3 (MDALM)	1.1145	25.036	24.2214	3.253715
F3 (MLM)	1.1931	26.2261	25.4389	3.00159

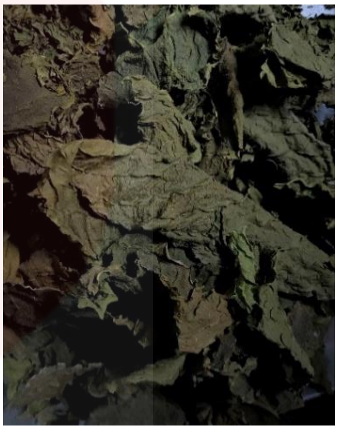
Table B5: Moisture Content Analysis for Feed Formulation

SAMPLE	SAMPLE WEIGHT (g)	MOISTURE (%)
F1 (MDALM)	1.107	12.74
F1 (MLM)	1.101	11.12
F2 (MDALM)	1.141	11.6
F2 (MLM)	1.105	10.33
F3 (MDALM)	1.088	11.71
F3 (MLM)	1.101	10.81

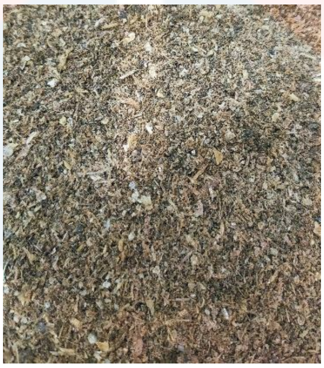
APPENDIX C


Fresh leaves Madre de agua


Fresh leaves Mulberry


Dried leaves


Rice bran


Fish meal

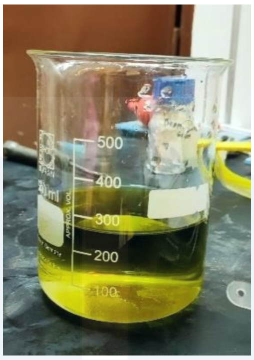

Broken rice

Boric acid

NaOH

1.25% NaOH and 1.25% Sulfhuric Acid for Crude Fiber analysis

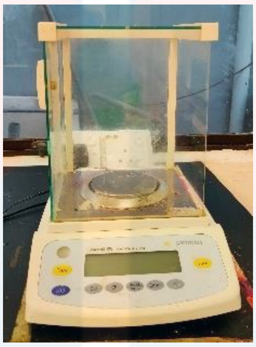
0.1M HCl and 40% NaOH for Crude Protein analysis



Bromocresol green solution

Bromocresol green and Methyl red solution as a receiver for distillation process in CP analysis


Magnetic Stirrer


Electronic Weighing Scale

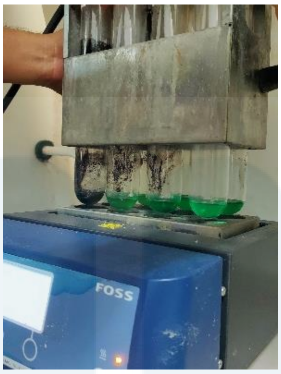

Moisture Balance machine


Process of grinding ingredient


Digestion machine for CP analysis


Process of digestion in CP analysis


Pouring acid solution into digestion tube after digestion process



Result of digestion process in CP analysis


Pouring 30 ml receiver solution in 250 ml conical flask

Distillation machine for CP analysis


Distillation process in CP analysis


Preparing receiver for distillation process

Titration in CP analysis


Process of titration to know the amount of N in sample

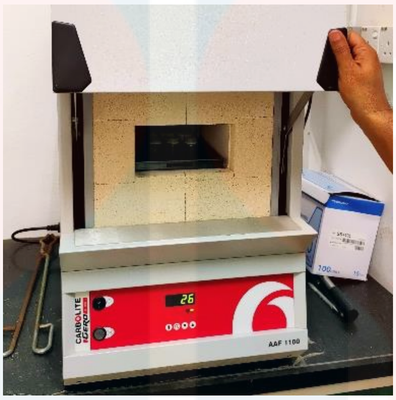

Result of titration for ingredients

Result of titration for feed formulation

Crude fiber analysis machine

Process of analysis crude fiber

Result of CF content in feed after run in the machine for 2 hr and half



CF furnace for sample after run at 2 hr and half

Cleaning the crucible of CF sample with acid sulfuric

Preparing for EE analysis

Muffle furnace for Ash analysis