Characterisation of Mulberry Leaf Pellet as Rabbit Feed

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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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Date :

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Supervisor Signature

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Date :

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CHARACTERISATION OF MULBERRY LEAF PELLET AS RABBIT FEED

ABSTRACT

Feed is one the essential factor for rabbit livestock production. Generally, commercial feed or concentrates are quite expensive although it has a significant impact on rabbit growth performance. However, there is abundance of tropical forages especially for multi-purpose trees such as Mulberry species (*Morus spp*.) which can act as alternative protein source in a partial or complete substitute for commercial feed. The objectives of this study were to assess and to compare the nutritive values and chemical composition of Mulberry leaf in pelleted form with commercial rabbit pellet using proximate analysis. The Mulberry leaf was formulated by mixing the leaves with binding agent and other materials to make Mulberry leaf pellet. The proximate composition of pellet was evaluated to check the dry matter, crude protein, crude fibre and crude fat and ash contents. Mulberry leaf contained 33.97±1.06 of DM content, 24.10±0.32 of CP, 41.55% of CF, 1.58±0.05 of EE and 10.15±0.09 of ash content. On the other hand, Mulberry leaf pellet contained 91.07±0.10 of DM content, 19.43±0.08 of CP, 8.17% of CF, 5.98%±0.04 of EE and 10.96±0.02 of ash content. In general, the chemical composition was shown a significant different between the groups except for ash and crude fiber analysis only. Hence, this finding also indicate that Mulberry leaf is an excellent feed for high yielding animal and can be offered either in fresh or dried in compounded feed. The data obtained will help the rabbit farmers in Malaysia to improve the utilization of underrated tropical forages especially for this multi-purpose tree as a lowly-cost of rabbit feed. Thus, Mulberry leaf pellet have potential to become as substitution for a pricey commercial rabbit pellet.

Keywords: Pelleted Mulberry leaf (*Morus spp.*), rabbit livestock production, commercial rabbit pellet, proximate composition

PENCIRIAN PELET DAUN MULBERI SEBAGAI MAKANAN ARNAB

ABSTRAK

Makanan haiwan adalah salah satu faktor penting untuk pengeluaran ternakan arnab. Secara umum, makanan komersial atau pelet agak mahal walaupun ia mempunyai kesan yang signifikan terhadap prestasi pertumbuhan arnab. Walau bagaimanapun, terdapat banyak makanan ternakan tropika terutama untuk pokok pelbagai guna seperti spesies Mulberi (*Morus spp.*) yang boleh bertindak sebagai sumber protein alternatif dalam pengganti sebahagian atau lengkap untuk makanan komersil. Objektif kajian ini adalah untuk menilai dan membandingkan nilai pemakanan dan komposisi kimia daun Mulberi dalam bentuk pelet dengan pelet komersil arnab menggunakan analisis proksim. Daun Mulberi dirumuskan dengan mencampurkan daun dengan agen mengikat dan bahan lain untuk membuat pelet daun Mulberi. Komposisi pelet proksimat dinilai untuk memeriksa bahan kering, protein mentah, serat mentah dan kandungan lemak dan abu mentah. Daun Mulberri mengandungi 33.97 ± 1.06 kandungan BH, 24.10 ± 0.32 PM, 41.55% SM, 1.58 ± 0.05 lemak dan 10.15 ± 0.09 kandungan abu. Sebaliknya, pelet daun mulberi mengandungi 91.07 ± 0.10 kandungan BH, 19.43 ± 0.08 PM, 8.17% SM, 5.98% ± 0.04 lemak dan 10.96 ± 0.02 kandungan abu. Analisis keseluruhan menunjukkan perbezaan yang signifikan antara kumpulan kecuali analisis serat dan serat mentah sahaja. Oleh itu, penyelidikan ini juga menunjukkan bahawa daun Mulberi adalah makanan yang baik untuk haiwan yang menghasilkan keuntungan tinggi dan boleh ditawarkan sama ada dalam makanan segar atau kering dalam makanan kering. Data yang diperolehi akan membantu para petani arnab di Malaysia untuk meningkatkan pemanfaatan makanan tropika yang kurang baik terutama untuk pokok pelbagai guna sebagai makanan rendah arnab. Oleh itu, pelet daun Mulberi mempunyai potensi untuk dijadikan sebagai pengganti untuk pelet komersil yang mahal.

Kata kunci : pelet daun Mulberi (*Morus spp.*), pengeluaran ternakan arnab, pelet komersil arnab, komposisi proksimat

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LIST OF SYMBOLS

^{O}C	Degree Celcius
G	Gram
H	Hour
m	Minute
s	Second
Kg	Kilogram
M	Metre
mL	Mililitre

LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ANOVA	Analysis of variance
CF	Crude Fiber
CP	Crude Protein
DM	Dry matter
EE	Ether extract
HCL	Hydrochloric acid
H_2SO_4	Sulphuric acid
H_3BO_3	Boric acid
NaOH	Sodium hydroxide
NFE	Nitrogen free extract
NPN	Non protein nitrogen

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CHAPTER 1

1.0 Introduction

1.1 Research background

Rabbit is known as a fast growing and productive non rodent animal that has potential to produce amount of meat in a shorter period of time. It can be raised at backyard barn and reproduce well from affordable forages. Thus, rabbit farming is one of the potential ways for small-scale backyard enterprises in order to generate profitable income source in developing countries as it is not only get demand for their meat, but also for their wool production and make as domestic pet. Although rabbit farming provides benefits, the farmers still have to face with problem related to lack of capital and knowledge as well as costly source of feed and management. Therefore, finding alternative source of feed from local forages is one of the effort that should be implemented as it can cut-cost of feed production for rabbit farmer in order to strengthen food security in developing country.

In tropical and developing country like Malaysia, there are abundance of woody plant in which can supply good potential for forage production in which later can be used as utilization for animal rations substituting a part of pricey protein source in livestock feed production. Martín *et al.* (n.d) reported that their ability to capture the light energy and in biomass production also are much effective than grasses. Mulberry (M*orus spp.*) is among likely species which can greatly adapt to tropical condition and easily integrated into livestock production system (Martín *et al.,* n.d). It also promotes elements of security in feed supply and maintain sustainability in environmental aspect (Benavides *et a*l., 1994). According to García *et al*. (2006), Mulberry also stands out from other multi-purpose trees due to its combination of good chemical composition and nutritional quality. Nutritionally, it contain about 15-25% of crude protein (CP), high energy level, good mineral composition and about 75-90% of dry matter digestibility (Benavides, 1999). Thus, Mulberry leaf pellet also have a great potential as an alternative protein source as well as replacing for a very costly commercial feed in rabbit feedstuff.

1.2 Problem statement

Feed is the major essential factor for rabbit livestock production. Generally, commercial feed or concentrates diet is expensive although it has a significant impact on rabbit growth performance. However, multi-purpose trees such as Mulberry species can act as alternative protein source in a partial or complete substitute for commercial feed. According to Bamikole *et al*. (2005) Mulberry leaf can supply feed intake and digestibility up to 50% and reduce the dependency toward commercial diet.

1.3 Hypothesis

H_O : The Mulberry leaf pellet cannot be partially substitute as commercial rabbit feed.

H_1 : The Mulberry leaf pellet can be partially substitute as commercial rabbit feed.

1.4 Aim and objectives

The general aim of this research was to study the characterization of Mulberry leaf in pelleted form as a rabbit feed. The specific objective of this study were:-

1. To assess of nutritive value Mulberry leaf in pelleted form
2. To compare the chemical composition of Mulberry leaf pellet and commercial rabbit pellet.

1.5 Scope of study

The uses of Mulberry (*Morus spp.*) leaf in the form of pellet and comparison of nutritive value with commercial rabbit pellet and by using proximate analysis.

1.6 Significance of study

Feed is the major contributing to the main expense in the livestock production especially when using the commercial pellet or concentrates diet. In order to maximize the meat production of rabbit as well as lowering the feed cost, the farmers need to apply tropical plant and forages as an utilization for rabbit nutrition. Thus, the Mulberry leaf have a great potential as feed pellet of rabbit due to their fast growing tree in a shorter time. Plus, the binding agent like cassava starch or molasses would improved the structure of pellet as well as reducing the dust. Dust can actually harm the respiratory tract of the rabbit.

1.7 Limitation of study

This study did not involve the digestibility of the rabbit and health performance when consuming the Mulberry leaf pellet. Then, unfavourable weather condition like raining would affect the Mulberry leaf pellet due to absorption of moisture in the surrounding area. The sick animals also can loss of appetite to the feeding trial which can effect data observation in the experiment.

CHAPTER 2

2.0 Literature review

2.1 New Zealand White Rabbit

McNitt, Lukefarhr, Cheeke and Patton (2013) reported that the origin and evolution of rabbits is still unclear to trace due their small and fragile bones which often destroyed by the predators. Originally, the rabbits were classified under rodents but now are placed under a separate order which is Lagomorpha because they have six incisor teeth instead of four incisor teeth like in rodents (McNitt *et al*., 2013). The lagomorphs are then separated into two major families which are pikas and rabbits and hares. Pikas or rock rabbits commonly inhabit high mountain areas while rabbits and hares usually living in the burrow. McNitt *et al* (2013) stated that, all the breeds of domestic rabbits are descendants of the European wild rabbit, *Orytolagus cuniculus*. Rabbit are being raised commercially for meat, wool, fur and also laboratory use as they are very useful in medical purpose. Plus, domestic rabbit can be made as pet as they are soft and delicate mammal.

New Zealand White rabbit is one of the well-known species among the meat production in rabbit species. Their origin was likely in United States (U.S). They can be recognized in white coat fur with upstanding ears and pinky red eyes. A male rabbit is called buck which can grow up to 4 to 5kg while doe is referring to female rabbit which can also grow up to 5 to 6kg (Lee & Wee, 2010). Next, their average life span can be up to 7 to 10 years as pet while 3 to 5 years in commercial production. The New Zealand White rabbit become stand out as premier meat rabbit due to its superior reproductive and growth performance as well demand for white-furred rabbits.

2.2 Digestive system of the rabbit

Rabbits are mono-gastric herbivores which only have a simple stomach and an enlarged hind-gut including caecum and colon. Their digestive system consists of oesophagus, liver, stomach, pancreas, small intestine, large intestine, caecum, colon and rectum. As bacterial protein synthesized in the hind-gut only make a little contribution to their protein need, the rabbit need to depend on dietary essential amino acids (McNitt *et al.*, 2013). *Bacteroides sp.* is the main microflora that living the gastrointestinal (GI) tract of the rabbit (Johson, 2006). McNitt *et al.* (2013) also mentioned about ruminants and rabbits do share a similar characteristic related to their gut bacteria which means that both of them can synthesize adequate amounts of B vitamins.

2.3 Caecotrophy in rabbit

The act of consuming any cecal content is called caecotrophy. Although it sound unsavory, but it is consider normal behaviour pattern for many kinds of animal including rabbit (McNitt *et al.*, 2013). Normally, rabbit excrete two type of feces either hard feces of soft feces. Hard feces are usually produce during day time in the large intestine while the soft feces or also known as caecotrophs are produce in the cecum during night time. According to Buseth and Saunders (2015), the hard feces are usually fibrous, brown droppings which are mainly consist of undigested hay and grass. Plus, they also should be round, odourless, firm with no liquid. In contrast, the caecotrophs are dark, mushy, smelly and grape like balls pressed together (Buseth & Saunders, 2015). Plus, the rabbit usually eat them directly from the anus via coprophagy during night. The principle nutritional regarding caecotrophy in rabbit is by providing the B vitamin requirements due to its high concentrations of B-complex vitamins. As a result, rabbits do not require vitamin B in their diet instead. Buseth and Saunders (2015) stated that, caecotrophs approximately contain twice amount of the protein and half the amount of fibre compared to the normal droppings. Some evidence shown that bacteria from these re-ingested caecotrophs help to complete the food digestion while in the stomach (Laura Tessmer *et al*, 1998).

2.4 Nutrition diet in rabbit

Rabbits have a special and delicate digestive system and thus it is essential to take this into consideration especially when planning their proper diet. Imbalance diet is a sure ticket to bad health and even a shortened life span of rabbit. Thus, a proper balance diet for rabbit should consist at least 85-90% of hay, grass and dried grass (Buseth, 2015). Buseth and Saunders (2015) also mentioned that a good guideline amount is about the volume of same size as the rabbit. Plus, a rabbit only need a limited amount of high-fibre of pellets for mineral and vitamin supplementary. Providing green leafy salad and fresh water also should be part of their daily diet (Buseth & Saunders, 2015).

2.4.1 Carbohydrate

Generally, carbohydrates are made up carbon, hydrogen and oxygen. They are synthesized by plants from carbon dioxide and water by using solar energy through a process called photosynthesis. Glucose is the simplest carbohydrate product. The most essential carbohydrates in rabbit feed are starch and cellulose. Both of them are consist entirely of glucose. Starch is usually found in cereal grain and tubers like potato or cassava while cellulose is the major structure components of plant fiber. According to McNitt *et al.* (2013), only certain animals that have bacteria in their gut can digest cellulose such as ruminant and rabbit. Although rabbit has bacterial population in their hind-gut, this animal can only digest minor amount of cellulose (McNitt *et al.,* 2013).

McNitt *et al.* (2013) also reported that the main function of carbohydrates in rabbit diets is to supply energy as well as maintaining the integrity of the digestive tract. Rabbit are dependent on nourishment derived from hay, various type of grass and leaves while sugar content in non-leafy vegetables and fruits like carrot is quite higher compared to their natural diet. Their digestive system is not designed for eating sugar and starch and thus carrot, apple or grain should be avoided or minimize in order to prevent digestive system disorder.

2.4.2 Crude protein (CP)

Protein is a major component of muscle tissue, cell membranes, certain hormones and all enzymes. They build up a basic units called amino acid. Essential amino acid is refer to incapable to manufacture by animal itself and must be consumed in the diet like arginine, histidine, or lysine. Protein is digested primarily takes place in small intestine by several enzymes secreted from pancreas like trypsin consist of process breaking the bond that join amino acid together in that protein. Plus, protein also plays a vital in bodybuilding, repair of tissue and protection of body surface for defense against invading organism (Akande, 2015). Halls (2010) also reported that rabbit can actually increase their amino acid intake by consuming their soft feces or cecotrophy. Thus, as the rabbits are low in diet protein quality, the microbial protein in cecotrophs will significantly improve the absorbance of amino acid.

Table 2.1: Crude protein requirements for rabbits

Rabbit stage (production type)	**Crude protein requirements (%)**	**Author (sources)**
Fur (hair) producing	17	Church (1991)
Lactating	18 17 16	Pond *et al.* (1995) NRC [National Research Council] (1977) Pond *et al.* (1995)
Growing (young) rabbits	16 15	NRC [National Research Council] (1977) Adegbola (1991)
Gestation (pregnancy)	18	NRC [National Research Council] (1977)
Breeding	17-18	FAO [Food and Agriculture Organization] (1997)
Maintenance	12	NRC [National Research Council] (1977)
Fattening	15-16	FAO [Food and Agriculture Organization] (1997)

In rabbit diet, the content of amino acid should be adequate. Higher dietary protein level should be supplied for young, gestation as well as lactation of stages in rabbit life for maintenance purposes. According to summarization in Table 2.1 stated that, the dietary protein requirement is about 16% for maximum growth and 18% for lactation stages. Another recommended crude protein levels of 12%, 18%, and 16% for maintenance, pregnancy and growth stages respectively. Soybean meal, cottonseed meal, alfalfa meal and other forages also good sources of protein that been used in rabbit diet as protein supplements (McNitt *et al.*, 2013).

2.4.3. Crude Fibre

Irlbeck (2001) demonstrated that gastrointestinal tract in rabbits features in a simple stomach, a well-developed caecum and a particle dependent separation mechanism with a proximal colon. It has commonly been assumed that enlarged hind-gut (caecum and colon) is an area that functionally similar to the rumen of cattle. However it is not true since there is no requirement for dietary amino acid in ruminant because bacterial protein synthesized in the rumen. In contrast, the bacterial protein synthesized in the hind-gut makes very little contribution to their protein need and thus the rabbit are depending on dietary essential acids. Plus, cattle can digest fibrous feeds, due to rumen bacteria produce cellulase, which breaks down cellulose while rabbit do not digest fiber efficiently (Mcnitt *et al,* 2013).

Cave (2012) has defined that dietary fibre as the edible portion of plants or analogous carbohydrates that are resistant to digestion and absorption in the small intestine, which are the available to be completely or partially fermented by resident microbiota in the distal small intestine and large intestine. Dietary fibre can be divided into slowly fermentable or rapidly fermentable in the colon. In fact, the fibre fraction of feed is poorly digested by rabbits. This can be proven by the illustrated in Table 2 on the digestibility of alfalfa hay fiber by various animals.

Table 2.2. Digestibility of the alfalfa hay fiber by various animals.

Animal	**Percent fiber digestibility**
Cattle	44
Sheep	45
Goats	41
Horses	41
Pigs	22
Rabbits	14

Adapted from Maynard, L.A *et al.* 1979. Animal Nutrition (7th ed.) McGraw-Hill Book Co. New York, p.31

Table 2 shown result that rabbit is lowest percent fibre digestibility compared to other animals. This contradiction can be explained by recognizing that fiber make up only 20-25 percent of forages. Thus, forage like alfalfa meal is 75 to 80 percent non-fibre. Macnitt *et al.* (2013) reported that European studies have suggested that there is separation of large and small particle in the caecum since rabbits can efficiently digest non-fiber fraction, such as the protein and soluble carbohydrates and excretes the fiber fraction. The small particles are retained for further digestion whereas the large particle such as fiber are rapidly excreted. The ability of rabbit to utilized high fibre dietary fiber levels of alfalfa and other forages is due to high intake of these low energy feeds with rapid excretion of fibre and efficient digestion of the non-fibre component (Macnitt *et al*, 2013).

Table 2.3. Nutrient requirements of crude fibre (CF) in rabbit

Rabbit stage (production type)	**Crude fibre requirements (%)**	**Author (sources)**
Growth	10-12 14 14-18	NRC [Nattional Research Council] (1977) Lebas (1980) Gidenne & Lebas (2002)
Maintenance	14 15 -16 14-18	NRC [National Research Council] (1977) F. Lebas (1980) Gidenne & Lebas (2002)
Gestation (pregnancy)	10-12 14	NRC [National Research Council] (1977) F.Lebas (1980)
Lactation	10-12 14	NRC [National Research Council] (1977) F.Lebas (1980)

Although fibre is not useful as an energy source of rabbit, it is very essential components of rabbit feeds. Numerous studies have shown that low fibre diets promote increased enteritis and prevent fur chewing (Macnitt *et al.,* 2013). Irlbeck (2001) and Camphbell-Ward (2012) further discussed about fibre provides an abrasive effect on teeth essential to prevent their overgrowth, which lead to malocclusion, maldigestion and other health problem. While increasing fibre levels reduces the soluble carbohydrate level, which aid in reducing carbohydrates overload of the hind-gut.

2.4.3 Fat

According to Institut Nationale de la Recherche Agronomique [INRA] (1989), rabbits have no specific fat requirement apart from small amount of essential fatty acid (EFAs). Thus, this requirement is easily to meet by the lipid contained in the conventional raw materials used in the feed formulation. Traditionally, rabbit feed is formulated based on low or moderate energy diet without adding any pure fats or oil as well as their crude fat content also does not exceed 30-35kg^{-1} (Xicatto, 2010). However, Maertens (1998) suggested that the addition of limited amount of fats about 10-30kg^{-1} is currently common under intensive rearing systems. In weaning rabbits, the dietary condition of fat may improve body condition, stimulate development of the immune system and boost health (Xicatto et al., 2003; Maertens et al., 2005).

2.4.4 Vitamin and Mineral

A group of complex organic compounds that are present in natural feeds is called vitamin and it is essential for nutrient metabolism and life. It is differ from trace mineral which is from inorganic compound. Generally, vitamins are classified based on their solubility which Vitamins A,D,E and K are fat soluble whereas all the other like B-complexes and vitamin C are soluble in water. According to Mateos, Rebollar and Blas (2010), fat soluble vitamins are stored in the body while water soluble vitamins are rapidly excreted, the exception being vitamin B_{12}. Since rabbit have a functional hind-gut, they prefer the need of supplementation for fat soluble vitamins much higher compared to water soluble vitamins. Mateos *et al.* (2010) reported that the deficiency of vitamin in the diet can cause a lower performance and often associated with pathological disease.

In term of mineral, macro-minerals are defined as those elements including calcium, phosphorus, magnesium, sodium, potassium, chloride and sulphur that required in grams per day and are expressed as g kg^{-1} (Mateos, 2010). Thus, Mateos et al. (2010) also discussed about dietary calcium and phosphorus level below requirements will lead to rickets in young rabbit, osteomalacia in adult rabbits, lack of fertility in does as well as abnormal behaviour. In contrast, trace mineral are those elements expressed as mg kg^{-1} or ppm in the diet including iron, copper,manganese, zinc, selenium, iodine and cobalt. Plus, these trace elements are routinely added to rabbit diet as salts through a premix (Mateos, 2010).

2.5 Mulberry leaves

According Sánchez (2002), the scientific name of Mulberry is known as *Morus spp.* and its genus belonging to Moraceae family of the Urticales subclass. Traditionally, the Mulberry is usually associated with the production of silkworm (*Bombyx mori*) or also known as sericulture. Food and Agriculture Organization [FAO] (1990) stated that the domestication of Mulberry started several thousand years ago as requirement for silkworm production. In origin, most cultivated Mulberry varieties are believed from Japan/China area and in the Himalayan foothills (Sánchez, 2002).

Singh and Makkar (2002) reported that Mulberry is monoecious, occasionally dioecious shrub or moderate-sized tree with a fairly cylindrical straight bole. It can be grown up to 3.0 m in high and 1.8 m in girth. Their leaves also variable, ovate or broadly ovate, serrate or crenate-serrate and often deeply lobed (Singh & Makkar, 2002). Plus, Mulberry tree also capable to grow rapidly in the early stages as well as reaching maturity at an early age. Thus, it can be potential as animal feed due to their fast growing tree and easily integrated into livestock production system.

In other hand, Mulberry can be categorized as an ideal supplement in most forages diets as their nutritional quality produced by Mulberry leaves itself is equivalent to the grain-based concentrates. As a result, many utilization of this tropical forages had been done for animal feed production as substitution for conventional commercial feed.

2.6 Pellet quality

A good rabbit feed should be in pellet form with a diameter of 3-4 mm and contain a low proportion of fines. According to Acedo-Rico, Mendez and Santoma (2010), pelleting is the process where to transform meal into compact pellets of cylindrical shape. Different factors associated within the pelleting process result the final pellet quality. Behnke (1996) reported that formulation, conditioning, pelleting,die or rolls and cooling are the main influences in pellet quality and their contribution percentage are shown in Figure 2.1.

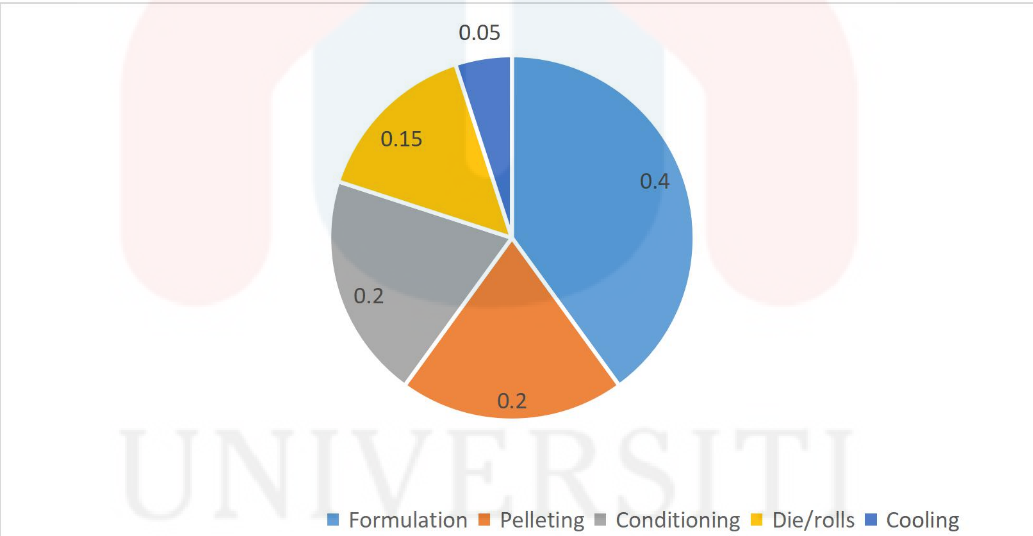

Figure 2.1. Main factors influences on pellet quality (Behnke, 1996)

Formulation is the major contribution as it is not only including raw material in the feed but also how these have been ground (Acedo-Rico, 2010). For instance, the large particle size which is above 1.5 mm can block the passage of pelleting whereas very small particle size can promote digestive and respiratory problems in the rabbit.

CHAPTER 3

3.0 Methodology

3.1 Formulation of Mulberry leaf pellet

The Mulberry leaf pellet were prepared from whole fresh leaves that were chopped , sun-dried for 2 to 3 days and grind and then mixed with other binder ingredients. The mixture of Mulberry leaf were put through pelleting machine and the pellets were sun dried again for 2 to 3 days in order to reduce the moisture.

Table 3.1. Feed formulation for pelleted Mulberry leaf

Ingredients	Percentages(%)
Mulberry leaf	90
Cassava starch/ tapioca starch	5
Molasses	5

3.2 Proximate analysis of feed sample

The Mulberry leaf, Mulberry leaf pellet and commercial pellet from Cargill brand sample were analysed for dry matter (DM), crude protein (CP), crude fibre (CF), Ether extract (EE), ash and nitrogen free extract (NFE) content. Different methods and equipment were conducted by using standard methods of Association of Official Analytical Chemists [AOAC] (1995).

3.2.1 Dry matter analysis

Two grams of each feed sample groups were taken for dry matter analysis with three replicates respectively. The fresh mulberry leaf were weighed using electronic balance in the laboratory right after the process of sun dried of the pellet finished. The process should be done quickly before the pellet absorb moisture from surrounding environment. Then, all the feed samples were oven dried for 24 hours at 105°C (AOAC International, 1995). The next day, the dry weight were measured in order to determine the dry matter content in feed samples were calculated using equation (3.1) :

$$\%DM = \frac{W_f}{W_i} \times 100 \qquad (3.1)$$

Where,

%DM = Percentage of DM

W_i = Initial weight of sample

W_f = Final weight of sample

3.2.2 Crude Protein analysis

The crude protein of the feed samples were analysed using Kjeldahl method which was divided into three part including (1) Digestion, (2) Distillation and (3) Titration. The equipment used in that method were Gerhardt Kjeldaterm and Gerhardt Vapodest.

3.2.2.1 Digestion

About 1gram of each sample was weighed and inserted to the digestion tube filled with a piece Kjeltab tablet in each tube respectively. Next, 12 mL of concentrated H_2SO_4 was added into each tube in the fume chamber before placed them into digestion rack. Before that, the digestion block of Gerhardt Kjeldaterm was turned on and set up to 400^o as pre-heating. The sample were put into digestion rack and the lids were closed tightly on the top of digestion tube in order to prevent the vaporised of H_2SO_4 from escaping. Then, the samples were left for digestion to be completely finish and let it cool. The result from digestion process shown that the colour of the sample turn blueish from dark colour.

3.2.2.2 Distillation

The preparation of receiver should be done before distillation process. The receiver was prepared by adding 250 mL of distilled water into a beaker. Next, 10 g of boric acid (H_3BO_3), 2.50 mL of bromocresol green and 1.75 of methyl red were added and placed on the hot plate for stirring purposes. Before use the Gerhardt Vapodest distillation machine, it need to run for clean the system. Thus, it must be repeated for each new samples. After the sample from digestion cooled down, the digested sample was diluted with 80mL of distilled water and 50 mL 45% of sodium hydroxide (NaOH) before transferred into distillation unit. An amount of 30 mL of receiver was put on the receiver platform and distilled for a few minutes. Next, the distillates which are green in colour are collected whereas receiving flask was removed from the unit for titration process.

3.2.2.3 Titration

The distilled receiver was then titrated with standard 0.1M hydrochloric acid (HCL) to reach pink colorization end point. The volume of HCL used for the titration was recorded. The determination of CP content in the sample was calculated using equation (3.2):

$$\%N = \frac{(T-B) \times N \times 1.4007 \times 100}{\text{Weight of sample (mg)}} \quad (3.2)$$

$$\%CP = \%N \times F$$

Where,

%N = Percentage of nitrogen in the sample

T = Volume of titrant used for feed sample

B = Volume of titrant used for blank sample

N = Normality of titrant

%CP = percentage of CP

F = Conversion factor for nitrogen to protein

3.2.3 Crude Fibre analysis

As for the crude fiber analysis, the samples were sent into Makmal Analisis MARDI at Serdang, Selangor. The sample was sent on 11th October 2019 through postage. The result from the analysis was sent through email one month later.

3.2.4 Ether extract/Crude Fat analysis

The fat content of the feed sample from mulberry leaves pellet were analysed by using the Soxtec method. The empty cups were firstly heated in a furnace for 30 minutes and let it cool in dessicator for 20 minutes to get stable heat before being weighed as initial cup. Then, samples were weighed about 2.5g before put in the sample tube containing folded cotton. The cups were filled with 80 mL of petroleum ether. The sample tube were inserted into the machine by moving up the thimble and attached the magnet with the top of sample tube while the cups containing petroleum ether were put below the sample tube with a cup holder. The temperature and program was set up properly and the water tap is opened for the reflux condensers each time before run the machine to prevent the petroleum ether evaporation from the condensers. The thimbles need to push down in each time of the beeping sound produce. After finished, the cups need to heated in furnace again for 30 minutes and let it cool for 20 minutes before weight as final weight of cup. The fat content also was determined and measured by sing equation (3.3):

$$\%EE = \frac{W_f - W_i}{W_s} \times 100 \quad (3.3)$$

Where,

%EE = Percentage of extract ether (EE)

W_i = Initial weight of the aluminium cup

W_f = Final weight of the aluminium cup

W_s = Weight of the samples

3.2.5 Ash content analysis

The crucibles were weighed as initial weight. The feed sample were weight about 1g each before placed in the crucible and incinerated in the furnace at temperature about 500-600°C for one hour and let it cooled in the desiccator for overnight along. Then, final weight of the crucible containing the ash was recorded. The determination of ash content were calculated using formula given as equation (3.4):

$$\% \text{ Ash} = \frac{W_f - W_i}{W_s} \times 100 \qquad (3.4)$$

Where,

% Ash = Percentage of ash

W_i = Weight of the crucible with sample

W_f = Weight of the crucible with ash

W_s = Weight of the sample

3.2.6 Nitrogen free extract analysis

The nitrogen free extract content of the feed sample were analysed using equation (3.5):

$$\%NFE = 100 - (\%CF + \%CP + \%EE + \%Ash) \qquad (3.5)$$

Where,

% NFE = Percentage of NFE

3.3 Data analysis

All the data obtained from the laboratory analysis were recorded in Microsoft Excel. An one way Anova was used to calculate the data to determine the significant different between the groups in each analysis.

CHAPTER 4

4.0 Result and Discussion

4.1 Chemical composition of Mulberry leaf pellet, commercial pellet and Mulberry leaf.

Based on the proximate analysis, Mulberry leaf pellet had the highest dry matter content (%) which were 91.07% ± 0.10 compared to commercial pellet and mulberry leaf only at 89.43% ± 0.04 and 33.97% ± 1.06 respectively. However, in term of crude protein content (%), Mulberry leaf contain 24.10% ± 0.32 which was the highest among the feed samples, followed by Mulberry leaf pellet was 19.43% ± 0.08 and commercial pellet is at 17.20% ± 0.16. Next, Mulberry leaf contained higher crude fibre content (41.55%) than commercial pellet and Mulberry leaf pellet which were 18.00% and 8.17% respectively. But ether extract (EE) and ash content (%) of the Mulberry leaf pellet were the highest compared to commercial pellet and Mulberry leaf which is 5.98% ± 0.04 and 10.96% ± 0.02 respectively. Commercial pellet contained 2.24% ± 0.04 of EE and 10.84% ± 0.37 of ash content whereas Mulberry leaf contain 1.58% ± 0.05 of EE and ash content of 10.15% ± 0.09 respectively. An one-way ANOVA revealed that all the analysis shown that there were a significant different ($p<0.05$) between the groups except for the ash determination analysis only obtained p value bigger than 0.05 and thus resulting no significant different among them.

Table 4.1.1: Chemical composition (%) of Mulberry leaves pellet, commercial pellet and mulberry leaves

(Mean±SE)

Constituents	Mulberry leaves	Mulberry leaf pellet	Commercial pellet	P value
Dry matter (DM) %	33.97 ± 1.06	91.07 ± 0.10	89.43 ± 0.04	0.00
Moisture (%)	66.02 ± 1.07	8.92 ± 0.10	10.57 ± 0.04	0.00
Crude protein (%)	24.10 ± 0.32	19.43 ± 0.08	17.20 ± 0.16	0.00
Crude fiber (%)	41.55	8.17	18.00	-
Ether extract (%)	1.58 ± 0.05	5.98 ± 0.04	2.24 ± 0.04	0.00
Ash content (%)	10.15 ± 0.09	10.96 ± 0.02	10.84 ± 0.37	0.08

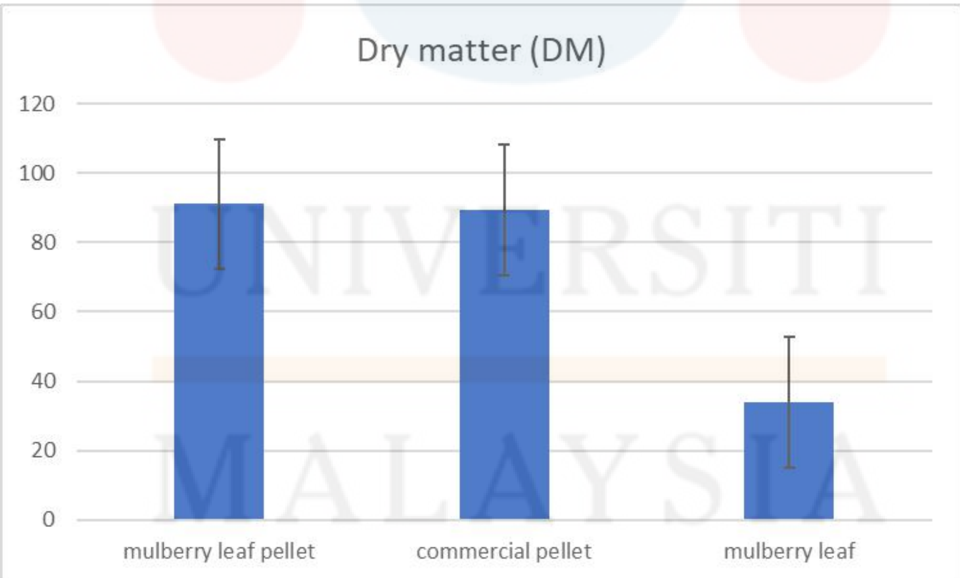

Figure 4.1 Mean of dry matter (DM) content between the groups

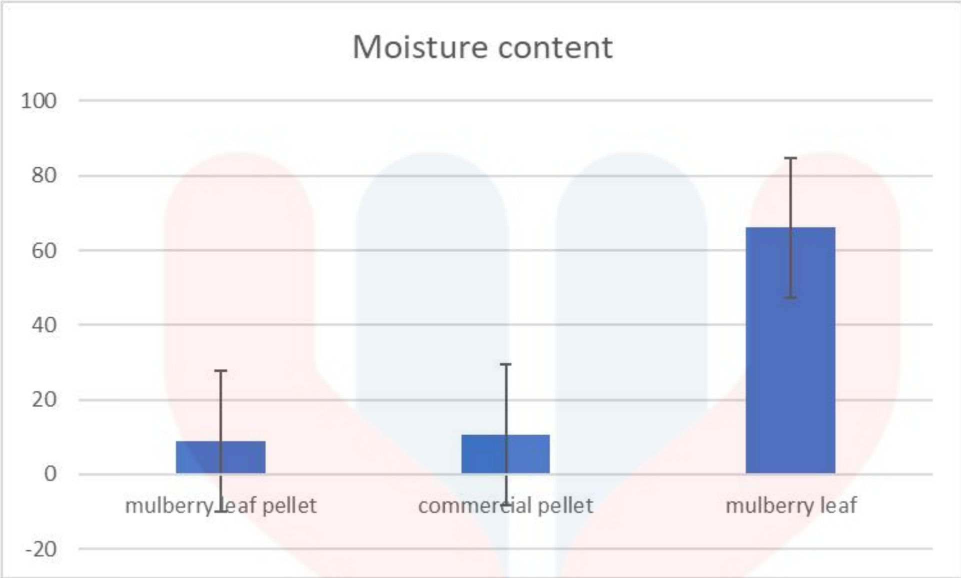

Figure 4.2 Mean of moisture content between groups

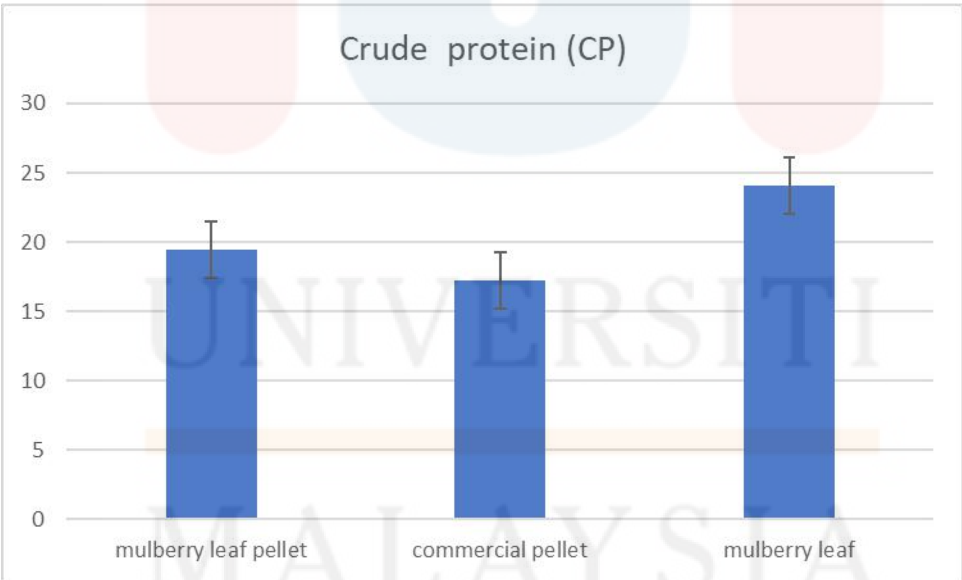

Figure 4.3 Mean of crude protein (CP) content between the groups

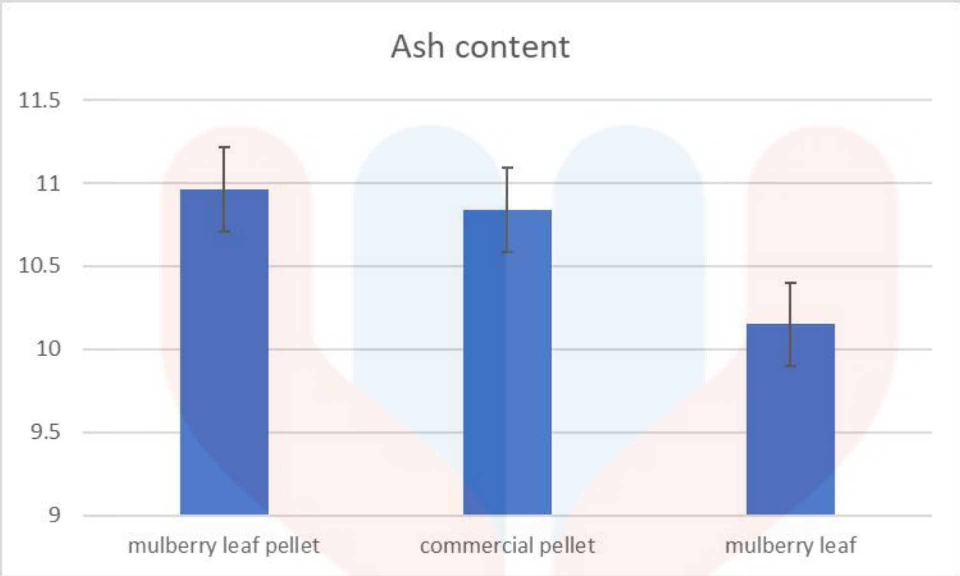

Figure 4.4 Mean of ash content between the groups

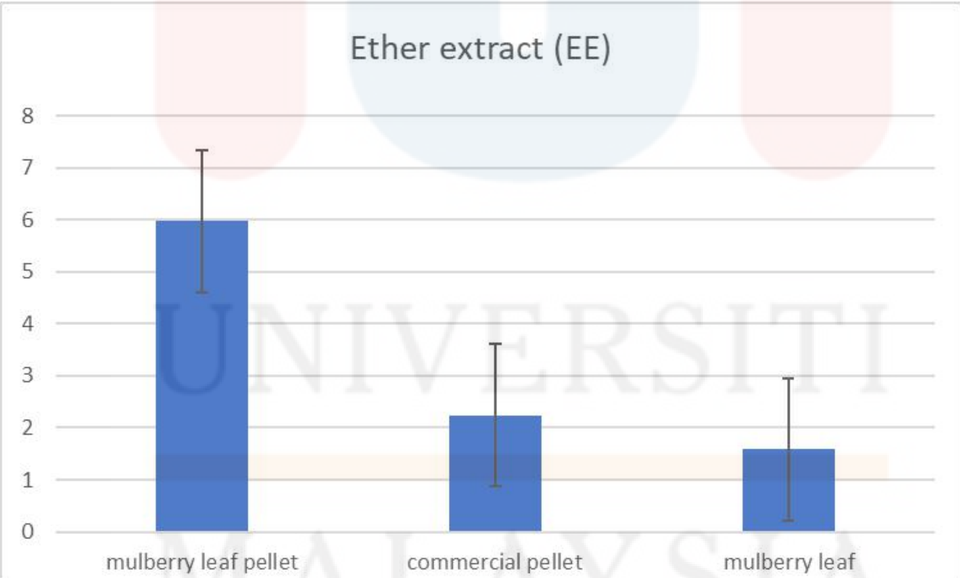

Figure 4.5 Mean of ether extract (EE) content between groups

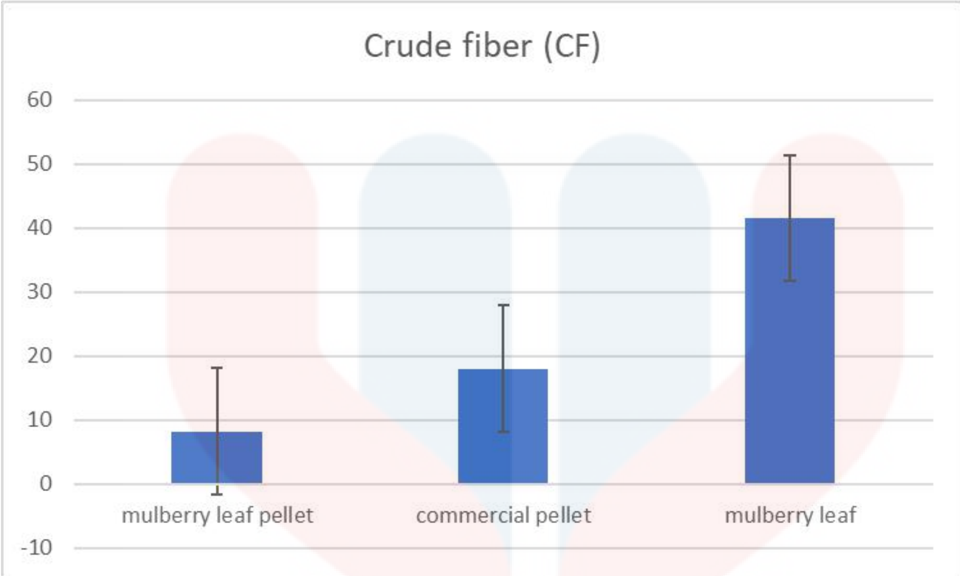

Figure 4.6. Mean of crude fiber (CF) content between the groups

The result from proximate analysis indicates that both mulberry pellet and commercial pellet contain higher dry matter content as well as lower in moisture content compared to mulberry leaf. Nowadays, most rabbits pellets sold commercially are nutritionally complete with the correctly balance protein, fiber, fat, vitamin and mineral. Previous study reported that commercial pellet was developed and formulated with low water content which was less than 13% in order to enhance for long term storage (Lou, 2018). This finding enhances our understanding about animal feed and concentrates which contained ingredients that can deteriorate over time. Many feed products and minerals are hygroscopic in nature and may bind and lump together after prolonged storage which can change the appearance of the product. To be exact, most of the animal feed should be consume within 3 months from the manufacturing date for the best result. Thus, the moisture content in rabbit pellet should not exceed 13% as it can inhibit the process of deterioration which can affect the quality and nutritive value of the pellet.

For commercial reasons, it is essential to control moisture content in order to compensate for losses that occur during grinding and cooling process. Hence, a sufficient moisture is important as it can reduce the energy usage during pelleting process as well as to ensure the production runs smoothly by lowering the risk of blockages.

On the other hand, Mulberry leaf was significantly higher in water content about 66% compared to the both pellets as it came from fresh forages. Among woody forages, Mulberry leaf itself is nutritionally contain higher CP which up to 20%. According to Boschini (2002), the CP in leaf was three times of that stem and the leaf cell wall and ADF were half. In this study, the result of CP analysis in Mulberry leaf was at 24.10%. A few similar findings conducted by Ouyang et al (2019), Lou (2018), Vu *et al.* (2011)

and Yao et al. (2000) had shown that CP level of the Mulberry leaf in their research were at 26.50%, 21.9%, 22.3% and 20.30% respectively. These results from previous researchers had proven that CP level in most Mulberry leaf in the literature is exceeded 20%. Therefore, Vu et al. (2011) further discussed a few factor in their finding research on why CP content of mulberry leaf is varied considerably due probably including maturation stage (Kabi and Bareeba, 2008), soil manure application and cutting frequency (Benavides et al., 1994; Espinoza and Benavides, 1996; Kabi and Bareeba, 2008), the value reported in the previous and present studies indicates that mulberry leaf could be a high protein forage for farm animals.

When comparing Mulberry leaf and Mulberry leaf pellet, the Mulberry leaf pellet seemed to be slightly lower CP content (19.43%). A reduction in nutritive value of crude protein in Mulberry leaf pellet is probably due to converting fresh Mulberry leaves into pelleted form which involving the process of sun drying, grounding, pelleting through a machine and sun drying the pellet again in order to reduce the moisture. Meanwhile, CP level in Mulberry leaf pellet is better than commercial pellet (17.20%). As mentioned in the literature review, both types of pellets, therefore are achieved the general crude protein requirement in rabbit nutrition which is exceed 16% of CP.

In term of crude fibre content, the Mulberry leaf pellet only contained 8.17% which was the lowest among Mulberry leaf (41.55%) and commercial pellet (18%). As mention in the literature review, the Mulberry leaf pellet is contain slightly lower than the minimum CF requirement in rabbit for growth according to the NRC is about 10-12 %, and these data are, however, old (1977) and refer to farm rabbits (NRC, 1977). The reason why Mulberry leaf pellet is low in fibre content because it was formulated by using the leaf part only. The fibre contain in stem part is higher compared to leaf part.

Van Soest (1992) found that Mulberry leaf contain 33% NDF and their stem was 64% whereas alfalfa has 40%, Orchard grass 55%, Pangola grass 70% and Elephant grass is 9%. However, the main limitation of the use of Mulberry stem in the formulation pellet is not related to their chemical quality but to the physical form in which very high moisture content as well as hard to ground and processed them into pelleted form. On the other hand, Mulberry leaf, can be properly dried and ground, is of excellent quality and its characteristics make it most suitable for inclusion in compounded feed for highly producing rabbit.

Next, the total fat content that had been extracted through Soxtec method for Mulberry leaf pellet is quite higher which is 5.98% compared to Mulberry leaf (1.58%) and commercial pellet (2.24%). This probably due to additional fat content from the binder in the formulation that make a contribution to the increasing level of ether extract of Mulberry leaf pellet. However, Halls (2010) reported that levels of 2 – 5% fat in the diet also helps promote a shiny coat, which is beneficial in show rabbits. Plus, the binder use in this pellet making only using tapioca starch and molasses and it is restricted not more than 5% binder in the formulation. Then, the raw material for coating, such as oil seeds, did not use in this formulation. In feed manufacturing industry, oils are not used in substantial amounts for any of the main farms including rabbits. According to Fernandez et al (2000), that recently the uses of whole seeds, especially from soy (full-fat soybean), has substantially increased. This trending will make a greater impact of level of unsaturated fatty acids in the carcasses of animals fed on this product, and a consequence is the number of related papers published in recent year. Carcass fat deposits are greatly affected by the ingestion of fat-added diets, although polyunsaturated fatty acids seem to increase body fat less than saturated ones. In the work of Fernández and Fraga (1996) reported that the main carcass traits were not influenced by fat inclusion in diets, but more body perirrenal and scapular fat was found in rabbits fed on the highest fat-added diets. The finding from Fernández et al. (2000) further discussed about the durability of the pellets deteriorates when a high level of fat is added. Plus, to avoid friable pellet, coating the pellets with the melted fat in a vertical mixer should be practiced. In addition, fiber material such as wheat bran, lecerne and a straw that are included in typical rabbit diets, probably do not allow a great percentage of fat to be added (Fernández *et a*l, 2000).

Lastly, in term of ash analysis, all the sample feed from this study shown an uniform result of mean value between the groups. Thus, all feed sample contain about a range of 10 to 11 percent of total ash content. Carlos Boschini (2000) found that Mulberry leaf had two or three time more ash in leaves than in stem. Plus, it is reported in similar finding by Gonzalez and Mejia (2014), the Mulberry leaf from Hacienda Lucerna contain about 17.3% ash which is quite higher from this study. Thus, the total ash obtained was used to prepared ash solution for the estimation of calcium by the researchers in previous or present findings (AOAC, 1995).

Overall analysis from the result that had statistically analysed, the alternative hypothesis is accepted since characterisation and nutritive value of Mulberry leaves pellet have a potential to substitute partially with commercial pellet as rabbit feed.

CHAPTER 5

5.0 Conclusion and Recommendation

Based on the current finding, Mulberry leaf pellet can contribute to an excellent protein source as the main diet in rabbit growth which can partially substitute with commercial pellet. However, a few important things need to be consider in order to improve the quality of Mulberry leaf pellet especially additional raw material which can contribute to fibre content in the pellet formulation as well as improving its coating and durability so that can prevent friable pellet. In general, the chemical composition was shown a significant differences between the groups except for ash and crude fibre analysis. Therefore, this finding also indicate that Mulberry leaf is an excellent feed for high yielding animals, and can be offered either fresh or dried in compound feed. Under tropical conditions, mulberry productions for compounded feeds need further research and findings.

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APPENDICES

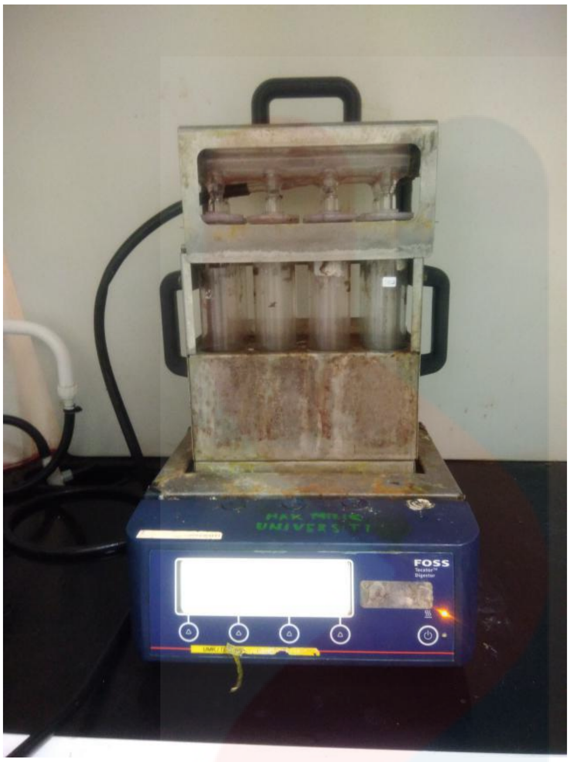
Appendix A.1 : The raw data of sample analysis

Feed	Mulberry leaves pellet 1	Mulberry leaves pellet 2	Mulberry leaves pellet 3	Mulberry leaves 1	Mulberry leaves 2	Mulberry leaves 3	Commercial pellet 1	Commercial pellet 2	Commercial pellet 3
Dry matter (%)	91.19	91.17	90.86	31.9	34.54	35.47	89.36	89.43	89.5
Moisture content (%)	8.81	8.83	9.14	68.1	65.45	64.53	10.64	10.57	10.5
Crude protein (%)	19.39	19.59	19.32	24.18	23.5	24.62	17.18	17.49	16.93
Crude fat (%)	5.99	6.05	5.91	1.67	1.58	1.49	2.17	2.33	2.22
Ash content (%)	10.93	10.95	11	9.97	10.24	10.25	11.54	10.73	10.26

Appendix B.1: The physical appearance of mulberry leaf pellet

Appendix B.2: Machine of moisture analysis

Appendix B.3: Machine of ether extract analysis



A

B

C

Appendix B.4: Crude protein analysis equipment; A:Machine for digestion; B: Machine for distillation; C: Titration process of crude protein analysis