Improvement of Rabbit Pellet Formulation Developed Using Pineapple Leaves

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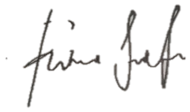
DECLARATION

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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Peningkatan Formulasi Pelet Arnab dibangunkan dengan Menggunakan Daun Nanas

ABSTRAK

Sisa nanas ialah hasil sampingan pembuatan nanas yang biasanya dibuang dan mencemarkan alam sekitar. Salah satu pendekatan yang berpotensi untuk menguruskan sisa ladang nanas adalah dengan mengubahnya menjadi makanan haiwan. Dalam penyelidikan ini, penambahbaikan formulasi pelet arnab yang dibangunkan menggunakan daun nanas telah dikaji. Nilai pemakanan dan sifat fizikal formulasi pelet arnab baru yang dibangunkan menggunakan daun nanas dibandingkan dengan pelet arnab komersial. Oleh itu, 40% daun nanas dengan bahan tambahan seperti jagung pecah, tepung soya, dedak padi, premix vitamin, gula merah, batu kapur dan garam digunakan untuk memenuhi keperluan nutrisi arnab. Hasilnya, 2 kg pelet dihasilkan pada akhir proses. Sifat fizikal pelet seperti ketumpatan pukal dan keliangan didapati kesan yang signifikan ($P<0.05$), manakala ketumpatan sebenar menunjukkan tidak terdapat kesan yang signifikan ($P>0.05$) antara pelet daun nanas dengan pelet komersial. Hasilnya, analisis kimia dilakukan untuk menentukan kandungan lembapan, protein, lemak, serat, abu dan gula bagi kedua-dua sampel. Analisis statistik kandungan lembapan menunjukkan tidak terdapat kesan yang signifikan ($P>0.05$) antara pelet daun nanas dengan pelet komersial. Walau bagaimanapun, terdapat kesan yang signifikan ($P<0.05$) dalam protein, lemak, serat, abu dan gula antara pelet daun nanas dengan pelet komersial. Sampel pelet daun nanans memperoleh serat dan kandungan protein tertinggi berbanding pelet komersial yang memberikan nilai kandungan serat dan protein yang paling rendah. Kesimpulannya, di antara kedua-dua sampel, 40% pelet daun nanas menunjukkan hasil yang baik dari segi penggunaan nutrisi kepada pertumbuhan arnab berbanding pelet komersial. Dapatan kajian menggambarkan potensi daun nanas bermanfaat dalam menghasilkan bahan makanan arnab, membantu mengurangkan kesan alam sekitar pemprosesan nanas dan membantu petani memperoleh lebih banyak wang, juga mewujudkan peluang pekerjaan.

Kata kunci: Daun Nanas, Pelet, Arnab, Nilai Pemakanan, Sifat Fizikal, pelet komersial.

Improvement of Rabbit Pellet Formulation Developed Using Pineapple Leaves

ABSTRACT

Pineapple waste is a by-product of pineapple manufacturing that is typically discarded and pollutes the environment. One of the potential approaches to managed pineapple field wastes is by transforming them into animal feeds. In this research, the improvement of rabbit pellet formulation developed using pineapple leaves was investigated. The nutritional value and physical properties of new rabbit pellet formulation developed by using pineapple leaves was compared with commercial rabbit pellets. Therefore, 40 % of pineapples leaves with additional ingredients such as cracked corn, soybean meal, rice bran, vitamin premix, molasses, limestone and salt was used to fulfil the nutritional requirements of rabbit. As a result, 2 kg of pellet was produced at the end of the process. Pellet physical properties like bulk density and porosity was found to be significantly different ($P<0.05$), while true density showed there was no significant difference ($P>0.05$) between pineapple leaves pellet with commercial pellet. Chemical analysis was performed to determine the moisture, protein, fat, fibre, ash and sugar content of both samples. The moisture content and fat statistical analysis showed that there was no significant difference ($P>0.05$) between pineapple leaves pellet with commercial pellet. However, there was significant difference ($P<0.05$) in protein, fibre, ash and sugar between pineapple leaves pellet with the commercial pellet. Sample of pineapple leaves pellet obtained the highest amount in fibre and protein content in comparisone to the commercial pellet which gives the lowest values of fibre and protein content. In conclusion, among both samples, 40% pineapple leaves pellet was superior in term of nutrient utilisation that is beneficial for rabbit growth in comparison to the commercial pellet. The findings of the study illustrated that the potential of pineapple leaves was beneficial in producing rabbit feedstuff, and also in helping to alleviate the environmental impact of pineapple processing while helping farmers to earn more money, and creating job opportunities.

Keyword: Pineapple Leaves, Pellet, Rabbit, Nutritional Value, Physical Properties, Commercial pellet.

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LIST OF UNITS AND SYMBOLS

cm	Centimetre
g	Gram
h	Hour
kg	Kilogram
mL	Millilitre
g/ml	Gram per Milligram
m	Metre
mg	Milligram
nm	Nano meter

LIST OF ABBREVIATIONS

CF	Crude fibre
CP	Crude Protein
CF	Crude fat
DE	Digestible energy
EE	Ether Extract
HCL	Hydrochloric acid
H2SO4	Sulphuric acid
ME	Metabolize energy
NaOH	Sodium hydroxide
KOH	Potassium hydroxide

CHAPTER 1

INTRODUCTION

1.1 Research background

Pineapples (*Ananas comosus*) is a tropical plant from the *Bromelioaceae* family that is believed to originate from the Eastern Part of South America. Pineapple was first introduced to Malaya by the Portuguese in the 16th century, coinciding with the growth of rubber plantations. In the year of 1921, the pineapple was planted in Johor and Selangor as a cash crop. Pineapple crops are still growing and being cultivated in peatlands mainly in Johor.

The pineapple hybrid variety 'MD2 pineapple variety' is currently the most common pineapple planted in Malaysia due to high demand from the global market, particularly in China, and domestic demand in Malaysia. According to the Minister of Agriculture and Agro- based Industry, the export of pineapples products from Malaysia increased by 56 % in 2019, following the increase in high demand from abroad. This is due to the success of the country's pineapple market in entering the Chinese market, as well as the industry's ability to expand its production and processing capability. It's a huge achievement that will boost the country's Gross Domestic Product (GDP), boosting farmers' and pineapple growers' incomes. It is reported that pineapple production is 434,811 metric tonnes covering a farm of 13,433 hectares with about 32.37 tonnes of pineapple per hectare.

However, the generation of waste from the growing pineapple farming industry in Malaysia in particular will affect the environmental balance and contribute to environmental pollution. This is because after fruit harvesting, the leaves will often be burned or left on the ground. Nowadays, by detecting and transforming by-products into value-added products, various attempts have been made to protect the environment. Therefore, there are various innovations that can be highlighted from pineapple waste that can add value to the pineapple waste itself. In addition, it can also preserve the environment and as an additional source of income for pineapple growers in Malaysia.

In Nigeria, pineapple waste was used to feed small ruminants. According to a survey, the feed can be used after proper processing (Onwuka et al., 1997). The best pellets can be made with strong physical properties, chemical properties and a high nutritional value for herbivore consumption. Pineapple leaves have a high Holocellulose content (85.7%), followed by Cellulose (66.2%) and Hemicellulose (19.5%) (Zawawi et al., 2014). Animal will use the fiber in pineapples waste as a source of energy as well as a healthy for digestive meal.

The scientific name of the rabbit is *Oryctolagus spp.* Rabbits are living things from the animal kingdom of the phylum chordata in the mammalian class in the order lagomorpha. Rabbits are a mammalia class in the animalia kingdom that can be found all over the universe. There are several other rabbit species in the order Lagomorpha, which also includes pikas and hares. Rabbit meat is thought to be leaner and healthier than beef and pork because it contains less fat and cholesterol (Enser et al., 1996).

The herbivores animal such as rabbit, only consume plants and vegetables. Rabbits have dietary needs that can be fulfilled by eating particular foods. Protein, fibre, fat, carbohydrate, vitamins, minerals, and water are the most basic essential nutrients for rabbits. The nutritional value of pellet must contain a sufficient nutritional content for rabbit consuming to promote growth performance of the rabbits. Animal malnutrition has been identified as a major factor affecting animal production around the world (FAO, 2000).

1.2 Problem Statement

In Malaysia, the rabbit industry is still relatively new. The majority of rabbit farmers operate on a micro- and small-scale (Raharjo, 2008). The pellets for rabbits are commonly made from hay or alfalfa as a main source of fibre and nutrients. However, the commercial feed is very expensive to produce and purchased. The commercial diets also are becoming more common on some medium-scale farms, but the forage availability is restricted due to competition from other livestock sectors that consume more. Alternatively, there are some biomass waste from agriculture industries that has the potential to be formulated as animal feed that can meet the rabbit requirement. One of the biomass waste that are easily available in Malaysia is pineapple leaves. Pineapple leaves have high nutrients content and it is a waste if it is discarded. It is possible that substituting pineapple leaves as a main source of fibre in making pellets as it would help in the rabbit weight gain and performance in same the time can help small-scale livestock farmers can afford it too.

The nutritional content in pineapple leaves in term of protein, fibre, ash, moisture, fat, and sugar was found to be 5.64-7.05%, 30.93-31.04%, 2.8-2.35%, 7.07-9.42, 2.53-3.15% and 10% respectively (Zainuddin et al., 2014). However, due to the high crude fibre content, low protein content in pineapple leaves, the use of pineapple leaves as rabbit feed is restricted. The nutritional requirements for rabbit growth must be met by improving the ingredient in the formulation by incorporating new formulations that make the pellet more nutritious while lowering the price which is affordable for small scale farmer and at the same time the additive ingredients as raw materials for new feed formulation also easily accessible.

1.3 Research aim and objectives

The objective of this study are:

1. To develop new rabbit pellet formulation using 40% pineapple leaves and additional ingredients to fulfil the nutritional requirements of rabbit.
2. To compare the nutritional value and physical properties of new rabbit pellet formulation with commercial rabbit pellet.

1.4 Hypothesis

H0: There is no significance difference between the new rabbit pellet formulation using 40 % pineapple leaves and the commercial rabbit pellets.

H1: There is significance difference between the relationship of new rabbit pellet formulation using 40% pineapple leaves and commercial rabbit pellets.

1.5 Scope of study

This study's scopes have been determined in order to meet the research objectives. The study's was focused on developing a new rabbit pellet formulation that uses pineapple leaves to meet the nutritional needs of rabbits. This research is based on producing new formulation pellets from agricultural products such as 40% pineapple leaves and other feed ingredient such as soybean meal, corn bran, rice bran, limestone, molasses, vitamin premix and salt. Following that, proximate analysis and sugar content analysis was performed using various techniques such as kjeldahl method to determine the levels of protein, fat, moisture, ash, and carbohydrates in the new rabbit pellet formulation. Physical properties of the pellets were determined, including true density, bulk density, and porosity. Mixing, grinding, drying, and pelleting are all steps in the palletisation process. The final year project (FYP) was last 3 months and was conducted in the animal lab and biology lab at the University of Malaysia Kelantan in Jeli.

1.6 Significance of study

Since there is a lack of information about the potential of the plants after the fruit is discarded, the pineapple plant is considered unprofitable after the fruit has been harvested. The primary goal of this study was to develop a new rabbit pellet formulation using 40 % pineapple leaves and additional ingredients to fulfil the nutritional requirements of rabbit. This is due to the fact that pineapple leaves are a good formulation for rabbit health, and with the right processing technology, these fibrous materials can be converted into herbivorous fibre feed sources. In the feed industry, this approach can be more cost effective while also being more environmentally friendly. This is a new way approach of utilized waste to worth (w2w). This innovative approach will not only help farmers earn more money, but it will also create jobs (Ahmed et al., 2002).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Rabbits are herbivorous and monogastric (they only have one stomach and only eat plants). They need specific amounts of nutrients to grow and perform optimally. Proteins, carbohydrates, lipids (or fats), minerals, and vitamins are examples of important nutrients that rabbits needed. The majority of domestic rabbits are fed a pelleted diet that contains all of the required nutrients that made up of plant-based ingredients such as alfalfa meal and wheat middling. They also have very specific dietary requirements due to sensitive digestive system of rabbits. A diet of 2,200 calories per kilogramme of body weight, or 2.2 calories per gram of body weight, is required for rabbits (Cheeke, 1994). A healthy adult rabbit requires minimum 12-18 % protein. Young rabbits which is less than five months old require a higher protein level to increased their rapid growth (Akande and Eunice, 2015). Rabbit teeth and digestive systems have evolved to consume a high-fiber, low-fat, low starchy-carbohydrate herbaceous diet. Rabbit feed can be found in a number of places, including wild native plants, cultivated forage, farm crop residue, agricultural by-products and market sources (Lukfahr et al., 1992). Feed should be cost effective, taking into account the cost of ingredients as well as their digestibility efficiencies to meet the animal's nutritional needs. On a per-unit protein basis, alternative protein sources may be cheaper than fish meal. The formulated feed should not have a negative effect on the animal's overall output or the consistency of the food (Gaye and Muammer, 2010). Sugar cane, cassava and various palm oil species can all be used to produce high-quality forage, extractable sugars or fruits, or roots, which are mainly used as rabbit feed.

In the 1980s, rabbits were first bred as pets and by the early 1990s, they were being raised as a meat substitute for livestock. In Malaysia, the rabbit industry is still relatively new, but it has expanded rapidly in popularity as a result of numerous government campaigns and incentives aimed at its growth. The demand for meat-based products is increasing dramatically, particularly in Selangor state, which has the highest population (5.46 million). According to the most recent industry estimates, the DVS District Office has 24,987 does and 600 farmers registered, indicating that rabbit meat is in high demand, with a few marketers involved in "satay" production demanding nearly 1,200 kg per month. The current price range for a carcass with an average weight of 1-1.2 kg is RM 35.00-RM 45.00/kg. The price of live weight at the farm gate is RM 13.50 per kg. According to a DVS study titled "Malaysian Preferences for Rabbit Meat," shows the price is considered expensive. Since, the cost of commercial feed remains high due to producer overhead costs and the amount of feed available is low, the price of meat is rising. Many companies are not interested in formulating or producing rabbit pellets, and if they are, the production costs are high.

Malaysia’s first commodity crop is pineapple, which has a strong export potential (Jaji, Man and Nawi, 2018). The pineapple, which belongs to the bromeliaceae family, is regarded as a crucial tropical fruit. The United Nations Conference on Trade and Development (UNCTAD) has put pineapple second in the ranking of commercial tropical fruits for global production after banana. Pineapple has long been a valuable commercial or commodity crop with a variety of benefits. Pineapple has a variety of dietary, medical and industrial benefits that help people feel better. The increase expansion of agro-industrial operation in recent years has resulted in a significant amount of lignocellulosic residue being accumulated all over the world. The estimated total amount of pineapple leaves grown in Malaysia in 2010 was 28,469 metric tonnes (Wan Mohd nad Zainuddin, 2013). As soon as possible, this residue must be converted into value-added goods.

Agriculture by products like pineapple leaves can be turned into unique products or used as raw materials in other industries. The use of agricultural by-products as a source of functional ingredients is a promising field that addresses environmental issues caused by agricultural by-product disposal in an indirect manner (Bustanul Ariffin et al., 2009). Before being converted into animal feed pellets, the nutritional content of pineapple leaf waste was determined (crude protein, crude fibre, ash, fat, and sugar). Pineapple waste contains 81.90% organic matter, 14.22% dry matter, 0.56 % nitrogen, 8.10% ash, 3.49 percent crude lipid, 3.50% crude protein, and 4,481.2 kcal kg-1 gross energy, whereas pineapple skin waste contains 16.7% crude fibre (Nurhayati et al., 2013). The production of pellet from pineapple waste will help in reduce animal feed costs and improve feed quality, which is reflected in animal's high production efficiency.

Table 2.1 Chemical composition of pineapple leaves

Constituents (%)	**Leaves**
Dry matter	89.21-90.32
Moisture	7.07-9.42
Ash	2.08-2.35
Crude fibre	30.93-31.04
Crude protein	5.64-7.05
Crude fat	2.53-3.15
Carbohydrate	33.31-34.57

Sources: Zainuddin et al., (2014)

Antinutritional factors are substances or chemical compounds found in fruits and food in general. These anti-nutritional factors are known to interfere with metabolic processes, affecting nutrient growth and bioavailability. Most fruits contain anti-nutritional factors such as alkaloids, tannins, phytate, cyanide, saponins, and oxalates. Anti-nutritional factors reduce nutrient utilisation and/or food intake of plants or plant products used as human foods or animal feeds, and they play an important role in determining plant use for humans and animals.

The leaves and fruit of pineapple species were rich in tannin or known as alkaloids. The phyto constituents such as flavonoids, phenols, tannins, glycosides are found in A. comosus (Sharma., et al 2016). Different phytochemical constituents which are present in samples are known to be biologically active compounds and they are responsible for different activities such as antimicrobial, antioxidant, antifungal, anticancer and antidiabetic activities. Tannins, glycosides, flavonoids, and glycosides have hypoglycaemic and anti-inflammatory activities. However, depending on the animal's tannin tolerance, tannins might impair feed intake, fibre and organic matter digestion, and production if taken in large amounts by a mammal. There is a possibility of kidney and gastrointestinal issues. A high tannin intake may also lead to an increase in faecal nitrogen. Tannin solubility decreases with drying, as does their capacity to bind to proteins (Shuhaidu and Soh, 2016).

To remove tannins from feed, alkali has long been employed. Sodium hydroxide (NaOH) has been recommended as the most effective pre-treatment for detannification of tree leaves, particularly oak leaves (Canbolat et al., 2007). Alkali pre-treatments have been shown to reduce tannins at higher pH levels due to phenolic oxidation by air oxygen (Makkar and Singh, 1992). Numerous studies on various phytochemicals have been undertaken, with the results indicating that when given in suitable doses, some phytochemicals may have favourable benefits on rabbits. Not all phytochemicals are healthy; in fact, some might be toxic if ingested in large amounts or under certain conditions. The sensitivity of individual rabbits varies. One rabbit may be unable to absorb high levels of oxalates or tannins in vegetables, but another may be able to do so without adverse effects.

Dietary fibre fractions are important in animal feeding because of their influence on passage rate, mucosal functionality, and role as a substrate for gut microbiota, which is related to performance and digestive health. To define appropriate rabbit feeding recommendations, it is necessary to understand the nutritional role of fibre (Gidenne, 1996 and 2000). Polysaccharides or complex carbohydrates are difficult to break down and are significant constituents of plant structural parts, particularly the cell wall which under the category of insoluble fibre. Insoluble fibres include hemicellulose, cellulose, and lignocellulose. In plant cell walls, cellulose and lignin frequently combine to generate lignocellulose. Lignin is a phenolic compound, not a carbohydrate, but it is often included in discussions of fibre because of its presence in cell walls between molecules of cellulose, hemicellulose, and pectin. In mature plants, lignocellulose is found in higher concentrations than in young plants (Moore et al., 2017).

Pineapple leaf shape resembles a sword that tapers at the end with a blackish-green color and on the edge of the leaf there is a sharp thorn. Pineapple leaves have a composed outer layer from the top and bottom layers. Between those layers there are many bundles or strands of fiber that are bound to each other by a type of adhesive (gummy substances) found in the leaves. According to hidayat (2008), the results of pineapple leaf chemistry research shows cellulose content ranges from 69.5% - 71.5%, lignin levels ranged between 4.4% - 4.7%, pectin levels 1.0% -1.2%, fat and waxes content 3% -3.3% while pentosan levels 17.0% - 17.8%.

Lignin is indigestible, the nature of the lignocellulose affected the performance and health of the rabbits. A lack or excessive of lignocellulose, increases the frequency of digestive problems (Maître et al., 1990; Blas et al., 1994). Lignin has been observed to reduce the digestibility of crude protein, Acid detergent fibre (ADF) and Neutral detergent fiber (NDF), gross energy, and dry matter content in rabbit diets. Simply said, diets high in lignocellulose are less digestible than diets low in lignocellulose, and the amount of nutrients and energy obtained by rabbits from their food is reduced. Foods containing lignocellulose with low lignin proportions caused reduction of the voluntary food intake, as light improvement of the food conversion but also higher health risk. In contrast, a high intake of lignins (over 6 g/day) over young rabbit can reduced the health risk due to digestive troubles and stimulated the transit of dgesta through the digestive system. An intake of lignin of about 6 g/day for growing rabbit appeared to ensure a good growth performance and health status (Gidenne et al., (2001). Rabbits fed diets containing less than 6-10 percent crude fibre are susceptible to digestive issues such as gastrointestinal hypomotility and enteritis. However, feeding rabbits a diet that contains more than 25 percent crude fibre can cause mucoid enteropathy and cecal impaction (Moore, 2017). For optimal digestive health, a balance of insoluble and soluble fibre is essential. Rabbits require balanced diets that contain all of the vital components.

Pre-treatments such as mechanical, physicochemical, chemical, and biological pre-treatments have all been employed to disrupt the complex structure of lignocellulosic biomass. Chemical modification is one method of improving the properties of agro-wastes derived from plant sources for composite production (Nachtigall et al., 2007). The chemical treatment aids in breaking down the gelly-like material found in agro-waste and releasing it in the chemical modifier solution, which can then be rinsed away with distilled water. Alkaline and acid treatment are two chemical treatments used in the purification of lignin and other unwanted parts from agro-based plants (Shuhaidu and Soh, 2016). Chemical pre-treatment using alkaline solution pre-treatment is one of the most commonly investigated treatment procedures. Alkaline pretreatment using sodium hydroxide (NaOH) has recently received a lot of attention as a potential pretreatment option because it is cheap, effective on a wide range of feedstocks, and requires less energy than other pretreatment options (Xu et al., 2010).

According to previous research, the main advantage of NaOH pre-treatment is the efficient breakdown of lignin from biomass without significant loss of structural carbohydarate materials such as glucose, xylose, arabinose, and so on from different types of lignocellulosic biomass such as, wheat straw, corn stover, switch grass, and sugarcane bagasse (Amin et al., 2017). Typically, NaOH or KOH are used for alkali pretreatment. Pretreatment with alkali accelerates the degradation of hemicellulose and lignin. The acetate group from the hemicellulose will be removed during alkali pretreatment, allowing hydrolytic enzymes to more easily access the carbohydrates (Kong et al., 1992). In addition, alkali treatment by NaOH also an alternative to these chemicals in many cases swell the cell wall of the matrix structure and give better penetration. NaOH as a modifier has frequently been used in the removal of unwanted components of lignocelluloses based material and has shown to be one of the best method for reduction of unwanted part of plant fiber composition (Cotanaa et al., 2015).

According to previous research, the optimum conditions for the entire process which is favourable to the responses (cellulose, hemicellulose and lignin content) for mango seed shell flour (MSSF) is at 6.09% NaOH concentration and soaking time of 5.22 hours. The cellulose content was increased to 94.8002%, while the hemicelluloses and lignin content were reduced to 2.2779 and 0.508502 percent, respectively, under these conditions (Mike et al., 2019). Previous study of 1% NaOH at 121°C for 1.5 hours showed increase in cellulose contents to 44%, decrease of hemicelluloses and lignin contents to 44% and 42% respectively (Xiong et al., 2017). In NaOH pre-treatment cellulose contents were increased the most which are beneficial because it increases the sample porosity, which serves as instrument for efficient enzymatic hydrolysis. Alkaline treatment of fiber produces smaller soaking time and temperature of reactant than any other treatment process (Kim and Han, 2012).

2.2 Nutritional component of rabbits feed.

2.2.1 Energy

Lebas (1975) investigated the growth performance of rabbits fed diets with varying energy contents. Regardless of diet energy content, an additional 9.5 kcal of digestible energy (DE) was required for each gram of body weight gain. According to the findings, 2,500 kcal of DE per kg of diet will meet the rabbit's energy requirements for rapid development, but lower levels may cause the rabbit to become ill. Protein and energy are both provided by Palm Kernel Cake (PKC). PKC is a medium-grade protein feed on its own, and because of its high fibre content, it is frequently considered suitable for ruminant feeding (Chin, 2009). Aduku et al. (1988) investigated the effects of palm kernel cake, peanut meal, and sun flower meal on the diets of weaner rabbits. Maize has been the chief sources of enegy in monogastric animal (Dada et al., 1998). Maize is high in energy where it consists of 9 percent protein and 3340 kcal ME/kg. This can be used as a sources of energy in the diets of growing rabbits because it has the potential of improving growth performance.

2.2.2 Amino acid and protein

Proteins are constructed from amino acids. Growing rabbits require specific amounts of 10 of the 21 amino acids that make up proteins, according to researchers. These are known as the basic or essential amino acids, and they are supplemented with two additional amino acids that can partially replace two of the essential amino acids. This is the complete list for rabbits, which includes arginine, histidine, leucine, and isoleucine.

The significance of protein quality in rabbit nutrition is widely acknowledged. Rabbits rely on adequate amounts of essential amino acids in their diet to grow quickly. Since rabbits depend on essential amino acids in their diet, non-protein nitrogen sources are ineffective. Several studies have shown this. Olcese and Pearson (1948) discovered that adding urea to a low-protein diet prevented growth. According to King (1971), substituting urea for a portion of the plant protein in a grower's diet resulted in less growth. When urea, or diammonium citrate were added to a low-protein diet, neither urea, or diammonium citrate improved growth, according to Cheeke (1972). These studies provide ample evidence that non-protein nitrogen sources cannot be used effectively in grower diets.

The effectiveness of various protein supplements for rabbits has been investigated. Cheeke and Amberg (1972) discovered that soybean meal promoted growth rates of 34 g per day at equivalent protein levels, while cottonseed meal promoted growth rates of 25 g per day. Cottonseed meal supplemented with lysine and methionine raised growth rate to the same degree as the other two supplements. Lebas (1973) discovered that soybean meal supported a higher growth rate because it consists of 45 % protein, 2130 kcal ME/kg than sesame meal. Colin and Lebas (1976) discovered that rapeseed meal, horse beans, and peas are acceptable protein supplements after methionine supplementation. The different reactions to different protein supplements are largely a result of their amino acid composition.

2.2.3 Carbohydrate and fibre

For rabbits, carbohydrates are a big source of nutrition. The majority of rabbits' carbohydrate needs are met by fibre. Foods high in indigestible fibre, such as timothy grass hay and alfalfa hay, can help prevent enteritis and obesity. Indigestible fibre does not ferment in the cecum, whereas digestible fibre does. Indigestible fibre is good for rabbit dental health because it stimulates the gums. Cheeke (1974) discovered in preference trials that rabbits prefer barley or wheat when given a choice. In Algeria, wheat bran is also one of the most widely available and widely used by-products in animal nutrition. Additionally, due to its chemical composition and nutritional value, it is widely used in rabbit feed (Blas et al., 2000). The best performance with the lowest energy grain suggests that factors other than energy content are involved, such as palatability.

2.2.4 Lipids

Rabbits consume fat for energy as well as to absorb fat-soluble vitamins. Most foods contain 2% to 5% DM fat, which rabbits can obtain from a vegetable diet. Rabbits do not require fat in their diet. Fat can improve palatability, but too much can increase the risk of obesity, hepatic lipidosis, and aortic atherosclerosis (Vicky, 2011). Rabbits have been shown to be deficient in essential fatty acids such as linoleic acid (n-6) and alpha–linolenic acid (n-3) (Ahluwalia et al., 1967). Symptoms include decreased growth, hair loss, and changes in the male reproductive system, such as degenerative changes in the seminiferous tubules, impaired sperm development, and decreased accessory gland weights.

2.2.5 Mineral (calcium and phosphorus)

Calcium absorption and excretion in rabbits differ from that of other domestic species. Rabbits absorb all of the calcium in their diet, with any excess excreted in the urine as calcium carbonate. Excess calcium carbonate can cause crystals and uroliths to form in the kidneys, ureters, and bladder. As a result, rabbits require a calcium level of 0.5 percent to 1 percent DM (Carpenter et al., 2010). The calcium can be obtained from alfalfa or alfalfa meal, as well as grass hay, though the levels are lower than in alfalfa. Phosphorus is found in grain by-products such as wheat bran. Calcium and phosphorus are also found in some fruits and vegetables. Bone meal, cereals, milk by products and commercial mineral mixtures are the main sources of Ca and P (Moore, 2017).

2.2.6 Vitamin

A rabbit's diet must include vitamins, particularly vitamins A, D, and E. Rabbits can synthesise their own B and K vitamins. They do not require large amounts of these vitamins, but their diet should include them. When feeding both a supplemented pellet and a high amount of alfalfa, care must be taken to avoid vitamin A toxicity (Moore, 2017).

2.3 Digestion in monogastric herbivores

The digestive system of a rabbit is very similar to a horse. Both have a "cecum," which means they are "hind-gut fermenters. It works similarly to a cow's rumen, but at the opposite end of the digestive tract, the various fibres and other feedstuffs that enter the cecum are broken down and digested by special microbes. Symbiotic microorganisms in the cecum produce cellulase, which is used to break down the cellulose walls of plant cells (Laughlin, 1990). Therefore, hemicelluloses and pectins are a major part of the polysaccharides present in rabbit diets. Because of the greatly expanded cecum and large intestine, which provide area for microbial digestion of fibre, the rabbit can utilise a large amount of roughage. They eat forages as well as grains and other concentrated feeds. The rabbit's cecum needs a fibre from the forage it eats, but since the rabbit's digestive tract is similar to that of a monogastric animal (like pigs and humans), the fibre quality must be good. High-quality fibre is an important source of energy for rabbits as well as an effective tool for maintaining the microbial population in the cecum. For proper digestion and gut health, healthy microbial communities in the cecum are essential (Moore, 2017).

Rabbits are unusual in that they produce two types of faeces which a rough, dry faecal pellet and caecotropes, which are soft faeces. The soft faeces are produced in the cecum (a pouch between the small and large intestines) and swallowed by the rabbit as they are excreted directly from its anus. This is known as coprophagy (ka-prof-a-gee) or caecotrophy and it occurs when the animal is alone. Some faeces have a mucus covering and are excreted as clumps rather than as individual pellets. This is the kind of faeces that is known as hard faeces. Coprophagy is a natural mechanism that provides B vitamins and protein to the rabbits, all of which are excreted in soft faeces. The protein and water content of soft faeces is significantly higher than that hard faeces, while the fibre content is significantly lower. The animal recirculates the components by ingesting the mixture and they remain inside the body. Lack of consumption in the long term may result in malnutrition and vitamin B deficiencies as well as a predisposition to digestive upsets.

The caecotrophs, which are packets of partially digested food, bacteria, and bacterial metabolites, including vitamins, ingested directly from the anus, are another source of nutrients. Cellulose, hemicellulose, pectin, and lignin are primarily digested by caecal bacteria, while precaecal digestion is hypothesised to occur in the stomach and small intestine by microbial pectinases and xylanases. Volatile fatty acids (VFA) produced by caecal bacteria's fermentative action are absorbed across the caecal epithelium and used as an energy source for rabbits (Fekete, 1989). Rabbits need at least 12 percent to 16 percent DM fibre to create volatile fatty acids, depending on their life stage. On the other hand, pet rabbits need more fibre to avoid obesity and fur chewing, as well as to keep their gastrointestinal systems healthy. Researchers found that adding hemicellulose and pectin led to higher weight gain, increased digestibility, and increased content of VFAs. The latter is considered a benefit because VFAs help the absorption of water and sodium, thereby possibly preventing diarrhea at times sugars are not fully digested in the stomach and ileum before they reach the cecum. It has been found in some studies that higher VFA concentrations lower the pH of the cecum, making the cecal environment less favourable to pathogenic bacteria.

2.3.1 Disease related to diet

Digestive ills are often the first sign of a serious problem with a rabbit's health. In general, digestive trouble decreases when both indigestible and digestible fibre are included in the diet and when fibre from multiple botanical origins is included in the diet. Too little fibre (less 6-10%) can alter GI tract conditions so that pathogenic organisms proliferate; excessive (20-22%) dietary fibre can alter the digestible energy of the diet, creating an energy deficit and a protein surplus (Vicky, 2011). Fibre also affects the mucosa of the gut, which is important in immune response. It has been found that including 20% soluble fibre (pectins, xylans) in diets that had been based on insoluble fibre improved the health of the mucosa and thereby improved the immune response. It is important that rabbits' diets be balanced. For optimal digestive health they need to receive indigestible fibre, digestible fibre, protein, and other nutrients in both the proper amounts and proper proportions (Moore,2017).

While, hairballs and Gastrointestinal hypomotility (stasis), it is no longer thought that hairballs are the primary condition, but rather occur secondary to gut stasis. If a rabbit is on a poor low-fibre diet then it can cause the hair to get stuck in the stomach and can cause slowdown of food passing through. A well-balanced high fibre diet (with lots of hay and good, fresh veggies) would prevent hair from getting lodged in the rabbit's stomach. A diet that has a balance of both indigestible and digestible fibre provided by plants of different botanical origin. Indigestible fibre slows the rate of transit of digesta in the stomach and intestine and speeds its time in the cecum while digestible fibre increases the rate of transit through the stomach and slows it through the cecum. Rabbits need both fibres for good digestive health. In addition, mucoid enteropathy is a diarrheal disease in which there is mucus in the diarrhea. In mucoid enteropathy large amounts of mucus are produced in the colon and impaction of the cecum and/or terminal part of the small intestine occurs in 75% of the cases. Young rabbits are most commonly affected by this condition, especially if they are receiving low-fibre high-carbohydrate diets. However, older rabbits under stress can also be affected by mucoid enteropathy. The chances of a rabbit becoming affected by mucoid enteropathy can be reduced by providing a diet with crude fibre over 15% but less than 25% of the diet (Lucile, 2017).

2.4 Utilisation of Pineapple plant waste

There have been numerous studies on the use of waste from the fruit and vegetable, dairy, and meat industries. Several attempts have been made in this regard to use pineapple waste collected from various sources. Pineapple cannery wastes have been used as a substrate for bromelain, organic acids, ethanol, and other compounds because they are a potential source of sugars, vitamins, and growth factor (Larraurietal et al., 2009). Several experiments have been conducted over the years to investigate the feasibility of using these wastes (Beohner and Mindler, 1949).

Several researchers have reported the presence of fibres in pineapple fruit (Lund and Smoot, 1982; Bartolome and Ruperez, 1995; Gorinstein et al, 1999). However, some studies have focused on utilising pineapple waste fibres. The high cellulosic content, availability, and low cost of pineapple leaf fibres are being investigated for use in fiber-reinforced polymeric composites (Devi et al., 1997).

Feed production is a relatively new industry. Tons of pineapple leaf fibres are produced each year, but only a small portion is used as feedstock or energy. Pineapple waste is rich in fibre and can be used as both an energy source and a good digestive feed for animals such as chickens, broilers, and cows (Suphalucksana et al., 2017). Feeding pineapple waste to dairy cows can increase milk output as well as increased the digestion rate. (Norman et al., 2019). Besides that, pineapple waste offers a number of advantages in terms of caloric density, digestibility, and feed consumption for animals. In addition, it boosts the rumen's dominant cellulolytic bacteria populations. Pineapple plants have a high fibre content, making them an excellent source of fibre for ruminant feed. To retain the quality of its nutrients, pineapple plants could be used as ruminant feed in silage form (Sayan, 2004). However, the use of pineapples waste as a poultry feed is limited due to the high crude fiber and water content, as well as the low protein content. The simple fermentation with locally available, natural beneficial microorganism solution, especially cellulolytic microorganisms can reduce the crude fibre content in pineapples waste. Rice waste, bamboo sprout and banana corms can all be used to obtain local microorgansims (Adrizal et al., 2017).

Bromelain is the most useful and researched component extracted from pineapple waste. However, a small amount of bromelain is also found in pineapple waste (Hebbar et al, 2008). Bromelain, a standardised complex of proteases from the pineapple plant, is consumed unchanged at a rate of 40% from the intestine of animals; it was found to have anti-edema, anti-inflammatory, and coagulation-inhibiting effects in animal studies.

Pineapple bran is high in vitamins and amino acids, making it ideal for making fruit vinegar. The production of pineapple bran into vinegar will increase the value content of pineapple fruit processing by 10% or more in China, where pineapple resources are abundant. This will significantly increase not only the use and conversion rate of pineapple bran, but also the extensive use of limited natural resources by making waste profitable, the effective use of pineapple by-products, resource waste reduction, and the impact of pineapple by-products on the ecosystem.

CHAPTER 3

METHODOLOGY

3.1 Materials

Fresh pineapple leaves were collected from local farm in Kelantan and brought to laboratory for testing. The new rabbit pellet formulation was made by mixing several kinds of raw materials, such as cracked corn, soy bean meal, limestone, vitamin premix, salt, rice bran and molasses obtained from local store. Meanwhile, commercial pellets were purchased from a nearby supermarket.

Figure 3.1: Pineapple leaves (*Ananas comosus*)

3.2 Sample preparation

15 kg of pineapple leaf waste were randomly collected from local farm in Kelantan. Collected pineapple leaf waste was cleaned thoroughly in water twice by removing foreign particles like, stones, and dirt. It was then cut into small pieces of size 10-15 cm long by a local chopper. Then, the pineapple leaves were placed into the tray for drying process. The trays were placed into the oven for 70°C for 72 hours. During the drying process, the leaves were flipped so that the leaves were not grown by fungi because after chopped, the leaves were wet. After finished drying process, the pineapple leaves were ready for grind by using grinder machine at a size of 1 mm. After that, the ground and sieved samples was dried for another 24 hours at 60°C in the oven. The result from grinding process turned the dried pineapple leaf into a very small particle and not so powdery since it contained high of fibre. From the process, 2.5 kg sample of pineapple leaves were obtained. The dry samples were stored in the box for further examination to keep it long lasting.

For pelleting process, 800g of pineapple leaves sample were used. Before making pellet, several raw materials such as cracked corn, rice bran, soybean meal, molasses, vitamin premix, limestone and salt are weight by using digital weighing balance. Then, all the ingredient was uniformly mix according to the formula that meeting the nutrition requirements of all living stage rabbits almost. A correct mixture can be obtained by hand or with mechanical mixing device. Next, the mixtures were examined in moisture analyser to maintain at a moisture content of 30%. Following that, all of the ingredients were placed in the pelletizer. Finally, the pellets were baked for 5 hours at 70°C in an oven to ensure that they were firm and in good shape. At the end of the process, 2 kg of pellet was produced.

Table 3.2 Formulation of pellets for rabbit diet.

Feed ingredient	Basal diet (%)
Pineapples leaves	40.00
Cracked Corn	15.00
Soybean meal	28 .00
Rice bran	8.00
Limestone	1.0
Salt	0.5
Vitamin premix	0.5
Molasses	7.00
Total	**100**

3.3 Determination of moisture content

Moisture is an important component of feed and a critical quality indicator in general since it has a direct impact on feed pellet quality. In the animal feed preparation process, proper moisture content influences shelf life and quality of feed which reduces energy consumption and increases output. In this experiment, moisture analyser mx-50 and a drying oven are commonly used in conjunction with a balance. Moisture analysis in a drying oven necessitates the use of an analytical balance with a readability of typically 0.1 mg (0.0001 g).

3.4 Palletization of Pineapples leaves with additional ingredient.

Extrusion, compression, pelletization, are all steps in the palletizing process (Supriya et al., 2012). In this experiment, the palletizing process was carried out using a pelletizer machine. Following that, at a moisture content of 30%, 2 kg of mixtures were compacted in the extruder. Each of the pellet sized around 2 cm long and shaped like a cylinder.

3.5 Physical analysis

The physical properties of pellets such as true density, bulk density and porosity were determined. The ranges of pellet's true density, bulk density and porosity were between 300.56 to 343.33 kg/m3, 1474.33 to 1513.67 kg/m3, and 76.71 to 80.14%, respectively.

3.5.1 True density

The true density of the pellets was determined using a gas pycnometer (Micromeritics, AccuPyc II 1340). The weight per unit volume of powder material was used to calculate true density. First of all, empty 100 ml of measuring cylinder was weight and recorded. Then, by using 100 ml measuring cylinder, the pellet sample was measured at the level of 50ml and the sample pellet weight was recorded using a digital balance. Then, 50 mL of distilled water was added to the measuring cylinder and the volume of water level and weight was recorded. Equation 1 demontrated a complete equation for true density determination.

$$(W2-W1) / W4-W2 \quad (1)$$

Where, this equation showed W1 (weight of empty specific bottle), W2 (weight of bottle and sample), W4 (weight of sample, bottle and water).

3.5.2 Bulk density

The mass of a dry sample (mass of solids) is divided by the volume of the sample to measure bulk density. First of all, empty 100 ml of measuring cylinder was weight and recorded. This test was carried out by filling a 100 ml measuring cylinder with a fixed sample from a predetermined height, and taping it twice to achieve uniform packing and minimise the impact on the wall. The sample was weight and recorded by using digital balance and the volume of sample level was recorded. Equation 2 demontrated a complete equation for bulk density determination.

$$D = \frac{M}{V} \quad (2)$$

Where, this equation showed D (density), M (mass), V (volume)

3.5.3 Porosity

Porosity in a material is defined as the volume of voids over total volume and voids space as a fraction. It typically ranges from 0 to 1 (Zainuddin, 2014). Equation 3 demontrated a complete equation for porosity determination.

$$porosity\,\% = 1 - \frac{Bulk\ Density}{True\ Density} \times 100 \qquad (3)$$

3.6 Chemical analysis

The AOAC protocol was used to determine the proximate component of the test ingredient, sample of the diet, and its nutritional value such crude fibre, crude protein, fat, ash and total sugar; AOAC 991.43 for crude fibre, AOAC 981.10 for crude protein, AOAC 991.36 for fat, AOAC 923.03 for ash, and AOAC 977.20 for total sugar.

3.6.1 Ash determination

The clean crucible and lid was placed inside the oven for 105°C for 20 minutes. After that, the crucible was placed in the desiccators for cooling about 20 minutes. The crucible was weighed (W1) and 1g of feed sample was put into crucible (Ws). Next, the crucible plus the sample was put into oven for 500 ° C for 8 hours by which the sample had turned into ash (whitish brown). The ash was removed from the oven and allowed to cool in desiccators for cooling so that it does not absorb any moisture. Lastly, the crucible containing ash was weight using analytical balance (W2). Equation 4 demontrated a complete equation for ash determination.

$$Ash\ \% = \frac{w2 - w1}{ws} \times 100 \qquad (4)$$

This equation showed W1 (weight of blank dish), W2 (weight of dish with ash after drying) and WS (weight of sample).

3.6.2 Crude fibre determination

The aim of determining crude fibre was to distinguish between carbohydrates that are more easily digestible and those that are more difficult to digest. The insoluble organic residue of the feed after successive boiling with acid and alkali solutions according to specified procedures is referred to as crude fibre.

Now, the collected fiber is dried in hot air oven at 130 °C for 2 hours. After 2 hours, take out the crucible from the oven and cool in a desiccator (20 minutes). After 20 minutes, weight the crucible containing fibre and noted the weight (W1). Then, for incineration of fiber, place the crucible inside of muffle furnace and burn the fibre at 550 °C for 2 hours. After 2 hours, open the furnace door after down the temperature below 250 °C, take out the crucible from the furnace and cool it in desiccator. After that, the ash of the collected fiber will appear. Take weight crucible that containing with ash (W2) and note the weight. Equation 5 demontrated a complete equation for fibre determination.

$$Crude\ fiber\ \% = \frac{W1 - W2}{WS} \times 100 \tag{5}$$

This equation also showed W1 (weight of crucible with fiber), W2 (weight of crucible) and WS (weight of sample).

3.6.3 Crude protein determination

The crude protein content is calculated using the micro-Kjeldahl technique, which determines the nitrogen (N) content of the food. This method consists of three major steps: sample digestion, digest distillation, and distillate titration. The food sample is placed in a digestion flask and heated in the presence of sulphuric acid, and a catalyst such as copper, selenium, or zinc (to speed up the reaction). At the boiling point, digestion took about 4 hours. The sample was then allowed to cool for 30 minutes. The 80 ml of distilled water and 50 ml sodium hydroxide were inserted in k-set, and the distillation process was carried out. For distillation, 30 ml of boric acid was added to the conical flask. Then, in the conical flask containing the boric acid, the 2.5 ml of bromo and 1.75 ml methyl red were added. The burette was then filled with hydrochloric acid, and the samples were titrated against a normal acid solution. Equation 6 demonstrated a complete equation for protein determination.

$$N\% = \frac{(T - B) \times N \times 14.007 \times 100}{Ws} \qquad (6)$$

This equation also showed T (Sample titration), B (Blank Titration), N (Normality of titrant), Protein percentage (N×F) and factor F is Protein factor which equal to 6.25.

3.6.4 Crude fat determination

The extraction beaker was put into the steel extraction beaker rack holder. The extraction cup was heat in the oven for 105°C for 30 minutes. Then, the extraction beaker was cooled down in the desiccator for 20 minutes. The extraction beaker was weight to obtain W1 reading. After that, 1 g of sample was weight in filter paper to obtain WS reading. After that, the filter paper was wrap and insert to the bottom of thimble. The sample with 2 cotton wool were insert in thimble. Next, 80 ml of petroleum ether was added into the extraction beaker. The extraction beaker with solvent was insert into soxthem and extraction started. After extraction is done, the extraction beaker from soxthem was took out. The extraction beaker was heat in the oven for 105°C for 30 minutes to make sure some residual solvents are removed. Then, the extraction cup was placed into the desiccator for 20 minutes. Next, the extraction beaker was weighed to obtain the W2 reading. Lastly, fat content can be calculated by using the data obtain and equation 7 demonstrated a complete equation for fat determination.

$$Fat\ content = \frac{(W2 - W1) \times 100}{WS} \tag{7}$$

Where, this equation showed WS (weight of sample), W1 (weight of extraction beaker) and W2 (weight of extraction beaker with oil).

3.7 Sugar content analysis

3.7.1 Preparation of standard glucose solution.

In a 100 ml volumetric flask, a standard solution of glucose was prepared with 0.1g. In a separate test tube, place 0.0,0.25,0.5,0.75,1.0,1.5 mL of standard glucose solution and distilled 2 mL of water. Then, 2 mL of Alkaline copper reagent was added to each test tube and boil for 8 to 10 minutes. The test tube was kept cool under running tap water without being shaken. After that, 2 ml of phosphomolybdic acid was added to each test tube and vortexed for 2 minutes. Then, wait for 2 minutes. Then, using distilled water, increase the volume to 10 mL. Next, using a spectrophotometer, measure the absorbance at 630 nm. Make a graph by plotting concentration versus optical density.

3.7.2 Extraction of new rabbit pellet formulation using 40 % pineapple leaves and commercial pellet.

1g of pellet was ground with 10 to 20 ml of distilled water in a mortar for pellet extraction. Then, place the sample in the falcon tube and tightly close the lid. The sample should then be centrifuged for 10 minutes at 26˚C and 4000 revolutions per minute. After 10 minutes, transfer the supernatant to a 100 ml volumetric flask. Using distilled water, make up to 100 mL. Using a 1000 micropipette, transfer 1 mL of the sample solution to each test tube and top off with distilled water to make up to 2 mL. Then, repeat the Alkaline copper reagent and phosphomolydic acid steps. Finally, using a spectrophotometer, measure the absorbance at 630 nm. The concentration glucose of sample was calculated by using data obtained and equation 8 demonstrated a complete equation for reducing sugar determination

$$(\boldsymbol{x}) = (\boldsymbol{y} + \boldsymbol{b})/\boldsymbol{m} \quad (8)$$

Where, this equation also showed x (concentration), y (Absorbance of sample), b (y-intercept) and m (Gradient).

3.8 Statistical analysis

To analyse the physical properties, proximate analysis, and reducing sugar, all the test was recorded in triplicate. This experiment's data was analysed using an unpaired T-test with SPSS software at a significance level of P less than 0.05.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Sample preparation

15 kg of pineapple leaf waste were randomly collected from local farm in Kelantan. Collected pineapple leaf waste was cleaned thoroughly in water twice by removing foreign particles like, stones, and dirt. It was then cut into small pieces of size 10-15 cm long by a local chopper. Then, the pineapple leaves were placed into the tray for drying process and were placed into the oven at 70°C for 72 hours. During the drying process, to prevent fungi from forming on the chopped leaves, they were flip upside down. The pineapple leaves were ready for grinding at a size of 1 mm once the drying process was done. After that, the ground and sieved samples was dried for another 24 hours at 60°C in the oven. The result from grinding process turned the dried pineapple leaf into a very small particle and not so powdery since it contained high of fibre. As a result of the process, a 2.5 kg sample of pineapple leaves were obtained. The dry samples were stored in the box for further examination to keep it long lasting.

For pelleting process, 800g of pineapple leaves sample were used. Before making pellet, several raw materials such as cracked corn, rice bran, soybean meal, molasses, vitamin premix, limestone and salt are weight by using digital weighing balance. Then, all the ingredient was uniformly mix according to the formula that meeting the nutrition requirements of all living stage rabbits almost. A correct mixture can be obtained by hand or with mechanical mixing device. Next, the mixtures were examined in moisture analyser to maintain at a moisture content of 30%. Following that, all of the ingredients were placed in the pelletizer. Finally, the pellets were baked for 5 hours at 70°C in an oven to ensure that they were firm and in good shape. At the end of the process, 2 kg of pellet was successfully produced. From the result, evaluation of pineapple leaves pellet according to physical and chemical properties was examined to compare with commercial pellets. The measurement of each pellet is about 2 cm long and is shaped like a cylinder.

4.2 Pelleting

2 kg of 40% Pineapple leaves pellet was produced. The pellet was finally made by pelletizer, also known as pellet presses or extruders which was used in the pelleting process. Pelleting was accomplished by using a great pressure to the mixture as it passes through the die’s holes. The temperature of the pellet rises as the pressure and friction increase. As the temperature rises during the pelleting process, 30 % moisture content

was used in 2 kg of pineapple leaves mixture to keep the pineapples leaves mixture bind when used in extruder machine. Based on the figure 4.2, the measurement of pineapple leaves pellet is about 2 cm long, whereas the commercial pellet is also around 2 cm long. Pellet length for rabbits should be between 0.8 and 1.0cm, as longer pellets are more prone to shatter and waste (Irlbeck, 2011). However, pellet size varies significantly between brands, target species, and, in some cases, life stages. Even when the nutrient content is the same, particle size has been proven to alter digestion rate, with tiny pellets breaking down significantly faster than bigger things. Pellet size influences the ease with which animals can access nutrients from their meal. This is because tiny things have a much higher surface area mass ratio, which may result in faster nutrient absorption (JohnPac, 2015).

a) 40% pineapple leaves pellet

b) Commercial pellet.

Figure 4.2: Measurement of 40% pineapple leaves pellet and commercial pellet.

4.3 Physical properties

The physical properties of the feed are an important consideration during the feed preparation procedure. A procedure for handling, processing, and storing feed in feed mills must include knowledge of the feed's chemical composition and nutritional content, as well as knowledge of the feed's physical properties, to minimize feed handling losses (Jasmal et al., 2015). Pellets must have good physical qualities to withstand processing, transportation, storage and packaging without generating considerable amounts of dust or particles. Table 4.3 shows the data obtained for the physical properties of 40% Pineapple leaves pellet and commercial pellets. There is a significant difference between the 40% pineapple leaves pellet and commercial pellet for all three physical properties at P 0.05.

Table 4.3 Result of physical properties

Samples	Bulk density ($g\ cm^{-3}$)	True density ($g\ cm^{-3}$)	Porosity (%)
40% Pineapple leaves	0.3974 ± 0.00036^{b}	0.4159 ± 0.00107^{a}	4.333 ± 0.57735^{b}
Commercial	0.5083 ± 0.00570^{b}	0.4219 ± 0.31371^{a}	15.3333 ± 0.57735^{b}

*Values are expressed as mean ± SD of triplicates measurements. Mean with different letter are significantly different at $p<0.05$ for each column.

4.3.1 Bulk Density

Based on the table 4.3 there were significant effect ($P<0.05$) on bulk density in all sample. The pineapple leaves pellet obtained 0.3974 ± 0.00036^{b} compare to commercial pellet 0.5083 ± 0.00570^{b}. The p value of both sample group is $p = 0.001$. Bulk density has a direct impact on pellet packing, transport equipment and conversion cost. In producing animal feed pellet, the durability of pelleted feed is essential in improving feed efficiency and feed quality. The higher bulk density of feed can reduce storage capacity and save transportation cost. In general, the bulk density is low due to the material's fluidity is poor during the granulation process and relatively difficult as the crude fibre content of pellet is high (Karuranithy et al., 2012).

4.3.2 True Density

True density is the important parameter for determined the physical properties of pellets. According to table 4.3 the true density of pellet was found not significant ($P>0.05$) between on sample. The p value of both sample group is $p = 0.977$. The sample of pineapple leaves pellet in this study was slightly higher 0.4159 ± 0.00107^{a} compare to commercial 0.4219 ± 0.31371^{a}. The comparison between the both sample shows good performance but at slow rate. Density is depended on process variables, type of feedstock and machinery. These density value were used to calculate the percentage of porosity.

4.3.3 Porosity

Porosity is the factor that indicates the frequency of pores in the bulk material. Based on the table 4.3, there were significant effect ($P<0.05$) on porosity in all sample. The p value of both sample group is $p = 0.003$. Sample 40% pineapple leaves pellet obtained the lowest 4.333 ± 0.57735^{b} compare to commercial pellet obtained 15.3333 ± 0.57735^{b}. From the result, low porosity of the pineapple leaves sample indicated that the void space was low and result in low compressibility. The high porosity of the commercial pellet indicated that the sample was compacted and result in higher compressibility.

4.4 Chemical analysis

For the purpose of animal feed, the 40% pineapple leaves with other feed ingredient pellet was tested using a series of AOAC analytical analysis methods. The outcome is as shown in Table 4.4 The primary goal of this formulation is to convert the 40% pineapple leaf into animal feed because it contains a high concentration of nutrients beneficial to animal growth. The results of this analysis were compared to the commercial pellet using two independent methods to determine whether there was a significant difference between both sample.

Table 4.4: Chemical Composition of 40% pineapples leaves pellet and commercial pellet.

Samples	Moisture (%)	CP (%)	CF or EE (%)	CF (%)	Ash (%)
40 % Pineapple leaves	13.3667±0.59911^{a}	12.9215±0.17136^{a}	1.9033±0.68850^{a}	23.2700±0.73750^{a}	9.3533±0.13503^{a}
Commercial	11.6433±0.84619^{a}	5.6409±0.15044^{b}	1.4400±0.17692^{a}	7.9400±0.73369^{b}	11.1667±0.28361^{b}

*Values are expressed as mean ± SD of triplicates measurements. Mean with different letter are significantly different at p<0.05 for each column.

Note: CP (Crude Protein), CF (Crude Fat), EE (Ether Extract), CF (Crude Fibre).

4.4.1 Moisture content

Moisture content is important because it can affect physio-chemical and stability of pellet (Mahapatra et al., 2010). Based on 4.4 table above, statistical analysis showed there was no significant effect (P>0.05) between pineapple leaves pellet with commercial pellet. The p value of both sample group is p= 0.084. The chosen range of moisture content is 30%. The sample with the desired moisture contents were prepared by adding an amount of distilled water which is used when moisture content of the mixed material is too low. From the figure, it can be seen that pineapple leaves pellet obtained the highest result 13.3667±0.59911[a] whereas, commercial pellet obtained 11.6433±0.84619[a] give the lowest values of moisture content. The is because the pineapples leaves were over dried during the sample preparation and the condensing steam in extruder which raises the mash's temperature and moisture content, was insufficient to soften the pellet before it is compacted, the frequency of blocked die increases due to less water loss in pellet processing (Angela, 2015).

4.4.2 Crude Protein

Table 4.4 shows that the mean of crude protein content for 40% pineapple leaves pellet obtained the highest result of 12.9215±0.17136[a] compare to commercial pellet obtained 5.6409±0.15044[b] which give the lowest values of protein content. There was significant different ($P<0.05$) between pineapple leaves pellet with commercial pellet. The p value of both sample group is $p = 0.000$ respectively. Protein is a fundamental in animal food requirement. Protein is important for growth, gestation, lactation and weight gain. The dietary protein requirement and recommended levels is between 12% to 17% for maintenance, growth, lactation and reproduction respectively (Akande, 2015). Protein deficiency can lead to slow growth, decrease feed efficiency lower milk yields and low birth weight.

4.4.3 Crude Fat

Table 4.4 shows that the mean of crude fat for pineapple leaves pellet result in slightly higher value of 1.9033 ± 0.68850^{a} compare to commercial pellet 1.4400 ± 0.17692^{a} give lowest values of fat content. There was not significant different ($P>0.05$) between pineapple leaves pellet with commercial pellet. The p value of both sample group is $p = 0.226$. Hence, generally the recommended amount of fat content of rabbit is range 2% to 5% (Vicky, 2011). Rabbits do not require fat in their diet. Besides, the quantities of fat content in plant is very small (Kawas et al., 2012). However, fat can improve palatability, but too much can increase the risk of obesity, hepatic lipidosis, and aortic atherosclerosis (Vicky, 2011).

4.4.4 Crude Fiber

According to table 4.4, the crude fibre of pellet was found significantly different ($P<0.05$) on both sample. The p value of both sample group is $p = 0.002$. The sample of 40% pineapple leaves pellet in this study was higher 23.2700 ± 0.73750^{a} compare to commercial pellet 7.9400 ± 0.73369^{b}. Pineapple pellet is rich in fibre and can be used as energy source and a good feed for animals (Suphalucksana et al., 2017). The high fibre is an important source of energy for rabbits as well as an effective tool for stimulate gut motility and maintaining the microbial population in the cecum. Non digestible fibre important to dental health because it helps wear rabbits's teeth (Vicky, 2011). However, low-fiber diets impede GI motility, resulting in food and hair retention, as well as the formation of hairballs. Obstacles can be fatal to rabbits because they are unable to spit hairballs like other animals. Rabbits require balanced diets that contain all of the vital components. Most forages supplied contain just 20 to 25 percent actual crude fibre, depending on forage maturity (McNitt et al., 1996). The higher the crude fibre concentration in a forage, the more mature pineapple leaves were used. In conclude, reducing the pineapple leaves may improve the crude fibre in rabbits digestion. The chances of a rabbit becoming affected by mucoid enteropathy can be reduced by providing a diet with crude fibre over 15% but less than 18% of the diet (Lucile, 2017).

4.4.5 Ash

The overall mineral content of a forage or diet is referred to as ash. Table 4.4 shows the mean of ash for 40% pineapple leaves pellet result slightly lower 9.3533 ± 0.13503^{a} compare to commercial pellet obtained 11.1667 ± 0.28361^{b} give the higher values of Ash. There was significant different ($P<0.05$) between pineapple leaves pellet with commercial pellet. The p value of both sample group is $p = 0.003$ respectively. Generally, the recommended amount of ash content of rabbit is 5 to 6.5%. When all of the food content is heated to a high temperature, the nutrients are incinerated out of the food, leaving an ash pile containing indigestible minerals such as calcium, phosphorous, and other minerals, some of which are required for our bodies to convert food into energy or to keep bones strong, such as calcium.

4.5 Reducing sugar

Figure 4.5 shows the standard curves developed using standard glucose solution. The standard curve was used to calculate the value of an unknown quantity by comparing its value to the value of a more easily quantifiable quantity. Absorbance is measured using a spectrophotometer. The result of the experiment was recorded in table form and plotted into a graph. The concentration of standard glucose obtained is 0.0, 0.5, 0.25, 0.75, 1.0, 1.5 and 2.0 mg/mL respectively. Based on the figures 4.5 above, the concentration of reducing sugar in 40% pineapple leaves is low 0.29, 0.31, 0.38 mg/mL compare to commercial pellet shows range 0.79, 0.89, 0.98 mg/mL. Both sample are significantly different ($P<0.05$).

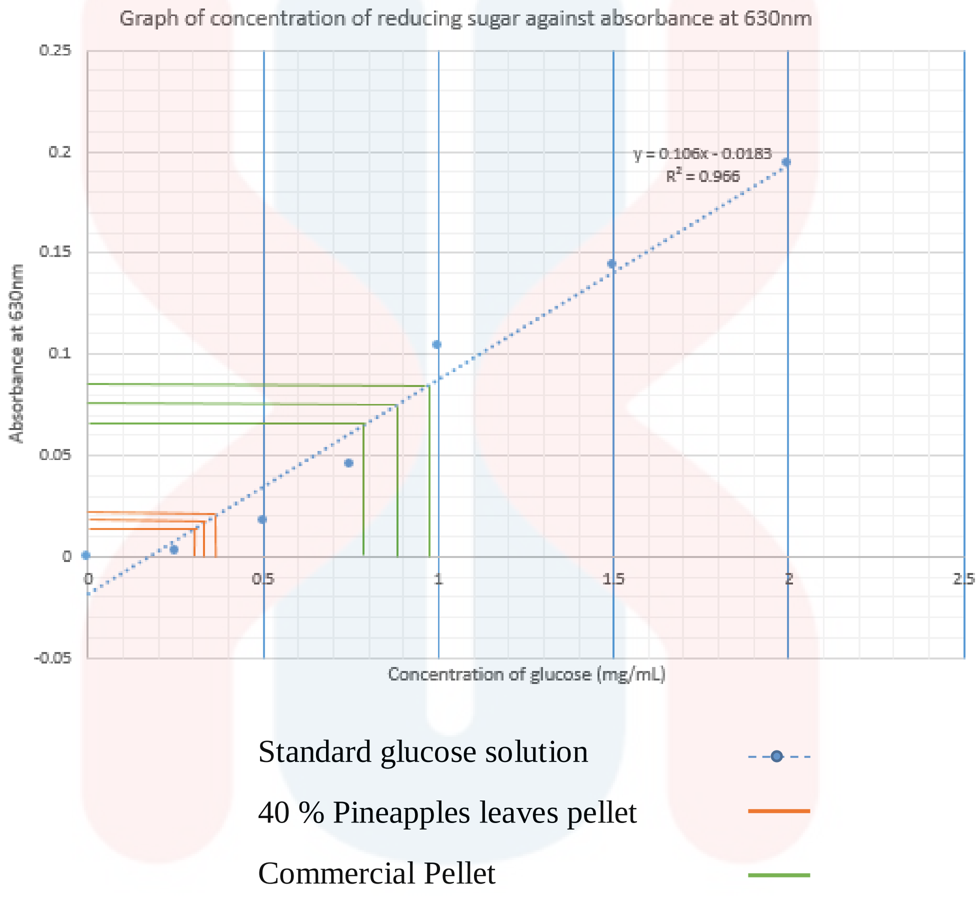

Figure 4.5 Graph of concentration of reducing sugar against absorbance at 630 nm

According to table 4.5, the reducing sugar was found to be significantly different ($P<0.05$) on both sample. The p value of both sample group is $p = 0.003$. The sample of 40% pineapple leaves pellet in this study was lower 0.3263 ± 0.04869^{a} compare to commercial pellet 0.8864 ± 0.09909^{b}. Simple sugar was made up of simple carbohydrates which produce brief spurts of energy. Complex carbohydrates such as fibre, on the other hand, take significantly longer to digest and hence supply more energy to rabbits over a longer period of time. Excess sugar consumption in rabbits can lead to dental issues, diabetes, and bloating. However, rabbits enjoy sweet foods such as sugar and molasses in their diets, hence the pineapple leaves pellet was mixed with molasses to make it more appealing (Cheeke et al, 2013). Molasses incorporation of 4-6% in the feed improved pellet quality and giving an incentive to introduce molasses even if no benefits on growth performance (Mendez et al., 1998).

Table 4.5: Result reducing sugar of 40% pineapples leaves pellet and commercial pellet.

Samples	Reducing Sugar
40 % Pineapple leaves	0.3263 ± 0.04869^{a}
Commercial	0.8864 ± 0.09909^{b}

*Values are expressed as mean ± SD of triplicates measurements. Mean with different letter are significantly different at $p<0.05$ for each column.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, the study showed that the pineapple leaves has the potential to be developed as animal feed pellet as suggested by the chemical analysis in comparison to the readily available commercial pellet. The pineapple pellet formulation developed contained significantly higher amount of protein and fibre in comparison to commercial pellet and no significant different in term of its moisture content and crude fat content.

Pellet quality is the key role to good animal performance, reducing feed cost, and increasing profitability to the small scale farmers that want an alternative solution to reduce feed cost by using agricultural waste. Pellet that contains complete nutrient as required by the rabbits is essential. It is found that the presence of additional ingredient in pineapples leaves give the improvement in nutrient content on the pellet. The crude protein content in pineapple leaves was higher compare to commercial pellet. The protein value follow the recommended levels which 12% that is suitable for rabbit's growth.

Overall, the pineapple leave pellet contains higher nutrient value which are crucial for rabbit's development as compared to the commercial rabbit pellet, which are poor in fibre and protein since they lack roughage that is restricted to rabbits.

5.2 RECOMMENDATION

The study show that the pellet has the potential to enter the feeding trials to test for its efficacy. Next, the process of pineapple leaves should be improved by lowering the crude fibre content of the pellet. Pre-treatment processes are necessary to overcome the complex structure of lignocellulosic biomass and make it accessible for enzymatic and microbial hydrolysis. A chemical pre-treatment with sodium hydroxide solution can be used to break down the lignin content in the pineapple leaves. The activation energy of the lignin can be evaluated and the impacts of numerous parameters on lignin degradation, such as NaOH content, temperature, and retention time can be further analyse .

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APPENDIX A

SPSS Analysis

Table A.1: T-test of physical properties by sample

BULK DENSITY

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pineapple Leaves	.3974	3	.00036	.00021
	Commercial	.5083	3	.00570	.00329

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Pineapple Leaves - Commercial	-.11090	.00599	.00346	-.12579	-.09601	-32.054	2	.001

TRUE DENSITY

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pineapple Leaves	.4159	3	.00107	.00062
	Commercial	.4219	3	.31371	.18112

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Pineapple Leaves - Commercial	-.00600	.31350	.18100	-.78478	.77277	-.033	2	.977

POROSITY

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pineapple Leaves	4.3333	3	.57735	.33333
	Commercial	15.3333	3	.57735	.33333

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Pineapple Leaves - Commercial	-11.00000	1.00000	.57735	-13.48414	-8.51586	-19.053	2	.003

Table A.2: T-test of chemical properties by sample

MOISTURE

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pineapple Leaves	13.3667	3	.59911	.34590
	Commercial	11.6433	3	.84619	.48855

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Pineapple Leaves - Commercial	1.72333	.92197	.53230	-.56697	4.01364	3.238	2	.084

PROTEIN

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pineapple Leaves	12.9215	3	.17136	.09894
	Commercial	5.6409	3	.15044	.08686

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Pineapple Leaves - Commercial	7.28053	.05285	.03051	7.14924	7.41182	238.601	2	.000

FAT

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pineapple Leaves	1.9033	3	.68850	.39751
	Commercial	1.4400	3	.17692	.10214

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	NewPellet - Commercial	.46333	.52548	.30339	-.84204	1.76871	1.527	2	.266

FIBRE

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pineapple Leaves	23.2707	3	.73757	.42584
	Commercial	7.9372	3	.73297	.42318

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Pineapple Leaves - Commercial	15.33347	1.21705	.70266	12.31015	18.35678	21.822	2	.002

ASH

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pineapple Leaves	9.3533	3	.13503	.07796
	Commercial	11.1667	3	.28361	.16374

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Pineapple Leaves - Commercial	-1.81333	.16289	.09404	-2.21798	-1.40869	-19.282	2	.003

SUGAR

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pineapple Leaves	.3263	3	.04869	.02811
	Commercial	.8864	3	.09909	.05721

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	95% Confidence Interval of the Difference Upper			
Pair 1	PineappleLeaves - Commercial	-.56010	.05327	.03075	-.69243	-.42777	-18.212	2	.003

APPENDIX B

Ingredients	Amount of Dry matter basis (100%)	CF (%)	CP (%)	ME (MJ /kg dry)
Pineapples leaves	40	12.40	2.6	460.8
Cracked corn	15	0.65	1.6	192.75
Soybean meal	28	1.85	12.7	312.48
Rice bran	8	0.84	1.2	60.72
Limestone	1	-	-	-
Salt	0.5	-	-	-
Vitamin premix	0.5	-	-	-
Molasses	7	0.05	0.01	97.72
Total	**100**	**15.79**	**18.11**	**1124.47 MJ in 100 kg**

B.1: Pineapple Leaves Pellet Formulation

Ingredients	Calculation 90 DM = 100g fresh	Kg
Pineapples leaves	$\frac{100 \times 40}{90}$	44.5
Cracked Corn	$\frac{100 \times 15}{90}$	16.7
Soybean meal	$\frac{100 \times 28}{90}$	31.1
Rice bran	$\frac{100 \times 8}{90}$	8.9
Limestone	$\frac{100 \times 1}{90}$	1.1
Salt	$\frac{100 \times 0.5}{90}$	0.6
Vitamin premix	$\frac{100 \times 0.5}{90}$	0.6
Molasses	$\frac{100 \times 7}{90}$	7.8
Total		**111.3 kg**

B.2: Air dry basis

Item	Quantity (g/kg)	Calculation
Raw material		
Pineapple leaves	$\frac{44.5 \times 2}{111.3} = 0.80\ kg$	800 g
Cracked Corn	$\frac{16.7 \times 2}{111.3} = 0.30\ kg$	300 g
Soybean meal	$\frac{31.1 \times 2}{111.3} = 0.56\ kg$	560 g
Rice Bran	$\frac{8.9 \times 2}{111.3} = 0.16\ kg$	160 g
Limestone	$\frac{1.1 \times 2}{111.3} = 0.02\ kg$	20 g
Salt	$\frac{0.6 \times 2}{111.3} = 0.01\ kg$	10 g
Vitamin premix	$\frac{0.6 \times 2}{111.3} = 0.01\ kg$	10 g
Molasses	$\frac{0.14 \times 2}{111.3} = 0.14\ kg$	140 g
TOTAL	**2.0 kg**	**2000 g**
Commercial pellets	1 kg	-

B.3: Budget

APPENDIX C

C.1: Pineapple leaves after chop

C.2: Dried Pineapple leaves

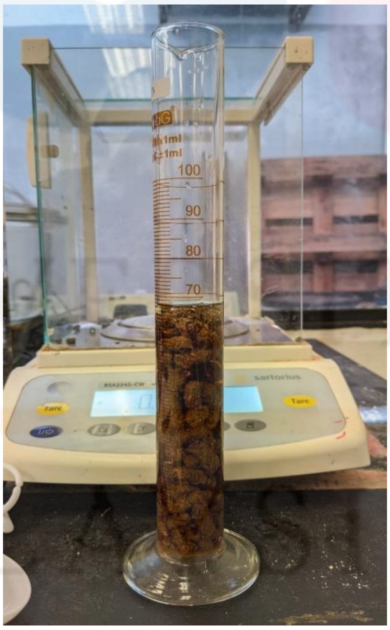
C.3: Pineapple leaves mixture

C.4: The pineapple leaves pellet was put into pelletizer machine.

C.5: The pineapple leaves pellet was stored in zipper bag.

C.6: The pineapple leaves pellet after dried in oven.

C.7: Bulk density test of pineapple leaves pellet

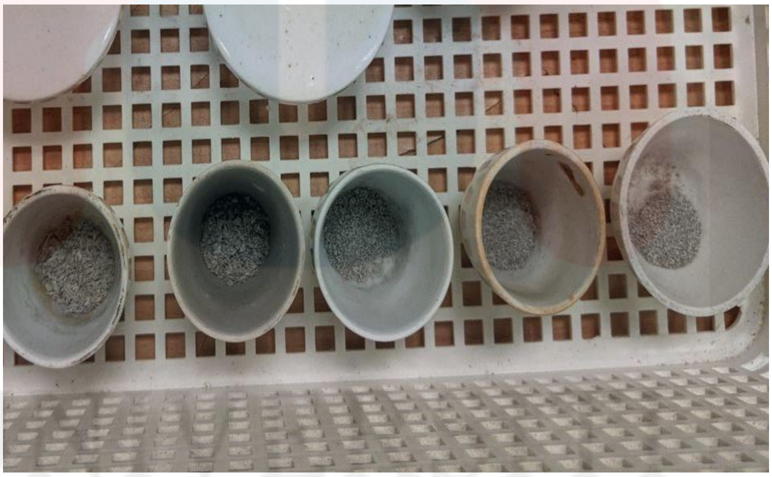


C.8: True density test of pineapple leaves pellet

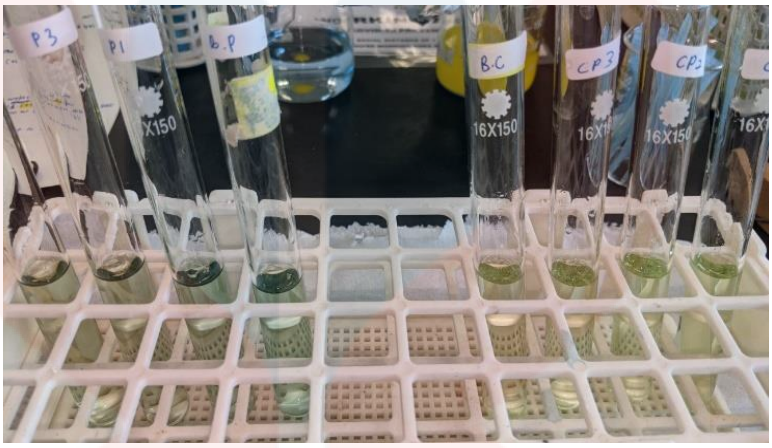
C.9: Protein Analysis for pineapple leaves and commercial pellet

C.10: Fat Analysis of pineapple leaves and commercial pellet

C.11: Fiber Analysis of pineapple leaves and commercial pellet



C.12: Ash analysis of pineapple leaves and commercial pellet



C.13: Reducing sugar analysis of pineapple leaves and commercial pellet

C.14: Extraction of pineapple leaves and commercial pellet supernatant