DIETARY EFFECT OF MULBERRY PELLET ON HAEMATOLOGY AND BIOCHEMICAL ANALYSES OF RABBITS

NURIN JAZLINA BINTI MOHAMAD FAUZI

F18A0282

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DECLARATION

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

Student's Name: Nurin Jazlina binti Mohamad Fauzi

Matric No: F18A0282

Date: 1 March 2022

I certify that the report of this final year project entitled "Dietary Effect of Mulberry Pellet on Haematology and Biochemical Analyses of Rabbits." By Nurin Jazlina binti Mohamad Fauzi with Matric Number F18A0282 has been examined all the correction recommend by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours, Faculty of Agro Based Industry, University Malaysia Kelantan.

Verified by:

Supervisor's Name: Ts. Dr Nor Dini Bt Rusli

Stamp:

Date:

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Nurin Jazlina binti Mohamad Fauzi

Animal Husbandry Science, University Malaysia Kelantan.

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ABSTRACT

Morus alba (White Mulberry) is high in quality, minerals, and nutrients. So Mulberry plant is suitable to be fed to monogastric animals and herbivores. Rabbit meat has a lot of potential as a beef and mutton substitute. Due to the rising cost of animal feed ingredients and the high demand for protein supplements, several efforts have been made to use non-traditional feed protein ingredients to help alleviate feed shortages while lowering feeding costs. Mulberry plant is a well-known medicinal plant that has long captivated researchers. This study aims to evaluate the effect of newly formulated Mulberry pellet on the haematological and biochemical parameters in growing New Zealand White (NZW) rabbits. The formulation of mulberry pellet consisted of mulberry leaves and stems (7:3) with 5% molasses, 5% tapioca starch, and 1% sodium bicarbonate. Diets were divided into three dietary groups: Control (100% commercial pellet), T1 (50% mulberry leaf pellet and 50% commercial pellet) and T2 (100% mulberry pellet). According to findings, there was no significant difference ($p>0.05$) in hematology and serum biochemistry during post-feeding trial. However, the T1 group shows the hematological results optimum within the normal range (WBC, LYM, GRA, RBC, HGB, HCT, RDW, PLT, MPV, PCT, and PDW). At the same time, biochemical parameters in T2 in BUN, GLU, and ALT have recorded superior values than C and T1. Red blood cells (RBC), haemoglobin (HGB) and hematocrit (HCT) percentages decreased with mulberry leaf-to-stem in the diets while mulberry pellets decreased serum glucose (normal range is 75.00-145.00 mg/dl). However, T2 outperforms C and T1 in biochemical analysis. The current finding may help the farmers to reduce the feed cost.

Keywords: Mulberry pellet, rabbits, blood profile.

KESAN PEMAKANAN DEDAK MULBERI TERHADAP HEMATOLOGI DAN ANALISIS BIOKIMIA ARNAB

ABSTRAK

Morus alba (Mulberi Putih) mempunyai kualiti, mineral dan nutrien yang tinggi. Jadi tumbuhan Mulberi sesuai untuk diberi makan kepada haiwan monogastrik dan herbivor. Daging arnab sangat berpotensi sebagai pengganti daging lembu dan kambing. Disebabkan oleh peningkatan kos bahan makanan haiwan dan permintaan yang tinggi untuk suplemen protein, beberapa usaha telah dilakukan untuk menggunakan bahan makanan protein bukan tradisional untuk membantu mengurangkan kekurangan makanan sambil mengurangkan kos pemakanan. Mulberi adalah tumbuhan ubatan terkenal yang telah lama memikat para penyelidik. Kajian ini menilai kesan rumusan makanan Mulberry terhadap perubahan histologi dalam beberapa organ dan parameter darah dalam tumbesaran arnab New Zealand White (NZW). Peratusan sel darah merah (RBC), hemoglobin (HGB) dan hematokrit (HCT) menurun dengan daun ke batang mulberi dalam diet manakala pelet mulberi menurunkan glukosa serum (julat normal ialah 75.00-145.00 mg/dl). Daun dan batang mulberi (7:3) dengan 5% molase, 5% kanji ubi kayu, dan 1% natrium bikarbonat adalah makanan tambahan yang baik untuk membesarkan arnab tanpa kesan buruk pada hati, buah pinggang atau fungsi organ lain. Matlamat kajian ini adalah untuk menentukan sama ada pelet mulberi boleh meningkatkan profil darah. Diet dibahagikan kepada tiga kategori: C (100% pelet komersial), T1 (50% pelet mulberi dan 50% pelet komersial) dan T2 (100% pelet mulberi). Menurut penemuan, T1 adalah formulasi terbaik untuk analisis hematologi. Walau bagaimanapun, T2 mengatasi C dan T1 dalam analisis biokimia. Penemuan semasa boleh membantu petani mengurangkan kos makanan.

Kata kunci: Pelet Mulberi, arnab, profil darah.

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LIST OF UNITS AND SYMBOLS

g	Gram
mm	millimetre
g/dL	grams per decilitre
µl	microlitre
µm	micrometre
pg	picogram
mg/dL	milligrams per decilitre

LIST OF ABBREVIATIONS

WBC	White blood cells
LYM	Lymphocytes
MON	Monocytes
GRA	Granulocytes
RBC	Red blood cells
HGB	Haemoglobin
HCT	Haematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
RDW	Red blood cells distribution width
PLT	Platelet count
MPV	Mean platelet count
PCT	Procalcitonin
PDW	Platelet distribution width
BUN	Blood Urea Nitrogen
GLU	Glucose
ALT	Alanine aminotransferase
TP	Total protein

CHAPTER 1

INTRODUCTION

1.1 Background of study

The mulberry is a hardy perennial woody plant that proliferates and belongs to the Morus Moraceae family (Pan and Lou, 2008; Yang 2010). According to Salbiah et al. (2021), mulberry is found worldwide because it is resilient enough to thrive in adverse conditions. Mulberry tree is widely distributed all over the regions and it is also suitable in tropical to temperate countries (Yonous Wani, 2017). In various climates, including warm temperate and subtropical regions of Asia, Africa, and Europe, with most species native to East and South Asia, many countries worldwide enjoy the tasty fruit that mulberries provide (Buhroo et al., 2018). As an inexpensive source of minerals, protein and fibre, mulberry could be an excellent option for those looking to save money (Sánchez, et al., 2017). Mulberries are valuable plants because of their high nutritional content and unique agronomic properties. The CP content of M. Alba's is more than 20% DM (Valdes et al., 2017). Because of their high biomass production, chemical composition, high ruminal degradability, adaptability to various climate and soil conditions, and availability, Morus species are widely used as forage (Valdes et al., 2017). Cai et al. (2019) suggested that the high-quality protein in mulberries could be used to feed livestock, particularly in the winter and spring. The mulberry foliage is highly nutritious and palatable and commonly used to feed silkworms (Sánchez, et al., 2017). Mulberry silage has a higher crude protein content than other forage crops, making it a

superior sheep food (Chandrashekhar and Hj, 2020). The inclusion of safety features and environmental protection in the feed supply chain is also encouraged (Gulab et al., 2020). Most forage diets can benefit from the addition of mulberry leaves, as their nutritional value in the leaves is comparable to that of grain-based concentrates (Dini et al., 2021). Mulberry leaves and stems may have 15% to 28% protein and a range of essential organic compounds edible and digestible to herbivores and even monogastric with 70% to 90% range of protein and organic compounds (Sánchez, 2017).

Before being widely domesticated throughout the world, rabbits (Oryctolagus cuniculus) trace their ancestry back to European regions. Diets high in fibre and low in nutrients have adapted their teeth and digestive systems. Grass or grass hay, vegetables, and small amounts of concentrates are essential for tooth and gut health, as well as weight maintenance. Rabbits are ground dwellers that live in desert, tropical forest, and wetland habitats. NZW is widely used in laboratory studies due to high fertility, ease of availability, feeding, and housing (Jeklova et al., 2009). A rabbit's diet is essential to its overall health. Low-fibre diets have been linked to gastrointestinal, dental, and urinary tract disease and other health issues. Infectious diseases of the digestive system make up 70% of all rabbit illnesses. Rabbit meat also advocates good health because it does not form urine acid during metabolism (Lyeghe-Erakptobor, 2017). To make the rabbit consumed by humans, the health of the rabbits must be a priority. Assessment of blood parameters level is one of the indicators of health status. The haematological parameter allows us to understand the normal and abnormalities of blood cell count such as Red Blood Cells (RBC), White Blood Cells (WBC), lymphocytes, granulocytes, Mean Platelet Volume (MPV), Hematocrit and Mean Corspular Volume (MCV). Blood abnormalities were determined by haematologic analysis. Biochemical testing can be used to evaluate a wide range of organ conditions. Haematological and biochemical parameters can yield

significant results (Melillo, 2007). Knowing the reference values for hematologic and biochemical parameters when assessing rabbit diseases is helpful for clinicians when evaluating rabbit health situations and provides important information (Archetti et al., 2008). Blood and biochemical parameters can be affected by a wide range of variables, including the animal's genetics (Chineke et al., 2006), age (Meillo, 2007), diet (Jeklova et al., 2009), stress (Abedl-Azeem et al., 2010), and even pregnancy (Chineke et al., 2006; Melillo, 2007). A sterile 26-gauge needle was used to draw blood from the rabbit's central ear vein and transfer it to tubes for analysis. Haematological samples were slowly withdrawn into K2-EDTA microtube, while biochemical samples were drawn into sterile Lithium Heparin microtube. Since the haematology and biochemical parameter are important for the rabbits, this current study aims to evaluate mulberry pellets as feed resources and feed alternatives to improve animal health and efficiency in sustainable animal production.

1.2 Problem statement

Rabbit production is highly profitable and not widely commercialized. However, rabbit meat is a good source for human consumption. Animal feed ingredients are becoming increasingly expensive, and there is a high demand for protein supplements, so several efforts were made to use non-traditional feed protein ingredients to help combat feed shortages while also reducing feeding costs. Because of this, it has never been more critical to find low-cost, locally sourced feed sources with similar feeding values to replace some of the common ingredients (Wong & Tan 2009). So, to improve livestock production, the mulberry pellet was tested on rabbits' blood profiles. The use of natural plant feed (mulberry) improved rabbit health. Feed cost plays a key's role, which makes

up most production costs. To reduce feed costs, mulberry can be used instead of high protein sources like diets in limited level commercial pellets. Mulberry is a nontoxic natural therapeutic agent plant for better diets. Studies on diabetic humans and mice showed mulberry reduces blood glucose levels (Kimura et al., 2007). Due to its high fibre content, it is used to feed silkworms and animals (Saurabh et al., 2013). Constipation is a common occurrence in animals due to intestinal blockage caused by poor animal nutrition. Mulberry is very digestible (Saddul et al., 2005). So a mulberry pellet is suitable for rabbit disposal. Incorrect nutrition caused health issues. Consider its teeth and guts. It was keeping healthy and working correctly will help maintain rabbit health. calcium ranged from 380.00 to 786.00 mg/100 g fresh leaves and 786.66 to 2226.66 mg/100 g dried leaf (Srivastava et al., 2006). Insufficient calcium intake caused eating and caecotrophy problems (stop grooming). Mulberry could help feed the world's growing population and improve nutrition. Dietary supplements of cultivated green foods should be given daily, and the only amount of concentration (Bourne, 2009). The mulberry diet's high fibre content is good for the intestines. Low GI mobility led to gas accumulation in the stomach and trichobezoar development in the caecum (Bourne et al., 2012). Non-digestible, good for teeth and the gastric system (Meredith, 2011). Obese rabbits are less likely to develop cardiac hypertrophy (Rebra, 2012).

1.3 Objectives

- To evaluate the effect of mulberry pellet on the haematology analysis in rabbits.
- To evaluate the effect of mulberry pellet on the biochemical analysis in rabbits.

1.4 Hypothesis

Ho: There are significant differences in mulberry pellet feeding of blood profile constituents in rabbits.

H1: There are no significant differences in mulberry pellet feeding of blood profile constituents in rabbits.

1.5 Scope of study

Mulberry plants were hand-picked from the tunnel garden Faculty Industry Agro-Based (FIAT) and Agro-Techno Park of University Malaysia Kelantan, Jeli Campus. In the present study formulation, the main ingredients of the mulberry pellet are the leaves and stem of the mulberry plant with a mulberry leaf-to-stem ratio (7:3). There are a few ingredients in the formulation of this mulberry pellet: cassava starch, molasses, and sodium bicarbonate as a new binder agent replaced from sodium alginate because of its lower price and ease of finding were mixed at University Malaysia Kelantan, Jeli Campus. Six (6) rabbits bought from rabbit farms in the Agro-Techno Park of University Malaysia Kelantan, Jeli Campus, were fed with different types of feed using mulberry pellet and commercial pellet.

1.6 Significance of study

Feed is one of the necessary elements in farm management. Animal feed ingredients have become much more expensive, causing commercial feed to be marketed to farmers at a higher price. Natural feed from plants (mulberry) aids in the production of

a more natural product that meets the needs of rabbits while also being low in cost. The quality feed comes with the balanced nutrition that equivalent New Zealand White (NZW) breed rabbits to ensure the highest standards in health and reduce the risk of diseases caused by food nutrition practice.

CHAPTER 2

LITERATURE REVIEW

2.1 Mulberry

Morus alba L., a dominant species in the Moraceae family, is found in a wide range of climates, from tropical to temperate (Yuan et al., 2015). The top health institutions in the US, India, France, and Asia have proven the scientific data about the mulberry leaf miracle. Mulberry is one of the world's oldest herbs used for healing and traditional medicine.

2.2 Mulberry nutrient composition

Monogastric, ruminant, and animal rabbits eat protein-rich mulberry forage (Benavides, 2020). In the spring and autumn, mulberries contain 21.1 and 20.9 percent crude protein (CP) and 88.2 and 85.8% true protein (TP). Mulberry is a good source of nitrogen, sulphur, and minerals for livestock (Singh et al., 2002). Foliage from mulberry trees is higher in protein and low in Leaf and stem protein content with excellent essential amino acid profile (Sanchez,2000). Highly palatable and digestible (70-90%) for

herbivores and monogastrics. (1992) The mulberry leaves are high in protein and minerals and are highly digestible and palatable to all livestock animals (Sujathamma et al., 2013).

2.3 Mulberry for health

Mulberry plants have been found to have a wide range of medicinal properties (Datta, 2000). Mulberry is famous inability to boost vitality and ensure the healthiness regarding the mineral content and vitamin sources themselves. Mulberry tea is made from mulberry buds and leaves. Mulberry tea's crude protein contains 17 amino acids, 15 essentials to human health. Dietary fibre, chromium and rutin are all found in mulberry tea; the body and beneficial to health quickly absorb all. Mulberry tea is a health drink for all ages that calms the liver, improves vision, and removes wind-heat (Wang et al., 2011). The mulberry tree has many medicinal uses in Chinese medicine. There are many clinical studies showing that mulberry chemical components can be used to treat bacterial, viral and tumour infections, as well as ageing (Su 2010; Asano et al. 1994; Andallu et al. 2001; Xu et al. 2005; Hua et al. 2007; Guo 2008). Mulberry fruit is also used in folk medicine in China, Korea, and Japan to treat fever, sore throat, liver, and kidney protection, improve eyesight, and lower blood pressure (Zhou et al., 2017).

2.4 Mulberry as animal feed

2.4.1 Silkworm

Mulberry silkworms' other unusual foods have long been consumed in Assam, India (Mishra et al., 2003). It is because the total protein (12 to 16%), total fat (11 to 20%), carbohydrate (1.2 to 1.8%), moisture (65 to 70%), and ash were all present in

mulberry silkworm pupae (0.8 to 1.4%). Silkworms (Bombyx mori L.) are known as poikilothermic and are the primary source of silk. It could be a good source of protein and fat. Mulberry leaves are the only food source for silkworms (Kandyli et al., 2009).

2.4.2 In poultry

They have a higher feeding value than other forages because of their high dry matter, protein, and mineral content, as well as a high level of available digestible energy. Because of the high concentration of lysine (1.88%) and leucine (2.55%) in the mulberry leaves, these two essential amino acids are readily available in the leaves (Al-Kirshi et al. 2009). Ascorbic acid, carotene, vitamin B1, folic acid, folinic acid, and vitamin D are all found in these foods. Nutrient-rich mulberry leaves may help offset the fibre's effect on monogastric animals such as broilers (Ayaoan and Baylan 2012; Freddie Simol et al. (2012). Dietary extracts of mulberry leaves have been shown to reduce the concentration of blood cholesterol in broilers, according to studies by Suwannee Saenthaweesuk (2009) and Sunder et al. (2011).

2.4.3 Ruminant

Mulberry leaves have recently been reported as an additional protein source for ruminants by Liu et al. (2001) and Yao et al. (2000). According to ruminant feeding experiments, mulberry leaves are as nutritious as some well-known high-quality fodders. Growing lambs (Benavides, 2000) and goats (Gonzalez et al., 2000) gained more weight when supplemented with mulberry leaves in their diets, while goats (Ba et al. 2005) produced more milk when given higher amounts of mulberry. Mulberry leaves, which are high in nitrogen, sulphur, and minerals, could supplement ruminant feeding systems. The use of mulberry leaves in livestock feed can improve productivity.

2.5 Mulberry pellet as rabbit feed

In terms of moisture content, the dried mulberry leaf had a range of 5.11–7.24%, 15.31–13.91%, 14.59–17.24%, 27.60–36.6%, 2.09–4.93%, and 9.70–29.64% carbohydrate content (Srivastava et al., 2006). According to research, the tree may be a viable option for increasing farm animal diets and reducing feed costs in a few production processes. Using a different perspective, the dry matter digestibility of mulberry leaves in vitro was 89%. It included 163 ash and 201 crude proteins; 120 crude fibres; 37 extracts of the ethers; 479 nitrogen-free extracts; 268 neutral detergent fibres; 148 acid detergent fibres; 41 lignin's; and 121 cellulose and 107 hemicelluloses. Scores for digestibility range from 70% to 90% for leaves and 37% to 44% for stems. Mulberry is an excellent rabbit food because of its digestibility, high concentration of essential amino acids and minerals, and lack of antinutritional factors (Sanchez, 2000). Herbal plants can be used in rabbit diets to improve nutrient utilisation, growth, and meat quality (Bosco et al.,2014). Regarding the pellet size, feeding pellets of small diameter (<0.25cm) will lessen the intake and weight gain because of the increased feeding time, while feeding larger diameter pellets (>0.5cm) results in greeter feed wastage. It is recommended the length of the pellet for rabbits is 0.8 to 1.0cm because the longer the pellet, it will cause more significant breakage. A solid and firm pellet of 0.63cm in length and 0.47cm in diameter is optimum for rabbit (Irlbeck, 2011), while this study shows that the diameter of the mulberry leaf pellet was 0.4mm and it indicates that the mulberry leaf pellet was the optimum diameter for the rabbit to consume.

2.6 Rabbit

The rabbit (*Oryctolagus cuniculus*) is a herbivore that lives 1–2 years. The shortest production period is for the small fluffy animal (20-50 cm). Rabbit meat is high in unsaturated fatty acids (FA), low in sodium, and low in cholesterol (Hernandez et al., 2006). In fact, the USDA statistical breakdown of several portions of meat. Rabbit meat is low in fat and high in protein compared to other meats. However, rabbit meat is low in fat and high in omega-3 fatty acids. There are many processes where temperature monitoring is critical, especially in the livestock industry. Temperature is a major factor in animal behavior. The health of rabbits may be harmed because of climatic change, making environmental monitoring essential to raising healthy livestock

2.7 Nutrient requirement of rabbit

Diet and supplement sources are vital in rabbit production. Providing a substrate for fermentation in the cecum to produce bacterial cells as a source of protein and B vitamins is essential for rabbits. Rabbit diets should contain at least 14% fibre. Rabbits are organisms that require vitamin A, D, E, and K, as well as water-soluble vitamins (B complex and C). Their teeth and digestive system have been adapted to their high-fibre, low-nutrient diet. Providing a balanced diet of grass or grass hay, vegetables, and small amounts of concentrates is essential for tooth and gut health and weight maintenance. Rabbits are suitable converters of fibrous plant materials, and high-quality forages are included in their diet. The nutrients that rabbits require in their diet are protein, energy (carbohydrates and fat), minerals, vitamins. Rabbit is particular with their food as they mostly prefer the green leaves resulting in higher protein and digestible energy and low in fibre (James et. al, 2013). Lack in fibre intake led to serious diarrhoea and mortality in growing animal (Bennegadi et.al, 2001).

2.8 Blood profile in rabbit

Male and older rabbits have higher RBC counts than female and younger rabbits. High RBC counts can be caused by dehydration or cold stress. High nucleated RBC counts can indicate bacterial infection, flea infestation, or internal bleeding. A slight increase in nucleated RBC counts is standard in rabbits. The HCT (Haematocrit) test calculates the

percentage of red blood cells. A low value indicates anaemia. Anaemia (low Hb) can be diagnosed by measuring haemoglobin concentration. High platelet counts are linked to anaemia and chronic bleeding. Cold stress and drugs like glucocorticoids and epinephrine can raise platelet counts. Aplastic anaemia, severe allergic reactions, and systemic bacterial or fungal infections can cause low levels. Too much time between storage and analysis can generate common values. White blood cells (WBC, leukocytes): WBC counts vary by age, sex, breed, and season. WBC counts can include monocytes, lymphocytes, neutrophils/heterophils, and basophils. Infectious diseases in rabbits are often detected by the proportion of different WBC types rather than the total WBC count. High monocyte counts indicate chronic infection, while high eosinophil or basophil counts indicate parasitic infection.

Dehydration or exposure to cold can raise RBC counts. RBC counts with a high nucleated RBC count can indicate bacterial infection, flea infestation, or internal bleeding. WBCs: White blood cells. According to age, gender, breed, and season, white blood cell counts vary. Mulberry has hypoglycemic, hypotensive, and diuretic properties in rabbits' blood profiles. Protein is found in animal tissue (muscles, cells), hormones, and enzymes. The haematological indices reflected the animals' dietary treatments in terms of feed type and quantity. They met the animal's physiological, biochemical, and metabolic needs (Ewuola et al., 2004). The blood contains numerous metabolites and other constituents that can be used to assess a person's or animal's nutritional status.

When blood sugar glucose (GLU) levels are elevated, it can indicate kidney disease, but stress can also play a role. The blood urea nitrogen (BUN) test is an essential tool for evaluating kidney function; for example, how much protein is in the diet and how well-hydrated the rabbit affects urea levels. The enzyme alanine aminotransferase (ALT) determines whether liver damage has occurred. Dehydration in rabbits can result in high

serum protein, total protein (TP) levels, while malnutrition or liver disease can cause low levels.

2.9 Normal values blood profile in rabbit

Table 2.9a: The reference value of haematological analysis in rabbit (Faculty of Veterinary Medicine, University Malaysia Kelantan, 2018).

No.	Parameter	Reference values
1.	WBC (10^3/µl)	**4.0-12.0**
2.	LYM	**1.0-5.0**
3.	MON	**0.1-1.0**
4.	GRA (10^3/µl)	**2.0-8.0**
5.	LYM%	**25.0-50.0**
6.	MON%	**2.0-10.0**
7.	GRA%	**50.0-80.0**
8.	RBC (10^6/µl)	**4.0-6.20**
9.	HGB (g/dl)	**11.0-17.0**
10.	HCT (%)	**35.0-55.0**
11.	MCV (µm^3)	**80.0-100.00**
12.	MCH (pg.)	**26.0-34.0**
13.	MCHC (g/dl)	**31.0-35.5**
14.	RDW (%)	**10.0-16.0**
15.	PLT (10^3/µl)	**150-400**
16.	MPV (µm^3)	**7.0-11.0**

17.	PCT (%)	**0.200-0.500**
18.	PDW (%)	**10.0-18.0**

Table 2.9b: The reference value of biochemical analysis in rabbit (Faculty of Veterinary Medicine, University Malaysia Kelantan, 2018).

No.	Parameter	Reference value
1.	BUN (mg/dL)	**10.0-24.0**
2.	GLU mg/dL)	**75-145**
3.	ALT (u/L)	**31-53**
4.	TP (g/dL)	**5.5-7.2**

2.10 Mulberry pellet feeding in blood constituents

Mulberry pellet can determine the health of the rabbit by evaluating the blood constituents' changes. It is because animal nutrition has significant relationship with the animal health. The mulberry pellet can determine the health status, hence consuming the right nutrition diet can hence the rabbit health status. This research focused on animal health; It is pivotal to evaluate the health status of rabbit for human consumption. Biochemical and hematological parameter can show the physiology by telling us the normal and abnormal reading of substances that abnormally higher level of reading blood such as total protein in blood serum and reading enzyme that secreted by liver to stabilize the toxicity that presence in the blood such as alanine aminotransferase (ALT).

CHAPTER 3

METHODOLOGY

3.1 Experimental design

- 70 days of New Zealand White breeds.

Control Group

- 100% commercial pellet
- 3 of rabbits
- 3 males

Group treatment 1

- 50% of mulberry pellet and 50% of commercial pellet
- 3 of rabbits
- 3 males

Group treatment 2

- 100% of mulberry pellet
- 3 of rabbits
- 3 males

3.2 Sample preparation

The controlled feed group were given commercial pellets. Commercial feed was easy to feed and store at 75% of production costs. Plant-based ingredients were used in commercial feed, grains (oats, wheat, barley, grain sorghum, corn, and hay), milled feeds (bran, middling, and shorts), and green feed (grass, weed, leafy vegetables) with the adequate amount of the required nutrients.

Fresh mulberry leaves and stems were collected. The dried mulberry leaves and stem were ground until powder formed. A 89% of mulberry formulation with 70% consist of mulberry dried leaves, and 30% dried stem. It was mixed with other ingredients such as 5% tapioca starch, 1. 0% sodium bicarbonate, and 5% molasses. The daily feed value was 100g per rabbit. Then, the mulberry leaves and stem were dried in the oven with 55^{o}c for 12 hours to remove some moisture. The mix of mulberry leaf, stem and other ingredients had been pelletized using pelletizer machines which the diameter of the pelletizer was 0.4mm. The quality of the mulberry pellet had been inspected before being given to the rabbits. 100% of commercial pellet was given for control group and 100% of the mulberry pellet was given for group treatment 1. The mix of 50% mulberry pellet and 50% commercial pellet was formulated for group treatment 2.

Table 3.2: The feed formulation ratio mulberry pellet for 2 months feeding trial.

Ingredients		Percentages (%)	Amount (27kg/month)
Mulberry plants	Leaves (70%)	89%	16.82
	Stem (30%)		7.209
Molasses		5%	1.35
Tapioca starch		5%	1.35
Sodium Bicarbonate		1%	0.27
Total		100%	27.0 g

3.3 Experimental site and animal

Nine (9), 2 months 10 days old; New Zealand White (NZW) for the meat production breed typed with average weight 1.5 kg or 2 kg in groups. There is a one-week 'adaptation period' (quarantine period). Adaptation to the environment and daily routine in the animal pens. During the feeding trial, six male rabbits participated and were randomly weighted and sorted by cage number. The feed trial would last 60 days if the adoption period were not included. Using the watered nipple to drip fresh water directly through a pipe, a cage was built. To allow excrement to fall into the galvanized mesh wire collection trays, the mesh cages (0. 90x0. 60x0. 45m) were stacked vertically at a height of 0. 8cm from the ground. Due to its circadian biorhythm, the colony required between 12 and 14 hours of light each day. UMKKJ's Agro-Techno parking lot had served as the test site for the experiments conducted at the rabbit house.

3.4 Experimental diets

Control group, treatment group 1 and treatment group 2 were the three (3) groups of rabbits that were used in the experiment. Diets for the study's participants included only commercial pellets. There was a 50% mulberry pellet and 50% commercial diet option for the first group of patients. Dietary treatment group 2 was given mulberry pellets containing 100% of the recommended daily allowance.

3.5 Evaluation of rabbit performance

3.5.1 Feed intake

Every morning and evening, the rabbits received 50g of weighed feed. Each rabbit was fed a different diet. The commercial pellet and a sample of mulberry pellet were combined in the feed for the treatment 1 groups. Treatment 2 fed with a sample of mulberry pellet.

3.5.2 Blood sampling

For serological analysis and haematology testing, serum and haematology samples were obtained from each rabbit in the groups. The entire circulating blood volume equals 5.5%-8.0% of the animal's body weight on average. Without extra monitoring, non-terminal blood collection should be limited to 10% of total circulating blood volume each collection or every two weeks for serial groups (Formulary for Laboratory Animals,2005).

Using a 0.5ml vacutainer blood tube, a 26G needle, and a 0.5ml syringe, blood samples were taken from the rabbits' central auricular artery ear (incision method). Central auricular artery easily visualized, accessible, does not require sedation/anaesthesia and rapid collection of larger volume of blood (Moore et al., 2015).

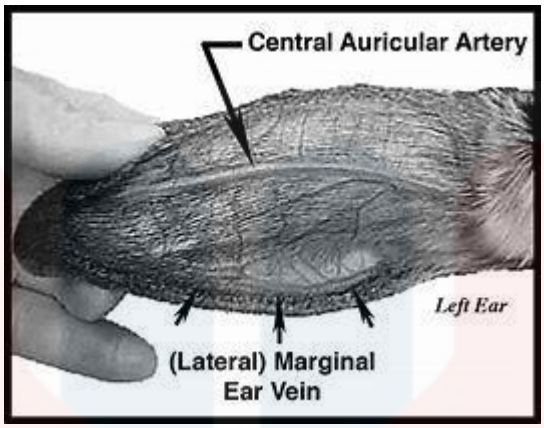

Figure 3.5.2: Vessels of the rabbit ear used for blood collection (The Medical University of Ohio and the University of Toledo, 2007).

Each rabbit blood was collected followed weight and 10% of total blood volume. The rabbit is restrained, and the rabbit's ear is cleaned with 95% percent alcohol. Blood was collected in a collecting tube after puncture drawn from the central auricular artery with a 26G needle. 30-60 seconds ear massage to dilate vessels (University Veterinarian and reviewed by Virginia Tech IACUC, 2017). Following the collection of blood, clean, sterile cotton is placed on the collection site and a finger pressure for 2 to 4 minutes to administered to halt the bleeding and prevent hematoma formation (Moore et al., 2015). A hematoma on the pinna may be irritating to the rabbit, causing head shaking and ear scratching, which can cause the hematoma to become even larger and more irritating.

Fresh blood should be chilled right once to prevent the clotting process from being slowed down. Samples can be kept at 4^{o}C before they are tested and quickly processed (Moore et al., 2015).

Rabbit Estimated Circulating Blood Volume = 56ml/kg (44-70ml/kg) Mean blood volume rabbit = 56 ml/kg.

Calculate using mean blood volume and body weight:

Total blood volume/ml = (mean blood volume ml/kg x body weight/kg)

Total blood volume may be collected = 0.01% of total blood volume/ml (University Veterinarian and reviewed by Virginia Tech IACUC, 2017). Maximum blood collection was 1% of body weight every two (2) weeks (Mheineke, 2013).

3.5.3 Blood sampling method

3.5.3.a Haematological analysis

Figure 3.5.3.a: Mythic CBC Test

This test can be analysed by using the haematology analyser (Mythic CBC test). The levelled red blood cell (RBC) and white blood cell (WBC) levelled could diagnose the infectious disease. Performing the haematological analysis can talk about red blood cells (RBC), white blood cells (WBC), leukocytes, haemoglobin (Hb), packed cell volume (PCV), Mean Corpuscular Volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) and Reticulocytes. If the number of RBC is high, it is known as erythrocytosis and polycythaemia, or the low level of RBC is called anaemia. Red blood cells (RBC) count values were influenced by stressed, age, gender, season, and genus in rabbits (Melillo, 2007). Blood should be placed in an anticoagulant vacutainer tube (a purple top tube containing Ethylenediaminetetraacetic acid [EDTA]). To reduce the risk of haemolysis, which could affect test results (Moore et al., 2015).

3.5.3.b Serum biochemistry

Figure 3.5.3b: Idexx Biochemistry Test Machine

The biochemistry test is vital to identify the substances that contain in rabbit blood that can affect their health. By running biochemistry test, the level of rabbit health can be determined especially the performance of internal organ of the rabbit. Biochemical analysis conducting to diagnose the disease caused by microbes and nutrition. Two types of protein in the blood called albumin and globulin. It could provide the information about the function of the liver, kidneys, and digestive systems. The two levels enzyme Alkaline Phosphate (ALP) and Alanine Aminotransferase (ALT), the enzymes typically found when the cells of the liver stressed and could diagnosed when the bile flowed in the liver reduced. Finally, serum biochemistry could measure to assess kidney function blood urea nitrogen (BUN), glucose (GLU) and total protein (TP). The dark green vacutainer tube for biochemistry contains sodium heparin, which acts as an anticoagulant (Watson, 2014).

3.6 Statistical analysis

By using IBM SPSS statistic 25, statistical analysis was performed in this study. One-way ANOVA and post-hoc Duncan test was used to distinguish treatment means at a 95% confidence level ($P>0.05$) and in the analysis of blood analysis data. Standard deviation mean were computed to represent the data (SE).

CHAPTER 4

RESULT AND DISCUSSION

4.1 Hematological parameters

According to the result from Table 4.1, the monocytes (MON) level in T1 and T2 were higher than the standard reference value (0.1-1.0). In the pre-feeding trial, the white blood cells (WBC), lymphocytes (LYM), granulocytes (GRA%), mean platelet volume (MPV), platelet count (PLT), mean corpuscular hemoglobin concertation (MCHC) were lower than the reference value in C group. However, the white blood cells (WBC), lymphocytes (LYM), hemoglobin (HGB), mean corpuscular volume (MCV), and red blood cells distribution width (RDW) level were in the normal range.

To our knowledge, this is the first study to examine the effect of feeding trials on the levels of white blood cells (WBCs) and lymphocytes (LYMs) in rabbit feeding trial following the Mulberry pellet feeding. Hot weather has been linked to an increase in WBC production because of stress from pathogens and increased blood viscosity, both of which can cause allergic reactions. According to Lee et al. (1976), neutrophils, monocytes, eosinophils, lymphocytes, and basophils are just a few of the many subtypes of white blood cells (WBC). These subgroups lowered the WBC and caused leucopenia in the rabbits. There was no significant difference in WBC before the feeding trial ($P<0.05$)). T2 had the highest MON levels compared to C and T1, with a significant difference

(P>0.05). The level of MON in the T2 group before and following the feeding trial decreased dramatically from2.600± 0.10 b to 0.850± 0.65 à respectively. However, the MON level in C and T1 increased after the feeding trial because of the stress caused by environmental factors and disease conditions (Melillo, 2007). Osteomyelitis caused by dental disease is common in rabbits with average monocyte counts (Melillo, 2007).

After a feeding trial with a significant difference (P>0.05), granulocytes (GRA) in the C and T1 groups increased slightly, except in T2, GRA levels were marginally lower after the feeding trial was held.

Reduced RBC levels were seen in all treatments during this period. No significant difference was found (P>0.05) in RBC before the feeding trial. However, after the feeding trial, there was a significant difference in RBC levels between the treatments (P>0.05). Blood counts (RBC and WBC) are lower in young rabbits under 12 weeks old (Melillo, 2007). The levels of haemoglobin (HG) and hematocrit (HCT) decreased for the various treatments. There was a significant difference between the HGB and HCT values (P>0.05). Hematocrit (HCT) is a test that determines how many red blood cells an individual has in their system. A low reading may indicate anaemia. Anaemia (low Hb) can be diagnosed by measuring the concentration of haemoglobin (HCT) in the blood. All treatments reduced mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) significantly (P>0.05). MCH and MCHC are derived from Hb, respectively, to determine the average haemoglobin concentration in the body's tissues (Wesche, 2014).

All treatments increased red blood cell distribution width (RDW). There was no significant difference between the RDW values of all treatments in terms of RDW % (10.0-16.00). The red cell distribution width (RDW) measures erythrocyte variation in

size. After the feeding trial, platelet (PLT) levels increased steadily from all treatments, with a significant difference at (P>0.05). Blood from rabbit's clots quickly (Wesche, 2014). Chronic bleeding and iron deficiency anaemia may be linked to high counts. The presence of low levels may indicate an allergic reaction, massive bleeding, aplastic anaemia, or systemic bacterial or fungal infections, all of which are potentially life-threatening medical problems. Procalcitonin (PCT) was slightly increased for all treatments at different periods. The MPV and PDW level were significantly difference at (P>0.05).

Table 4.1a: Hematological parameters of New Zealand White (NZW) rabbits fed with different types of feed for two months.

Parameters	Pre-Feeding Trial				Post-Feeding Trial			
	C	T1	T2	P-value	C	T1	T2	P-value
WBC	2.405 ± 0.705 [à]	5.650 ± 1.15 [àb]	8.150 ± 0.45 [b]	0.036	5.000 ± 2.90 [à]	8.70 ± 7.00 [à]	5.100 ± 4.10 [à]	0.844
LYM	1.050 ± 0.150 [à]	2.700 ± 0.70 [à]	3.150 ± 0.85 [à]	0.194	1.850 ± 0.85 [à]	4.150 ± 3.050 [à]	2.950 ± 2.450 à	0.795
MON	0.800 ± 0.40 [à]	2.100 ± 0.30 [àb]	2.600 ± 0.10 [b]	0.047	1.000 ± 0.60 [à]	1.800 ± 1.60 [à]	0.850 ± 0.65 [à]	0.804
GRA	0.365 ± 0.34 [à]	0.900 ± 0.10 [à]	2.350 ± 1.35 [à]	0.332	2.050 ± 1.450	2.750 ± 2.350 [à]	1.350 ± 0.950	0.849

					à		à	
LYM%	47.400 ± 8.40 à	46.550 ± 3.05 à	39.150 ± 12.15 à	0.779	42.20 ± 7.50 à	54.45 ± 8.55 à	52.40 ± 6.60 à	0.545
MON%	31.800 ± 5.50 à	37.200 ± 1.80 à	32.400 ± 3.00 à	0.596	20.15 ± 0.35 à	16.25 ± 5.65 à	15.90 ± 0.50 à	0.641

à Indicates the means that with significant different (P>0.05). C: control group (100% commercial pellet), T1: treatment 1 group (50% commercial + 50% mulberry pellet), T2: treatment 2 group (100% mulberry pellet).

WBC white blood cells (4.0-12.0), LYM lymphocytes (1.0-5.0), MON monocytes (0.1-1.0), GRA granulocytes (2.0-8.0), LYM% lymphocytes (25.0-50.0), MON% monocytes (2.0-10.0), GRA granulocytes (50.0-80.0), RBC red blood cells (4.0-6.2), HGB hemoglobin (11.0-17.0), HCT hematocrit (35.0-55.0), MCV mean corpuscular volume (80.0-100.00), MCH mean corpuscular hemoglobin (26.0-34.0), MCHC mean corpuscular hemoglobin concentration (31.0-35.5), RDW red blood cell distribution width (10.0-16.0), PLT platelet count (150-400), MPV mean platelet volume (7.0-11.0), PCT procalcitonin (0.200-0.500), PDW platelet distribution width (10.0-18.0).

Table 4.1b: Hematological parameters of New Zealand White (NZW) rabbits fed with different types of feed for two months (continuation).

Parameters	Pre-Feeding Trial				Post-Feeding Trial			
	C	T1	T2	P-value	C	T1	T2	P-value
GRA%	20.80 ± 2.90 [à]	16.25 ± 1.25 [à]	28.35 ± 15.25 [à]	0.668	37.65 ± 7.15[à]	29.30 ± 2.90[à]	31.60 7± 0.00[à]	0.643
RBC	4.755 ± 0.245 [à]	5.805 ± 0.015[b]	6.060 ± 0.160[b]	0.024	4.390 ± 2.54[à]	4.370 ± 2.37[à]	3.850 ± 2.44[à]	0.985
HGB	10.60 ± 0.90 [à]	12.40 ± 0.40 [à]	12.60 ± 1.00 [à]	0.300	7.950 ± 4.45[à]	7.850 ± 4.45[à]	6.85 ± 4.55[à]	0.982
HCT	42.70 ± 3.00 [à]	50.70 ± 1.10 [à]	51.95 ± 3.15 [à]	0.153	39.68 ± 23.28[à]	38.25 ± 21.55[à]	33.30 ± 21.80[à]	0.978
MCV	89.70 ±	87.80 ±	85.65 ±	0.574	89.70 ± 1.10[à]	86.10 ± 2.60[à]	84.60 ± 3.00[à]	0.411

	1.70 [à]	2.60 [à]	2.95 [à]					
MCH	22.50 ± 0.75 [à]	21.45 ± 0.85 [à]	20.80 ± 0.49 [à]	0.589	18.40 ± 0.50[à]	17.60 ± 0.60[à]	17.20 ± 0.90[à]	0.531
MCHC	23.70 ± 0.70 [à]	25.8 ± 1.60 [à]	24.25 ± 0.45 [à]	0.440	20.50 ± 0.80[à]	20.50 ± 0.10[à]	20.35 ± 0.35[à]	0.972
RDW	13.20 ± 0.60 [à]	11.95 ± 0.150 [à]	12.40 ± 0.30 [à]	0.226	14.65 ± 0.05[à]	13.60 ± 0.20 [àb]	12.90 ± 0.40[b]	0.039
PLT	130.50 ± 87.50 [à]	60.50 ± 15.50 [à]	91.00 ± 16.00 [à]	0.673	437.50 ± 362.50[à]	321.00 ± 237.00[à]	117.50 ± 81.50[à]	0.699
MPV	5.60 ± 0.20 [à]	5.70 ± 0.20 [à]	5.40 ± 0.00 [à]	0.502	6.00 ± 0.30[à]	6.50 ± 0.90[à]	5.90 ± 0.40[à]	0.766
PCT	0.0715 0.047 [à]	0.0345 0.007 [à]	0.0495 0.009 [à]	0.673	0.2515 0.2045[à]	0.2215 0.1745[à]	0.0725 0.0525[à]	0.720
PDW	10.0	10.85	10.05	0.825	13.35	14.80	11.75	0.702

0.70 [à]	1.55 [à]	0.65 [à]	0.75[à]	2.40[à]	3.35[à]

[à] Indicates the means that with significant different (P>0.05). C: control group (100% commercial pellet), T1: treatment 1 group (50% commercial + 50% mulberry pellet), T2: treatment 2 group (100% mulberry pellet).

4.2 **Biochemical Parameters**

Table 4.2 shows the biochemical parameters such as blood urea nitrogen (BUN), glucose (GLU), alanine aminotransferase (ALT), and total protein (TP). According to Table 4.2, blood urea nitrogen (BUN) was increased gradually for all treatments. different periods. BUN was significantly difference at (P>0.05). BUN for all treatments after feeding trial was normal in range reference value (10.0-24.0 mg/dl). Circadian rhythm, protein quality in the diet, nutritional status, liver function, intestinal absorption, and hydration are all factors that influence urea levels (Melillo, 2007).

Serum glucose at normal range is 75.00-145.00 mg/dl. According to table 4.2, GLU from C has recorded the highest value than T1 and T2. GLU level was significantly different at (P>0.05) before and after the feeding trial. In clinical practice, most cases of hyperglycemia overdue to stress such as transport, handling, and poor environment. However, serum glucose in rabbits is difficult to obtain because rabbits ingest faecal pellets and may continue to ingest cecotrophy if starvation for a long period.

Alanine transferase (ALT) increased from C, except T1 and T2 was intensely reduced from before the feeding trial to after the feeding trial. However, ALT levels from T1 and T2 recorded the normal value range ALT (31.00-35.00 u/L) after feeding trial from (T1: 54.5 ± 22.5 à, T2: 64.5±24.5 à) to (T1: 29.0±8.00 à, T2: 30.5±20.5à). ALT is another test to assess liver damage. Mildly elevated levels have been found in healthy rabbits and are caused by low toxins like aflatoxins in food or compounds in a wood litter. High levels indicate hepatic lipidosis or coccidiosis-induced liver damage.

Total protein (TP) value for all treatments was increased gradually at a different time with significantly different (P>0.05). Normal values of TP (5.5-7.2 g/dL), thus T2

group was slightly increased difference than C and T1 with 7.65± 2.05à after feeding trial was held. Nevertheless, hemolysis during blood collection can induce several artefacts, decrease the total protein (TP). Dehydrated rabbits may have high total protein levels due to gastrointestinal hypomotility (stasis) and malnutrition or liver disease can cause low levels. Physiological variations due to age, reproductive status, pregnancy, breed, and strain have been reported (Wesche, 2014). Venipuncture compression may cause falsely enhanced TP readings (Wesche, 2014). A significant difference from all treatments ($P>0.05$).

Table 4.2: Biochemical parameters of New Zealand White (NZW) rabbits fed with different types of feed for two months.

Pre-Feeding Trial					Post-Feeding Trial			
Parameter	**C**	**T1**	**T2**	**P-value**	**C**	**T1**	**T2**	**P-value**
BUN	10.0 ± 0.00[à]	6.00 ± 0.00 [à]	7.50 ± 3.50 [à]	0.465	13.0 ± 0.00 [à]	10.5 ± 1.50 [à]	10.5 ± 3.50 [à]	0.685
GLU	148.00 ± 1.00 [à]	115.5 ± 5.50 [àb]	107.5 ± 13.5[b]	0.082	141.50 ± 3.50[à]	123.5 ± 0.50 [à]	119.5 ± 9.50[à]	0.142
ALT	10.0 ± 0.00 [à]	54.5 ± 22.5 [à]	64.5 ± 24.5 [à]	0.250	12.0 ± 2.00 [à]	29.0 ± 8.00 [à]	30.5 ± 20.5[à]	0.583
TP	8.50 ± 1.10 [à]	6.00 ± 0.10 [à]	5.75 ± 0.25 [à]	0.101	7.50 ± 0.50 [à]	6.55 ± 0.35 [à]	7.65 ± 2.05[à]	0.805

[à] Indicates the means that with significant different (P>0.05). C: control group (100% commercial pellet), T1: treatment 1 group (50% commercial + 50% mulberry pellet), T2: treatment 2 group (100% mulberry pellet).

BUN blood urea nitrogen (10-24), GLU glucose (75-145), ALT alanine aminotransferase (31-53), TP total protein (5.5-7.2).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This research indicates that a new type of rabbit pellet has excellent potential and a suitable feed diet that improves the parameters level of blood constituents. In hematological analysis with the tremendous potential formulation, T1 is most excellent than C and T2. However, T2 stated the superior in biochemical analysis. However, all parameters are found non-significant among all treatments (except RDW). The cost of producing this pellet is significantly lower than that of the commercial pellet, and it is much easier to obtain. In addition, this pellet provides the rabbits with the necessary nutrients they need to thrive.

5.2 Recommendation

To ensure that the specific composition of the mulberry plant is maintained on the UMK, Jeli Campus, mulberry plants should be planted and treated with fertilizer or any other nourishment nutrient. Mulberry trees need to be replanted regularly to maintain their maximal fruit production, maximum leaf density, and overall health at their highest levels. For the sample to run smoothly, the formulation feeds to be maintained, and quality control to be effective, the apparatus and machines in the UMK Laboratory must be operated regularly and kept up to date with the newest. A grinder, mixer, and pelletizer are among the machines that will be required to complete this research. The housing system's facilities must be acceptable for the feeding trial to be successful.

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APPENDIX A

Figure A.1: Collecting mulberry plant

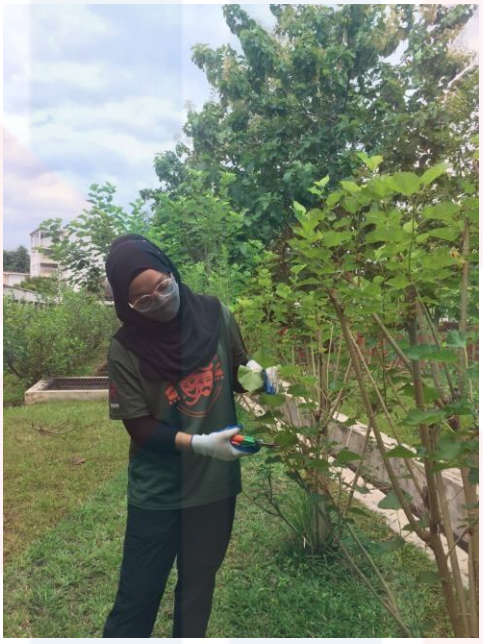


Figure A.2: Dried mulberry plants

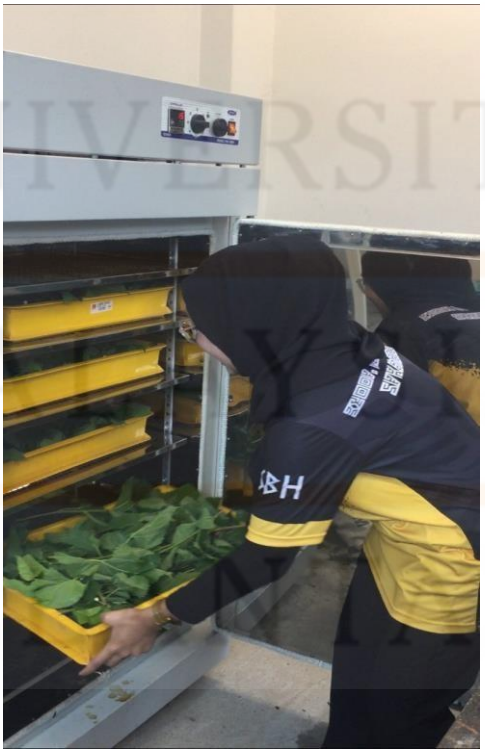


Figure A.3: Blend mulberry leaves

Figure A.4: Grinding mulberry stem

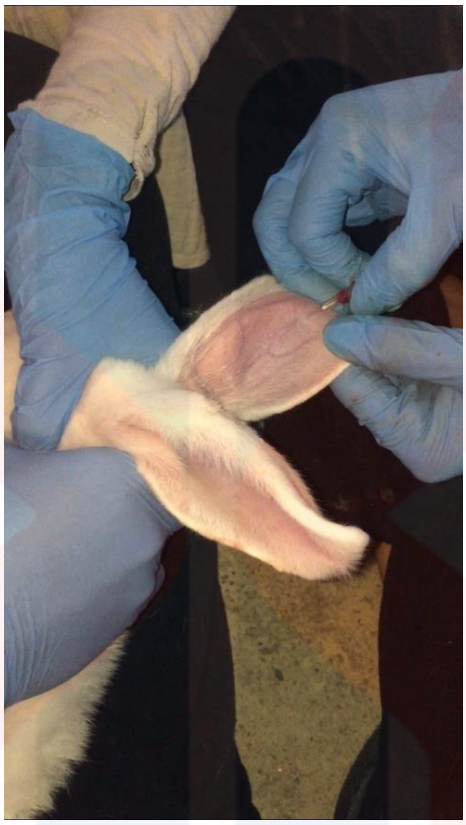
Figure A.4: Mulberry pellet using pelletizer

Figure A.5: Mixed mulberry formulation

Figure A.7: Feeding trial

Figure A.8: Weighed rabbits for blood collection.

Figure A.8: Weighed rabbits for blood collection



APPENDIX B

Table B.1: The post hoc test hematological analysis on pre-feeding trial.

WBC

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Control	2	2.4050	
	Treatment1	2	5.6500	5.6500
	Treatment2	2		8.1500
	Sig.		.132	.226
Duncan[a]	Control	2	2.4050	
	Treatment1	2	5.6500	5.6500
	Treatment2	2		8.1500
	Sig.		.068	.120

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

LYM

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Control	2	1.0500
	Treatment1	2	2.7000
	Treatment2	2	3.1500
	Sig.		.197
Duncan[a]	Control	2	1.0500
	Treatment1	2	2.7000
	Treatment2	2	3.1500
	Sig.		.104

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MON

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Control	2	.8000	
	Treatment1	2	2.1000	2.1000
	Treatment2	2		2.6000
	Sig.		.103	.529
Duncan[a]	Control	2	.8000	
	Treatment1	2	2.1000	2.1000
	Treatment2	2		2.6000
	Sig.		.052	.316

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

GRA

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Control	2	.3650
	Treatment1	2	.9000
	Treatment2	2	2.3500
	Sig.		.326
Duncan[a]	Control	2	.3650
	Treatment1	2	.9000
	Treatment2	2	2.3500
	Sig.		.179

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

LYM%

LYM_P

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	39.1500
	Treatment1	2	46.5500
	Control	2	47.4000
	Sig.		.796
Duncan[a]	Treatment2	2	39.1500
	Treatment1	2	46.5500
	Control	2	47.4000
	Sig.		.548

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MON%

MON_P

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Control	2	31.8000
	Treatment2	2	32.4000
	Treatment1	2	37.2000
	Sig.		.619
Duncan[a]	Control	2	31.8000
	Treatment2	2	32.4000
	Treatment1	2	37.2000
	Sig.		.382

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

GRA%

GRA_P

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Treatment1	2	16.2500
	Control	2	20.8000
	Treatment2	2	28.3500
	Sig.		.650
Duncan[a]	Treatment1	2	16.2500
	Control	2	20.8000
	Treatment2	2	28.3500
	Sig.		.409

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RBC

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Control	2	4.7550	
	Treatment1	2		5.8050
	Treatment2	2		6.0600
	Sig.		1.000	.593
Duncan[a]	Control	2	4.7550	
	Treatment1	2		5.8050
	Treatment2	2		6.0600
	Sig.		1.000	.365

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

HGB

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Control	2	10.6000
	Treatment1	2	12.4000
	Treatment2	2	12.6000
	Sig.		.325
Duncan[a]	Control	2	10.6000
	Treatment1	2	12.4000
	Treatment2	2	12.6000
	Sig.		.179

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

HCT

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Control	2	42.7000
	Treatment1	2	50.7000
	Treatment2	2	51.9500
	Sig.		.165
Duncan[a]	Control	2	42.7000
	Treatment1	2	50.7000
	Treatment2	2	51.9500
	Sig.		.086

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MCV

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	85.6500
	Treatment1	2	87.8000
	Control	2	89.7000
	Sig.		.549
Duncan[a]	Treatment2	2	85.6500
	Treatment1	2	87.8000
	Control	2	89.7000
	Sig.		.329

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MCH

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	20.8000
	Treatment1	2	21.4500
	Control	2	22.2500
	Sig.		.565
Duncan[a]	Treatment2	2	20.8000
	Treatment1	2	21.4500
	Control	2	22.2500
	Sig.		.340

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MCHC

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Control	2	23.7000
	Treatment2	2	24.2500
	Treatment1	2	25.8000
	Sig.		.434
Duncan[a]	Control	2	23.7000
	Treatment2	2	24.2500
	Treatment1	2	25.8000
	Sig.		.248

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RDW

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment1	2	11.9500
	Treatment2	2	12.4000
	Control	2	13.2000
	Sig.		.212
Duncan[a]	Treatment1	2	11.9500
	Treatment2	2	12.4000
	Control	2	13.2000
	Sig.		.112

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

Sum of Squares			df	Mean Square	F	Sig.
WBC	Between Groups	33.190	2	16.595	12.311	.036
	Within Groups	4.044	3	1.348		
	Total	37.234	5			
LYM	Between Groups	4.890	2	2.445	2.970	.194
	Within Groups	2.470	3	.823		
	Total	7.360	5			
MON	Between Groups	3.453	2	1.727	9.962	.047
	Within Groups	.520	3	.173		
	Total	3.973	5			
GRA	Between Groups	4.219	2	2.110	1.627	.332
	Within Groups	3.889	3	1.296		
	Total	8.109	5			
LYM_P	Between Groups	82.363	2	41.182	.272	.779
	Within Groups	454.970	3	151.657		
	Total	537.333	5			
MON_P	Between Groups	35.040	2	17.520	.618	.596
	Within Groups	84.980	3	28.327		
	Total	120.020	5			
GRA_P	Between Groups	149.410	2	74.705	.462	.668
	Within Groups	485.070	3	161.690		
	Total	634.480	5			
RBC	Between Groups	1.914	2	.957	16.718	.024
	Within Groups	.172	3	.057		
	Total	2.085	5			
HGB	Between Groups	4.853	2	2.427	1.848	.300
	Within Groups	3.940	3	1.313		

	Total	8.793	5			
HCT	Between Groups	100.750	2	50.375	3.753	.153
	Within Groups	40.265	3	13.422		
	Total	141.015	5			
MCV	Between Groups	16.423	2	8.212	.671	.574
	Within Groups	36.705	3	12.235		
	Total	53.128	5			
MCH	Between Groups	2.110	2	1.055	.634	.589
	Within Groups	4.990	3	1.663		
	Total	7.100	5			
MCHC	Between Groups	4.743	2	2.372	1.094	.440

	Within Groups	6.505	3	2.168		
	Total	11.248	5			
RDW	Between Groups	1.603	2	.802	2.545	.226
	Within Groups	.945	3	.315		
	Total	2.548	5			
PLT	Between Groups	4927.000	2	2463.500	.453	.673
	Within Groups	16305.000	3	5435.000		
	Total	21232.000	5			
MPV	Between Groups	.093	2	.047	.875	.502
	Within Groups	.160	3	.053		
	Total	.253	5			
PCT	Between Groups	.001	2	.001	.454	.673
	Within Groups	.005	3	.002		
	Total	.006	5			
PDW	Between Groups	.910	2	.455	.206	.825
	Within Groups	6.630	3	2.210		
	Total	7.540	5			

Table B.2: The post hoc test hematological analysis on post-feeding trial.

WBC

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Control	2	5.0000
	Treatment2	2	5.1000
	Treatment1	2	8.7000
	Sig.		.865
Duncan[a]	Control	2	5.0000
	Treatment2	2	5.1000
	Treatment1	2	8.7000
	Sig.		.632

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

LYM

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Control	2	1.8500
	Treatment2	2	2.9500
	Treatment1	2	4.1500
	Sig.		.778
Duncan[a]	Control	2	1.8500
	Treatment2	2	2.9500
	Treatment1	2	4.1500
	Sig.		.529

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MON

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	.8500
	Control	2	1.0000
	Treatment1	2	1.8000
	Sig.		.812
Duncan[a]	Treatment2	2	.8500
	Control	2	1.0000
	Treatment1	2	1.8000
	Sig.		.567

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

GRA

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	1.3500
	Control	2	2.0500
	Treatment1	2	2.7500
	Sig.		.836
Duncan[a]	Treatment2	2	1.3500
	Control	2	2.0500
	Treatment1	2	2.7500
	Sig.		.595

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

LYM%

LYM_P

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Control	2	42.2000
	Treatment2	2	52.4000
	Treatment1	2	54.4500
	Sig.		.557
Duncan[a]	Control	2	42.2000
	Treatment2	2	52.4000
	Treatment1	2	54.4500
	Sig.		.334

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MON%

MON_P

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	15.9000
	Treatment1	2	16.2500
	Control	2	20.1500
	Sig.		.669
Duncan[a]	Treatment2	2	15.9000
	Treatment1	2	16.2500
	Control	2	20.1500
	Sig.		.424

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

GRA%

GRA_P

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment1	2	29.3000
	Treatment2	2	31.6000
	Control	2	37.6500
	Sig.		.635
Duncan[a]	Treatment1	2	29.3000
	Treatment2	2	31.6000
	Control	2	37.6500
	Sig.		.396

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RBC

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	3.8500
	Treatment1	2	4.3700
	Control	2	4.3900
	Sig.		.987
Duncan[a]	Treatment2	2	3.8500
	Treatment1	2	4.3700
	Control	2	4.3900
	Sig.		.885

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

HGB

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	6.8500
	Treatment1	2	7.8500
	Control	2	7.9500
	Sig.		.984
Duncan[a]	Treatment2	2	6.8500
	Treatment1	2	7.8500
	Control	2	7.9500
	Sig.		.872

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

HCT

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	33.3000
	Treatment1	2	38.2500
	Control	2	39.6800
	Sig.		.978
Duncan[a]	Treatment2	2	33.3000
	Treatment1	2	38.2500
	Control	2	39.6800
	Sig.		.851

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MCV

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	84.6000
	Treatment1	2	86.1000
	Control	2	89.7000
	Sig.		.400
Duncan[a]	Treatment2	2	84.6000
	Treatment1	2	86.1000
	Control	2	89.7000
	Sig.		.226

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MCH

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	17.2000
	Treatment1	2	17.6000
	Control	2	18.4000
	Sig.		.515
Duncan[a]	Treatment2	2	17.2000
	Treatment1	2	17.6000
	Control	2	18.4000
	Sig.		.303

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

MCHC

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Treatment2	2	20.3500
	Control	2	20.5000
	Treatment1	2	20.5000
	Sig.		.976
Duncan[a]	Treatment2	2	20.3500
	Control	2	20.5000
	Treatment1	2	20.5000
	Sig.		.846

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RDW

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Treatment2	2	12.9000	
	Treatment1	2	13.6000	13.6000
	Control	2		14.6500
	Sig.		.282	.126
Duncan[a]	Treatment2	2	12.9000	
	Treatment1	2	13.6000	13.6000
	Control	2		14.6500
	Sig.		.153	.065

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

Sum of Squares			df	Mean Square	F	Sig.
WBC	Between Groups	33.190	2	16.595	12.311	.036
	Within Groups	4.044	3	1.348		
	Total	37.234	5			
LYM	Between Groups	4.890	2	2.445	2.970	.194
	Within Groups	2.470	3	.823		
	Total	7.360	5			
MON	Between Groups	3.453	2	1.727	9.962	.047
	Within Groups	.520	3	.173		
	Total	3.973	5			
GRA	Between Groups	4.219	2	2.110	1.627	.332
	Within Groups	3.889	3	1.296		
	Total	8.109	5			
LYM_P	Between Groups	82.363	2	41.182	.272	.779
	Within Groups	454.970	3	151.657		
	Total	537.333	5			
MON_P	Between Groups	35.040	2	17.520	.618	.596
	Within Groups	84.980	3	28.327		
	Total	120.020	5			
GRA_P	Between Groups	149.410	2	74.705	.462	.668
	Within Groups	485.070	3	161.690		
	Total	634.480	5			
RBC	Between Groups	1.914	2	.957	16.718	.024
	Within Groups	.172	3	.057		
	Total	2.085	5			
HGB	Between Groups	4.853	2	2.427	1.848	.300
	Within Groups	3.940	3	1.313		

	Total	8.793	5			
HCT	Between Groups	100.750	2	50.375	3.753	.153
	Within Groups	40.265	3	13.422		
	Total	141.015	5			
MCV	Between Groups	16.423	2	8.212	.671	.574
	Within Groups	36.705	3	12.235		
	Total	53.128	5			
MCH	Between Groups	2.110	2	1.055	.634	.589
	Within Groups	4.990	3	1.663		
	Total	7.100	5			
MCHC	Between Groups	4.743	2	2.372	1.094	.440

	Within Groups	6.505	3	2.168		
	Total	11.248	5			
RDW	Between Groups	1.603	2	.802	2.545	.226
	Within Groups	.945	3	.315		
	Total	2.548	5			
PLT	Between Groups	4927.000	2	2463.500	.453	.673
	Within Groups	16305.000	3	5435.000		
	Total	21232.000	5			
MPV	Between Groups	.093	2	.047	.875	.502
	Within Groups	.160	3	.053		
	Total	.253	5			
PCT	Between Groups	.001	2	.001	.454	.673
	Within Groups	.005	3	.002		
	Total	.006	5			
PDW	Between Groups	.910	2	.455	.206	.825
	Within Groups	6.630	3	2.210		
	Total	7.540	5			

Table B.3: The post hoc test biochemical analysis on pre-feeding trial

PRE_BUN

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Treatment1	2	6.0000
	Treatment2	2	7.5000
	Control	2	10.0000
	Sig.		.444
Duncan[a]	Treatment1	2	6.0000
	Treatment2	2	7.5000
	Control	2	10.0000
	Sig.		.255

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PRE_GLU

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Treatment2	2	107.5000	
	Treatment1	2	115.5000	
	Control	2	148.0000	
	Sig.		.084	
Duncan[a]	Treatment2	2	107.5000	
	Treatment1	2	115.5000	115.5000
	Control	2		148.0000
	Sig.		.551	.072

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PRE_ALT

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Control	2	10.0000
	Treatment1	2	54.5000
	Treatment2	2	64.5000
	Sig.		.257
Duncan[a]	Control	2	10.0000
	Treatment1	2	54.5000
	Treatment2	2	64.5000
	Sig.		.138

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PRE_TP

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Treatment2	2	5.7500
	Treatment1	2	6.0000
	Control	2	8.5000
	Sig.		.115
Duncan[a]	Treatment2	2	5.7500
	Treatment1	2	6.0000
	Control	2	8.5000
	Sig.		.059

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PRE_BUN	Between Groups	16.333	2	8.167	1.000	.465
	Within Groups	24.500	3	8.167		
	Total	40.833	5			
PRE_GLU	Between Groups	1840.333	2	920.167	6.465	.082
	Within Groups	427.000	3	142.333		
	Total	2267.333	5			
PRE_ALT	Between Groups	3367.000	2	1683.500	2.282	.250
	Within Groups	2213.000	3	737.667		
	Total	5580.000	5			
PRE_TP	Between Groups	9.250	2	4.625	5.409	.101
	Within Groups	2.565	3	.855		
	Total	11.815	5			

Table B.4: The post hoc test biochemical analysis on post feeding trial.

POST_BUN

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment1	2	10.5000
	Treatment2	2	10.5000
	Control	2	13.0000
	Sig.		.727
Duncan[a]	Treatment1	2	10.5000
	Treatment2	2	10.5000
	Control	2	13.0000
	Sig.		.477

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

POST_GLU

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment2	2	119.5000
	Treatment1	2	123.5000
	Control	2	141.5000
	Sig.		.148
Duncan[a]	Treatment2	2	119.5000
	Treatment1	2	123.5000
	Control	2	141.5000
	Sig.		.077

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

POST_ALT

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Control	2	12.0000
	Treatment1	2	29.0000
	Treatment2	2	30.5000
	Sig.		.613
Duncan[a]	Control	2	12.0000
	Treatment1	2	29.0000
	Treatment2	2	30.5000
	Sig.		.378

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

POST_TP

	Treatment	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Treatment1	2	6.5500
	Control	2	7.5000
	Treatment2	2	7.6500
	Sig.		.816
Duncan[a]	Treatment1	2	6.5500
	Control	2	7.5000
	Treatment2	2	7.6500
	Sig.		.570

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
POST_BUN	Between Groups	8.333	2	4.167	.431	.685
	Within Groups	29.000	3	9.667		
	Total	37.333	5			
POST_GLU	Between Groups	549.333	2	274.667	4.010	.142
	Within Groups	205.500	3	68.500		
	Total	754.833	5			
POST_ALT	Between Groups	422.333	2	211.167	.649	.583
	Within Groups	976.500	3	325.500		
	Total	1398.833	5			
POST_TP	Between Groups	1.423	2	.712	.233	.805
	Within Groups	9.150	3	3.050		
	Total	10.573	5			

APPENDIX C

Table C.1: The reference value of haematological analysis.

Parameter	Reference values
WBC (10^3/µl)	4.0-12.0
LYM	1.0-5.0
MON	0.1-1.0
GRA (10^3/µl)	2.0-8.0
LYM%	25.0-50.0
MON%	2.0-10.0
GRA%	50.0-80.0
RBC (10^6/µl)	4.0-6.20
HGB (g/dl)	11.0-17.0
HCT (%)	35.0-55.0
MCV (µm^3)	80.0-100.00
MCH (pg)	26.0-34.0
MCHC (g/dl)	31.0-35.5
RDW (%)	10.0-16.0
PLT (10^3/µl)	150-400
MPV (µm^3)	7.0-11.0
PCT (%)	0.200-0.500
PDW (%)	10.0-18.0

Table C.2: The reference value of biochemical analysis value.

Parameter	Reference value
BUN (mg/dL)	10.0-24.0
GLU mg/dL)	75-145
ALT (u/L)	31-53
TP (g/Dl)	5.5-7.2

Table C.3: The maximum blood volume collection (ml) of rabbit according to weight.

Pre-feeding trial					Post-feeding trial							
Group	C2	C3	T2	T3	T5	T6	C2	C3	T2	T3	T4	T5
Weight (kg)	1.886	1.290	1.706	2.076	1.098	2.016	2.234	1.614	1.918	2.252	1.262	1.08
Blood volume collection (ml)	1.05	0.72	0.96	1.2	0.62	1.13	1.25	0.90	1.07	1.26	1.08	0.71
Average weight (kg)	1.588		1.891		1.557		1.924		2.085		1.171	