IN VITRO, IN SACCO AND *IN VIVO* DIGESTIBILITY OF RUMINANT FEEDS SUPPLEMENTED WITH HERBS

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ABSTRACT

Animal feeds with added herb tested in *in vitro*, *in sacco* and *in vivo* techniques are the potential use combined with commercial pellets or PKE pellets to enhance the nutritive value of the feeds. Two types of herb mixture were tested. The mixture of local herbs (named as DD herbs): formulated in the Animal Biotechnology Laboratory University of Malaya (UM) and imported herb. The objective of the research was to compare DD herb potential in improving ruminant digestibility against imported herb. The experimental diets were formulated into 3 combination doses: 25 gm of herbs added to 975 gm of pallets (2.5% herbs added), 50 gm of herbs added to 950 gm of pallets (5.0% herbs added) and 75 gm of herbs added to 925 gm of pallets (7.5% herbs added). Each combination of mixed herb was tested separately but the same experimental procedures were employed. The highest digestibility of herbs in the *in sacco* and *in vitro* technique was tested using *in vivo* technique. *In sacco* experiments were conducted in the Institute Sciences of Biological Mini Farm (ISB), UM and the Animal Biotechnology Laboratory. Eleven replicates were tested in *in sacco* technique using six local goats with average age of 12 months fitted with rumen cannulae. The commercial pellet with DD herb animal feeds had the highest rate of digestion. The percentage of *in sacco* digestibility in 5.0% DD herb added commercial pellets and Napier grass (*Pennisetum purpureum*) was 67.47% at 24 hours, 69.61% at 48 hours, 69.83% at 72 hours and 74.63% at 96 hours. Same pellets and herb combination showed the highest rate of digestion results using *in vitro* digestibility technique. The 5.0% DD herb added commercial pellets and Napier grass *in vitro* digestibility was 68.39% at 24 hours, 73.50% at 48 hours, 78.29% at 72 hours and 80.22% at 96 hours. In the gas production experiments, the

5.0% DD herb gas production was higher than the other herb combination. The increase of DD herb produced a significant ($p<0.5$) increase in the digestibility percentage. However, increasing of the treatment to 7.5% DD herb had no significant effect on the percentage of digestibility. The laboratory findings of the *in vitro* and *in sacco* experiments were put to test in a field in vivo experiment using goats on a farm in Bidor, Perak to test digestibility of feeds and growth performance in goats. The experiment compared a group of kids fed diets containing DD herb versus fed control diets kids.

ABSTRAK

Tambahan herba ke dalam makanan haiwan ruminan diuji dalam teknik *in vitro*, *in sacco* dan *in vivo* dan potensi dipantau dengan herba ditambahkan dengan pelet komersial atau pelet PKE untuk meningkatkan nilai suapan pemakanan haiwan. Dua jenis campuran herba telah diuji. Campuran herba tempatan (dinamakan sebagai herba DD): ia dirumuskan dalam Makmal Bioteknologi Haiwan, Universiti Malaya (UM) dan juga herba yang diimport. Objektif kajian ini adalah untuk membandingkan potensi herba DD dalam meningkatkan penghadaman haiwan ruminan terhadap herba yang diimport. Eksperimen diet pemakanan telah dirumuskan ke dalam tiga kombinasi dos: 25gm herba ditambah kepada 975gm pelet (2.5% tambahan herba), 50gm herba ditambah kepada 950gm pelet (5.0% tambahan herba) dan 75gm herba ditambah kepada 925gm pelet (7.5% tambahan herba). Setiap gabungan campuran herba telah diuji secara berasingan dengan menggunakan prosedur uji kaji yang sama. Penghadaman tertinggi herba dalam teknik *in sacco* dan *in vitro* telah digunakan dan diuji dalam teknik *in vivo*. Eksperimen *in sacco* telah dijalankan di Ladang Mini Institut Sains Biologi (ISB), UM dan Makmal Bioteknologi Haiwan. Sebelas replikasi telah diuji dalam teknik *in sacco* menggunakan enam kambing tempatan dengan purata umur 12 bulan yang telah dilengkapi dengan Kanula. Pelet komersial dengan tambahan DD herba menunjukkan kadar suapan penghadaman tertinggi haiwan. Peratusan penghadaman *in sacco* dalam campuran 5.0% DD herba, pelet komersial dan rumput Napier (*Pennisetum purpureum*) adalah 67.47% pada 24 jam, 69.61% pada 48 jam, 69.83% pada 72 jam dan 74.63% pada 96 jam. Gabungan formulasi pelet dan herba yang sama menunjukkan kadar pencernaan tertinggi dalam teknik penghadaman *in vitro*. Peratusan 5.0% DD herba ditambah dengan

pelet komersial dan rumput Napier dalam penghadaman *in vitro* adalah 68.39% pada 24 jam, 73.50% pada 48 jam, 78,29% pada 72 jam dan 80.22% pada 96 jam. Dalam uji kaji pengeluaran gas, rawatan 5.0% DD herba dengan komersial pellet adalah lebih tinggi daripada gabungan herba yang lain. Herba DD menunjukkan signifikan ($p < 0.5$) yang ketara dalam peratusan penghadaman. Walau bagaimanapun, peningkatan rawatan kepada 7.5% herba DD tidak mempunyai kesan yang besar ke atas peratusan penghadaman. Hasil tertinggi dalam kajian makmal dan eksperimen teknik *in vitro* dan *in sacco* telah diuji bagi eksperimen *in vivo* menggunakan kambing di sebuah ladang di Bidor, Perak. Ia menguji penghadaman suapan dan prestasi pertumbuhan kambing. Eksperimen yang telah dijalankan adalah perbandingan sekumpulan diet anak-anak kambing memakan makanan mengandungi campuran DD herba dengan komersial pelet sebagai makanan tambahan berbanding diet kawalan makanan pellet komersial.

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"In the name of Allah, the most beneficent and most merciful" gracious

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ADF	Acid Detergent Fibre
ATP	Adenosin-Three Phosphate
CF	Crude Fibre
CO_2	Carbon Dioxide
CP	Crude Protein
Cu	Copper
DM	Dry Matter
EE	Ether Extract
GE	Gross Energy
gm	Gram
H	Hour
Kg	Kilogram
ME	Metabolizable Energy
mg	Milligram
ml	Mililiter
NDF	Neutral Detergent Fibre
N	Nitrogen
OM	Organic Matter
VFA	Volatile Fatty Acids
°C	Degree of Celcius
%	Percentage

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CHAPTER 1: INTRODUCTION

1.0 Introduction

The agricultural sector in Malaysia is important for the production of meat and dairy products primarily for domestic consumption. Most of the livestock industry products such as beef, lamb and dairy products are imported to meet the growing of the growing Malaysian population. A total of 131,026 tonnes of beef was imported in 2012 at a value of RM1,285 million to meet the needs of the local population. In the same year, imports of live goats were 45,351 tonnes at a value of RM22.92 million and import of mutton was 18.531 tonnes at a value of RM304.04 million (Agrofood Statistics, 2012).

Ruminants produce approximately one-third of meat produced worldwide (FAO, 2011). The most common ruminant species in Malaysia are goats, sheep and cattle. To support the local industry and reduce reliance on imports, more research is needed, to measured beef and mutton production. The National Agrofood Policy (NAP) 2011-2020, which was launched by the Prime Minister of Malaysia on January 12, 2012 was enacted to achieve three main objectives: to ensure adequate food supply and food safety, to develop the agrofood industry into a competitive and sustainable industry and to improve the income level of the target groups (Agrofood Statistics, 2012).

Shortage of feed is one of the main problems facing the ruminant industry in Malaysia. Productivity of green feed or pasture is low mainly due to low soil fertility (Ahmed *et al.*, 2012). Malaysia spends more than RM5.14 billion to import animal feed (Agrofood Statistics, 2012).

Poor digestion in ruminant's digestive system affected the feed costs and burden the small farmer (Devandra, 1991). Existing supplies of agro-industrial by-products and non-conventional feeds should be used effectively to achieve better performance in animals as well as to reduce cost.

Nutritious feed constitutes the largest cost for with raising livestock, by ruminants, which accounted for about 60% or more of total production costs (Susan, 2009). Nutrition has an enormous influence on ruminant reproduction, milk production, and also growth of offspring's. Young ruminants with higher growth potential have higher nutritional needs, especially for protein.

Animals receiving inadequate diets are more prone to diseases and usually fail to achieve their genetic potential. Many factors affect the nutritional requirements of ruminants for maintenance, growth, pregnancy, lactation, fibre production, activities and their environment. It varies according to the size or weight of these animals. Small animals might require a higher percentage of intake to maintain their weight (Schoenian, 2007).

Grazing alone is usually not sufficient to provide the nutrient requirements of ruminant diet because grass has a low nutritional value (Moore *et al*., 1973). Concentrate pellets as a supplement in the diet of ruminants not only increases profits, but also improves feed efficiency (Lindah and Terril, 1963). Commercial pellets as supplements promote ruminant growth, improve their health and their weight and commercial pellets suitable for use in low-quality pastures or during drought.

Apart from the commercial pellets, palm kernels are also an alternative feed ingredient for animals. Malaysia is a major producer and exporter of palm kernel products. Palm kernels are an extremely good source of protein and energy for livestock and commonly used in ruminant feed (Rahman *et al*., 2013). They have been accepted as

a component in animals feed for their food nutritious value and attractive price (Zulfadhli, 2012).

Palm Kernel Expeller (PKE) is a palm kernel by-product from crushing and pressing for palm kernel oil. This product is known for its high energy and protein, high fibre, good level of residual oil and high nutrition. Wyngaard *et al.* (2014) reported that PKE can sustain milk yield and milk fat components at a level of up to 400 g/kg of concentrate when fed at 6 kg/cow/d to cows grazing Kikuyu-rye-grass pastures. Over the years, it has been used in compound feeds for adult ruminants such as dairy cows, beef cattle's, goat and sheep. However, this product is high in fibre, low in palatability, deficient in several amino acids, and low in lysine availability (Raghavan, 2002; Onuoha, 2014). It is widely large available from the oil palm sector, which is one of the major industries in Malaysia. These alternative pellets can be enhanced by adding herbs to improve its nutrition value.

Although PKE pellets can improve the nutrition status and well-being of ruminant animals, the cost of feed among ruminants is also affected. One way to reduce the cost is by adding herbs in the ruminant feeding. Herbs can help improve in ruminant digestion, growth and reproduction. Up to now, no study has been conducted to investigate the effect of addition of herbs in ruminant feed either in laboratory or in the field in Malaysia. However, many studies have been conducted on ruminants in other countries (Christaki *et al*., 2012). This research focuses on benefits on nutrition and digestion when herbs are added to ruminant feed in order to develop muscles and weight in ruminant animals. Commercially available pellet is good for ruminant animals, but it will increase the cost of feed. The alternative to reduce the cost by adding herbs in ruminant feeding (Nik Fatihah & Noraida, 2012).

Laboratory methods are cheaper and faster as alternatives to measure *in vivo* digestibility. These methods involve either predictive digestion of the chemical composition or *in vitro* and *in sacco* simulation of the digestive process. *In vitro* gas production technique offers no advantages in prediction of the total digestive tract, but is useful for screening treatments for the rate of digestibility in the rumen (Kitessa *et al.,* 1999; Brouček, 2014).

All treatments in this research were tested using *in vitro* and *in sacco* digestibility methods. These methods used artificial fibre bags made of nylon which is available in a variety of pore sizes (Mack, 2011). The best treatments that have been tested in both digestion methods were selected and tested by *in vivo* method.

Both *in vitro* or *in sacco* methods have extensive applications in ruminant nutrition as they allow determining degradability and testing quality of forages and ruminants' diet faster and cheaper compared with *in vivo* methods. *In vitro* digestion technique is proposed to determine the effects of rumen and post-ruminal digestion on the viability of foods. This technique is cheaper and can be conducted in the laboratory to obtain valid results in the ruminant digestive system. However, there is limited information on the characteristics of dry matter (DM) and crude protein degradation in the rumen and digestibility in the lower digestive tract of protein sources used for domestic livestock in the tropics (Promkot *et al*., 2003; Riasi *et al*., 2014). The food is then weighed to determine the mass lost due to digestion and then tested for viability.

Another common technique used to observe the effect of ruminant digestion on the viability of food is the *in sacco* digestibility technique. Nylon bag technique is not only a powerful tool for indexing the relative degradability of feedstuffs, but may also be used to study the rumen processes, as it is possible to vary the factors within the bag, or within the rumen (Ørskov *et al*., 1980; Promkot *et al*., 2003). Feed is sealed in a small

nylon bag and suspended into the rumen of a cannulated animal for varying incubation periods. Nylon bags containing the incubated feeds are suspended in the fistulated ruminants to determine the extent of the breakdown of feed protein degraded in the rumen (Rao and Prasad 1989; Aghajanzadeh-Golshani *et al*., 2015).

After identifying the best treatment from the *in vitro* and the *in sacco* digestibility, the treatment will be subsequently tested *in vivo*. This technique will give the best results to test the samples used in this research. The research was conducted on a farm over a period of nine months to assess the ruminant digestion. The body weight is recorded to see the effects of the feeds on the growth of the ruminants.

This research is expected to help farmers reduce the cost of ruminant feed and gain maximum weight for their livestock's (goats, sheep, cattle etc.). Adding herbs in ruminant feed, especially local herbs formulated feeds (DD herbs), it is expected that these animals will gain weight faster with less feeding (Noraida & Nik Fatihah, 2012), as well as can improve the reproductive and the health states of the animals. It is hoped that the ruminant industry in Malaysia can be developed to a commercial scale with the inclusion of the herbs in their feeds.

The overall objective of the research is to evaluate the digestibility of the herbs formulated feeds and to test *in vitro* and *in sacco* feeds in goats at a selected farm. A series of experiments were designed using the *in vivo* technique on a group of goats on a farm which not only focused on digestion, but also the growth performance of the goats fed on the experimental feeds.

This outcome of the research is expected to provide many benefits to the animal feed industry such as:

(i) increase the rate of digestion in ruminants;

(ii) reduce the quantity of ruminant feeds being used to feed the animals;

(iii) reduce the quantity of ruminant feeds being used to feed the animals.

1.1 Objectives of the Research

This research was conducted to investigate the potential use of herbs combined with the commercial pellets or PKE pellets to enhance the nutritive value of the feeds. The objectives of the research are:

(1) To evaluate and compare the nutritive values between local herbs formulated feeds (DD herbs) and imported herbs formulated feeds;

(2) To evaluate and compare the *in vitro* digestibility of herbs formulated feeds when added with pellets;

(3) To evaluate and compare the *in sacco* digestibility of herbs formulated feeds when added with pellets in goat's rumen;

(4) To evaluate the growth performance *in vivo* of goats when fed with the herbs formulated feeds.

CHAPTER 2: LITERATURE REVIEW

2.0 Introduction

The farming industry occupies about 30 percent of the land surface area of the globe. It is a significant global asset with a value of at least USD 1.4 trillion (Steinfeld *et al*., 2006). The livestock sector employs at least 1.3 billion people worldwide and directly supports the livelihoods of 600 million smallholder farmers in the developing countries (Thornton *et al*., 2006). Keeping livestock is important for communities because livestock is a provider of essential nutrients. Even though differences exist between rich and poor countries, livestock products still contribute 17 percent to 33 percent kilocalorie consumption as well as protein in the world (Rosegrant *et al*., 2009). The ruminants have a potential to provide meat and milk production with demand in the future due to human health concerns. Their product contains essential compounds such as high protein and iron (Wanapat and Chanthakhoun, 2015).

Feed cost is important in the management of national economies. Therefore, it is become a major concern to develop livestock industry in developing countries by increasing the use of indigenous feed resources is expected to reduce the cost of importation (Devendra, 2013). Feed cost is a burden on the national budget of Asian countries, and also has become a burden to farmers because it constitutes approximately 60% of the total production cost (Deininger and Byerlee, 2011).

Malaysia faces a similar challenge to reduce livestock feed cost. The high cost of agricultural land to produce feed has become a primary concern in our ruminant industry due to smallholder farmers operating with limited land, animals and resources.

Another main problem is the shortage of suitable feeds or forages, both in terms of quantity as well as quality of feed available (Frison *et al*., 2011). At present, the majority of Malaysian livestock products such as beef, mutton and dairy products are imported in order to meet the growing demand of the population (MARDI, 2009). To support the local industry, many studies are needed to reduce our dependence on the import of agriculture products, especially beef and mutton.

Livestock production is an important source of livelihood resource for poor farmers in Asia and other countries including the developing world. Livestock productivity in many countries in the region, however, is below the genetic potential of the livestock, mainly due to the lack of adequate nutrition (IAEA, 2010). To overcome this problem, a study conducted in Mongolia reported that biotechnological approaches have been applied in search of alternatives to produce desirable bioactive compounds from herbs and herbal extracts selected from indigenous plants. Garlic has been shown to inhibit enzymes involved in lipid synthesis, decrease platelet aggregation, prevent lipid peroxidation of oxidized erythrocytes and low-density lipoprotein (LDL), increase antioxidant status, and inhibit angiotension-converting enzyme (Ali *et al*., 2008). An active compound of garlic showed significantly lowered formation of fatty streaks in the aortic sinus (Vasanthi & Parameswari, 2010). This should help digestion of feeds and reduce the internal parasites which burden ruminants (Phatak, 2013).

To determine the technical and economic viability of adding herbs to ruminant feeds in the application of this biotechnology experiment: *in vitro*, *in sacco* and *in vivo* techniques were used. In the present study, the aim was to investigate the benefits of using herbs in ruminant feeds through rather simple and economical methods which could be applied in commercial production. However, in order to achieve this, factors and

parameters that are likely to influence the herbs added to ruminant feedstuff need to be studied (Castro *et al*., 2000).

Some potential herbs that could be added to the feeds are discussed in this thesis; including on preferably local herbs (DD herbs) as well as some imported herbs. This study is also expected to help reduce dependence on importation of beef and mutton, thus saving cost in terms of foreign exchange. Furthermore, there is a need to ensure that consumers receive a steady meat supply at affordable prices and also to enhance the nutritional diet adequacy in Malaysia (Nik Maheran, 2009). With increased and excess production along commercial lines, Malaysia might be able to export 'halal' ruminant meat products to the world market.

2.1 Ruminants

Ruminants are grazing animals that have the ability to digest and metabolise cellulose. Microbes in the rumen ferment grass to form volatile fatty acids (VFA) and protein (Krehbiel, 2014). Ruminants such as cattle, sheep and goats have the ability to convert carbohydrates and protein of plant source into nutrients that can be used by humans (Jane *et al*., 2009a). Among the important features for the sustainability of agricultural production systems pastures have the capacity to produce millions of tonnes of resources (Lee, 2008).

2.1.1 Basis of the Digestive System in Ruminants

The ruminant digestive system is unique when compared to other animals. Ruminant has four compartments in their stomachs and subsist on roughage, grass and shrubs made up largely from cellulose (Brooker *et al.*, 2008). Ruminants are able to digest most of the nutrients in fibrous plant material due to their unique digestive system, which integrates a large microbial population in their digestive system (Mohamad Noor, 2012). Although this system is remarkably efficient, proper feeding management of food is required to keep ruminant healthy and productive. Mismanagement of their diet can be disastrous (Cronjé and Boomker, 2000).

An understanding of the ruminant digestive system is very useful in order to appreciate their dietary requirements. When ruminants eat, the food initially goes into the first stomach. The rumen which is the largest compartment (Russell, 2002). Due to fermentation by microbes in the rumen, the animals are able to utilise high fibre feeds, such as grass. From there, the feed moves gradually into the reticulum where it is broken down by bacteria and acid to form cud (Reece & Campbell, 2005). Muscles in the reticulum push the cud back into the animal mouth for a second chewing to help break down the food material (rumination). Once ruminants swallow the cud, it then enters the omasum for further digestion before it enters the abomasum (Dijkstra *et al*., 2005).

Generally low level of production is common in many places in the tropics which is consistent with low efficiency in nutritional management of ruminant (Devendra, 1980). Many studies have been conducted to improve the efficiency of digestion of these animals. Some evidence for significant improvement in performance due to better nutrition was reported in the West Indies (Chenost and Geoffrey, 1971; Devendra, 1972;

Sachdeva *et al.*, 1974; Devendra, 1979) similar studies in sheep were carried out in the eastern Mediterranean region (Demiruren, 1972; Devendra, 1986).

2.1.2 Ruminants' Digestive Processes

Nutrients absorbed from the digestive tract include volatile fatty acids (VFA), amino acids, fatty acids, glucose, minerals, and vitamins. They are used in the synthesis of different compounds in meat, milk and wool, and to replace nutrients that are used to maintain life processes including reproduction (Minson, 1990). Digestion begins when the animal takes a bite from the pasture. As the animal chews, the feed is formed into a bolus similar to a packet of food ready to be swallowed. Saliva is excreted and serve as a further aid to swallow and as a pH buffer in the stomach (Reece, 2009).

Once in the rumen, the feed will begin to undergo fermentation. Millions of microorganism will ingest the feed when feed is swallowed, turning into the final products that serve as a major source of nutrients for these animals.

Ammonia, methane, carbon dioxide, and VFA form and are absorbed to be used as energy source by the animal during this phase (Sjersen *et al.*, 2008). In the rumen, microbes help digest cellulose but their digestion rate is not efficient. According to Minson's report (1971b), the rate of digestion of tropical grass species are less efficient. Digestibility in tropical grasses decreased when the animal getting older (Milford and Minson, 1966). High digestion is sustained for mature grasses. The difference in the rate of digestion is also reported between difference grasses species (Strickland and Haydock, 1978).

The nutritional values of tropical grasses were reviewed by Miller and Rains (1963) and Hardison (1966) and Butterworth (1967). Tropical legumes were also reviewed by Minson (1971b) and tropical hays by Marshall *et. al.* (1961). Minson (1971b) reported that tropical grasses decrease in dry matter digestibility at a daily rate of 0.1 to 0.2 digestibility units (Devendra, 1986).

Butterworth (1967) has reported that 60-70% of the ruminant digestion, encourages the increasing of dry matter intake (DMI). This is because in tropical forages, protein content is generally low (French, 1957; Bredon and Horrell, 1961). The level of protein in the diet can affect the voluntary food intake (Campling *et al*., 1962; Blaxter and Wilson, 1963) whereby low protein diets are not easily ingested by ruminants (Elliott and Topps, 1963).

2.2 Ruminant Industry in Malaysia

The Malaysian livestock industry is an important component of the agricultural sector, having provided employment and generating useful animal protein for the human population (Fairuz *et al*., 2010). The ruminant industry is largely operated by smallholder farmers with limited land, animals and resources (Mahyuddin, 1993). It is considered small in comparison to that in Thailand and Indonesia. The Malaysian domestic ruminant population consists predominantly of Malaysian indigenous breeds such as Malin (sheep), Katjang (goats) and Kedah-Kelantan (cattle) plus imported purebreds and their crossbred offspring (Rosli *et al.*, 2001).

The local ruminant industry is not well developed compared with western countries. Issues related to the feed supply are the major factors which limit ruminant

production. Improving the livestock product quality and production is one of the objectives of the Malaysian government. The focus is currently on the agriculture and biotechnology sector as the engine of economic growth (Tunku Mahmud, 2004) with emphasis towards developing practical and low-cost feeds for various classes of ruminant species. Hence the current emphasis is towards the development of practical and low-cost feedstock for various classes of ruminant's species.

There are some limitations in the development of local feeds for ruminant's availability of fibre, low consumption, lack of practical techniques to convert local groceries to feed quality, the collection and harvesting of low efficiency raw materials, raw materials drying is costly, inefficient and storage forage-based feed operation and the lack of quality assurance protocols established for existing feeds. Appropriate strategies to enhance rumen function and the means to administer supplements are essential aspects for ruminant, as well as increasing the utilization of potential feed resources under the plantation environment (Wan Zahari *et al*., 2013).

Ruminant breeders in Malaysia, use a variety of methods depending on the size of livestock they breed. The factors that affect the farming method include the availability of large scale food organised by commercialisation efforts. These systems are extensive, intensive, semi-intensive and the animal-tree crop integrated systems (Wong and Chen, 2002).

The extensive system is the most popular in Malaysia. It is used in small herds of five to ten animals which are allowed to forage alongside road verges, hedges or wherever waste vegetation is available. Little management is required, as the animals are allowed to roam free in the mornings and return home in the evenings (Rajion *et al*., 1993).

The intensive system requires substantial inputs in animal's management. Only a few large farmers adopt this system, whereby the animals are confined in sheds and feeds that are provided by a cut-and-carry system. Supplements and drinking water have to be provided as well. Animals maintained under these systems are frequently sold as breeds or for fattening purposes, as their performance and health can be conveniently monitored. Animals reared under this system have a higher average daily gain than those kept under extensive systems (Devendra, 1996).

The semi-intensive system is widely practiced in many teaching and research institutions as well as in government farms (Wong and Chen, 2002). The animals are allowed to graze in identifiable paddocks carrying improved pasture for about six to seven hours daily and housed in sheds at night. Feed supplements and drinking water are provided (Dahlan *et al*., 2000).

The animal-tree crop integration system is widely practiced for goat, sheep and cattle production. In this system animals are reared in tree crop plantations such as rubber, oil palm and fruit orchards (Rosli *et al.*, 2001). The system allows maximum utilization of the available land area and the primary advantage of this system is a significant reduction in chemical weeding costs and improved soil fertility through the return of animal wastes. This system appears to be the most practical under the current Malaysian plan for large-scale ruminant production (Academy of Sciences Malaysia, 2010). It is one of the fattening-to-slaughter farming schemes practiced by some-large scale commercial entrepreneurs in Malaysia.

Livestock in Malaysia comprise mainly small ruminants (goats, sheep and cattle) which rely on unimproved indigenous grasses, as the cost of raising a pasture amongst the tropical rain forest is prohibitive (Halim, 1993). Moreover, ruminants need

specific feed that are cheap and readily available. Studies need to be conducted to help improve the quality of feed for cheap feeding cost with maximum output.

2.3 Ruminant Feeds

Ruminants are able to acquire nutrients from plant-based feed by fermenting it in a specialized stomach prior to digestion, principally through bacterial actions. The process typically requires the fermented ingesta (cud) to be regurgitated and chewed again. The process of re-chewing the cud to further break down plant matter and stimulate digestion is called rumination (Vaughan *et al*., 2000). These animals may spend up to eight hours per day in rumination, depending on the type of feed provided (Julio, 2007). A variety of food is available for ruminant animals (Raghavan, 2000). Feed can also be classified as livestock conventional feedstuffs or non-conventional feedstuffs.

Conventional feedstuffs have been traditionally used for decades and are normally abundant and are purposely cultivated to support animal production. Non-conventional feedstuffs are defined as by-products derived from industrial processing of the main products. They are normally feeds which have not been traditionally used in animal feeding (Tunku Mahmud, 2004).

Grasses serve as the major source of feed for livestock to provide nutrients required for maintenance and production. The major constituents of grasses are water, carbohydrates, protein, fat, minerals and vitamins (Tilden *et al*., 1999).

Energy values are usually expressed in feed composition tables as total digestible nutrients, metabolisable energy, net energy for maintenance and net energy for gain or lactation (Mohamad, 1987). These values are based on digestion or balance trials

for certain feedstuffs. For others, values are calculated by formulae derived from basic digestion or energy balance data. Due to this and the fact that many feeds deviate from averages, energy values should not be considered absolute. When a feed appears to deviate substantially from normal, adjusted energy values should be employed in formulating rations (Allen and Denis, 1983).

Forage energy value is best determined by forage maturity, density, and availability. Protein in forage is mostly correlated with forage maturity, as more mature forages have lower percentages of crude protein (Lee, 2008).

There is a shortage availability of good-quality forages and conventional concentrates. However, large amounts of crop residues and agro-industrial by-products are available. At present, these feed resources are underutilised, but with the development of improved supplementation strategies they may substantially increase animal productivity beside utilisation of available roughages. For ruminants, the use of crop by-products is restricted by their relatively low capacity to handle poorly digestible fibre. If possible, the basal diet for ruminants should consist of fresh forages with a neutral detergent fibre (NDF) concentration below 55-60% (Devendra, 1986). Many nutritionists consider energy value and intake of forages to be more important than crude protein (Robinson *et al.*, 1998).

The best estimate of the energy value of a feed is derived from determining *in vitro* dry matter (DM) digestibility where this analytical service is available. Energy values estimated from protein and crude fibres are useful if appropriate formulae are used in their calculation. The additional cost of a proximate analysis does not appear justified for calculating the energy value of a feedstuff (Khan and Chaundhry, 2010).

2.3.1 Grass

A variety of existing agro-industrial by-products and non-conventional feeds can be used as an effective way of obtaining good performance in animals as well as improving efficiency in terms of saving time and money (Loh, 2002). However, poor digestion in the ruminant's digestive system raises the cost of feeds. Especially with the limited resource of a small-scale farmer (Devendra, 1991).

Feeding grass alone is usually not adequate to meet the nutrient requirement of ruminants due to the variability of the quality of grasses and their relatively low digestibility. According to Moore and Mott (1973), tropical grasses and crop residues are low in nutritional value and are required to be supplemented with other ingredients to improve their nutritive values.

Besides feeding ruminants with indigenous grasses, the use of hybrid Napier grass (*Pennisetum purpureum*) as a forage source is popular throughout Southeast Asia because of its potentially high-yielding source of digestible fibre (Devendra, 1989). It also has high dry matter, reasonably quality, drought tolerance in addition to persistence to frequent harvesting (Goswami *et al.*, 2013). It is particularly suited for cultivation by smallholder farmers and can also be used as silage (Zafar, 2006). Napier grass requires at least three months of growth before the first cut; there after it can be cut at intervals of 6-8 weeks and should be replanted every 5-6 years.

Napier grass grows in the tropical and sub-tropical regions, depending on rain fall for high dry matter productivity (Yokota *et al*., 1998). To prepare for feeding, fresh grass should be chopped (3-5 cm) and mixed with concentrate feed and fed at the rate of 15-30% of the whole ration (dry feed basis) or about 55-65% as feed. Yokota *et al*. (1991) reported that the grass could provide good quality silage when it is

supplemented as fermentation is not affected by high temperature (40ºC). Enhancing soil fertility can improve the productivity of Napier grass (David, 2011).

Various factors on the use of Napier grass as a staple feed for ruminant animals have been presented. Napier grass has also been used in the treatment of *in vitro* and *in sacco* digestibility experiments for the selection of herbs and the best pellet supplements which can help improve the digestibility system in ruminant animals.

2.3.2 Pellets

Pellets are suitable for feeding ruminants as a supplementary feed within poor pasture or during drought. This is because pellets can be equipped with a high nutrient content in accordance with nutritional requirements of ruminant. Pellets as food supplement in ruminant diet can increase gains and improve feed efficiency (Lindahl and Terril, 1963).

The pellets in the diets of ruminants have been widely used since Lindahl and Reynolds (1960) reported. Several feeding experimental trials have been conducted in which pellets were compared with whole or ground forms. Increased productivity from pellet feeding could result from increase in the retention of digestible nutrients. It is also capable of reducing food wastage, increasing feed consumption and improving utilisation of nutrients (Reynolds and Lindahl, 1960).

The use of pellets for fattening ruminants can lead to increased profits and better feed efficiency. Pelletised supplements are also easily handled as well as can be stored longer. Various materials can be pelletised as feeds for ruminant animals as food supplements (Elly Roza *et al*., 2013). For feedstuffs which have been dried (hay) they can

be used as an additional supplier in ruminant protein, particularly in the dairy and meat ruminant animals (Wanapat, 2000a; Khang *et al*., 2005).

2.3.2.1 Commercial Pellets

Alternative supply of nutrients is necessary when grass production is limited. It is equally important to the grass as a staple food intake of ruminant animals. This is because grass does not necessarily contain adequate nutrients. According to Devendra (1988), protein energy malnutrition instead, is the main factor limiting the use of forage protein. Thus, additional protein is necessary to help the growth of ruminant animals.

Good quality protein sources such as groundnut cake and soybeans meal are generally expensive and in short supply, which means that the bulk of them ought to be processed for non-ruminant feeding (Tilden *et al*., 1999). In the present study, commercial pellets and PKE pellets purchased from local suppliers have been used. Both types of pellets were added with local herbs supplied by the local company to test its effectiveness to improve digestion in ruminant feeds. The formulation was used to test the effectiveness of the herbs to improve the digestibility of the pellets.

2.3.2.2 Oil Palm Products

Hence utilisation of agricultural by-products in livestock feeding is becoming more important since recycling agricultural animal feeds is found to improve the livestock production in the tropics (Dahlan *et al*., 2000). At present, shortage of proper grazing ground hinders the livestock industry, particularly in Malaysia (Manan, 1987).

In oil palm plantations, two important species can be found, they are *Elaeis guineensis* that has its centre of origin in Africa and *Elaeis oleifera* which is a species found in South America. *Elaeis guinensis*, is an important crop of Malaysia and is a major contributor to the national economy (Ebrahimi *et al*., 2014). There are various by products which can be produced from this plant (Dias *et al*., 2008).

During the fruit development in oil palm, oil synthesises and accumulates predominantly in the mesocarp tissue (Khalid Ghazi *et al*., 2011). Oil palm waste products consist of fibrous materials such as empty fruit bunches (EFB), palm pressed fibre (PPF) and palm kernel expeller (PKE), and less fibrous materials such as palm kernel cake (PKC) and liquid discharge palm oil mill effluent (POME) (Abdullah and Sulaiman, 2013).

Many of these by products may be used for feeding livestock. Palm oil is the second largest source of edible oils in the world and it has been recognised as one of the most usable consumer oil compared to other plant oils (Basiron, 2007; Basri *et al.*, 2005). It is one of the leading traded vegetable oils garnering nearly 50 percent of the world trade of about 36 million tonnes (Agricultural Data and Research, 2004; Sethupathi, 2004). Accordingly, the Malaysian palm oil industry emerged during in the last few decades as a leading agricultural industry.

2.3.2.2.1 Palm Kernel Expeller (PKE)

Palm Kernel Expeller (PKE) is the product of palm kernel crushing. It provides energy-balanced, high in fibre and protein, and is easily introduced in various fields of ruminants (Dahlan *et al*., 2000).

Dias *et al*. (2008) reported that the use of PKE as a food supplement for dairy cows can provide a beneficial protein source in the diet. The by-product from palm oil mills is known as palm kernel expellers, whereas the by-product from the coconut oil mills is known as copra expellers (Ng and Khan, 2012). Owing to its richness in energy, protein, oil and fibre, the expeller meal is suitable to be used as ruminant feed for growth and fattening of farm animals.

Cell wall component consists of more than 600 g/kg PKE and the fibrous component mainly comprises element based on mannose-soluble polysaccharide (Alimon, 2004). Palm oil also contains free fatty acids (FFA). FFA is uncombined of glycerol fatty acid in triglyceride molecule. The virtual absence of linoleic acid results in better stability against oxidation compared with other highly unsaturated vegetable oils (Andrew, 2001).

PKE is a better substrate than grass in terms of supporting growth and as a source of energy and protein to ruminants (Hishamuddin, 2001). Researches since the late 1980s showed that the PKE can be used as ruminant feed. However, when used singularly as feed pellets, the quality of food will deteriorate (Goh and Rajion, 2006). To solve this problem, the addition of herbs to ruminants feed is expected to improve digestion in these animals.

2.5 Herbs in Ruminant Feeds

In early civilizations such as Mesopotamia, Egypt, India, China and Greece, herbs and their extracts were used for culinary, cosmetic and medicinal purposes, where they were appreciated for their specific aroma and various properties (Frankič *et al.*, 2009; Ben-Yehoshua *et al*., 2012). Some herbs are believed to be able to improve health due to their active components. Technological progress has enabled more easily determination of the structures and functions of active molecules of plant origin (Frankič *et al*, 2009).

A number of studies have highlighted tremendous medical concerns through the systematic investigation of herbal remedies and their adverse effects on the vital organs of animals (Elham *et al*., 2013). Herbs have antibacterial and anti-microbial aspects as they contain antioxidant and anti-inflammatory agents (Lai and Roy, 2004).

Herbs have been added to food since early times, not only as a flavouring agent, but also for therapeutic or medicinal value and as a food preservative (Cutler, 1995). Herbal plants contain phytochemicals which are chemicals that act on the body. Spices and certain herbs prolong the storage life of foods by preventing odour through antioxidant activities or through bacteriostatic or bactericidal activity (Shana *et al.*, 2007).

Many vitamins are found from the study of herbs. Herbs contain antioxidant vitamins, ascorbic acid (vitamin C) and tocopherol (vitamin E). Antioxidants are also extremely strong in activity such as phenols, thiols (such as sulphur compounds) and carotenoids. Phenolic compounds which are present in herbs can also play a major role in their antimicrobial effects (Hara-Kudo *et al*., 2004; Sarah Behran, 2011). Herbs not only contribute in terms of antioxidants but also have good pharmaceutical characteristics (Wu *et al.*, 2006; Sarah Behran, 2011).

Several studies have shown the theoretical benefit of adding herbs to pellets. Some local herbs have been found to be effective against helminths (Jaafar, 2005). Adding herbs to pellets may help to increase the digestibility of ruminant feeds and the absorption of nutrients to support growth. In turn, an increase in the digestibility will lead to an increase in body weight, muscle mass and total body fat (Huntington, 1997).

Previous research showed that feed supplements with growth promoting activity increase the stability of feed and beneficially influence the gastro-intestinal ecosystem mostly through the growth inhibition of pathogenic microorganism with improved health status of the digestive system; animals are then less exposed to the toxins of microbiological origin. Consequently, herbs may help to increase the resistance of the animals exposed to different stress situations and enhance their growth (Windisch *et al.*, 2008).

Herbal extraction can provide several beneficial components affecting feed degradation and fermentation. Herbs rich in minerals could be used as an alternative source to compensate for deficits in forages. The mineral compositions of these herbs were found comparable by several researchers (Ozkutlu *et al.*, 2007; Ozcan and Akbulut, 2008), whereby they have moderate to high content of nutrients to be used as supplements in forage. Several herbs and spices have been shown to manipulate rumen degradation (Khan and Chaudhry, 2010).

Cardozo *et al.* (2006) reported six natural plant extracts (garlic, cinnamon, anise, yucca, oregano and capsicum extracts) and three secondary plant metabolites (cinnamaldehyde, eugenol, anethole) at five doses and two different pH (7.0 and 5.5) to determine their effects on *in vitro* microbial fermentation using ruminal fluid of heifers. The effects differed depending on the ruminal pH. At pH 5.5, garlic, capsicum, yucca and

cinnamaldehyde altered ruminal fermentation in favour of propionate, which is more energetically efficient (Cardozo *et al.*, 2005: Frankič *et al*, 2009).

Mixtures of cinnamaldehyde, capsicum oleoresin and carvacrol enhanced the growth of lactobacilli, and also increase the ratio of lactobacilli to enterobacteriaceae (Castillo *et al.,* 2006). Hence herbs and spices not only possess antimicrobial activity but also modulate the composition of microbial population through prebiotic activity (Frankič *et al.*, 2009).

2.4.1 Local Formulated Herbs (Cinnamon, Garlic, Mas Cotek, Ginger-DD Herbs)

In this study, herbs (cinnamon, garlic, mas cotek, ginger) were selected and formulated to produce new supplements, referred to as DD herbs. These herbs were expected to help improve the ruminants' digestibility.

Feed additives are generally used to improve feed intake and to increase the growth rate in animals (Fadlalla *et al*., 2010, Bali *et al*., 2011 and Abouelfetouh *et al*., 2012). For many years feed additives have been widely used to increase animals' performance. More recently, they have been used in animal industry to improve feed efficiency and growth (Khan *et al*., 2007).

Some farmers have used local herbs to control worms in ruminants (Chandrawathi and Nurul Aini, 2012). The use of herbal remedies has been traditionally practised all over Asia including Malaysia as each country has its unique herbs.

2.4.2 Cinnamon (*Cinnamon zeylanicum*)

Plate 2.1 Cinnamon (*Cinnamon zeylanicum*)

The name cinnamon (*Cinnamon zeylanicum*) is derived from a Greek word meaning "sweet wood". Cinnamon is typically used in food preparation. It is derived from the inner bark of the cinnamon tree, an evergreen tree of the family Lauraceae (Sara, 2010). Cinnamon has a long history both as a herb and as a medicine. It is not only known for its aroma and flavour, but is a source of manganese, fibre, calcium and iron (Wood, 1988; Sara, 2010).

The antimicrobial activity of cinnamon has been studied extensively (Friedman *et al*., 2002). Compared to other herbs, cinnamon had the strongest antimicrobial activity (Zaika *et al*., 1988). The main antimicrobial components of the bark extract of cinnamon are eugenol and cinnamaldehyde (Friedman *et al*., 2002). These compounds are active against many pathogenic bacteria (Suresh, 1992; Shashidar, 2002) and viruses (Pacheco *et al*., 1993). Phytochemicals in cinnamon, called chalcone polymers, can increase glucose metabolism in the cells by 20 times or more and are considered powerful antioxidants. Cinnamon also contains flavonoids called anthocyanins that act to repair capillaries and act as antioxidants (Wilkonson, 2008).

2.4.2.1 Medicinal Value of Cinnamon

Cinnamon was widely used as a medical ingredient for sore throat and coughs in medieval times. Cinnamon is also effective to overcome gastrointestinal disorders such as indigestion, stomach cramps, intestinal spasms, nausea, and diarrhoea (Charles, 1998). Cinnamon's anti-flammatory, anti-spasmodic and anti-clotting properties are attributed to its cinnamaldehyde components (Puangpronpitag and Sittiwet, 2009).

Cinnamon extract is active against *Candida albicans*, which is responsible for vaginal yeast infection. It is also active against *Helicobacter pylori*, the bacterium responsible for stomach ulcers (Tabak *et al*., 1999). Cinnamaldehyde also inhibits nitric oxide production, which has been implicated in the inflammatory disease process (Lee *et al*., 2005).

According to Roussel *et al*. (2009), the mechanisms underlying the beneficial effects may be related to the insulin potentiating and antioxidant effects of the cinnamon polyphenols resulting in decreased free radical production. Their study supports the hypothesis that inclusion of cinnamon extracts in the diet of overweight or obese human would reduce oxidative stress and impaire fasting glycemia which are risk factors associated with diabetes and cardiovascular diseases. This is because the polyphenols found in cinnamon appear to reverse the signs and symptoms of insulin resistance (Anderson *et al*., 2008). Researchers found that people with metabolic syndrome who took the water-based cinnamon extract had lower fasting blood glucose level; lower percentage of body fat and higher lean body mass (Anderson, 2008).

Inclusion of cinnamon in the diet of patients with Type-2 diabetes has been shown to reduce risk factors associated with diabetes (Khan *et al*., 2003; Baker, 2006).

The study published in Diabetes Care (2003) reported that a 40-day study involving 60 patients with Type-2 diabetes showed that cinnamon reduced blood sugar level (18-29%), triglycerides (23-30%), total cholesterol (12-26%) and LDL cholesterol (7-27%) after daily consumption of 1-6 g cinnamon.

Therefore, the beneficial effect of cinnamon on blood glucose and HbA1C [%] observed in the present study could be due to decrease of insulin resistance secondary to the observed cinnamon induced increase in adiponectin. The histopathological changes after cinnamon in mice fed high fat diet and injected with dexamethasone is confirmatory to the beneficial effect of cinnamon (Mahmoud *et al.*, 2013).

Crude cinnamon extract also inhibited the growth of cultured tumour cells and this effect was suggested to be due to procyanidins and eugenol in the bark extract (Shahidar, 2002; Sara, 2010). Cinnamon is also useful as a food preservative to inhibit the growth of common food-borne bacteria such as *Salmonella* and *E.coli* (Suresh, 1992).

2.4.3 Garlic (*Allium sativum*)

Plate 2.2 Garlic bulbs *(Allium sativum)*

Garlic *(Allium sativum)* belongs to the Liliaceae family and is widely used with a long medicinal history (Ali *et al*., 2000). In the nineteenth century, Louis Pasteur scientifically proved that garlic contains antibiotic properties. His discovery led to the initiation of hundreds of studies which have substantiated his findings (Song and Milner, 2001).

Garlic is well known as a spice and herbal medicine for the prevention and treatment of various diseases from infections to heart diseases (Javandel, 2008). What was thought to be nothing more than a culinary ingredient has immense medicinal value. Garlic can effectively kill bacteria, fungi, viruses and parasites (Mindell, 1994). Bacteria and protozoa population size decreased when garlic powder was included in ruminant feed. Based on these results, it can be concluded that there are no negative effects on rumen fermentation characteristics and nutrient utilisation by plants and herbal supplements which then can be used as a food additive to enhance the efficiency of rumen fermentation (Wanapat *et al*., 2013).

There are about 200 chemical compounds in garlic. Some of the more important ones include: volatile oil with sulphur-containing compounds such as allicin,

alliin, ajoene, and enzymes such as allinase, peroxidase and myrosinase (Mowrey, 1986). Moreover, Adibmoradi *et al.* (2006) reported that garlic possesses antimicrobial activity and has antibiotic, anticancer, antioxidant, immune modulatory, anti-inflammatory, hypoglycaemic and cardiovascular-protecting effects (Reuter *et al*., 1996). Garlics are high in flavonols and organo-sulphur compounds with antioxidant and anti-inflammatory properties.

After chopping or crushing the garlic, allinase enzyme convert allicin (a cysteine-sulphoxide) in garlic to allicin (allyl 2-propene thiosulphate) (Banerjee *et al*., 2003). According to Benkeblia (2004), this enzyme is responsible for many of garlic's medicinal effects. This compound can be metabolised to a number of additional organo-sulphur compounds (Khanum *et al*., 2004).

Allicin is the main active antimicrobial component of garlic and is responsible for its strong odour (Sara, 2010). The anti-bacterial properties of garlic can be eliminated by inhibition of the allinase enzyme and prevention of allicin formation (Wilson and Adams, 2007). The anti-bacterial effect of garlic is due to the interaction of sulphur compounds (e.g. allician) with the sulphur (thiol) groups of microbial enzymes (such as trypsin and other proteases), leading to an inhibition of microbial growth (Bakri and Douglas, 2005). Allicin is further broken down into a compound called ajoene which can contribute to the anti-coagulant action of garlic (Eoghan, 2010). Mindell (1994) reported that garlic also contains citral, geraniol, linalool, A-phellandrene and B-phellandrene, which is what gives garlic its antibiotic properties (Kim Pezza, 2014). Also, garlic is an excellent source of vitamin B1, B6, selenium and calcium.

There are many gram-positive and gram-negative bacteria that can be inhibited by garlic. Some strains were inhibited much more strongly by allicin or garlic extract compared to antibiotics (Lai and Roy, 2004). In addition, garlic can exert a

differential inhibition between useful intestinal microflora and harmful enterobacteria (Ruiza *et al*., 2010).

2.4.3.1 Medicinal Value of Garlic

Harris *et al*. (2001) reported that garlic has a range of actions including being anti-bacterial, anti-viral, anti-fungal and antiprotozoal. Recent studies have demonstrated garlic's pharmacological effects, such as antibacterial, anti-fungal, hypolipidemic, hypoglycaemic, anti-thrombotic, anti-oxidant and anticancer properties (Song and Milner, 2001).

Qureshi *et al.* (1983) stated that garlic has the tendency to lower serum and liver cholesterol. Garlic is also reported to have a positive effect on the immune system, as well as many biological activities, such as a protective role in cardiovascular function serving as an anti-hypertensive (Song and Milner, 2001). Garlic also has a positive impact on the performance of different animals. This is not surprising because garlic has been used for centuries in various cultures to fight against communicable diseases (Lanzotti, 2006).

Positive effects of herbal supplements on production performance and carcass quality have been demonstrated in animals (Tekeli *et al*., 2008). Demir *et al*. (2003) stated that garlic can improve the performance of broilers and has been used for 50 years as a growth promoter antibiotics and has improved the growth performance of poultry and pigs (Demir *et al.*, 2008). Elagib *et al*. (2011) reported that inclusion garlic in the diet at a 3% level had significantly enhanced growth performance of young broiler without any side effects.

Recent studies showed that garlic contains good nutrients with antimicrobial, anti-inflammatory, anti-oxidant and immune stimulant in animal nutrition as well. Garlic oil and active components tested at high doses prevented the rumen microbial fermentation, which confirmed their antimicrobial activity (Mirzaei-Aghsaghali *et al*., 2012). Garlic oil is a potential option for applications as additives for ruminants. It contains several compounds, including sulphur compounds (thiosulphates, allyl sulphides, glutamylcysteines, allicin), enzymes, sterols, steroids, triterpenoid glycosides, flavonoids, phenols and compounds containing organo-selenium (Lawson, 1996; Reuter *et al*., 1996).

However, their potential effects on rumen microbial fermentation has not been studied until recently. In a continuous culture system, 300 mg L^{-1} garlic oil reduces the ratio of acetate and branched-chain volatile fatty acids and increases the ratio of propionate and butyrate concentration of small peptides, amino acids plus N (nitrogen) (Busquet *et al*., 2005a). This change is consistent with the inhibition of methane fermentation profile and has the potential to produce better rumen microbial fermentation (Mirzaei-Aghsaghali and Maheri, 2011).

The anti-carcinogenic effects of garlic may be well originated from the organo-sulphur compounds responsible for its scent and flavour (Wargovich, 2006). Laboratory research has shown that both water and lipid soluble sulphur compounds from garlic provide anticarcinogenic benefits (Schaffer *et al*., 1997). Further animal studies have shown that the protection offered by the garlic is not limited to only certain tissues or certain carcinogens, but can occur in some tissues as a result of the treatment of various types of carcinogens (Dion *et al*., 1997). Garlic with a high content of flavanols, especially kaempferol, is suggested as a crucial element in the detoxification of carcinogenic compounds (Bilyk and Sapers, 1985).

2.4.4 Mas Cotek (*Ficus deltoidea*)

Plate 2.3 Mas Cotek (*Ficus deltoidea*)

Ficus deltoidea is a member of Moraceae family (Hasan *et al*., 2012). It is an epiphytic shrub, which is widely distributed in Southeast Asian countries. In Malaysia, *Ficus deltoidea* is locally known as mas cotek (Zunoliza *et al*., 2009). Its local name is given due to the presence of golden spots on the upper surface of the leaf (Draman *et al.*, 2012). *Ficus deltoidea* is in the division Magnoliophyta, class Magnoliopsida, order Rosales, and Moraceae family (Hasan *et al*, 2012).

Ficus deltoidea is native to Southeast Asia to Borneo, and the Philippines (Brickell and Zuk, 1997). Riffle (1998) describes this species as indigenous to the southern Philippines southward and westward to Southeast Asia, Malaysia, and Indonesia. *Ficus deltoidea* is one of many species of *Ficus* cultivated in various parts of the world as a house plant or as an orna mental shrub (Starr, 2003). In Malaysia, *Ficus deltoidea* is a wild plant which can be found in Kelantan, Pahang and Terengganu (Nurafida, 2008). It grows well in the forest near the beach but can also thrive in the hilly areas up to 700 m from sea level (Imanuel, 2008).

2.4.4.1 Medicinal Value of Mas Cotek (*Ficus deltoidea*)

Mas Cotek (*Ficus deltoidea*) contains flavonoids, which has an antioxidant activity (Draman *et al.*, 2012). Flavonoids also give the yellow pigmentation, and help the plant to protect itself from microorganisms and insects. Herbs that contain flavonoids also have the ability to act as anti-allergy, anti-inflammatory, antimicrobial, and anti-cancer agents (Buhler, 2007). Flavonoids in Mas Cotek are reported to improve wound healing and to protect tissues from oxidative damage (Saurez *et al*., 1996).

The anti-oxidant activity and its potency in certain plants depend on the existence of various compounds found in the plants. Several of the phenolic compounds (anthocyanidin, catechines, flavones, flavonols and isoflavones), tannins (ellagic acid, gallic acid), phenyl isopropanoids (caffeic acid, coumaric acids, ferulic acid), lignans, catchol and many others are antioxidants. The anti-oxidative and radical scavenging activities of the above antioxidants are well studied (Imanuel, 2008). Mas Cotek has been also reported as not causing death or visible signs of toxicity in any animals (Elham *et al*., 2013).

Traditionally, this plant has been used in the treatment of inflammation and to relieve pain. It is also used to treat several diseases, including gout, high blood pressure, pneumonia, diarrhea, and skin infections (Hakiman and Mazziah, 2009). In addition, Mas Cotek has been used as an aphrodisiac and to increase male fertility (Adam *et al*., 2007). Decoctions of the leaves of Mas Cotek have been extensively utilised in folk medicine to decrease the symptoms of diabetes mellitus, hyperlipidemia, and hypertension. Herbal medicine practitioners often recommend the leaves of both male and female plants as libido boosters and postpartum depression treatments to help in contracting the muscles and strengthen the uterus (Sulaiman *et al*., 2008; Bodeker, 2009). It is popularly known

as the female Viagra and also served as a health tonic which can help to harmonise the body. Studies have shown that Mas Cotek leaves possess antinociceptive, wound-healing, and anti-oxidant properties (Zahra *et al*., 2009; Abdulla *et al*., 2010).

Mas Cotek is an alternative therapeutic plant which contains a high content of polyphenolic compounds (Shahidi, 2008) including vitex in that may possess anti-hypertensive effects (Abdullah *et al*., 2009). It is a medicinal herb and its leaves are enriched with phytol-constituents having various pharmacological activities (Shafaei *et al*., 2011).

The natural products from plants may have therapeutic potential in the treatment of cardiovascular disease, including high blood pressure (Abdullah *et al*., 2009). This study may provide clues to the role of Mas Cotek and more through *in vivo* studies, identify the mechanisms needed involving vascular activities. It is conceivable that a lot of effort is still needed not only in the validation of plants but also in identifying the active principles of medicinal plants (Nadiah *et al*., 2013).

The beneficial effects of Mas Cotek have been found to prevent inflammation and ulcers too. Its ability to inhibit carbohydrate-hydrolysing enzymes, and its wound-healing, hepato-protective, and anti-nociceptive activities have been verified (Abdullah *et al*., 2010; Farsi *et al*., 2011). It is a well-known herb whereby women take it leaves after childbirth to constrict the womb, to improve blood circulation and to treat problems of the menstrual cycle (Nurafida, 2008). Furthermore, the plant also contains tannins, triterpenoids and phenols (Shafaei *et al*., 2011). Tannins are astringent, bitter-tasting plant poly-phenols that bind and precipitate proteins. Tannins may be employed medicinally in anti-diarrheal, hemostatic, and antihemorrhoidal compounds (Vattem *et al.*, 2005; MARDI, 2008).

Mas Cotek has long been used by natives to treat high blood pressure and gout. Some of the traditional medicine practitioners believe Mas Cotek is good for improving blood circulation and rejuvenation (Abdul Hamid, 2007). It has gained increasing attention among groups practising traditional medicine (Imanuel, 2008). The increase is due to its values in treating various diseases and for healthcare maintenance.

2.4.5 Ginger (*Zingiber officinale*)

Plate 2.4 Ginger (*Zingiber officinale*)

Ginger (*Zingiber officinale*) is one of the more commonly used herbal supplements. It belongs to the Zingiberaceae family. Its root stocks are smooth and thick which possess strong aromatic traits consumed as a delicacy, medicine, or herb. All parts of the ginger plant are consumable and have therapeutic significance (Arfeen *et al.*, 1995).

Ginger is grown primarily in Asia and tropical areas. It is used popularly as a culinary function. Ginger has been used since ancient times for a variety of conditions, including for treatment of colds, fevers, and digestive problems, and as an appetite stimulant. It is categorised by the U.S. Food and Drug Administration as a food additive but has been studied as a treatment for nausea and vomiting, as well as for arthritis (Brett,

2007). Rhode (2007) reported that dietary prevalence of foods such as ginger is thought to contribute to the decreased incidence of colon, gastrointestinal, prostate, breast and other cancers in Southeast Asian countries. There are signs that ginger is a source of nutrients, and mineral elements, amino acids, and phytol-chemicals (Olubunmi *et al*., 2013), therefore, its use as nutritional supplements is highly encouraged.

2.4.5.1 Medicinal Value of Ginger

Ginger has been traditionally used from time immemorial to treat various human diseases in different parts of the world. It is not only to aid digestion and treat stomach upset, but a remedy for diarrhoea and nausea as well. Ginger has been shown to be effective for nausea caused by pregnancy and vomiting after surgery (Jewell and Young, 2003). Grontved *et al*., (1988) reported that 1 gm of ginger is effective in reducing the severity of subjective sea-sickness in naval cadets at sea.

Spicy constituents are present in ginger and other zingiberaceous plants to have potent antioxidant and anti-inflammatory activity (Shukla and Singh, 2007). This herb has been identified as a medicinal product with a pharmacological effect. Ginger suppresses prostaglandin synthesis via inhibition cyclooxygenase- cyclooxygenase- 1 and 2 (Eleazu *et al*., 2013). It also contains several interesting bioactive constituents and possesses health-promoting properties. The proximate, mineral, antinutrient, amino acid, and phytol-chemical components of two varieties of ginger were investigated (Olubunmi *et al*., 2013). According to Yoshikawa *et al*. (1993), the consumption of ginger led to reduction in blood cholesterol and also served as a potential anti-inflammatory and antithrombotic agent (Zomrawii, 2012; Ferreira, 1999).

Although ginger is often used for culinary purposes, it is taken by many patients to treat a variety of conditions. However, there are mixed results from limited studies of ginger for the treatment of arthritis symptoms (Brett,2007).

In history, the traditional medicines of both India and China have valued ginger against arthritis and rheumatic complaints. This herb is also found to enhance the digestion of both carbohydrates and protein, and stimulates bile secretion. Ginger actually possesses a remedial effect for ulcers as well (Science Daily, 2012). Ali *et al*. (2008) reported the positive effect of ginger on blood circulation, gastric secretion, and enterokinetic. In addition, ginger has been found to enhance digestive enzyme activities (Platel and Srinivasan, 2000).

According to Herawati (2010) who reported on broiler fed 2% dried supplementary red ginger meal had significantly lower feed intake than those on the control diet. Meanwhile Doley *et al.* (2009) and Al-Homidan (2005) found an increase weight gain of broiler when fed 2% and 6% ginger. Feed conversion ratio were not affected among all groups, and these results concur with findings of Wafaa *et al*. (2012), who reported that there was no difference among birds fed on 0.5%, 1% and 1.5% ginger root powder in feed conversion ratio.

Moorthy *et al.* (2009) and Herawati (2010) reported that the birds which were fed with diets containing ginger up to 2% recorded better feed conversion ratio than un-supplemented ones. These have favourable effects on animal productivity. It showed that inclusion of ginger root powder in the animal diet at level of 1% improved performance, although level 2% ginger root powder decreased serum cholesterol concentration and had adverse effect on performance and blood constituents (Zomrawi *et al*., 2013).

2.5 Forage Development in Malaysia

Animal feed refers to herbal ingredients including grass, legumes, fibrous plants and trees. The main limitation is the lack of production of green food and lower nutritional quality forages (Jones and Wilson, 1987). The low nutritive quality of the forage during the growth period is mainly due to environmental stresses such as high temperatures (Van Soest, 1988) and infertile soils (Roberts, 1987).

Native rangelands provide the cheapest source of nutrients for ruminants and for a greater part of the year, grasslands do not supply sufficient nutrients to stock for greater productivity (Ndlovu, 1992). It has been emphasised that most tropical forage species have low dry matter digestibility and intake (Aregheore, 2001). Hence it is essential for supplementary food to produce high quality forages.

Some of the main issues in the Malaysian ruminant industry is the shortage of suitable feeds or forages, both in terms of quantity as well as quality of feed available. Even though Malaysia has an equatorial climate with adequate rainfall and sunshine all year around, it is still characterised by a lack of high quality natural pastures (Kong, 1989).

Our present choice of improved forage grasses is limited to grass genera, the grasses that have been shown to be most adaptable and productive under Malaysian conditions (Wong *et al*., 1982). Grasses like Napier (*Pennisetum purpureum*) also have been used widely as fodder grasses, as grazed grass-legume or nitrogen fertiliser pastures.

Research should focus on the problems faced by smallholders in livestock, particularly raising livestock productivity through improved nutrition, management and greater precision in the utilisation of available natural resources. It is necessary to take

into account of practical situation of smallholder livestock on the use of food resources effectively.

2.6 Growth

Growth is often measured as an increase in live weight per unit of time (Berg and Butterfield, 1976). It is measured by reference to the weight of the non-linear function such as a quadratic equation or allometric growth (Butterfield, 1988). Commercial growth involves only the amount of product that can be eaten. As the weight increases to the weight of mature animals, the percentage increase in fat, the muscles decline slightly or remained constant and the bone is reduced.

Growth path or rate of growth at specific periods of development has historically been used as a method of altering carcass composition (Ball *et al*., 1997). Dietary factors that affect the important reactions in body composition during growth is time and nutrition. After eating, a common point of intervention is food restrictions involving the change of body composition. It can be classified into a situation where growth restriction reduces relative to normal growth or weight loss (Schutt *et al*., 2009).

Two aspects of growth are:

i) measured as an increase in mass (weight) per unit time;

ii) involving changes in form and composition resulting from differential growth of the component parts of the body (Fowler, 1968; Noraida, 1987).

Body weight of the animal taken from an earlier stage of growth could be used to express and can also be used to predict the future performance of the animal

(Nagacenkar and Rao, 1983; Noraida, 1987). By taking the birth weights of ruminants, the sizes of the adult animals could be determined as several researchers have reported positive correlations of adult live weight with birth weight (Mukunden *et al*, 1981; Noraida, 1987).

2.6.1 Growth Curve

Growth is defined as an increase in tissue mass. The massive increase of hyperplasia (cell multiplication) early in life and hypertrophy (enlarged cells) have occurred. Hyperplasia of adipose tissue continues to grow throughout life (Owens *et al*., 1993).

Growth is usually defined as the production of new cells. It is usually measured as an increase in mass due to growth which includes not only hypertrophy and hyperplasia but also the incorporation of specific components of the environment (Philips *et al*., 1992). By definition, the deposition of fat in spite of the growth of muscle mass is of primary importance in the production of meat (Buttery *et al*., 1986).

Mass or growth curve is plotted against the cumulative weight of age, is the sigmoid, which consists of a pre-maturity accelerated phase coupled with the slow phase after reaching maturity. Mathematically, this curve can be described as a function of the mass of maturity, growth rate and age breakdown (Lawrence *et al*., 2002). By using the growth curve, the average daily gain is calculated as the difference between successive weight every month and divided by 30 days. A spreadsheet program is recorded and graphs cria growth compared to the standardised growth curves to see the growth of ruminant animals (Van Saun, 2007).

The median weight of raw data showed that the average daily gain increased from one to two months of age where it peaked and then decreased logarithmically until a plateau is reached around 30 gm/day (Jarrige *et al*., 1986). If one takes the issue of the growth curve model, the average daily gain is reflected as a decrease in a linear (y = 127.8-3.56x). Daily profit linear model does not show high levels of profitability. Logarithmic curve is consistent with the observed growth rates and indicate the maximum rate of profit during the peak lactation, or two months of age (Riek *et al*., 2007).

For certain levels of maturity, body composition seems to be fixed. If the mature mass amended, body composition will change at some point (Scanes, 2003). Alternatively, slow deposition of fatty compounds or estrogen administration may increase the mass of the mature protein. Animals with higher weight require more energy for maintenance. Thus, greater maturity is improper for breeding group (Dayton and Hathaway, 1991). However, young ruminants with retarded growth often make less efficient feedlot after weaning. Hence manipulation of specific nutrients and hormones is needed for growth of young ruminant animals (Owens *et al*., 1993).

Although animal growth encompasses many diverse fields such as biochemistry, physiology, endocrinology, genetics, and animal management, this study will emphasise factors that influence growth by adding herbs in the animals' daily supplementary diet. The result will focus on effects of growth on the body and the primary goal of animal meat production (Batt, 1980).

Mature size is generally considered the point where the muscle mass reaches its maximum. Net growth is the difference between synthesis and degradation of body tissue. Both are continuous processes; total protein synthesis six to ten times net retention protein (Eisemann *et al.*, 1989). Muscle hyperplasia occurs primarily in the prenatal stage

(Allen *et al*., 1979), and muscle fibre increases in global growth after birth (Bergen and Merkel, 1991). Post-natal growth of muscle mass occurs through hypertrophy and through satellite cell replication and incorporation, numerous factors have been elucidated that regulate the number of muscle fibres and of nuclei (Goldspink, 1991).

2.6.2 Factors That Affect Body Composition and Growth Rate

Research on the growth and body composition of ruminant animals has contributed significantly to the knowledge regarding body composition methodology, the effects of severe malnutrition, compensatory growth following a period of malnutrition, and factors that influence the partitioning of nutrients into various tissues. This has resulted in improvements in the growth, body composition and feed efficiency of meat-producing animals, to the benefit of both the producers and consumers (Mitchell, 2007). Basic to advance in the study of the growth and nutrition of animals is the ability to measure body composition. Nutritional approach to improve lean growth has involved changes in the level of dietary protein and energy ratio in the diet (Scanes, 2003).

2.6.2.1 Mature Body Size

Although the maximum body size is genetically determined, it can be altered by nutritional and hormonal factors. Differences between breeds in size are due to differences in size of the skeleton and in the number but not the size of muscle cells (Hammond, 1961). Early fetal growth largely is genetically regulated, although blood

flow later in pregnancy will alter fetal weight and maturity. Growth rate at later fetal stages and after birth but before maturity can be influenced greatly by factors such as plane of nutrition, hormonal status, and environment (Owens *et al.*, 1993).

Many factors may inhibit cell division to the point of maturity. Ruminants mature size can be altered readily by nutritional restriction remains (Berg and Butterfield, 1976). Depending on the severity of the restriction and the specific nutrient involved, size of an animal when it becomes mature has been reported variously to be decreased, unchanged, or increased (Pond *et al.*, 1990). Discrepancies among reports may relate to the timing or severity of deprivation or the amount of nutrient involved (Widdowson and Lister, 1991).

According to Guererro *et al.* (1988), the genetic potential of cattle to determine the composition of their carcasses for maximum weight loss will be achieved in a short or long period of time. Some researchers (Lake *et al.*, 1974; Lewis *et al.*, 1990b) have stated that limiting energy intake during early pubertal post will significantly reduce body fat by prepubertal. Livestock producers may be able to change the weight of mature animals to meet user specifications and packaging plant for carcass composition and meat weight (Owens *et al.*, 1993).

2.6.2.2 Nutrition

Tissues grow and develop chronologically in specific during growth waves. Certain tissues grow and malnutrition will influence the growth of these tissues. Certain tissues grow and proceed to bone, muscle tissue, and finally adipose tissue (Callaway *et al*., 2003).

For each tissue, development depends on its location in the body. Hence, body shape and composition change as an animal matures. Dietary nutrients supply should be coordinated with this progression to maintain optimum growth rates (Owens *et al.*, 1993). Adequate nutrition is also necessary for optimum growth and to achieve the genetic potential of animals (Hossner, 2005).

Ruminants productivity is influenced primarily by feed intake, in turn, is determined by feed digestibility and the capacity of the diet to provide the correct balance of nutrients required by animals in different productive states. Thus the two major variables that need to be considered are:

i) the amounts and balance of nutrients required;

ii) the quantitative availability of nutrients from the diet (FAO, 2005).

These help to predict the nutrients required by ruminant livestock. These match can be obtained from the digestion of nutrients, because many interactions exist between animals, diet and microbial ecosystem in the rumen (Beetz, 2002). Forage diet depends on plant maturity, species, season, moisture, and grazing system (Minson, 1990). Otherwise supplementation may be necessary when grass is short, too mature, dormant, or if the animal's needs require it but an excessive supplementation may reduce the ability of the rumen microbes to use forage (Lee, 2008).

2.6.2.3 Hormones

Growth is a very complex process, and requires coordinated action of several hormones. The major role of growth hormone in stimulating body growth is to stimulate the liver and other tissues to secrete IGF-I (Velloso, 2008). IGF-I stimulates proliferation

of chondrocytes (cartilage cells), resulting in bone growth. Growth hormone does seem to have a direct effect on bone growth in stimulating differentiation of chondrocytes (Bowen, 2006).

Growth hormone expands the size of internal organs (Early *et al.*, 1990) and increases feed intake. Cascades of hormones may be involved with growth. Some growth promoters or inhibitors are also involved in controlling the growth of cells in muscle or muscle fibres (Dayton, 1988).

Hormone can stimulate feed intake at the hypo-thalamic level (Kong *et al*., 2004). The quantity and quality of food eaten are a major factor determining plasma concentrations of hormones such as thyroid hormones (Dauncey, 1990). Blood thyroid hormone levels are considered to be good indicators of the nutritional status of an animal (Riis and Madsen, 1985) and is associated with food intake in ruminant species (Rhind *et al*., 1998).

Thyroid hormone concentrations correlates with adiposity status (Caldeira *et al*., 2007a). The hormone is present in blood circulation, effective genetic selection based on the assumption that the level of systemic gene activity is reflected by the concentration of hormones in the blood stream and effectively alter growth, growth rate and metabolism (Hossner, 2005). Changes of thyroid hormone concentrations in blood is an indirect measure of the changes in thyroid gland and extra thyreoid alde-iodination activity. Many factors act simultaneously, modulating the activity of the thyroid gland and peripheral mono-deiodination (Caldeira *et al*., 2007b). Besides endogenous and environmental climatic factors, nutrition plays a primary role on thyroid gland activity and on blood thyroid hormone concentrations (Todini, 2007).

2.6.2.4 Compensatory Growth

Compensatory (catch-up) growth can be defined as a physiological process whereby an organism accelerates its growth after a period of limited development usually due to reduced food intake (Hornick *et al*., 2000). Compensatory growth represents hypertrophy of muscle tissue. If the nutrient intake of growing animals is restricted, growth rate is subnormal. The extent of compensatory growth is greater when it follows energy restriction rather than protein restriction (Drouillard *et al.*, 1991a).

This compensatory growth is often measured by determining the change in body weight (BW) of animals over several weeks. Unfortunately, body weight (BW) is an inaccurate predictor because it is biased lean body mass (Drouillard *et al.*, 1991b). The empty weight of digestive tract including the liver can be changed within a few weeks (Williams *et al.*, 1992).

Ruminants usually are bought and sold on live weight basis; compensatory weight can have a sizeable economic effect on livestock production. However, if compensatory growth does not represent lean carcass mass, it should be irrelevant for system based on the sale of animal's carcass (Owens *et al.*, 1993). Decreased maintenance cost, increased feed intake, a significant increase in the efficiency of growth, genetic background, and under certain circumstances increased digesta has been linked as a key mechanism in the phenomenon of compensatory growth (Sampelayo *et al*., 2003; Joemat *et al.,* 2004).

The magnitude of compensatory growth depends on a number of factors (Hogg, 1991). Compensatory growth seems to alter composition most during growth but has limited impact on body composition at maturity (Owens *et al.*, 1993). Animal

undergoing compensatory growth were found to be more efficient in utilising dietary protein for body protein than growing normally (Mahyuddin, 2004).

2.6.3 Measurements of Growth and Development

Knowing the body mass of small ruminants is very useful for good animal management, including understanding medication doses, adjusting feed supply, monitoring growth and choosing replacement males and females. Live weight (LW) is only an estimation of body mass because it varies continuously with feeding, watering, dropping, urinating, breathing and sweating (Mahieu, 2011).

Live weight plays an important role in determining the characteristics of the farm animals, especially those having economic interests. Birth weight, early growth, feed conversion ratio as well as feed requirements could be predicted by knowing the live weights directly (Eker and Yavuz, 1960). Estimating the size using body measurements is practical, faster, easier and cheaper in the rural areas where the sources are insufficient for the breeder (Nsoso *et al*., 2003).

Body measurements are important data sources in terms of reflecting the breed standards (Riva *et al*., 2002) and are necessary in giving information on morphological structure and development ability of the animals. Body measurements differ according to factors such as breed, gender, yield type and age (Pesmen and Yardimci, 2008). The most common parameters used for measure in ruminants are: head length, head depth, frontal, ear length, and body length, withers height, and rump height, body depth, and heart girth, width at withers, shank circumference, tail length and width.

Body weight estimation performed using a different statistical analysis (Gurcan, 2000). Empty body mass measurement is better than a shrink or full body weight, an increase in empty body mass does not necessarily represent an increase in empty body protein which fully reflect changes in protein mass or body energy content (Williams *et al.*, 1992). Laurenz *et al.* (1992) reported, during the summer months, mature Simmental cows, while losing 5.4 kg of empty body protein, gained 5.6 kg of empty body weight (BW) because of deposition of added fat (30.1 kg). It shows how a change in the weight directly or empty body mass would have no change in the mass of protein or energy body content (Owens *et al.*, 1993).

Body measurements are also used to predict weight gain in ruminants (Thys and Hardouin, 1991). In goats, similar contributions have been made (Singh and Mishra, 2004). However, several attempts were made to identify the circumstances of the heart as it is considered the most reliable measure of the goat (Alade *et al.*, 2008).

According to Carstens *et al.* (1991), the mature weight is the point of maximum lean body mass and any extra weight gained beyond this point is fat. The weight of mature animals alone may not reflect the weight of the fat content. It is fixed on the animal and matured lean body mass can be predicted by the commercial slaughter weight (Owens *et al.*, 1993).

2.6.3.1 Alternatives to Linear Measurement

Animal selection for fast growth rates or low content of fat often causes weight gain maturity. This is because certain animal body with a large bone and large mature weight is early in their growth curve. Mature size is desirable to increase the production

efficiency of lean tissue and reduce fat content by weight of slaughter animals in particular (Greathouse, 1986). However, a large mass increases both weight and maintenance costs (Owens *et al.*, 1993).

The method to circumvent the problem of selecting animals mature size is greater when selecting for growth. Mathematically, the final weight subtracts by initial weight and divided by feeding period will result in the breakdown of growth rate. Although both measures of relative and absolute growth rate have been shown, but the index is equally effective for rapid growth after weaning ruminants' (Brown *et al.*, 1988) selection by relatively more than absolute growth rate might enhance metabolic efficiency at other stages of growth.

Another index is the rate of growth of mature weight fraction. It proves preferable to both absolute and relative growth rate (Brethour, 2004). The growth rate in the period is divided by the weight of maturity, this index should avoid weight related to the mass and size of the body being more mature and therefore it is more appropriate as metabolic efficiency index (Owens *et al.*, 1993).

2.6.3.2 Growth Rate Measurements

The growth period of young generation until the puberty age can be divided into three phases:

(i) maternal phase: from birth to weaning;

(ii) phase of development of bio-physiological mechanisms of growth and individual response to environmental conditions: from weaning to sixmonths old;

(iii) growth phase: from the age of six months to puberty (Kristaq and Juan, 2010).

Rate of gain is usually calculated as change in weight during a specified time interval. Weight change from the initial to the final date typically is used to calculate growth rate. In contrast to calculating rate of gain from only two fixed time points, regression across the various time points growth curve (Tolley *et al.*, 1988). The animal nutrient requirements and pasture can change over time; it often proves informative to subdivide experiments into specified time periods to determine when treatment responses are evident (Owens *et al.*, 1993).

The convenient way to appraise responses in various time intervals is to calculate and plot weight gain as a percentage of the control. Growth rate can be defined mathematically in terms of mature size, the rate of growth deceleration, and age (Baker *et al.*, 2006). Although maximum growth rate is set genetically, nutritional and hormonal factors can limit growth rate. Altering mature size, may reduce or increase fat content of the carcass. Heavy mature weights are not desirable for ruminants maintained for reproduction (Garcia *et al.*, 2008).

The estimate of the body composition of live animals is essential to develop production systems that are more efficient as well as using less resources (Gerrits and Dijkstra, 2000). The initial body composition is important to predict energy requirement for growth, but its assessment is deficient and not trivial in live animals (Brethour, 2004).

The use of biometric measures (BM) as an indicator of animal type has been proposed for a long time (Brody, 1945; Fisher, 1975). Its limitation is associated with the accuracy of the measures, such as the identification and location of reference points, anatomical distortion generated due to changes in either position or posture or by

changing muscle tone, and errors involved in taking measurements at any position, which can vary depending on the device use (Fisher, 1975).

2.6.4 Effect of Sex

Growth distribution of gender has a significant effect on weight which is the tissue and subsequently impacts carcass composition (Berg and Butterfly, 1976). It is most noticeable by the composition of the body through the process of fattening. Muscle growth influenced by gender also has a significant effect on carcass composition. In the development of muscle weight distribution of animals that thrive, gender is a factor as well. Thus it affects some management decisions of ruminants.

The method allows manufacturers to decide whether they get the advantage of the growth that is affected by gender. Livestock producer must feed the animals of different sexes in a manner consistent with the characteristics of their growth. It is to achieve the best combination of slaughter weight and carcass composition.

In the present study the *in vivo* technique in Bidor Farm was carried out through the analysis of data of 10 kids managed with two different diets:

(a) semi-intensive production system in farm with over six kids (mixed; males and females) given commercial pellets added with local herbs as their supplement;

(b) semi-intensive production system in farm with over five kids (mixed; males and females) given commercial pellets as control experiment.

Analysis of the growth data was carried out according to the procedure of General Fixed Factor Linear Model (GLM). Sex and mode of birth are factors that must

be taken into account during growth of kids 4-9 months old. The live weight as well as average daily gain according to Sodiq (2012) are influenced by their gender.

Male kids are always heavier than females. These results agree with previous studies in several breeds in ruminants by several researchers: Mioč *et al*. (2011)'s study on Croatian goat, Sodiq *et al*. (2010)'s study on Katjang goat, Zhang *et al*. (2009)'s study on Boer goat, and Vargas *et al*. (2007)'s study on Native Creole goat. They reported that growth rate is influenced by animal's gender. Average daily gain could be explained by the influence of sex hormones on animal development affecting body dimensions and fat deposits, as well as muscle and bone. Sex differences were between 97 gm and 228 gm on average daily gain from birth to weaning (Sodiq, 2012).

Significant differences in sex chromosomes, probably in the position of genes are related to growth, and physiological characteristics, while differences in endocrinal system lead to differences in animal growth. In relation to endocrinal system, estrogen hormone has a limited effect on the growth of long bones in females (Vargas *et al*., 2007). Therefore, it causes females to have smaller body and lighter weight than males (Baneh and Hafezian, 2009).

In addition, there are sex influence on the birth weight, where males are heavier (2.07±0.02 kg) than females (1.95±0.02 kg) (Sodiq *et al*., 2010). This may be attributed to the anabolic effect of male sex hormones (Hafez, 1962). Sodiq (2004) reported goats' weight increased with the advance in parity up to the fourth parities and male kids tended to be higher in weight than those of female kids. These factors should be accounted for in genetic evaluations for the improvement of ruminant animals (Mioč *et al*., 2011).

2.7 Research Techniques to Estimate Feed Digestibility

Knowing energy value of ruminant feeds and their bio-availability are important for animal feed manufactures. The amount of available energy in feeds for ruminants is described either by its metabolised energy or by its organic matter digestibility (Barber *et al.*, 1990), since the value of organic matter digestibility is very close to the corresponding digestibility of energy (Thomas, 2004). The most accurate way of measuring digestibility of organic matter of feeds for ruminants is by conducting *in vivo* digestibility experiments.

This method is expensive and time-consuming therefore, it is not suited for routine analysis, there has been a constant search for alternative laboratory methods for routine prediction of the *in vivo* organic matter digestibility of ruminant feeds in order to implement an adequate system of quality control in the feed industry. *In vitro* methods used to predict the *in vivo* digestibility of organic matter of feeds for ruminants are generally based on chemical analysis, fermentation with rumen micro-organisms and hydrolysis with enzymatic preparations.

Organic matter digestibility prediction of forage using chemical analysis by the Weende method or acid detergent fibre method results in variable correlations (Aufrère & Michalet-Doreau, 1988). Methods for predicting *in vivo* organic matter digestibility using rumen liquor fermentation techniques have become well established, but there are limitations on the use of rumen liquor for digestibility studies. There must be fistulae animals and it is not available to all laboratories for the collection of fresh rumen liquor. There must be use of surgically modified animals in experimentation to fistulae these animals (Jones & Theodorou, 2000).

The use of incubation of feeds with exogenous enzymes to predict the *in vivo* organic matter digestibility has the aim to mimic the digestive process in these animals. Most enzymatic methods for organic matter digestibility estimation have been developed for forage feedstuffs, with a few used for other feedstuffs, as grains and compound feeds (Aufrère & Michalet-Doreau, 1988). Weisberg and Hvelplund (1993) developed a multi-enzymatic incubation method for estimation of enzymatic digestibility of organic matter for use on compound feeds. This procedure also showed an ability to estimate the organic matter digestibility of straws (Hvelplund *et al*., 1999), which demonstrated the potential of this method to predict the *in vivo* organic matter digestibility of grains and forage.

Besides *in vitro* and *in vivo* techniques, another technique used to estimate digestibility in ruminant animal is *in sacco* technique. This technique needs animal to be fistulated and the treatment needs to be incubated in ruminant rumen for a certain time. All these three techniques have their advantages and disadvantages, for the best digestibility estimation in ruminant animals. In the present study, all three techniques were used to gain accurate results.

2.7.1 *In Vitro* Technique to Estimate Feed Digestibility

Research should be conducted to evaluate the rate of digestion of ruminants. In determining the adequacy of food to meet the needs of the animal, it must be measured qualitatively and quantitatively.

The ruminant feed energy and bio-availability are important for food producers of ruminants and ruminant feed end users. The amount of energy available in the feed for ruminants can be described either through metabolically energy or by

digestion of organic material (Barber *et al*., 1990). This is because the digestion of organics is very close to the digestive energy (Thomas, 2004).

Kriszan *et al*. (2015) reported that *in vitro* technique is to determine the digestion of organic material (OMD). It uses the technique of rumen fermentation liquor which is used to determine the quantity of digestion of food in the laboratory (Palić and Leeuw, 2009). It has been widely adopted to determine the digestion of organic ruminant feed. Two-stage method of Tilley and Terry (T & T) are the rate of digestion of food in the rumen (Tilley and Terry, 1963) and techniques to measure gas production (GP) developed by Menke and Steingass (1988).

In vitro experiments objective is to mimic the process of digestion in ruminant animals. In the *in vitro* digestion technique, liquor acts as a microbial (Tilley and Terry, 1963), it has proven useful in assessing the relative feed digestion (Minson, 1990). The techniques involved, such as buffers, solvents, chemicals, and enzymes need to be prepared in the laboratory. Rumen fluid is extracted from the rumen of ruminants while another approach is the use of gas production as an indirect measure *in vitro* digestion techniques.

This technique is also considered among the most inexpensive techniques recommended. The results obtained from the experimental technique can also be adopted. OMD forecast compound feed for ruminants by using enzymes have been updated mainly for forage. Enzymatic methods in this technique has been studied with energy and protein feeds (Aufrere and Michalet-Doreau, 1988). Although performed on a small number of samples, the results of *in vitro* studies using the technique will result in a clear reference and indicates that the digestion of organic material (OMD) matter in complete diet for ruminants can be successfully determined.

A by-product of the technique is *in vitro* gas production. Production of this gas can be used to measure the volume of gas produced by the fermentation of foodstuffs (Schofield, 2000; Krisnamoorthy *et al*, 2005). It helps researchers to look at the rate of digestion of food items tested (Raab *et al*., 1983). The resulting gas is reported to be obtained from the fermentation of carbohydrates that are digested by rumen microbial activity (Khan and Chaudhry, 2010). However, gas production is not always available for analysis in the laboratory. Further studies, with the inclusion of more samples of incomplete diet, is needed to confirm these findings.

2.7.1.1 Advantage of the *In Vitro* Technique to Estimate Feed Digestibility

The technique for determining *in vitro* digestion of forage developed by Tilley and Terry (1963) has been widely used because of its convenience and high degree of correlation to *in vivo* digestibility and offers advantage over the *in sacco* technique. It is also found to be an easy and fast experiment (Khan and Chaundhry 2010).

Mehrez *et al*. (1977) studied the rate of rumen fermentation and found that the loss of dry matter from the bag was positive with increasing levels of rumen ammonia nitrogen 10-24 mg. Many research reports have been published on the degradation of protein from food for ruminants (Stern *et al.*, 1983; Gralak *et al*., 1997; Wanapat *et al*., 2000; Islam *et al*., 2002) and intestinal digestion (Maiga *et al.*, 1996). Ørskov *et al*. (1980) observed that the nylon bag technique was not only a powerful tool for indexing the relative degradability feedstuffs, but it may be used to study the rumen processes, as it is possible to vary the factors in the bag, or within the rumen (Khan and Chaundhry 2010).

However, there is limited information available on the degradation of the dry matter (DM) and crude protein (CP) in the rumen. To study the nutritive potential of pastures, a simple method for *in vitro* estimation of feed digestibility was introduced (Mabjeesh *et al.*, 2000) as an alternative to the traditional method of Tilley and Terry (1963).

2.7.1.2 Disadvantages of the *In Vitro* Technique to Estimate Feed Digestibility

Spencer *et al.* (1988) reported that the analysis of digesta of nylon bags incubated in *in vitro* digestibility showed that certain nutrients have been escaped from the nylon bags before being digested. The microbial attachment to feed incubated *in vitro* is frequently not measured and several studies have shown that the high level of food contamination incubated with rumen microbes (Huntington and Givens, 1995).

All these source of forecast errors increase diversity among laboratory degradability. Although widely used and standardised, the use of these methods need to address two points: first, a breakdown fully considered, and the loss of dry matter (DM) and nitrogen (N). During this step the value obtained can only be a matter of DM or N washed out of the bag; The second form is microbial contamination of feed within the bags. It will estimate the first point and the second point will degrade under the budget (Madsen and Hvelplund, 1994). Rumen fluid for incubations were still usually obtained from animal rumen-fistulated (Khan and Chaundhry, 2010).

Therefore, alternative methods are needed to estimate rumen destruction of feed which should be available without involving surgically prepared animals. The recent review will also explore the potential and problems that may arise in the development of

alternative *in vitro* methods and their main applications for the food industry and the animal. It requires the use of rumen fluid, obtained from rumen fistulated animals or freshly slaughtered animals (Krishnamoorthy *et al*., 2005).

2.7.1.3 The *In Vitro* Gas Production Method

The relationship between rumen fermentation and gas production, has long been known. There was an initial effort in 1939 where researchers attempted to record gas production directly through the rumen cannula in sheep (Pedraza, 1998). This technique was found to be too difficult to implement at the time (Blümmel *et al*., 1997).

In 1974, Menke and Ehrensvard (Theodorou *et al*., 1994) studied the stoichiometry of rumen fermentation with a syringe closed system where gas production data was obtained when the same substrate was incubated in the same quantity in different runs. In the system established by Menke *et al*. (1979), substrate incubated in gas-tight glass syringe calibrated via a plunger, the gas produced can be recorded within 96 hours, including the incubation media and buffers with rumen liquor. The main innovation in gas production method is recorded from degradation. Postulation then goes on to this day, but the method has been simplified and improved.

Theodorou *et al.* (1994) described the Menke gas production technique that uses a pressure transducer to monitor gas production efficiently. At certain times, gas pressure in the bottles was measured using a pressure transducer. The gas produced is then removed, disposed of and the amount of gas produced is measured. With sequential measurement of gas produced, cumulative gas production curve can be built on time.

Various numbers of equations have been used to interpret the data in terms of the constant rate and the final point. As yet there is little consensus on which is most appropriate. This method has been proposed as a simple method to investigate the rate of fermentation (Theodorou *et al*., 1994). Gas production technique is responsive to supplement nitrogen in a way consistent with earlier laboratory work.

The gas production technique may have the potential to obtain data reading in *in vitro* digestibility. It is also capable of providing data on rates of digestion to compare with nylon bag technique. To date, it can be used in combination with other food assessment techniques to assess the quality of food. Until now, the gas production technique seems useful as a tool for feed position, but it is not clear the extent to which this technique is sensitive to the anti-nutritional factors in feed. It also does not reflect the relative performance of animals in *in vitro* technique with an accurate reading.

2.7.2 Advantages and Limitations of the Gas Production Technique

The major advantage of this technique is the capability to non-destructively monitor fermentation and to measure the degradation of the soluble material (Decruyenaere, 2009). In the gas production technique each incubation is monitored non-destructively at regular intervals, so that the full fermentation process can be measured. This method is suitable for obtaining degradation rates data. The technique is potentially far cheaper to implement than the other techniques. *In vitro* gas production technique is suitable for the study of the degradation of the solute; and it is important to consider supplements (Dual Yulistiani *et al*., 2007). It is used for estimating microbial protein production and for investigating interactions between feeds.

One disadvantage of gas production technique is that it generates a data unit with the volume of gas. This technique has also been used to assess the presence of antinutritives compounds in different foodstuffs (Pedraza, 1998). Menke *et al*. (1979) have developed equations to equalise gas production of organic matter digested, but similar equations are no longer developed in Theodorou methods. However, various studies have been developed in science, for purposes of application of assessment and laboratory techniques tropical food. Despite all these, gas production technique is an alternative that still prevails.

2.7.3 *In Sacco* Technique to Estimate Feed Digestibility

Information on the rumen degradation of different feeds before their use is essential to formulate nutritious diets for ruminant animals. The *in sacco* method has been found useful for decades. The technique was first suggested by Quin *et al.* (Broderick and Merchen, 1992). This technique requires the use of fistulated animals and is widely applied by researchers (Khan and Chaundhry, 2010) with actual simulation in ruminant digestibility. It is also cheaper as compared with the *in vivo* technique. Therefore, this technique emerges as the best method to test the treatment for ruminants compared to the *in vivo* technique to reduce cost in this research.

The *in sacco* technique involves the sealing of feed samples in nylon bag, which is then suspended in the rumen of the ruminant for varying periods of time followed by determination of dry matter (DM) in the washed residues. The technique allows the test feed to be incubated in the ruminal environment in a limited situation because the feed is not subjected to mastication and rumination.

2.7.3.1 Advantages of the *In Sacco* Technique to Estimate Feed Digestibility

The *in sacco* technique was used to estimate utilisation of either forage (Van Keuren and Heinemann, 1962) or concentrates and high-protein feeds (Mehrez and Ørskov, 1977). It involves the measurement (Khan and Chaundhry, 2010) with actual simulation in ruminant digestibility.

Interest in techniques that have arisen since Mehrez and Ørskov (1977) critically assessed the factors that led to change in the degradability of dry matter and nitrogen. They concluded that as long as the bag is large enough to allow free movement of the substrate in this technique, it can be useful as a quick guide for determining the loss of nutrients, especially the rate and extent of rumen nutrient loss. All modern systems of ruminant feed (Agriculture and Food Research Council, 1993: National Research Council, 1996) require an estimation of the amount of protein food. This estimate is obtained by the *in sacco* technique, which is probably the best-known, simple and reliable method to assess the degradability of dry matter and protein in the rumen (Thomas, 2004).

2.7.3.2 Disadvantages of the *In Sacco* Technique to Estimate Feed Digestibility

Due to the *in sacco* method requiring surgically modified animals, its routine use to study degradation of ruminant feed has undesirable implications for animal welfare and management (Khan and Chaundhry, 2010). Synthetic fibre bag containing the test feed has to be incubated in the rumen for a period of time to evaluate the degradation of foodstuffs for DM and N. It will cause the flow rate of particles out. Between reproducibility of the method laboratories is low due to changes in proteolytic

activity between animals due to variable diet and physiological status of the animal (Khan and Chaudhry, 2011).

The results obtained for this method does not apply to all situations except in standardised methods for the same protocol. In addition, this method requires surgery, but it involves only a limited sample size and only apply to healthy animal.

2.7.3.3 Nylon Bag (*In Sacco*) Method

Basically, the *in sacco* technique for nylon bag method has the same characteristics as that used by the laboratory in *in vitro* methods. It involves the incubation of samples in a small size, covered nylon bag in fistulated animal rumens and material will be lost from the bag during the incubation period.

The method can be used to give end-point degradability data, or by incubating bags for different lengths of time, giving data on the rate of feed degradation (Decruyenaere, 2009). It can be used to measure the rumen degradation of dry matter or organic matter and, by weighing and analysing the residues, different components of the feed (Ørskov and McDonald, 1979). This method can be used to estimate the protein degradability of feeds in the rumen. It has become widely used for such measurements which are required by the metabolism of protein feed evaluation system.

The nylon bag technique has been shown to be a potentially useful predictor of animal growth, intake and digestibility in ruminants. The technique is found to be versatile and applicable to a wide range of feeds (Ørskov *et al*., 1980). Prediction equations, including the composition and parameters of nylon bags, have been reported in various studies. Larger pools of data are probably required to facilitate the development of robust prediction equations (Osuji *et al.*, 1993). This type of method does not require

sophisticated laboratory facilities, higher electricity and its uses have been conducted in many of the less developed countries (Preston, 1995).

2.7.4 *In Vivo* Technique to Estimate Feed Digestibility

The experimental design required a period of animal adaptation to the experimental diet and to the new maintenance conditions from the digestibility cages, followed by an experimental period when nutrient balances were performed and feed intake and excreta were recorded with this technique, the ruminant will be given the supplements pellet and supplements added with herbs. Every 2 weeks, the body weight is measured and recorded to estimate the growth curve in ruminant. The diet will be given to ruminant after weaning age (four months) until the goat is nine months old. Then estimation of the efficiency added herbs in ruminant feed is made.

2.7.4.1 Advantages of the *In Vivo* Technique to Estimate Feed Digestibility

Grass is the most economical herbivore feed for ruminant animals. Therefore, *in vivo* digestibility and voluntary intake should be estimated simultaneously in order to optimise grazing ruminant production (Decruyenaere, 2009). *In vivo* technique represents the actual study in digestibility. The experimental diet is given to ruminant in the farm and the growth curve is recorded along the experiment period. No fistulated animal is needed in this technique (Scales, 1974). Hence, the are no contaminations of incubated feed with rumen microbes in this procedure.

This technique is the best to analyse body physical measurements of ruminants grazing on grass supplemented with commercial pellets and added with herbs. It can also be used to estimate the best herbs to increase digestibility in ruminants and to develop better body weight in ruminants by using a new diet added to herbs by measuring tested ruminant body weight fortnightly after weaning of the kids until the kids turn nine months old (Nik Fatihah & Noraida, 2012).

2.7.4.2 Disadvantages of the *In Vivo* Technique to Estimate Feed Digestibility

Ruminant animals are dependent mainly on high forage. It is essential to maintain their health and production at various stages of their development and growth. Cost is one of the limitations hence considered a disadvantage of *in vivo* technique (Dragan and Klaas-Jan, 2009). Preparing the experimental diet and extracts from plants is costly as the extraction process requires expensive instruments. The experimental diet is required to feed at least four kids, both females and males in each treatment group (Calsamiglia and Stern, 1995).

It is thus hard to get the farmer's collaboration for the purpose of this research. The *in vivo* technique also needs a substantial period of time, at least three months are required to plot the estimate growth curve in ruminants. To get a better growth curve, the kids are tested until nine months old. This period is a burden to the farmers as they need to sell their products and this becomes a limitation in the research.

CHAPTER 3: MATERIALS AND METHODS

3.0 Introduction

This research on the *in vitro, in sacco* and *in vivo* digestibility of herbs formulated animal feeds was conducted using three techniques to test the effectiveness of using local herbal formulation (DD herbs) in the diet of ruminants. The present research evaluated the digestibility of commercial and PKE pellets when added with DD herbs.

The techniques used were:

i) *in vitro* technique;

ii) *in sacco* technique;

iii) *in vivo* technique.

Feed digestion can be estimated in the laboratory through biological methods known as *in vitro* techniques. It carries out a simulation system of the digestive process in animal (Getachew *et. al*., 2004) using the rumen liquor.

The other biological and laboratory method is *in sacco* technique. It is conducted inside the rumen using the nylon bags. In the *in sacco* technique, feeds are placed in nylon bag which are incubated in fistulated ruminants. A rumen fistula is surgically made by transecting the skin and the rumen, suturing the rumen to the skin and allowing the rumen to heal, creating a permanent opening into the rumen. This method helps to gain a better knowledge of the digestive processes and rumen functionality of livestock.

The herbs tested in this technique was mixed with commercial pellets or PKE pellets. The highest digestibility and the best results of herbs in the *in vitro* and *in*

sacco techniques will determine the herbs and dose selected to be used in the *in vivo* technique. These herbs will be finally chosen and tested in *in vivo* technique.

The *in vivo* technique is used to estimate digestibility and is an evaluation of treatment of ruminant animal to test in the field. The technique is currently running in local farms in the palm oil plantation area in Bidor.

3.1 Materials Research

There are several materials needed in this research. The materials used in this research can be categorised to the following :

i) herbs (DD herb-herbs formulated by University of Malaya researcher and imported herb from SeoulFeed, Co., Ltd., Korea);

ii) pellets (commercial pellets bought from Central Feedmills Sdn. Bhd and Palm Kernel Expeller-PKE pellets from FELDA);

iii) Napier grass (*Pennisetum purpureum*);

iv) treatments (for the digestibility tests);

v) equipment (equipment, apparatus used for the research);

vi) chemical, reagent and rumen juice;

vii) lab wares and disposables;

viii) animals (goats).

3.1.1 Herbs Formulated Feeds

Herbs used in this research were local herbs formulated feeds (DD herbs) and imported herbs. Imported formulated herbs were bought from SeoulFeed, Co., Ltd., Korea. The imported powdered herbs were used in this research to evaluate the herbs added with the pellets as comparison to DD herbs which formulated by University of Malaya researcher.

The DD herbs were formulated by the researcher team and prepared in the Animal Biotechnology Laboratory in the Institute of Graduate Study, University of Malaya. Some local herbs were selected and purchased from the local markets.

They were :

i) Garlic;

ii) Ginger (old ginger);

Other powdered herbs purchased from the local supplier are :

iii) Cinnamon;

iv) Mas Cotek.

The raw herbs were dried in the oven at 70ºC for 72 hours and grinded into powdered form using kitchen blender. All these herbs were formulated, mixed together, added with 50 gm of salt, stored in a bottle and kept in a refrigerator.

Plate 3.1 Pellet and Herbs Measured in Laboratory

3.1.2 Pellets

Two types of pellets were used for this research. Commercial pellets were purchased from a local supplier (Central Feedmills Sdn. Bhd) and PKE pellets were provided by The Federal Land Development Authority (FELDA). All pellets were grinded using a kitchen blender into a powdered form at the Animal Biotechnology Laboratory in IGS, University of Malaya.

3.1.3 Napier Grass (*Pennisetum purpureum*)

Napier grass (*Pennisetum purpureum*) was collected from the Institute of Biological Sciences Farm. It was cut and shredded into small pieces.

The Napier grass was then oven dried at 70ºC for 48 hours followed by grinding using a kitchen blender until it was in powdered form. These powdered Napier grass was stored in a bottle in a refrigerator for further use.

3.1.4 Treatments

Twelve treatments were used in *in vitro* technique. In *in sacco* technique, eleven treatments were used the same way as in *in vitro* technique except there was no control treatment in *in sacco* technique. The *in vivo* technique used DD herbs and commercial pellet as treatment. The experimental treatments were :

i) commercial pellets;

ii) commercial pellets added with imported herbs;

iii) commercial pellets added with DD herbs;

iv) commercial pellets added with DD herbs and powdered Napier grass (*Pennisetum purpureum*);

v) commercial pellets added with imported herbs and powdered Napier grass (*Pennisetum purpureum*);

vi) PKE pellets;

vii) PKE pellets added with imported herbs;

viii) PKE pellets added with DD herbs;

ix) PKE pellets added with imported herbs and powdered Napier grass (*Pennisetum purpureum*);

x) PKE pellets added with DD herbs and powdered Napier grass (*Pennisetum purpureum*);

xi) powdered Napier grass (*Pennisetum purpureum*);

xii) Control treatment (none was added).

3.1.5 Equipments for the Digestibility Tests

The most important equipment which used for the *in vitro* digestibility method :

i) Water Bath: used to incubate the treatments at 39ºC in the *in vitro* technique;

ii) Water Bath: used to incubate the treatments at 39ºC in the *in vitro* technique;

iii) Balance Weighing:

a. Digital Analytical Balance : 0.40gm of treatment was measured using weighing scale before placing into syringes in *in vitro* technique;

b. Digital Analytical Balance : 2.00gm treatment was measured using weighing scale before placing into nylon bags in *in sacco* technique;

c. Spring Balance : weighing the sample (goat) body weight in *in vivo* technique;

iv) Drying Oven : used to dry the treatments and nylon bags after

v) Drying Oven : used to dry the treatments and nylon bags after incubation in the *in vitro* and the *in sacco* technique;

vi) Carbon dioxide tank : supplied carbon dioxide for gas production experiment in the *in vitro* technique;

vii) Reagent Bottle : 1000 ml - used for preparing buffer solution in *in vitro* technique;

viii) Pipette: used to measure chemical solution for preparing buffer solution;

ix) Kitchen Blender : used for grinding herbs and pellets for preparing treatments in the *in vitro*, the *in sacco* and the *in vivo* technique;

x) Thermal Flask : to keep the rumen juice fresh after the animal is slaughtered and before starting the experiment for the *in vitro* technique;

xi) Nylon bags :

a. used in the *in vitro* technique for transferred incubated treatment from syringe to nylon bag for dried purposes;

b. used in the *in sacco* technique for incubation in goat's rumen.

xii) Nylon string : to tide the nylon bags before inserted to goat rumen for incubation purposes in *in sacco* technique.

xiii) Cannula (Model #8C, Bar-Diamond, Inc.): for easy access of the nylon bag into the rumen for incubation purposes in *in sacco* technique.

xiv) Spatula : used to insert the measured treatments into the nylon bags.

Plate 3.2 Drying Oven

Plate 3.3 Carbon diOxide Tank

Plate 3.4 Reagent Bottle

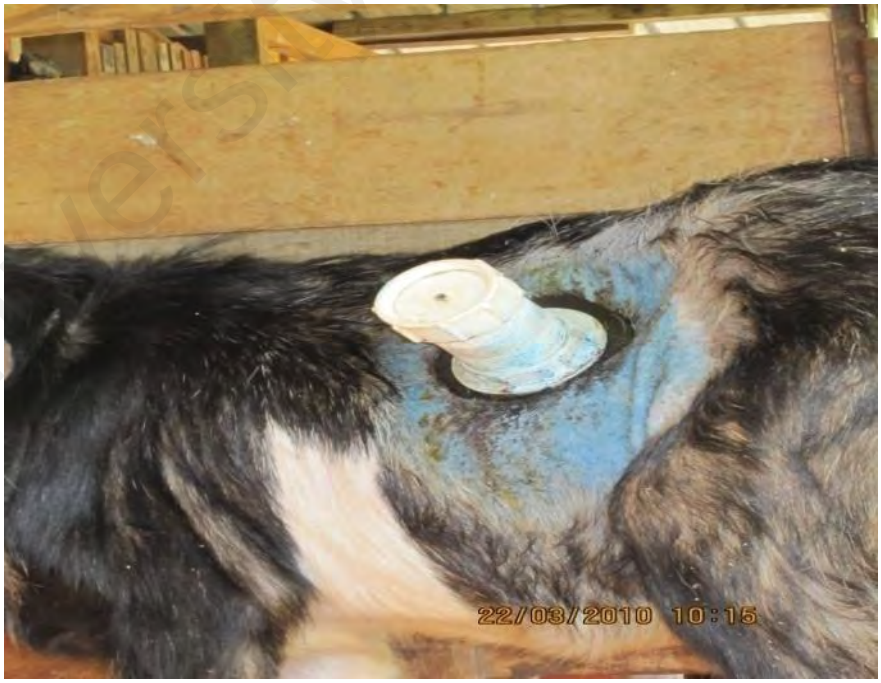


Plate 3.5 Cannula-Fistulae at Goat's Rumen

3.1.6 Chemicals, Reagents and Rumen Liquor

Several chemicals and reagents were used in the *in vitro* technique include :

i) Gas Test Medium :

a. HFT (Hohenheimer Futterwert Test) buffer;

b. Macro minerals;

c. Micro mineral;

d. 0.4% Resazurine;

e. 6M NaOH;

f. dH_2O;

g. Sodium sulphide;

h. Rumen fluid;

ii) HFT (Hohenheimer Futterwert Test) buffer :

a. Ammonium bicarbonate;

b. Natrium bicarbonate;

c. dH_2O;

iii) Macro minerals:

a. diNatrium Hydrogen Phosphate;

b. Potasssium diHydrogen Phosphate;

c. Magnesium Sulphate 7x H_2O;

iv) Micro minerals:

a. Calcium Chloride $2xH_2O$;

b. Mangan Chloride $4xH_2O$;

c. Cobalt Chloride $6xH_2O$;

d. Ferric triChloride $6xH_2O$;

v) 0.4% Resazurine;

vi) 6M NaOH :

a. NaOH;

b. dH_2O;

vii) Rumen liquor was collected from freshly slaughtered goat at a local abattoir (Shah Alam, Selangor). About 15 minutes post-slaughter, the rumen of each animal was cut open with a kitchen knife to collect the contents which were immediately strained into pre-warmed thermal flasks and transported to the Animal Biotechnology Laboratory, University of Malaya.

3.1.7 Lab wares and Disposables

In vitro technique was conducted at the University of Malaya's Animal Biotechnology Laboratory and this technique required some lab wares and disposables such as :

i) 40 syringes have a volume of 100 ml;

ii) 40 nylon bags (measurement of these bags are about 10 x 15 cm with 50 µm pore size);

iii) 40 plastic clips;

iv) 40 rubber tubes.

Plate 3.6 Syringes

3.1.8 Animal – Goats

i) Six local goats with an average 12 months of age and an average live weight of 22kg were used for the *in sacco* digestibility tests;

ii) Two groups of kids (average of four months old) were selected for the *in vivo* technique. The first group was a control group fed commercial feed, while the other group was fed with commercial pellet added with DD herbs. Each group comprised of five males and five females.

Plate 3.7 Kids for *in vivo* Technique

3.2 Methodology

3.2.1 *In Vitro* Technique:

(i) Preparation of Syringes: Weighing Treatments

All mixed pellets with herbs including Napier and control treatment were prepared for twelve treatments. The experimental diets were formulated into 3 groups of doses which contained 2.5% of herbs (DD or imported) added to 97.5% of commercial pellets, 5.0% of herbs (DD or imported) added to 95.0% of commercial pellets and 7.5% of herbs (DD or imported) added of 92.5% of commercial pellets. Each group of mixed herbs was tested separately employing the same experimental procedures.

The syringes was filled with 0.40gm of treatment and weighed using balancing as a measuring tool. There were 40 syringes filled with treatments and a gas test medium solution prepared in the laboratory before initiating the experiment. Each experiment had 40 syringes with at least 5 replicates of each.

Plate 3.8 Weighing the Treatment Process Using Balancing and Spatula

Plate 3.9 Preparing the Syringe

(ii) Preparation of Stocks and Media

(a) Preparation of Rumen Liquor and Gas Test Medium Solution

The rumen liquor was collected in thermos flasks and taken immediately to the laboratory and kept at 39°C under a CO_2 atmosphere until use within approximately 20 min. The rumen liquor content was then filtered to obtain only the rumen juice used in the gas test medium solution.

Gas Test Medium solution; this solution was prepared using the IAEA training procedure (2002) such as:

Table 3.1 The Amount Needed to Prepare Gas Test Medium Solution

Components	MW	final concentration	Amount unit
HFT buffer		16%	333ml
Macro minerals		16%	333ml
Micro minerals		0.016%	0.333ml
0.4% Reszurine		0.008%	0.417ml
dH_2O			732ml
Sodium sulphide		0.1984mg/ml	417mg
6N NaOH		0.0013N	0.444ml
Rumen fluid			700ml
total volume	of syringe	60	2100ml
	Volume/syringe	30 ml	

Sodium sulphide and NaOH are added just before collection of the rumen

(b) Preparation Other Solutions

Before conducting the experiment, the gas test medium was prepared, the reagent as follows :

i) Macro nutrient; this solution was prepared using the IAEA training procedure (2002) as shown below:

Table 3.2 The Amount Needed to Prepare Macro Nutrient Solution

Components	MW	Final concentration	Amount unit
diNatrium Hydrogen Phosphate	142.0	0.040M	11.4g
Postassium diHydrogen Phosphate	136.1	0.046M	12.4g
Magnesium Sulphate 7x H_2O	246.5	0.002	1.2g
Total volume	**Make up to with dH_2O**		**2000ml**

ii) Micronutrient; this solution was prepared using the IAEA training procedure (2002) as shown below :

Table 3.3 The Amount Needed to Prepare Micro Nutrient Solution

Components	MW	Final concentration	Amount unit
Calcium Chloride 2xH_2O	147.0	0.45M	3300mg
Manganese Chloride 4xH_2O	197.9	0.25M	2500mg
Cobalt Chloride 6xH_2O	237.9	0.02M	250mg
Ferric triChloride 6xH_2O	270.3	0.15M	2000mg
Total volume	**Make up to with dH_2O**		**50ml**

iii) Buffer Solution; was prepared using the IAEA procedure (2002) as shown below:

Table 3.4 The Amount Needed to Prepare Buffer Solution

Components	MW	Final concentration	Amount unit
Ammonium bicarbonate	79.1	0.05M	8.0g
Natrium bicarbonate	84.0	0.42M	70.0g
dH_2O			2000ml
Total volume			**2000ml**

iv) NaOH; this solution was prepared using the IAEA training procedure (2002) shown below :

Table 3.5 The Amount Needed to Prepare NaOH Solution

Components	MW	Final concentration	Amount unit
NaOH	40.0	6.0N	7.2g
Make up to with dH_2O			30ml

v) Resazurine; this solution was prepared using the IAEA training procedure (2002) shown below:

Table 3.6 The Amount Needed to Prepare Resazurine Solution

Components	MW	Final concentration	Amount unit
Resazurine		0.4%	200mg
Total volume			50ml

(iii) Water Bath Incubation

All stocks and media were mixed together following IAEA laboratory procedure into a reagent bottle. The laboratory handling of rumen juice was carried out under a continuous flow of CO_2. The syringes containing with treatments filled with 40ml of medium consisting of gas medium test solution mixed with rumen juice as described by Menke and Steingass (1988) were accurately prepared using the pipette.

The syringes filled with treatments and gas medium test solution were incubated in water bath at temperature of 39ºC. The gas production was recorded every 2, 4, 6, 12, 24, 48, 72 and 96 hours.

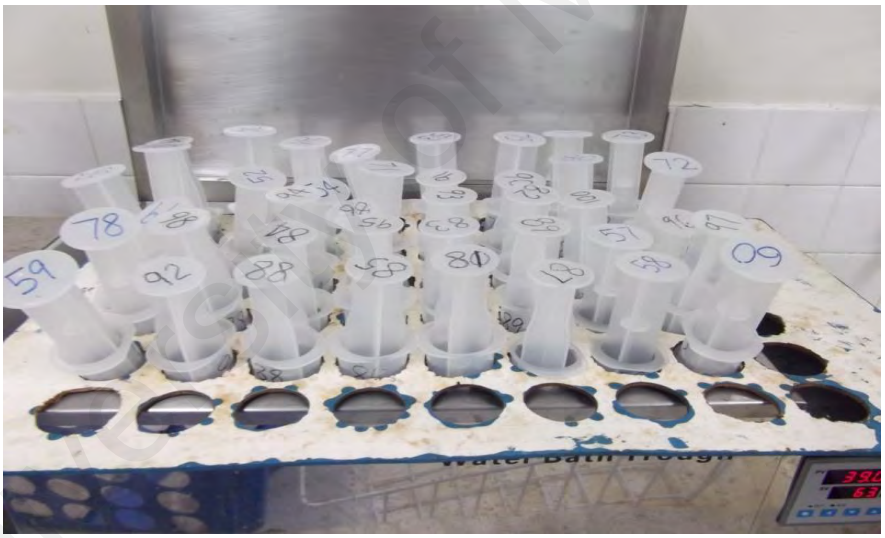


Plate 3.10 Syringes Incubated in Water Bath

(iv) Time Interval Specification

The 0 hour time represented the moment when the mixture of starter gas medium test solution with treatment was initiated which was placed in the water bath at 39ºC of temperature.

(v) *In Vitro* Nylon Bag Technique

At the end of incubation, after a 24 hour interval, treatments were taken out from the syringes and transferred into the nylon bags. Nylon bags, about 10 x 15cm in size with 50 µm pore size was weighed using the balance before used.

Plate 3.11 Weighing the Nylon Bag Using a Balance in the *In Vitro* Technique

Nylon bag containing incubated treatment was washed and rinsed at least four times in cold water until clear. They were then squeezed gently by fingers and dried at 70 °C for 48 hours, cooled at room temperature and weighed again. Same process was employed for 48 hours, 72 hours and 96 hours in incubated period. The digestibility percentages for each treatment were calculated by measuring the differences between pre-incubation and post-incubation dry matter contents.

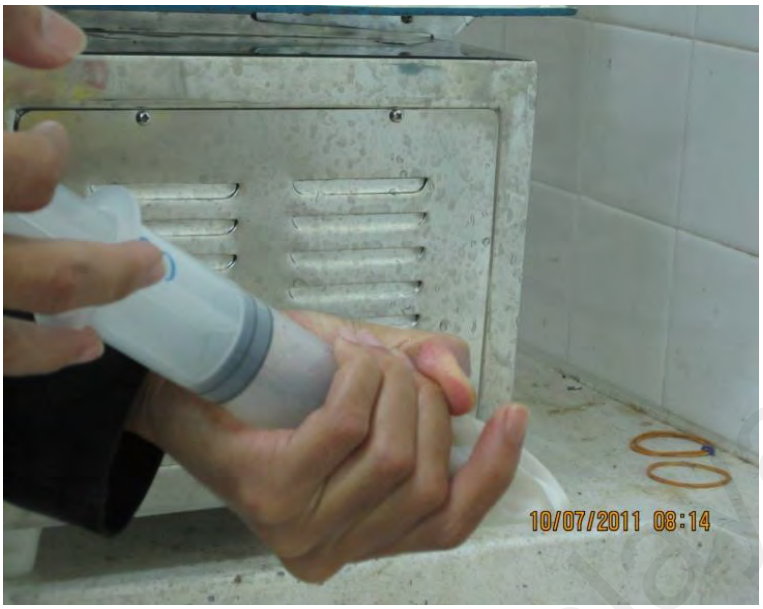


Plate 3.12 Transferring Treatments into the Nylon Bag

3.2.2 *In Sacco* Technique :

(i) Preparing Fistulae Goat

Starting from March 2010 (several months prior to the start of this search), first sample: 11 months old Jamnapari with live weight: 22 kilograms were surgically fitted with 3-inch ruminal cannula (Model #8C, Bar-Diamond, Inc.). Rumen fistulation procedure followed as described and modified by Hecker (1969).

After the surgery, the goats were housed in a 6m × 12m pen until the beginning of the trial at the Institute of Biological Sciences Mini Farm, University of Malaya. All the animals received 2 to 3 kilogram Napier grass with 250 gram commercial pellets, fed twice per day during this experiment.

The nylon bag technique (Ørskov *et al*., 1980) was used to determine Dry Matter (DM) degradability in the rumen. There were 11 treatments with at least 5 replications. Every experiment used 5 nylon bags containing the treatments that were inserted into the rumen.

(ii) Preparation of Nylon Bags : Weighing Treatments

All mixed pellet with herbs including Napier grass were prepared as same as in the *in vitro* technique treatments in 3 doses which contained 2.5% of herbs, 5.0% of herbs and 7.5% of herbs. There were eleven treatments for each dose similar to the *in vitro* technique except there is not any control treatment in the *in sacco* technique. Each group of mixed herbs were tested separately but following the same experimental procedures.

The nylon bags were about 10 x 15 cm in size with 50 μm pore of size and were filled with 2.00gm of each treatment and weight using balancing scale. Five nylon bags were filled with treatments and tied with nylon strings about 30 cm long, which were connected to the plug of the rumen cannula. The string was then attached to a wire loop inserted through the cannula top and the bags are pushed gently into the rumen.

Plate 3.13 Nylon Bags Filled with Treatments

Plate 3.14 Nylon Bags were Sealed with Nylon String

(iii) Incubation Period

The nylon bags were introduced into the rumen just before the morning feeding and incubated for 24 hours. At the end of the incubation, the bags were removed from the rumen, the string was then cut in order to clear debris trapped by the folded material, and the bag and contents were immediately washed and rinsed at least four times under running cold water until the wash water is clear. They were then squeezed gently by hand and dried at 70 °C for 48 hours.

Plate 3.15 Nylon Bags Inserted into the Ventral Part of the Goat's Rumen

The dried bags were weighed to determine the amount of residual dry matter. All the data were recorded to compute the digestibility of dry matter. Same procedure was repeated for 48 hours, 72 hours and 96 hours incubated samples.

Plate 3.16 After Taking Out from Rumen, the Nylon Bags were Washed

Plate 3.17 Nylon Bag Dried in Oven After Washed

The dry matter digestibility for each treatment diet was calculated by determining the difference between pre-incubation and post-incubation of dry matter.

3.2.3 *In Vivo* Technique:

(i) Preparation for Treatments in *In Vivo* Technique

Treatments consisted of varying ratios of commercial pellets to pellets added with local herbs of concentrate 50gm (DD herbs) : 950gm commercial pellet. Then each sample was fed with 500gm mixed pellet and herbs on a daily basis. All supplementary diets were formulated with 70% concentrate (cereal, soybean meal, and vitamin and mineral premix) and grass as main feed. For control group, animal were fed with commercial pellet of 500gm per day for each sample.

(ii) Experimental Procedure in *In Vivo* Technique

The first group (control group) was fed with commercial feed as their primary diet. The second group was fed with commercial pellet added with DD herbs. Each group consisted of ten kid (five males and five females, respectively).

Feeding was done very morning all. All these kids were allowed to graze in the plantation in the afternoon. This research on the growth performance for the kids were conducted when the kids were at the age of 4 months until 9 months.

Body Weight (BW) of the animals for the *in vivo* technique was measured every 2 weeks at 9.00 am to record their growth rates. The comparison was made between the control group and the group fed with DD herbs and pellets.

Plate 3.18 Measuring Weight Every Two Weeks

3.3 Statistical Analysis

Statistical analysis was conducted using SAS 9.2. All data obtained in the *in vitro* and *in sacco* techniques were presented as mean ± standard deviation (mean ± SD). *In vitro* gas production data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between the means (n=5) was determined by Duncan's Multiple Range Test at 95% least significance difference ($p<0.05$).

In the *in vivo* technique, all data were presented as means±standard deviation. Data recorded for *in vivo* technique were be analysed using SAS 9.2 to evaluate performance of the kids. Significant difference among treatments was determined where the *p* value was less than 5% ($p<0.05$). All data were analysed using the MIXED procedures of the statistical analysis system (SAS, 2009).

3.3.1 Treatments Analysis

All treatments were analysed for the chemical composition in the treatments used in this study at the Agricultural Chemical Analysis Laboratory, Malaysian Agricultural Research and Development Institute (MARDI). The analysis comprised energy, fibre, major elements and proximate content in all treatments.

3.3.2 Digestibility and Growth Calculation

i) Digestion Calculation

In *in vitro* and *in sacco* techniques, the digestibility percentages for each treatment diet were calculated by computing the differences between pre-incubation and post-incubation of dry matter (Givens *et al*., 2000).

The calculation of digestibility from Givens *et al*. (2000) was as follows :

$$\% \text{ Digestibility } = \left[1 - \left(\frac{(Wb1+Wb2\ldots+Wb5)-(Wa1+Wa2\ldots Wa5)}{(Wb1+Wb2\ldots+Wb5)}\right)\right] \times 100\ \%$$

Where:

Wb1, Wb2....Wb5 = before incubation, nylon bag and treatment weight;

Wa1, Wa2....Wa5 = after incubation, nylon bag and treatment weight;

% = percentage

ii) Growth Rate Calculation

To compare the growth rate in the *in vivo* technique, the average body weight in the group was calculated (Hamayun *et al*., 2006). From this information a customized growth chart was generated to track the growth.

The growth rate calculation of digestibility from Hamayun *et al*. (2006) was as follows :

$$\text{Average Body Weight (kg)} = \frac{BW1+BW2+BW3+BW4+BW5}{5}$$

Where:

BW1 .....BW5 = Body weight animal 1 until Body weight animal 5;

kg = kilogram

CHAPTER 4 : RESULTS

4.1 The Chemical Composition for Experimental Feeds

Local formulated herbs (DD herbs) and imported herbs were selected and tested in the *in vitro* and *in sacco* techniques while the best herbs with higher digestibility was chosen to be tested in the *in vivo* technique. The *in vitro* used 11 treatments and 1 control, whereas the *in sacco* techniques used 11 treatments. All the treatments used either *in vitro* or *in sacco* techniques included commercial pellets and PKE pellets as supplementary feed.

All the treatments used in this study and the herbs selected (DD herbs and imported herbs) were sent to MARDI laboratory for proximate analysis purposes to determine their nutrient contents before they were used in the *in vitro*, *in sacco* and *in vivo* techniques. DD herbs is formulated herbs from local herbs: Mas Cotek *(Ficus deltoidea),* cinnamon, garlic *(Allium sativum)*, and ginger *(Zingiber officinale)*. The results from this study are tabulated and reported in the following tables and figures.

Table 4.1 The Chemical Composition Herbs Component for All Treatments

Components	Gross Energy (Cal/gm)	Crude Protein (%)	Crude Fibre (%)	Dry Matter (%)	Ether Extract (%)	Neutral Detergent Fibre (%)
DD Herbs	4005.33	9.64	18.73	91.79	1.05	45.76
Cinnamon	4155.16	3.50	16.98	94.06	0.62	51.27
Mas Cotek (*Ficus deltoidea*)	4168.44	6.51	35.31	91.82	3.29	55.42
Ginger (*Zingiber officinale*)	3881.68	8.80	23.55	92.55	4.71	99.29
Garlic (*Allium sativum*)	3815.89	15.76	3.03	98.22	0.54	7.03
Imported Herbs	4116.01	15.19	26.68	88.68	5.49	45.17
Commercial Pellets	4129.59	16.12	9.93	90.89	4.87	41.98
PKE Pellets	3902.43	13.62	23.00	90.93	0.48	73.46
Napier Grass (*Pennisetum purpureum*)	3898.02	11.97	29.47	92.65	2.52	71.38

Table 4.2 The Mineral Contents of Herbs Used as Treatments in *In Vitro*, *In Sacco* and *In Vivo* Digestibility

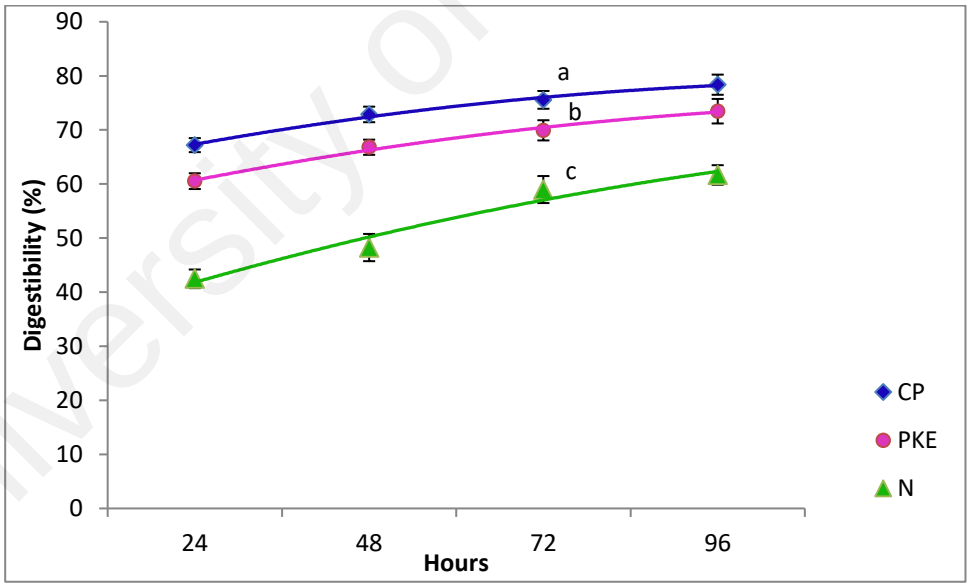
Components	Ca (%)	Cu (ppm)	Fe (ppm)	K (%)	Mg (%)	Mn (%)	Na (%)	P (%)	S (%)	Zn (ppm)
DD Herbs	0.96	4.95	281.53	1.72	0.33	0.06	0.49	0.21	0.29	-
Cinnamon	1.26	2.96	42.35	0.38	0.05	0.03	0.01	0.06	0.12	-
Mas Cotek	1.98	3.95	411.09	0.77	0.53	0.06	0.02	0.14	0.30	-
Ginger	0.12	4.77	105.37	0.88	0.20	0.02	0.06	0.18	0.61	-
Garlic	0.06	4.07	14.66	1.15	0.09	-	0.05	0.43	0.90	-
Imported Herbs	0.10	9.76	157.34	0.79	0.34	0.01	0.01	0.74	0.22	26.37
Commercial Pellets	1.46	12.86	640.70	0.88	0.31	0.02	0.20	0.71	0.26	103.96
PKE Pellets	0.56	21.27	1503.26	0.62	0.24	0.02	0.67	0.52	0.25	32.52
Napier Grass (*Pennisetum purpureum*)	0.75	8.01	1959.83	1.99	0.13	-	0.06	0.28	0.17	11.42

4.2 *In Vitro* Digestibility Technique

The results from this technique are reported in the following tables and figures.

4.2.1 *In Vitro* Digestibility of the Commercial Pellets, PKE Pellets and Napier Grass (*Pennisetum purpureum*)

The *in vitro* digestibility of commercial pellets, PKE pellets and Napier grass were recorded from 24 hours to 96 hours of incubation. The results are as follow :



Note : (a) CP = Commercial Pellets
(b) PKE = PKE Pellets
(c) N = Napier Grass (*Pennisetum purpureum*)

Figure 4.1 *In vitro* Digestion Rates for Commercial Pellet, PKE Pellet and Napier Grass within 24 Hours to 96 Hours in Goat Rumen. Different alphabet shows significantly different (p<0.05).

The highest digestibility during incubation from the 24 to 96 hour was commercial pellets and showing the digestion percentage as 67.19% (24 hours), 72.87% (48 hours), 75.56% (72 hours) and 78.37% (96 hours). PKE pellets result showed significant in digestibility when compare to Napier grass from 24 to 96 hours of incubation.

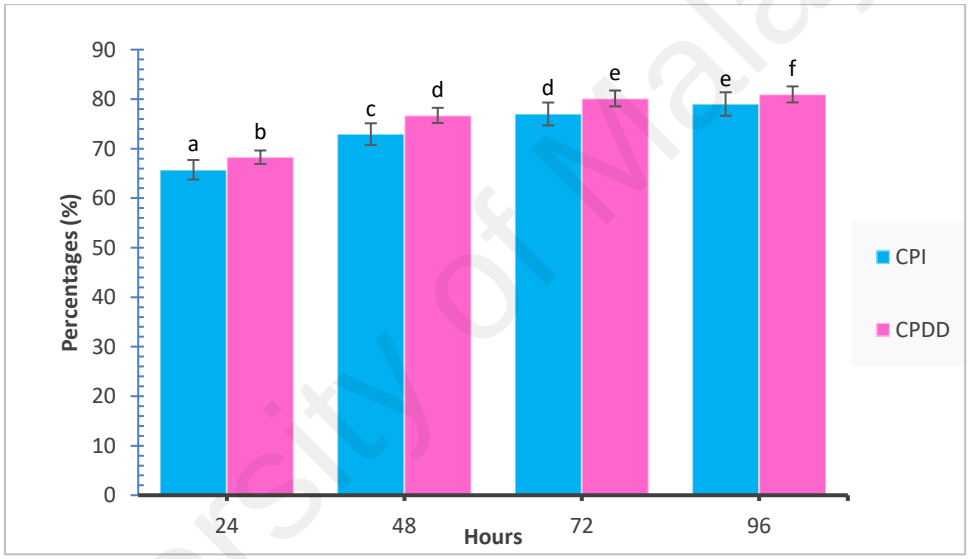
4.2.2 *In Vitro* Digestibility of Commercial Pellets Added 2.5% Herbs

2.5% of herbal mixture was added with 97.5% of commercial pellets, incubated in the syringes and water bath. The percentages of digestibility were recorded from 24 hours to 96 hours. Two types of herbs which were tested :

1) DD herbs, and;

2) imported herbs.

The results as in the figure below:



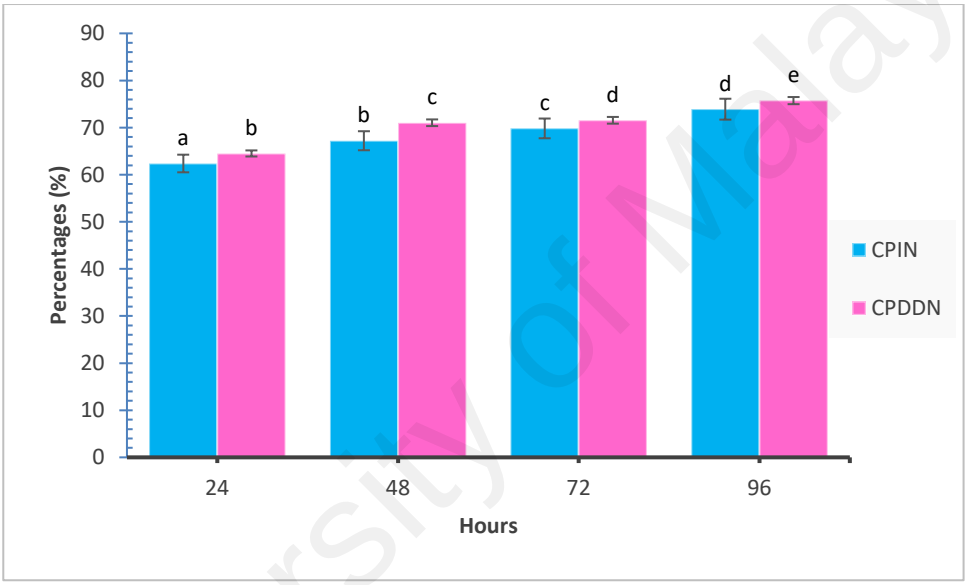
Note : Values with the different alphabets are considered significant (p<0.05)
CPDD = Commercial Pellets Added with DD Herbs
CPI = Commercial Pellet Added with Imported Herbs

Figure 4.2 *In Vitro* Digestibility of Commercial Pellets Added 2.5 % Herbs from 24 to 96 Hours of Incubation

The results showed that digestibility of commercial pellets added 2.5% DD herbs was significant compared to commercial pellets added 2.5% imported herbs in 24 to 96 hours of incubation.

4.2.3 *In Vitro* Digestibility of Commercial Pellets Added 2.5% Herbs and Napier Grass (*Pennisetum purpureum*)

Napier grass is a balanced diet for ruminants and was added in the treatments to study the digestibility in ruminant animals. The results of incubation in the water bath for 24 hours to 96 hours were recorded. The figure illustrates the percentages of *in vitro* digestibility from 24 to 96 hours.



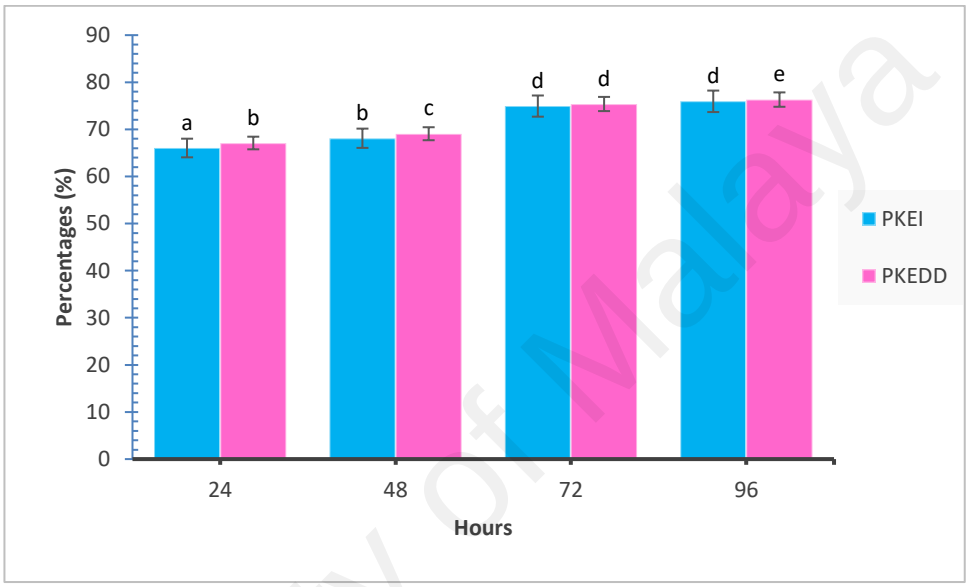
Note : Values with the different alphabets are considered significant (p<0.05)
CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass
CPIN = Commercial Pellet Added with Imported Herbs and Napier Grass

Figure 4.3 *In Vitro* Digestibility of Commercial Pellets Added 2.5 % Herbs and Napier Grass from 24 to 96 Hours of Incubation

The results showed that commercial pellets added 2.5% DD herbs was significant compared to commercial pellets added 2.5% imported herbs at 24 and 96 hours of incubation.

4.2.4 *In Vitro* Digestibility of PKE Pellets Added 2.5% Herbs

2.5% of herbs (0.20 gm) were added with PKE pellets (0.20 gm). The results were recorded after incubation for 24 hours to 96 hours. The percentages of digestibility were calculated and illustrated as figure below :



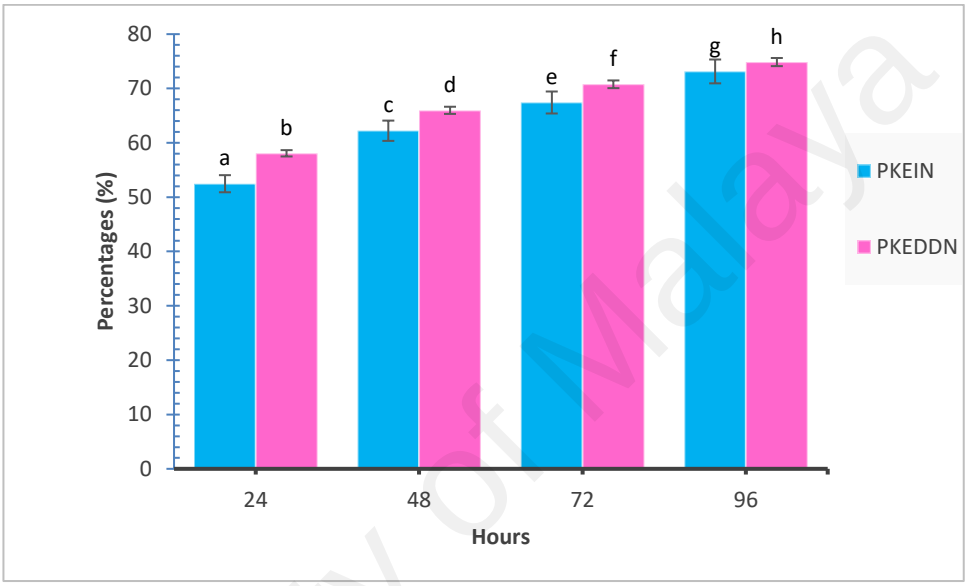
Note : Values with the different alphabets are considered significant (p<0.05)
PKEDD = Commercial Pellets Added with DD Herbs
PKEI = Commercial Pellet Added with Imported Herbs

Figure 4.4 *In Vitro* Digestibility of PKE Pellets Added 2.5 % Herbs from 24 to 96 Hours of Incubation

The results showed that digestibility of PKE pellets added 2.5% of DD herbs was significant compared to PKE pellets added with 2.5% of imported herbs at 24 and 96 hours. The digestion percentage of PKE pellets added with 2.5% of DD herbs was as follows : 67.10% (24 hours), 69.07% (48 hours), 75.38% (72 hours) and 76.32% (96 hours).

4.2.5 *In Vitro* Digestibility of PKE Pellets Added 2.5% Herbs and Napier Grass (*Pennisetum purpureum*)

0.20 gm of PKE pellets added with herbs were added to 0.20 gm of Napier grass (*Pennisetum purpureum*) in this experiment. Digestibility from 24 to 96 hours incubation was recorded and the figure illustrated as following :



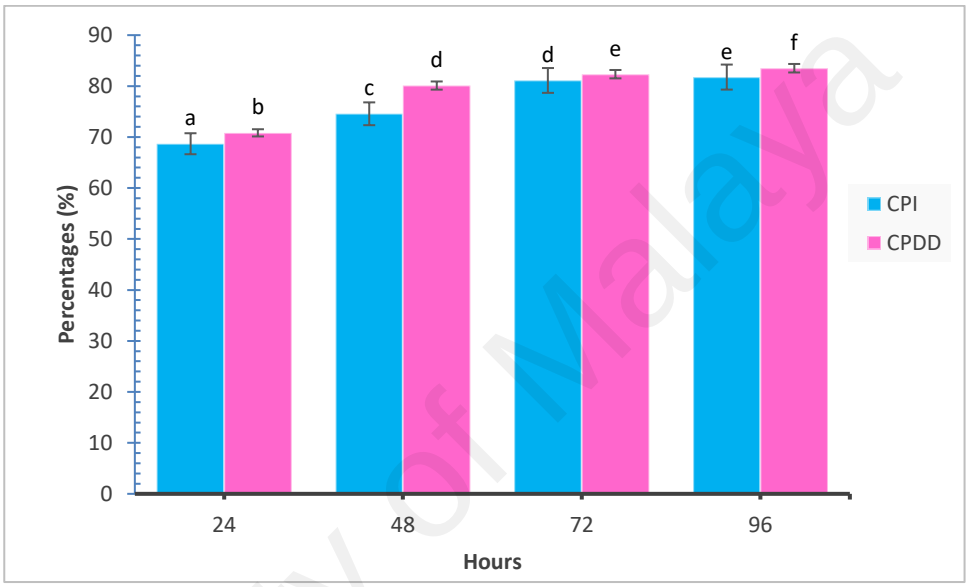
Note : Values with the different alphabets are considered significant (p<0.05)
PKEDDN = Commercial Pellets Added with DD Herbs and Napier Grass
PKEIN = Commercial Pellet Added with Imported Herbs and Napier Grass

Figure 4.5 *In Vitro* Digestibility of PKE Pellets Added 2.5 % Herbs and Napier Grass from 24 to 96 Hours of Incubation

PKE pellets added 2.5% of DD herbs and Napier grass showed significant compared to PKE pellets added with 2.5% of imported herbs and Napier Grass form 24 to 96 hours of incubation. The digestion percentage of PKE pellets added with 2.5% of DD herbs and Napier grass was as follows : 58.07% (24 hours), 65.98% (48 hours), 70.77% (72 hours) and 74.86% (96 hours).

4.2.6 *In Vitro* Digestibility of Commercial Pellets Added 5.0% Herbs

Herbs were upgraded to a dose of 5.0%. The results of incubation in the water bath for 24 hours to 96 hours were recorded. The figure illustrated the percentages in *in vitro* digestibility from 24 to 96 hours as following :



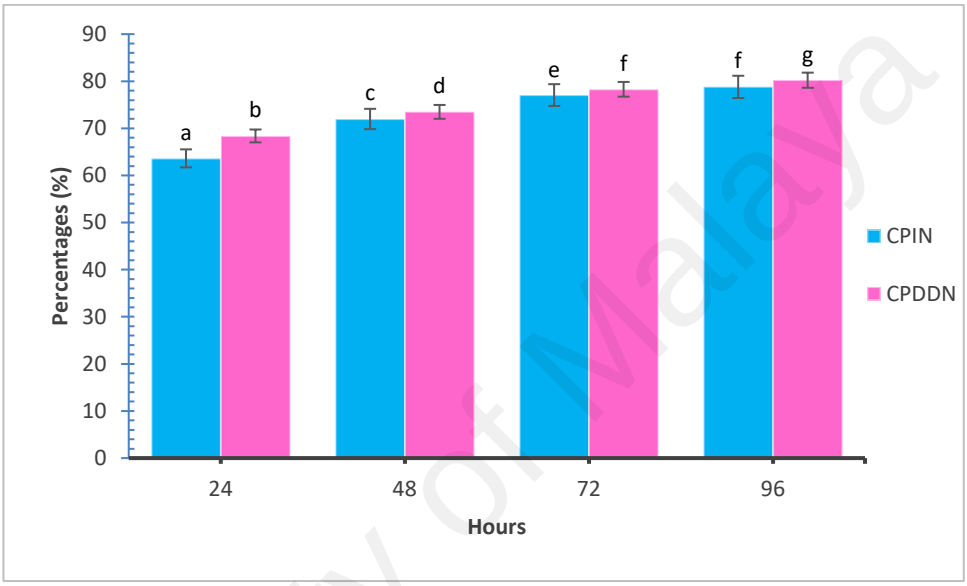
Note : Values with the different alphabets are considered significant (p<0.05)
CPDD = Commercial Pellets Added with DD Herbs
CPI = Commercial Pellet Added with Imported Herbs

Figure 4.6 *In Vitro* Digestibility of Commercial Pellets Added 5.0 % Herbs from 24 to 96 Hours of Incubation

The results showed that digestibility of commercial pellets added with 5.0% of DD herbs was significant compare to commercial pellets added with imported herbs at 24 and 96 hours of incubation. The digestibility percentage of commercial pellets added with 5.0% of DD herbs was as follows : 70.83% (24 hours), 80.11% (48 hours), 82.34% (72 hours) and 83.51% (96 hours).

4.2.7 *In Vitro* Digestibility of Commercial Pellets Added 5.0% Herbs and Napier Grass (*Pennisetum purpureum*)

0.20 gm of Napier grass was added to 0.20 gm of herbs in this *in vitro* digestibility. The results of incubation were recorded and indicated as below :



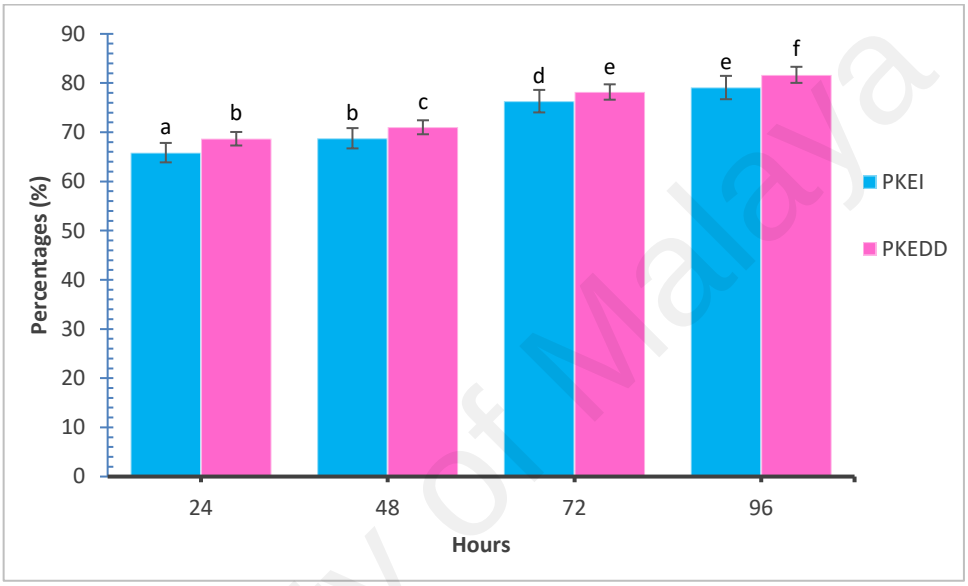
Note : Values with the different alphabets are considered significant ($p<0.05$)
CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass
CPIN = Commercial Pellet Added with Imported Herbs and Napier Grass

Figure 4.7 *In Vitro* Digestibility of Commercial Pellets Added 5.0 % Herbs and Napier Grass from 24 to 96 Hours of Incubation

The results showed that commercial pellets added with 5.0% of DD herbs and Napier grass was significant compare to commercial pellets added with imported herbs and Napier grass at 24 hours and 96 hours. The digestibility percentage of commercial pellets added with 5.0% of DD herbs and Napier grass was as follows: 68.39% (24 hours), 73.50% (48 hours), 78.29% (72 hours) and 80.22% (96 hours).

4.2.8 *In Vitro* Digestibility of PKE Pellets Added 5.0% Herbs

Herbs were upgraded to a dose of 5.0% and added to 95.0% of PKE pellets as a 5.0% formulation. The results of incubation in the water bath for 24 to 96 hours were recorded. The results obtained are illustrated in the figure below :



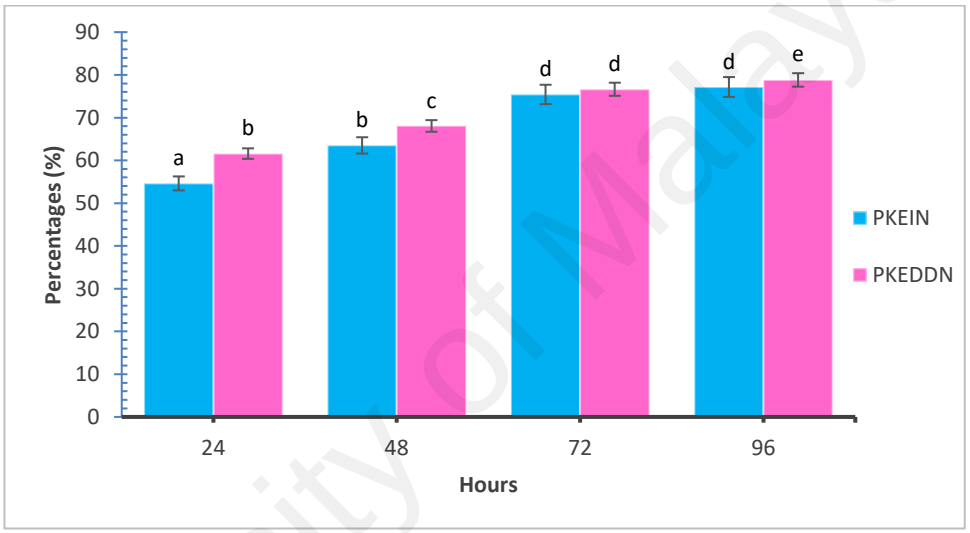
Note : Values with the different alphabets are considered significant (p<0.05)
PKEDD = Commercial Pellets Added with DD Herbs
PKEI = Commercial Pellet Added with Imported Herbs

Figure 4.8 *In Vitro* Digestibility of PKE Pellets Added 5.0 % Herbs from 24 to 96 Hours of Incubation

The results showed that digestibility of PKE pellets added with 5.0% of DD herbs was significant compare to PKE pellets added with imported herbs at 24 and 96 hours of incubation. The digestibility percentage of PKE pellets added with 5.0% of DD herbs was as follows : 68.68% (24 hours), 71.01% (48 hours), 78.18% (72 hours) and 81.67% (96 hours).

4.2.9 *In Vitro* Digestibility of PKE Pellets Added 5.0% Herbs and Napier Grass (*Pennisetum purpureum*)

0.20 gm of PKE pellets added with 5.0% DD herbs and 0.20 gm of Napier grass, then 0.20 gm of PKE pellets added with 5.0% imported herbs and 0.20 gm of Napier grass were incubated at 24 to 96 hours. Percentages of digestibility are illustrated in the figure below :



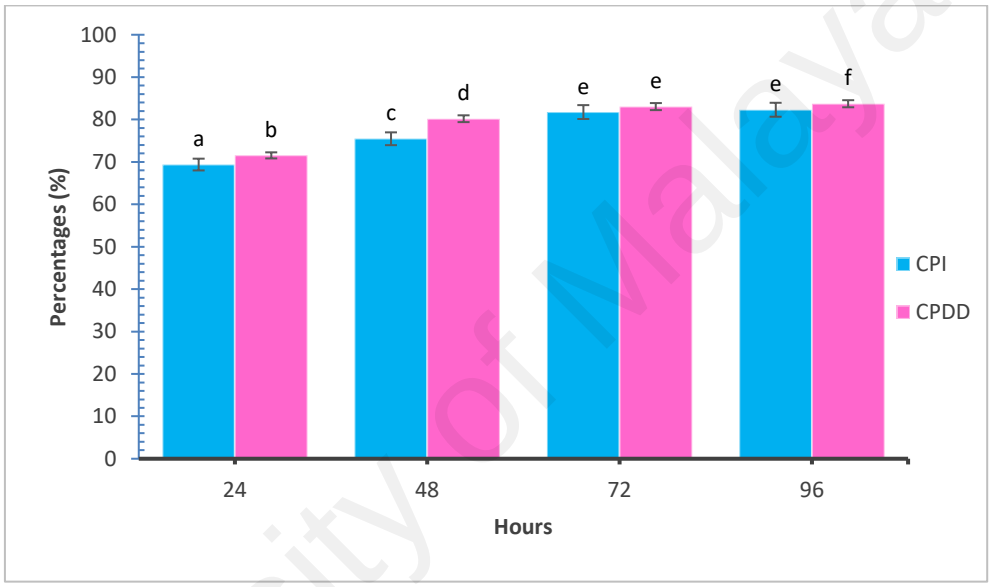
Note : Values with the different alphabets are considered significant (p<0.05)
PKEDDN = Commercial Pellets Added with DD Herbs and Napier Grass
PKEIN = Commercial Pellet Added with Imported Herbs and Napier Grass

Figure 4.9 *In Vitro* Digestibility of PKE Pellets Added 5.0 % Herbs and Napier Grass from 24 to 96 Hours of Incubation

PKE pellets added with 5.0% of DD herbs and Napier grass showed significant digestibility at 24 and 96 hours incubation compared to PKE pellets added with 5.0% of imported herbs and Napier grass. The digestibility percentage of PKE pellets added with 5.0% of DD herbs and Napier grass was as follows : 61.59% (24 hours), 68.06% (48 hours), 76.64% (72 hours) and 78.82% (96 hours).

4.2.10 *In Vitro* Digestibility of Commercial Pellets Added 7.5% Herbs

The experiment continued with DD and imported herbs by increasing the dose up to 7.5% and 92.5% of commercial pellets respectively. This treatment was then incubated in a water bath from 24 to 96 hours. *In vitro* digestibility results are shown in the following figure :



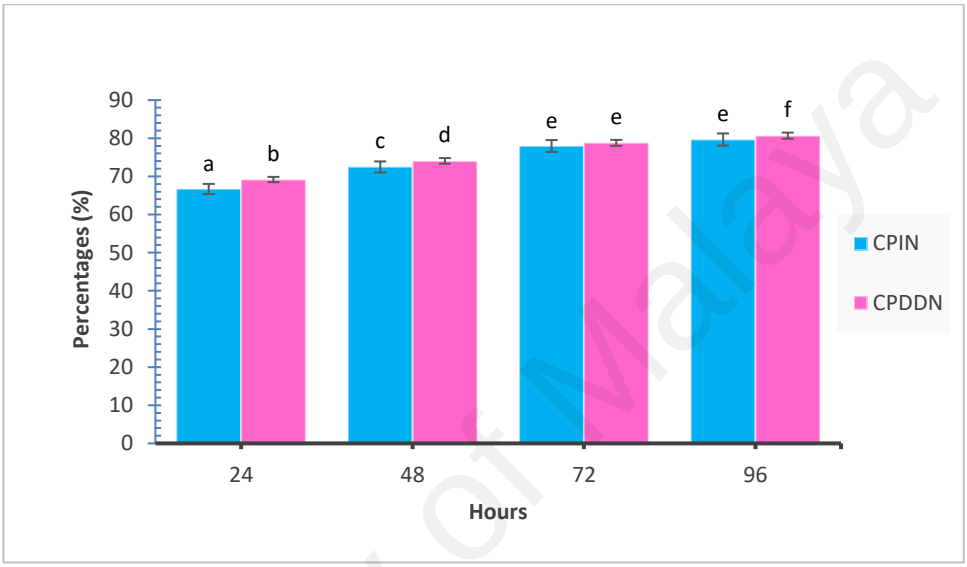
Note : Values with the different alphabets are considered significant (p<0.05)
CPDD = Commercial Pellets Added with DD Herbs
CPI = Commercial Pellet Added with Imported Herbs

Figure 4.10 *In Vitro* Digestibility of Commercial Pellets Added 7.5 % Herbs from 24 to 96 Hours of Incubation

The results showed that digestibility of commercial pellets added with 7.5% of DD herbs was significant compare to commercial pellets added with imported herbs at 24 and 96 hours of incubation. The digestibility percentage of commercial pellets added with 5.0% of DD herbs was as follows : 71.55% (24 hours), 80.19% (48 hours), 83.06% (72 hours) and 83.73% (96 hours).

4.2.11 *In Vitro* Digestibility of Commercial Pellets Added 7.5% Herbs and Napier Grass (*Pennisetum purpureum*)

The 0.20 gm amount of commercial pellets added with 7.5% then added to 0.20 gm of Napier grass. The results are as illustrated below :



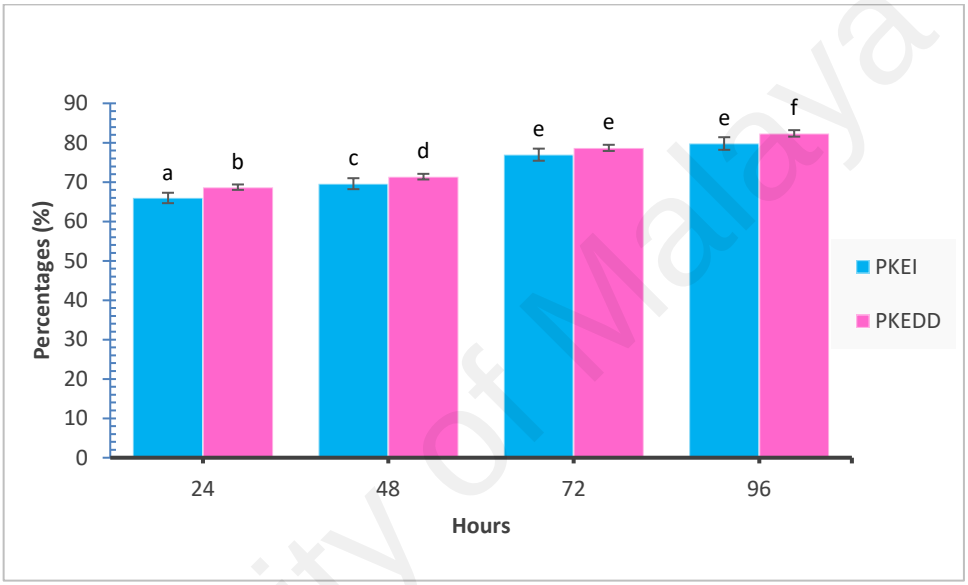
Note : Values with the different alphabets are considered significant (p<0.05)
CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass
CPIN = Commercial Pellet Added with Imported Herbs and Napier Grass

Figure 4.11 *In Vitro* Digestibility of Commercial Pellets Added 7.5 % Herbs and Napier Grass from 24 to 96 Hours of Incubation

The results showed that the in vitro digestibility of commercial pellets added with 7.5% of DD herbs and Napier grass was significant at 24 and 96 hours compare to commercial pellets added with 7.5% of imported herbs and Napier grass. The digestibility percentage of commercial pellets added with 7.5% of DD herbs and Napier grass was as follows : 69.18% (24 hours), 74.06% (48 hours), 78.78% (72 hours) and 80.68% (96 hours).

4.2.12 *In Vitro* Digestibility of PKE Pellets Added 7.5% Herbs

Herbs were upgraded to a dose of 7.5% and added to 95.0% of PKE pellets as a 7.5% formulation. The results of incubation in the water bath for 24 to 96 hours were recorded. The results below shows the digestibility percentages from 24 hours to 96 hours:



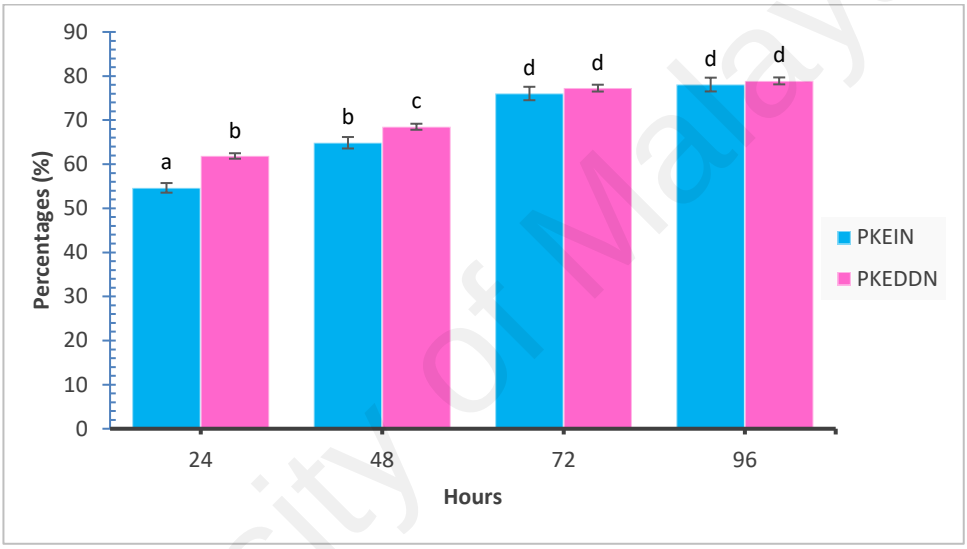
Note : Values with the different alphabets are considered significant (p<0.05)
PKEDD = Commercial Pellets Added with DD Herbs
PKEI = Commercial Pellet Added with Imported Herbs

Figure 4.12 *In Vitro* Digestibility of PKE Pellets Added 7.5 % Herbs from 24 to 96 Hours of Incubation

The results showed that digestibility of PKE pellets added with 7.5% of DD herbs was significant compare to PKE pellets added with imported herbs at 24 and 96 hours of incubation. The digestibility percentage of PKE pellets added with 7.5% of DD herbs was as follows : 69.18% (24 hours), 74.06% (48 hours), 78.78% (72 hours) and 81.98% (96 hours).

4.2.13 *In Vitro* Digestibility of PKE Pellets Added 7.5% Herbs and Napier Grass (*Pennisetum purpureum*)

0.20 gm of PKE pellets added with 7.5% of DD herbs and 0.20 gm of Napier grass, then 0.20 gm of PKE pellets added with 7.5% imported herbs and 0.20 gm of Napier grass were incubated from 24 to 96 hours of incubation. Percentages of digestibility are illustrated in the figure below :



Note : Values with the different alphabets are considered significant (p<0.05)
PKEDDN = Commercial Pellets Added with DD Herbs and Napier Grass
PKEIN = Commercial Pellet Added with Imported Herbs and Napier Grass

Figure 4.13 *In Vitro* Digestibility of PKE Pellets Added 7.5 % Herbs and Napier Grass from 24 to 96 Hours of Incubation

PKE pellets added with 7.5% of DD herbs and Napier grass showed significant digestibility at 24 and 96 hours incubation compared to PKE pellets added with 7.5% of imported herbs and Napier grass. The digestibility percentage of PKE pellets added with 5.0% of DD herbs and Napier grass was as follows : 61.87% (24 hours), 68.50% (48 hours), 77.26% (72 hours) and 78.90% (96 hours).

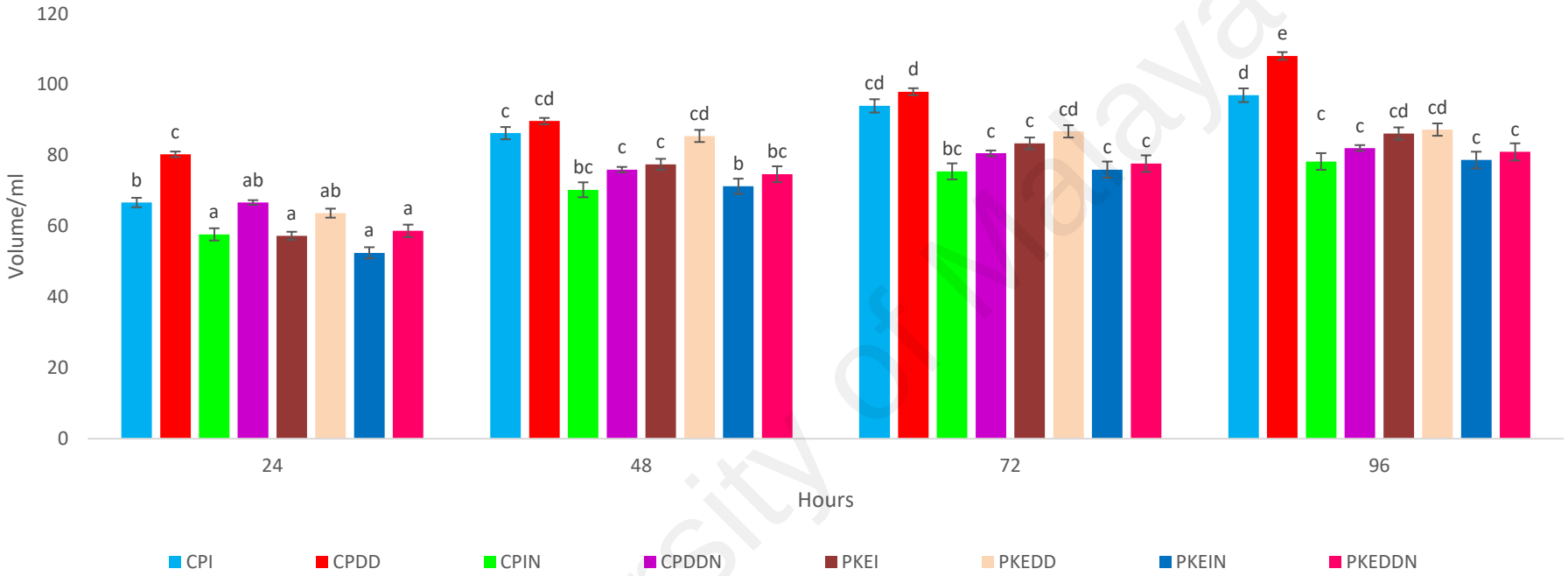
4.3.1 *In Vitro* Gas Production of All Treatments Added with 2.5% of Herbs and Napier Grass

Gas production readings in the treatment using 2.5% herbs were recorded. The results were illustrated in the table and figure below :

Table 4.3 *In Vitro* Gas Production of Pellets either Added 2.5% Herbs or Added 2.5% Herbs and Napier Grass from 24 to 96 Hours

Volume/ml	Hours			
	24	48	72	96
CPI	67.25±1.86b	78.30±1.30c	87.10±1.08cd	97.00±1.12d
CPDD	80.30±1.28c	89.70±0.59cd	98.00±1.24d	108.10±0.56e
CPIN	57.70±1.27a	70.30±1.28bc	75.50±0.87bc	78.30±1.92c
CPDDN	66.70±1.79ab	76.00±1.08c	80.60±0.41c	82.10±1.01c
PKEI	57.30±0.86a	77.50±1.80c	83.40±1.52c	86.20±0.84cd
PKEDD	63.70±1.86ab	85.50±1.18cd	86.80±0.71cd	87.30±1.60cd
PKEIN	52.50±1.29a	68.20±1.49b	76.00±1.16c	78.70±1.32c
PKEDDN	58.70±1.17a	73.80±0.83bc	77.70±1.92c	81.00±1.87c

Note : $^{a-h}$Mean (n=5) within the same column with different superscript are significantly different ($p<0.05$)
CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs,
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass, CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass,
PKEI= PKE Pellets Added with Imported Herbs, PKEI = PKE Pellets Added with Imported Herbs,
PKEDD = PKE Pellets Added with DD Herbs, PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass,
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass



Note : The *in vitro* gas production data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between the means (n=5) was determined by the Duncan's multiple range test at 95% least significance difference ($p<0.05$)
CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs,
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass, CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass,
PKEI= PKE Pellets Added with Imported Herbs, PKEI= PKE Pellets Added with Imported Herbs,
PKEDD = PKE Pellets Added with DD Herbs, PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass,
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass

Figure 4.14 *In Vitro* Gas Production Pellets Added 2.5% Herbs or Added 2.5% Herbs and Napier Grass During Incubation

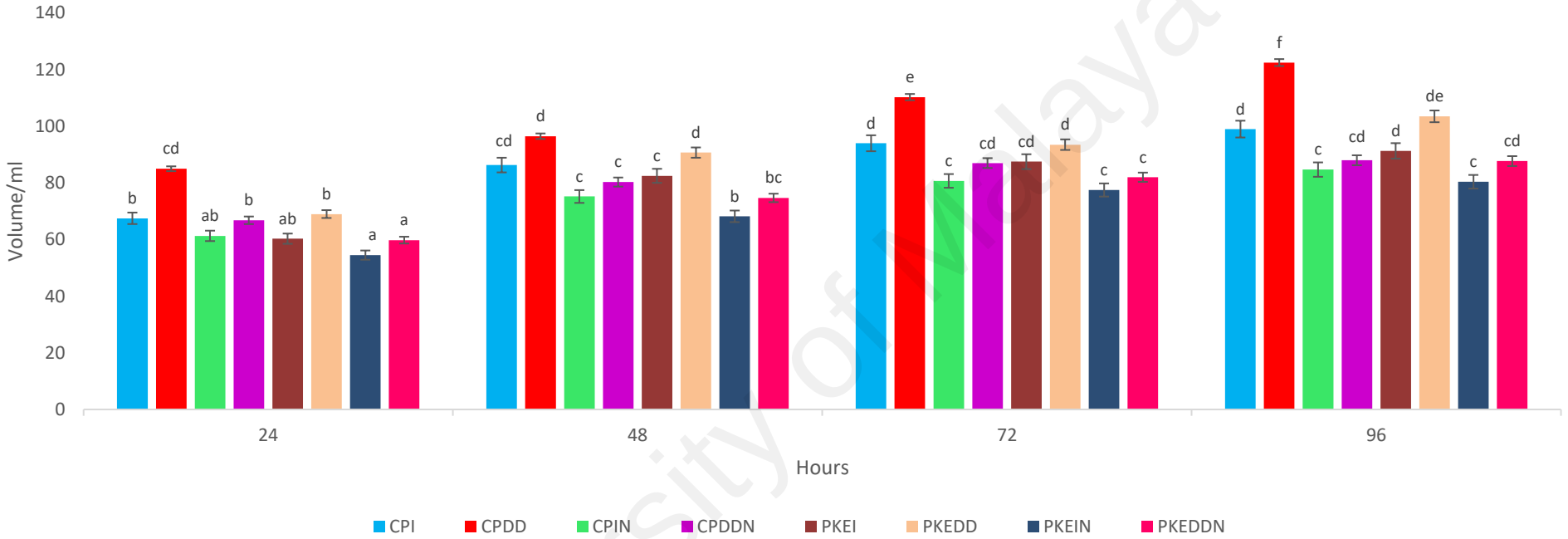
4.3.2 *In Vitro* Gas Production Technique of All Treatments Added with 5.0% of Herbs and Napier Grass

Herbs dosage was upgraded increased into 5.0% added with pellets. The results are as shown in the table and figure below :

Table 4.4 *In Vitro* Gas Production of Pellets either Added 5.0% Herbs or Added 5.0% Herbs and Napier Grass from 24 to 96 Hours

Volume/ml	Hours			
	24	48	72	96
CPI	67.50±1.40b	86.30±1.30cd	94.00±1.56d	99.00±1.50d
CPDD	85.00±1.56cd	96.50±1.55d	110.30±1.73e	122.50±1.58f
CPIN	61.30±1.63ab	75.20±1.16c	80.70±1.30c	84.70±1.77c
CPDDN	66.80±1.50ab	80.30±1.30c	87.00±1.86cd	88.00±1.42cd
PKEI	60.30±1.29ab	82.50±1.29c	87.50±1.71cd	91.30±1.56d
PKEDD	69.00±1.31b	90.70±1.40d	93.50±1.62d	103.50±1.51de
PKEIN	54.50±1.65a	68.20±1.60b	77.50±1.50c	80.40±1.36c
PKEDDN	59.80±1.10a	74.70±1.54bc	82.00±1.12c	87.70±1.38cd

Note : $^{a-h}$Mean (n=5) within the same column with different superscript are significantly different ($p<0.05$)
CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs,
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass, CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass,
PKEI= PKE Pellets Added with Imported Herbs, PKEI= PKE Pellets Added with Imported Herbs,
PKEDD = PKE Pellets Added with DD Herbs, PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass,
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass



Note : The *in vitro* gas production data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between the means (n=5) was determined by the Duncan's multiple range test at 95% least significance difference ($p<0.05$)
CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs,
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass, CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass,
PKEI= PKE Pellets Added with Imported Herbs, PKEI= PKE Pellets Added with Imported Herbs,
PKEDD = PKE Pellets Added with DD Herbs, PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass,
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass

Figure 4.15 *In Vitro* Gas Production Pellets Added 5.0% Herbs or Added 5.0% Herbs and Napier Grass During Incubation

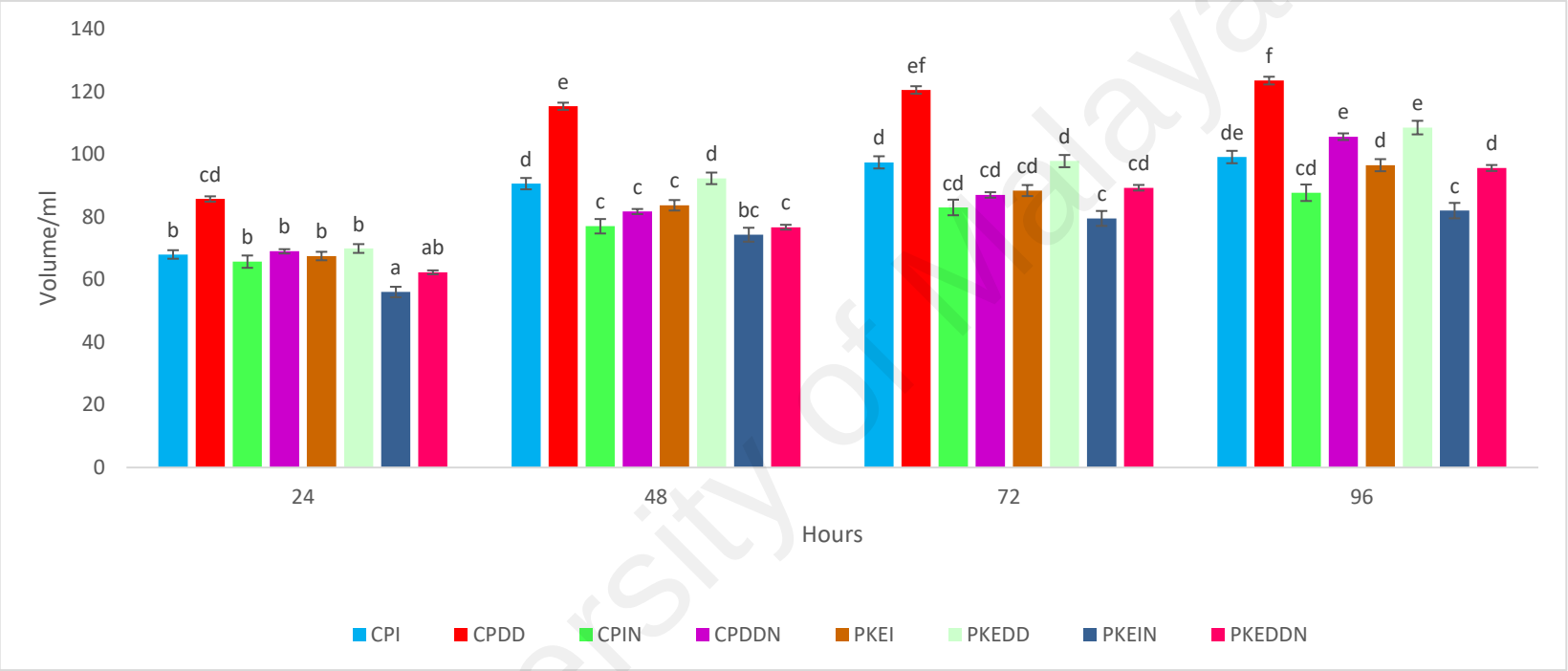
4.3.3 *In Vitro* Gas Production Technique of All Treatments Added with 7.5% of Herbs and Napier Grass

The comparative results of all treatments using 7.5% herbs added are as shown in the table and figure below:

Table 4.5 *In Vitro* Gas Production of Pellets either Added 7.5% Herbs or Added 7.5% Herbs and Napier Grass from 24 to 96 Hours

Volume/ml	Hours			
	24	48	72	96
CPI	68.00±1.58b	90.60±1.78d	97.40±1.71d	99.10±1.29de
CPDD	85.70±1.57cd	115.30±1.05e	120.50±1.06ef	123.50±1.69f
CPIN	65.70±1.63b	77.00±1.55c	83.00±1.87cd	87.70±1.77cd
CPDDN	69.00±0.71b	81.70±0.68c	87.60±1.86cd	105.60±1.42e
PKEI	67.50±1.30b	83.70±0.95c	88.40±1.71cd	96.50±1.56d
PKEDD	69.90±1.31b	92.30±1.95d	97.80±1.62d	108.50±1.51e
PKEIN	56.00±0.55a	74.30±1.39bc	79.50±1.69c	82.00±1.08c
PKEDDN	62.30±0.68ab	76.70±1.17c	89.30±1.12cd	95.60±1.38d

Note : $^{a-h}$Mean (n=5) within the same column with different superscript are significantly different ($p<0.05$)
CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs,
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass, CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass,
PKEI= PKE Pellets Added with Imported Herbs, PKEI= PKE Pellets Added with Imported Herbs,
PKEDD = PKE Pellets Added with DD Herbs, PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass,
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass



Note : The *in vitro* gas production data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between the means (n=5) was determined by the Duncan's multiple range test at 95% least significance difference ($p<0.05$)
CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs,
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass, CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass,
PKEI= PKE Pellets Added with Imported Herbs, PKEI= PKE Pellets Added with Imported Herbs,
PKEDD = PKE Pellets Added with DD Herbs, PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass,
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass

Figure 4.16 *In Vitro* Gas Production Pellets Added 7.5% Herbs or Added 7.5% Herbs and Napier Grass During Incubation

4.4 *In Sacco* Digestibility

In Sacco technique is a laboratory and field experiment. This experiments were conducted in the Mini Institute Sciences Biological Farm and Animal Biotechnology Laboratory, University of Malaya. The result are tabulated and reported in the following tables and figures.

Table 4.6 *In Sacco* Digestibility of Pellets Added with 2.5% of Herbs or Added with 2.5% Herbs and Napier Grass from 24 to 96 hours

Treatments/Hours	24	48	72	96
CP	66.36±0.65b	72.36±1.57c	74.88±0.95c	77.35±0.50c
CPI	66.43±0.30b	71.26±0.53c	75.41±0.61c	77.65±1.21c
CPDD	67.07±1.26b	73.67±2.17c	76.95±1.36c	77.82±0.97c
CPIN	60.63±0.38ab	62.62±2.66b	66.20±1.83b	68.31±1.96b
CPDDN	63.13±1.12b	67.62±1.49b	67.72±2.40b	72.98±1.24c
PKE	62.53±2.06b	68.80±2.91b	68.93±±2.51b	75.47±1.20c
PKEI	62.71±1.32b	69.00±0.53b	72.69±1.85c	73.75±0.74c
PKEDD	64.19±1.25b	69.11±2.37b	73.00±1.96c	76.82±1.28c
PKEIN	54.72±0.54a	61.75±0.68ab	62.74±1.47b	65.87±1.52b
PKEDDN	60.22±0.81ab	66.46±1.12b	68.62±1.02b	70.26±0.87bc
N	52.06±1.48a	60.09±0.93ab	64.68±3.32b	66.32±2.08b

Note : $^{a-c}$Mean (n=5) within the same column with different superscript are significantly different ($p<0.05$)
CP = Commercial Pellets, CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs,
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass, CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass,
PKE = PKE Pellets, PKEI= PKE Pellets Added with Imported Herbs, PKEI= PKE Pellets Added with Imported Herbs,
PKEDD = PKE Pellets Added with DD Herbs, PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass,
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass, N = Napier Grass

4.4.1 *In Sacco* Digestibility of Pellets and Napier Grass

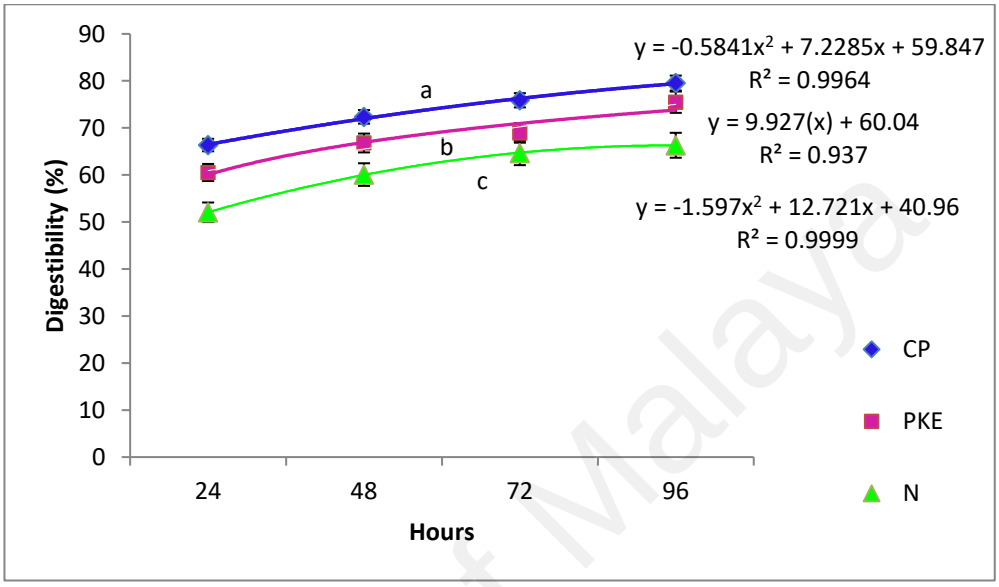
In sacco digestibility of commercial pellets, PKE pellets and Napier grass was recorded from 24 to 96 hours of incubation. The results as follow :

Table 4.7 *In Sacco* Digestibility of the Commercial Pellets, PKE Pellets Added 2.5% Herbs either DD Herbs or Imported Herbs and Napier Grass (*Pennisetum purpureum*) from 24 to 96 Hours Incubation

Treatments/Hours	24	48	72	96
CP	66.36±0.65b	72.36±1.57bc	74.88±0.95c	77.35±0.50c
PKE	62.53±1.06ab	68.80±0.91b	68.93±±0.51b	75.47±1.20c
N	52.06±1.48a	60.09±0.93ab	64.68±3.32b	66.32±1.08b

Note : $^{a-c}$Mean (n=5) within the same column with different superscript are significantly different (p<0.05)
CP = Commercial Pellets
PKE = PKE Pellets
N = Napier Grass

The main treatments used in *in sacco* digestibility is Napier grass and two types of pellets, which were commercial pellets and PKE pellets. The results obtained are shown as figure below :



Note : Mean ± SD for 3 treatments are significantly different ($p<0.05$)
(a) CP = Commercial Pellets
(b) PKE = PKE Pellets
(c) N = Napier Grass (*Pennisetum purpureum*)

Figure 4.17 *In Sacco* Digestibility of Commercial Pellets, PKE Pellets and Napier Grass (*Pennisetum purpureum*) from 24 to 96 hours in goat rumen. Different alphabet shows significantly different (p <0.05).

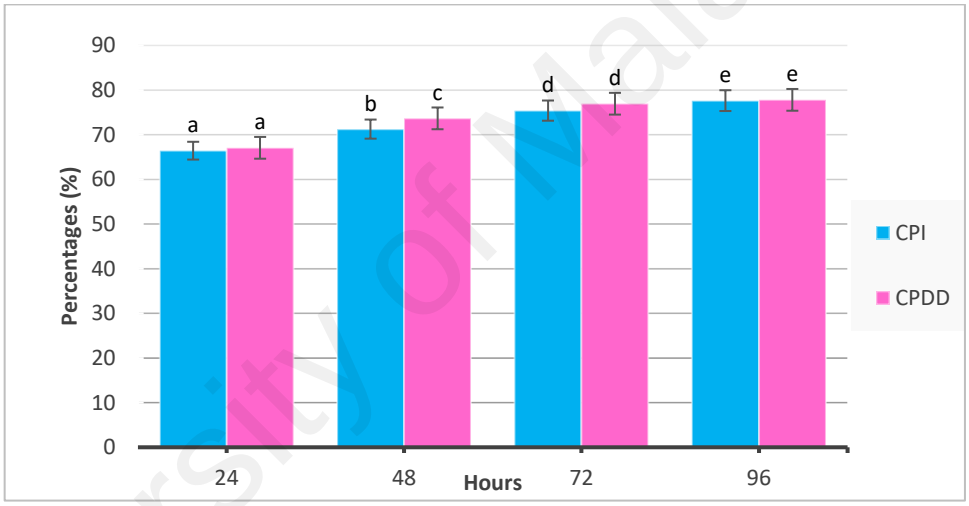
In sacco digestibility showed that commercial pellets were significant compared to Napier grass (*Pennisetum purpureum*). Commercial pellets were highest in digestibility from the 24 to 96 hour of incubation and the percentages were 66.36% (24 hours), 72.36% (48 hours), 74.88% (72 hours) and 77.35% (96 hours). The lowest digestibility was Napier grass (*Pennisetum purpureum*) with 52.06% (24 hours), 60.09% (48 hours), 64.68% (72 hours) and 66.32% (96 hours).

4.4.2 *In Sacco* Digestibility of Commercial Pellets Added with 2.5% Herbs

25gm of herbal mixture was added with 975gm of commercial pellet, incubated in the goat rumen. The percentages of digestibility were recorded from 24 hours to 96 hours. Two types of herbs which were tested :

1) DD herbs, and;
2) Imported herbs,

The percentages were calculated and the results as in the figure below :



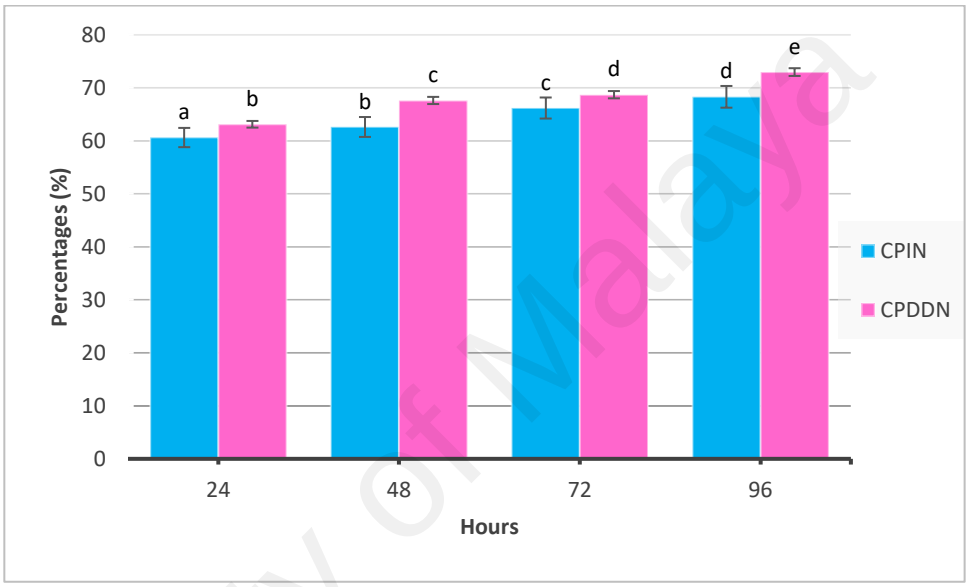
Note : Values with the different alphabets are considered significant (p<0.05)
CPDD = Commercial Pellets Added with DD Herbs
CPI = Commercial Pellet Added with Imported Herbs

Figure 4.18 *In Sacco* Digestibility of Commercial Pellets Added 2.5 % Herbs from 24 to 96 Hours of Incubation

The digestibility of commercial pellets added 2.5% DD herbs was significant compared to commercial pellets added 2.5% imported herbs at 24 and 96 hours of incubation.

4.4.3 *In Sacco* Digestibility of Commercial Pellets Added with 2.5% Herbs and Napier Grass (*Pennisetum purpureum*)

In Sacco digestibility was recorded from 24 to 96 hours of incubation. The percentages of digestibility figure illustrated as follow :



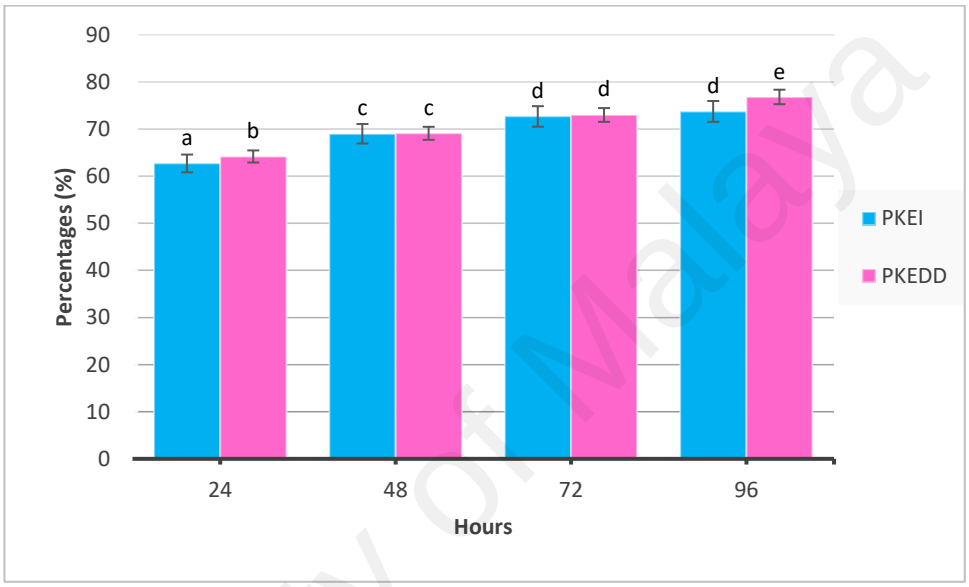
Note : Values with the different alphabets are considered significant (p<0.05)
CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass
CPIN = Commercial Pellet Added with Imported Herbs and Napier Grass

Figure 4.19 *In Sacco* Digestibility of Commercial Pellet Added 2.5 % Herbs and Napier Grass (*Pennisetum purpureum*) from 24 to 96 Hours

The result showed that the digestibility of commercial pellets added with 2.5% DD herbs and Napier grass was significant compared to commercial pellets added 2.5% imported herbs and Napier grass at 24 and 96 hours of incubation.

4.4.4 *In Sacco* Digestibility of PKE Pellets Added with 2.5% Herbs

The 25gm of DD herbs were added to PKE pellets and 25gm of imported herbs were added to PKE pellets. Results were recorded after incubation for 24 to 96 hours. The percentages of digestibility were calculated and illustrated as figure below :



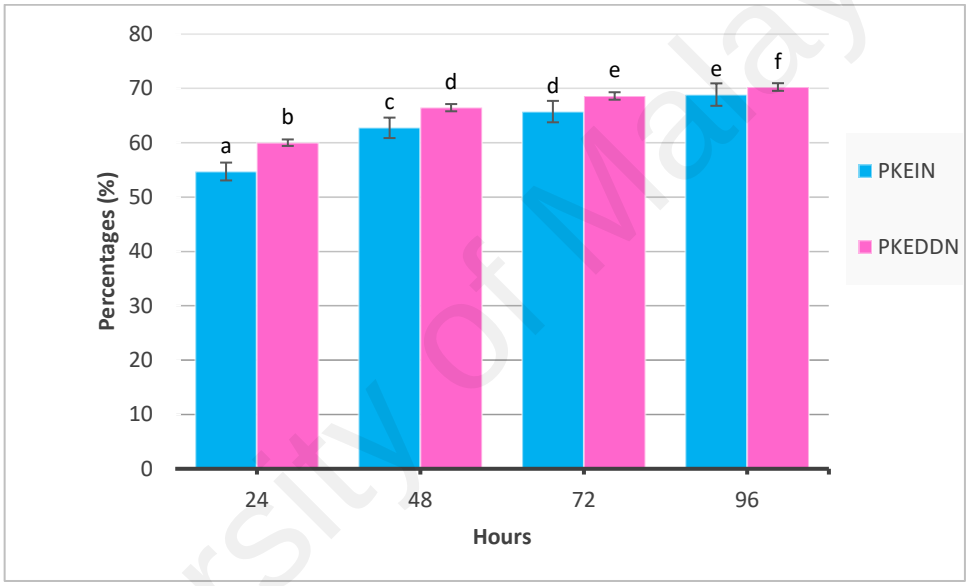
Note : Values with the different alphabets are considered significant (p<0.05)
PKEDD = Commercial Pellets Added with DD Herbs
PKEI = Commercial Pellet Added with Imported Herbs

Figure 4.20 *In Sacco* Digestibility of PKE Pellets Added with 2.5 % of Herbs from 24 to 96 Hours

The results showed that digestibility of PKE pellets added with 2.5% of herbs was significant compared to PKE pellets added 2.5% imported herbs at 24 and 96 hours of incubation.

4.4.5 *In Sacco* Digestibility of PKE Pellets Added with 2.5% Herbs and Napier Grass (*Pennisetum purpureum*)

0.20gm of PKE pellets added with 2.5% DD herbs and 0.20gm of Napier grass, another treatment was 0.20gm of PKE pellets added with 2.5% imported herbs and added 0.20gm of Napier grass in *in sacco* digestibility. Digestibility from 24 to 96 hours incubation was recorded and the figure illustrated as follows :



Note : Values with the different alphabets are considered significant (p<0.05)
PKEDDN = Commercial Pellets Added with DD Herbs and Napier Grass
PKEIN = Commercial Pellet Added with Imported Herbs and Napier Grass

Figure 4.21 *In Sacco* Digestibility of PKE Pellet Added 2.5 % Herbs and Napier Grass (*Pennisetum purpureum*) from 24 to 96 Hours

PKE pellets added 2.5% DD herbs and Napier grass, the digestibility was significant compared to PKE pellets added 2.5% imported herbs and Napier grass at 24 and 96 hours of incubation.

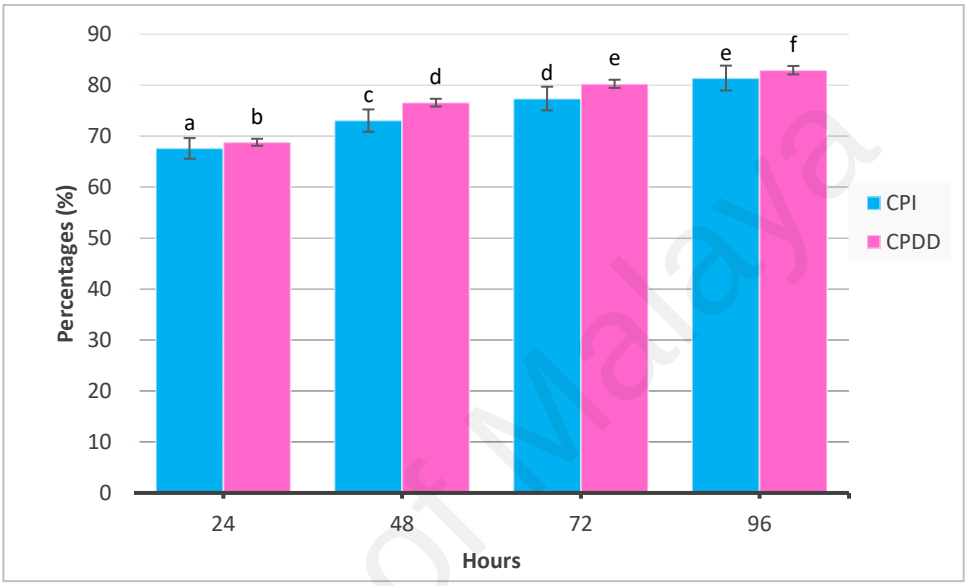
Table 4.8 *In Sacco* Digestibility of Pellets Added with 5.0% of Herbs or Added with 5.0% of Herbs and Napier Grass from 24 to 96 hours

Treatments/Hours	24	48	72	96
CP	66.36±0.65b	72.36±1.57c	74.88±0.95c	77.35±0.50c
CPI	67.59±0.84b	73.06±1.89c	77.38±1.13c	81.40±1.05d
CPDD	68.81±2.81b	76.57±1.89c	80.27±0.73cd	82.95±0.48d
CPIN	63.90±3.19b	66.67±1.27b	69.12±1.99b	69.88±1.59b
CPDDN	67.47±0.90b	69.61±1.56b	69.83±2.75b	74.63±2.77c
PKE	62.53±2.06b	68.80±2.91b	68.93±2.51b	75.47±1.20c
PKEI	64.23±1.22b	68.93±0.98b	76.22±0.46c	76.62±2.90c
PKEDD	68.39±3.05b	71.27±3.29bc	76.77±0.62c	78.64±1.24c
PKEIN	55.81±2.83a	61.48±0.63ab	64.07±1.12b	68.56±0.72b
PKEDDN	63.07±0.77b	68.86±1.30b	69.95±3.19b	71.89±0.88bc
N	52.06±1.48a	60.09±0.93ab	64.68±3.32b	66.32±2.08b

Note : $^{a-d}$Mean (n=5) within the same column with different superscript are significantly different ($p<0.05$)
CP = Commercial Pellets, CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs,
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass, CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass,
PKE = PKE Pellets, PKEI= PKE Pellets Added with Imported Herbs, PKEI= PKE Pellets Added with Imported Herbs,
PKEDD = PKE Pellets Added with DD Herbs, PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass,
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass, N = Napier Grass

4.4.6 *In Sacco* Digestibility of Commercial Pellets Added with 5.0% Herbs

Herbs were upgraded to a dose of 5.0%. The results of incubation in the water bath for 24 hours to 96 hours were recorded. The results are as follow :



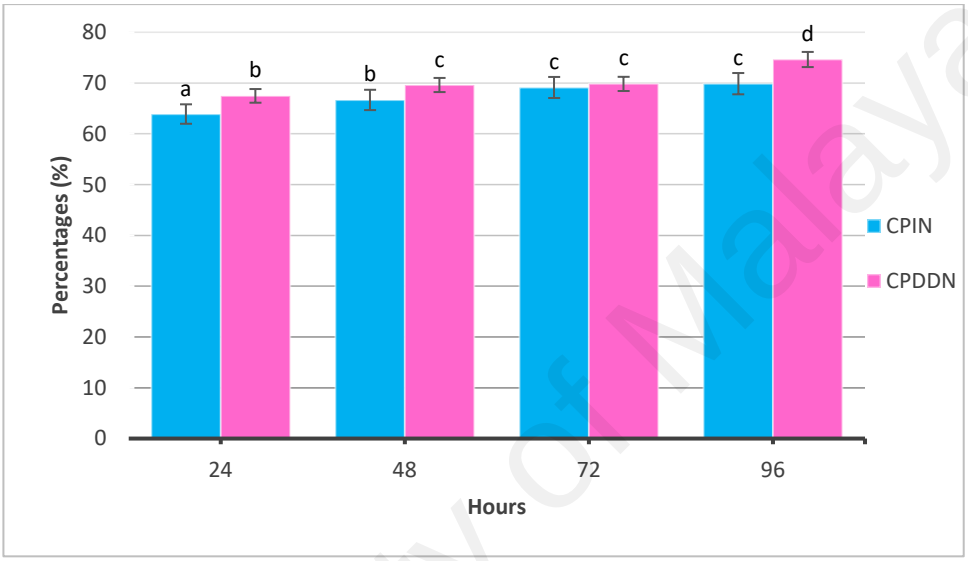
Note : Values with the different alphabets are considered significant (p<0.05)
CPDD = Commercial Pellets Added with DD Herbs
CPI = Commercial Pellet Added with Imported Herbs

Figure 4.22 *In Sacco* Digestibility of Commercial Pellets Added with 5.0% DD Herbs from 24 to 96 hours of Incubation

The results showed that digestibility of commercial pellets added with 5.0% DD herbs was significant compare to commercial pellets added with 5.0% imported herbs at 24 and 96 hours of incubation. The digestibility percentage of commercial pellets added with 5.0% DD herbs was as follows : 68.81% (24 hours), 76.57% (48 hours), 80.27% (72 hours) and 82.95% (96 hours).

4.4.7 *In Sacco* Digestibility of Commercial Pellets Added with 5.0% Herbs and Napier Grass (*Pennisetum purpureum*)

5.0% herbs added with Napier grass were tested and incubated in the goat rumen for 24 hours to 96 hours. The results were recorded and as indicated as below :



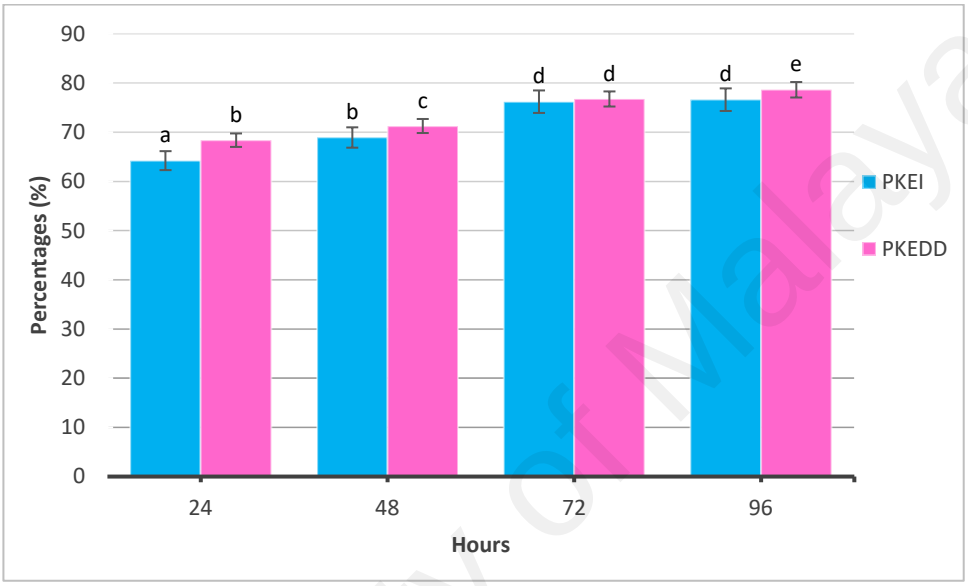
Note : Mean ± SD for 2 treatments are significantly different ($p<0.05$)
CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass and
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass

Figure 4.23 *In Sacco* Digestibility of Commercial Pellets Added with 5.0% Herbs and Napier Grass (*Pennisetum purpureum*) from 24 to 96 hours

The results showed that commercial pellets added with 5.0% DD herbs and Napier grass was significant compared to commercial pellets added with 5.0% imported herbs and Napier grass at 24 and 96 hours. The digestibility percentage of commercial pellets added with 5.0% DD herbs and Napier Grass was as follows : 67.47% (24 hours), 69.61% (48 hours), 69.83% (72 hours) and 74.63% (96 hours).

4.4.8 *In Sacco* Digestibility of PKE Pellets Added with 5.0% Herbs

Herbs were upgraded to a dose of 50gm and added to 950 gm of PKE as 5.0% formulation. The results of incubation for 24 to 96 hours were recorded. The results obtained are illustrated in the figure below :



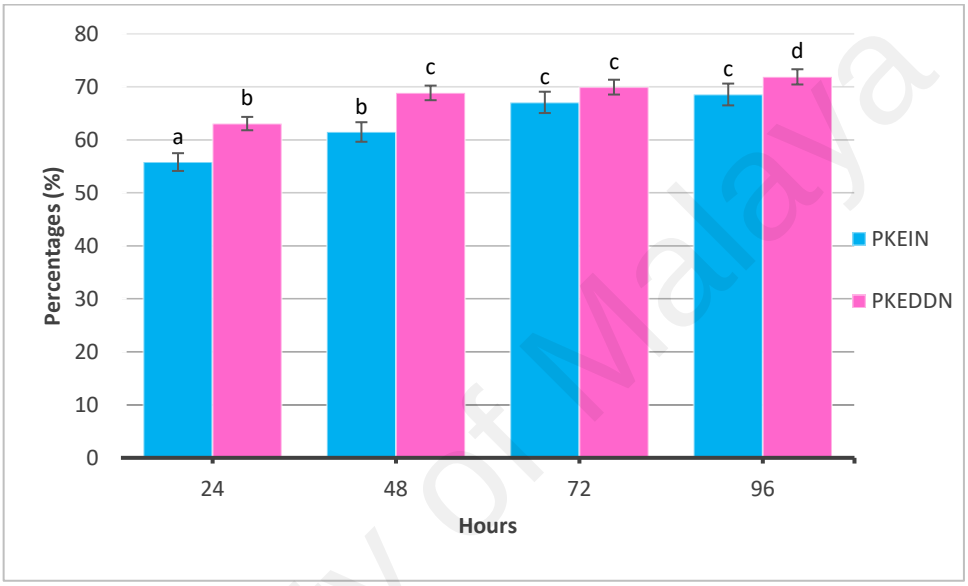
Note : Mean ± SD for 2 treatments are significantly different ($p<0.05$)
PKEDD = PKE Pellets Added with DD Herbs and
PKEI= PKE Pellets Added with Imported Herbs

Figure 4.24 *In Sacco* Digestibility of PKE Pellets Added with 5.0% Herbs from 24 to 96 hours

The results showed that digestibility of PKE pellets added with 5.0% DD herbs was significant compared to PKE pellets added with 5.0% imported herbs at 24 and 96 hours of incubation. The digestibility percentage of PKE pellets added with 5.0% DD herbs was as follows : 68.39% (24 hours), 71.27% (48 hours), 76.77% (72 hours) and 78.64% (96 hours).

4.4.9 *In Sacco* Digestibility of PKE Pellets Added with 5.0% Herbs and Napier Grass (*Pennisetum purpureum*)

Herbs added with PKE pellets and Napier grass were incubated in the rumen from 24 to 96 hours. The results obtained are illustrated in the figure below:



Note : Mean ± SD for 2 treatments are significantly different ($p<0.05$)
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass and
PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass

Figure 4.25 *In Sacco* Digestibility of PKE Pellets Added with 5.0% Herbs and Napier Grass (*Pennisetum purpureum*) from 24 to 96 hours

The results showed that digestibility of PKE pellets added with 5.0% DD herbs and Napier grass was significant compared to PKE pellets added with 5.0% imported herbs and Napier grass at 24 and 96 hours of incubation. The digestibility percentage of PKE pellets added with 5.0% DD herbs and Napier grass was as follows : 63.07% (24 hours), 68.86% (48 hours), 69.95% (72 hours) and 71.89% (96 hours).

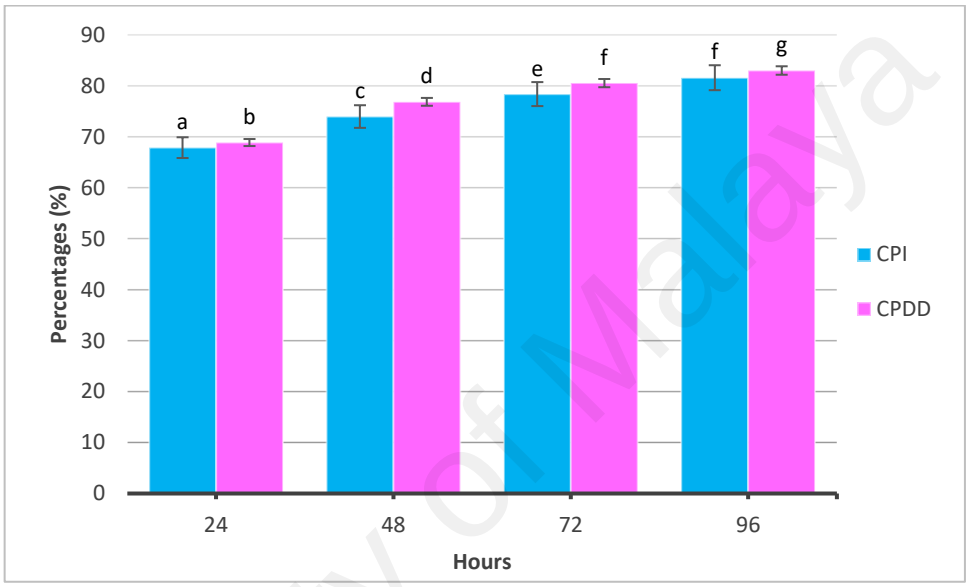
Table 4.9 *In Sacco* Digestibility of Pellets Added with 7.5% Herbs or Added with 7.5% Herbs and Napier Grass from 24 to 96 hours

Treatments/Hours	24	48	72	96
CP	66.36±0.65b	72.36±1.57c	74.88±0.95c	77.35±0.50c
CPI	67.87±0.86b	73.97±1.83c	78.38±2.15c	81.59±1.24cd
CPDD	68.87±1.34b	76.85±1.32c	80.54±0.37cd	83.01±0.85d
CPIN	64.08±0.69b	66.69±2.05b	69.27±2.00b	70.83±1.42bc
CPDDN	67.69±0.36b	69.73±1.05b	70.06±2.34bc	75.23±2.14c
PKE	62.53±2.06b	68.80±2.91b	68.93±2.51b	75.47±1.20c
PKEI	64.80±0.69b	69.81±0.56b	76.82±0.88c	76.96±0.37c
PKEDD	69.02±0.39b	71.98±2.17bc	77.09±0.81c	78.88±2.22c
PKEIN	56.06±2.33a	62.31±1.06b	64.57±0.82b	68.77±1.31b
PKEDDN	64.50±1.19b	69.64±1.47b	70.10±1.63bc	72.18±1.91c
N	52.06±1.48a	60.09±0.93ab	64.68±3.32b	66.32±2.08b

Note : $^{a-d}$Mean (n=5) within the same column with different superscript are significantly different ($p<0.05$)
CP = Commercial Pellets, CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs,
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass, CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass,
PKE = PKE Pellets, PKEI= PKE Pellets Added with Imported Herbs, PKEI= PKE Pellets Added with Imported Herbs,
PKEDD = PKE Pellets Added with DD Herbs, PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass,
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass, N = Napier Grass

4.4.10 *In Sacco* Digestibility of Commercial Pellets Added with 7.5% Herbs

Herbal dose was upgraded to 7.5% added with commercial pellets. The treatment was incubated in the goat rumen for 24 to 96 hours. *In sacco* digestibility results are shown in the following figure:



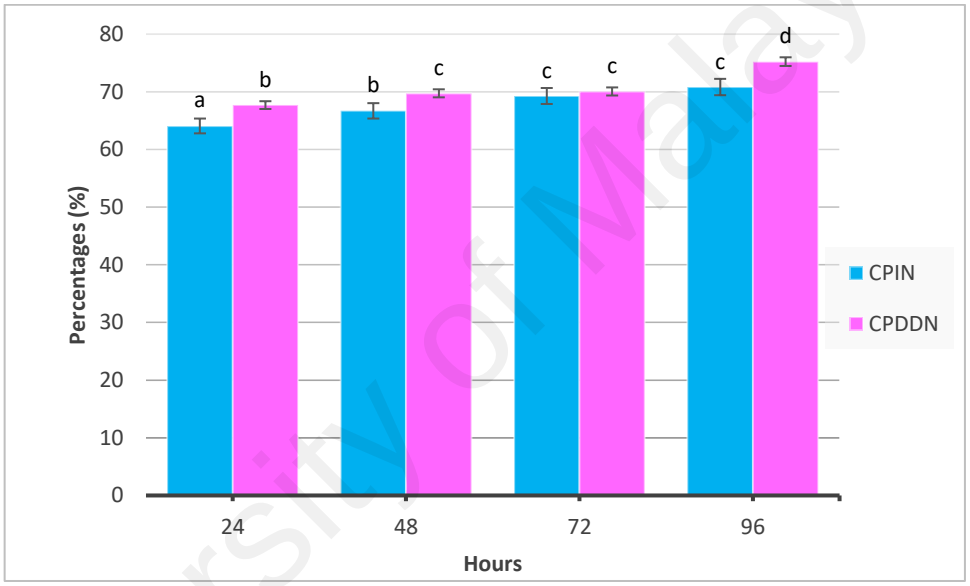
Note : Mean ± SD for 2 treatments are significantly different ($p<0.05$)
CPDD = Commercial Pellets Added with DD Herbs and
CPI= Commercial Pellets Added with Imported Herbs

Figure 4.26 *In Sacco* Digestibility of Commercial Pellets Added with 7.5% Herbs from 24 to 96 hours

The results showed that digestibility of commercial pellets added with 5.0% DD herbs was significant compare to commercial pellets added with imported herbs in 24 to 96 hours of incubation. The digestibility percentage of commercial pellets added with 50 gm DD herbs was as follows : 68.87% (24 hours), 76.85% (48 hours), 80.54% (72 hours) and 83.01% (96 hours).

4.4.11 *In Sacco* Digestibility of Commercial Pellets Added with 7.5% Herbs and Napier Grass (*Pennisetum purpureum*)

0.20 gm of herbs upgraded to 7.5% dose added with commercial pellets and 0.20 gm of Napier grass. The treatments were incubated in goat rumen and were recorded from 24 to 96 hour incubation period. The *in sacco* digestibility percentages were calculated and the results are as follow:



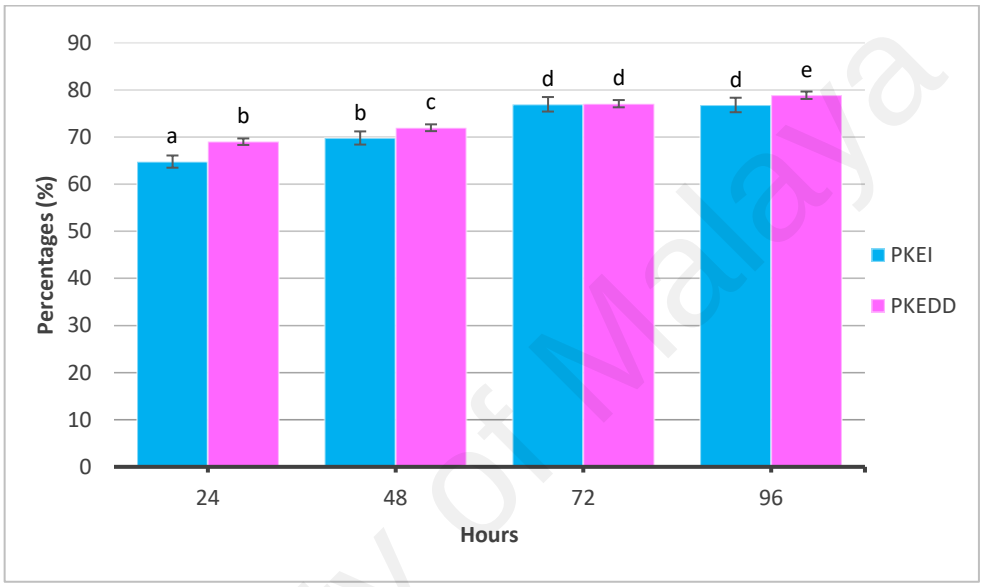
Note : Mean ± SD for 2 treatments are significantly different ($p<0.05$)
CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass and
CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass

Figure 4.27 *In Sacco* Digestibility of Commercial Pellets Added 7.5% Herbs and Napier Grass (*Pennisetum purpureum*) from 24 to 96 hours

The results showed that commercial pellets added with 7.5% DD herbs and Napier grass was significant compared to commercial pellets added with 7.5% imported herbs and Napier grass at 24 and 96 hours of incubation.

4.4.12 *In Sacco* Digestibility of PKE Pellets Added with 7.5% Herbs

Herbal formulation from local and imported herbs was upgraded to 7.5%. The *in sacco* digestibility were recorded from 24 until 96 hour of incubation. The digestibility results are as follow :



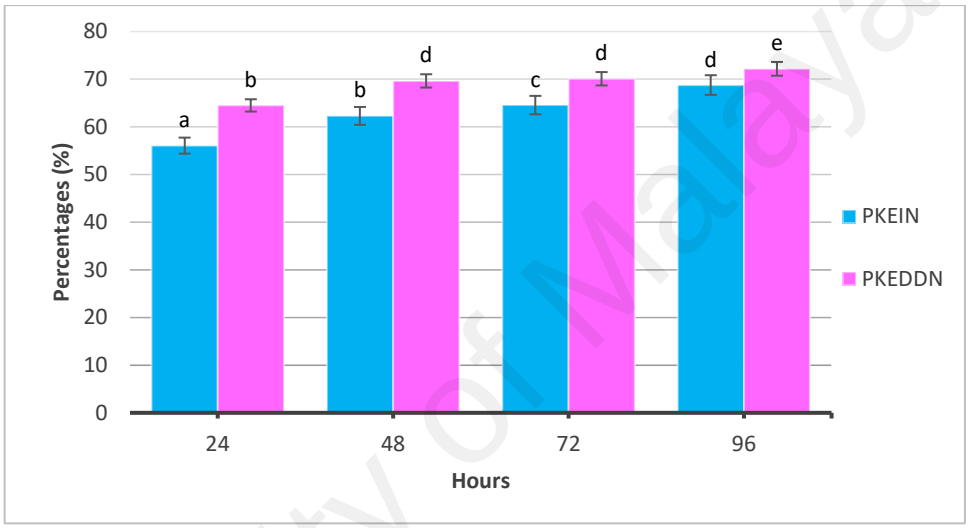
Note : Mean ± SD for 2 treatments are significantly different ($p<0.05$)
PKEDD = PKE Pellets Added with DD Herbs and
PKEI= PKE Pellets Added with Imported Herbs

Figure 4.28 *In Sacco* Digestibility of PKE Pellets Added with 7.5% Herbs from 24 to 96 hours

The results showed that digestibility of PKE pellets added with 7.5% DD herbs was significant compared to PKE pellets added with 7.5% imported herbs at 24 and 96 hours of incubation. The digestibility percentage of PKE pellets added with 7.5% DD herbs was as follows : 69.02% (24 hours), 71.98% (48 hours), 77.09% (72 hours) and 78.88% (96 hours).

4.4.13 *In Sacco* Digestibility of PKE Pellets Added with 7.5% Herbs and Napier Grass (*Pennisetum purpureum*)

The treatment containing 7.5% herbs and was added with PKE pellets and Napier grass. The *in sacco* digestibility percentages were calculated and the results are as follow :



Note : Mean ± SD for 2 treatments are significantly different ($p<0.05$)
PKEDDN = PKE Pellets Added with DD Herbs and Napier Grass and
PKEIN = PKE Pellets Added with Imported Herbs and Napier Grass

Figure 4.29 *In Sacco* Digestibility of PKE Pellets Added 7.5% gm Herbs and Napier Grass (*Pennisetum purpureum*) from 24 to 96 hours

The results showed that digestibility of PKE pellets added with 7.5% DD herbs and Napier grass was significant compared to PKE pellets added with 7.5% imported herbs and Napier grass at 24 and 96 hours of incubation. The digestibility percentage of PKE pellets added with 7.5% DD herbs was as follows : 64.50% (24 hours), 69.64% (48 hours), 70.10% (72 hours) and 72.18% (96 hours).

4.5 *In Vivo* Digestibility Technique

In vivo techniques were used in this study to evaluate the effective of herbs added to pellets on the ruminant digestive system. Finding from the *in vitro* and *in sacco* techniques indicated that findings, 5.0% local herbs (DD herbs) supplemented 95.0% commercial pellets was produced the best result. Therefore, this was used in the *in vivo* experiment. This *in vivo* trial was conducted on a goat farm located in Bidor, Perak which is surrounded by oil palm plantations.

4.5.1 Body Weight Increase of Goats' Kid Group When Fed Commercial Pellets Supplemented with 5.0% DD Herbs and without DD Herbs from 4 Months to 9 Months Old

The feeding was initiated at age 4 months and continued until the kids reached 9 months old. Control group was fed commercial pellets only. The results of body weight goats' kid gain when feed with commercial pellets added 5.0% DD herbs and commercial pellets from 4 months to 9 months of kids ages are as stated below:

Table 4.10 Body Weight of Goats When Fed with Commercial Pellets Added with 5.0% DD Herbs and Commercial Pellets without Herbs

Sex / Month	Body Weight (kg)						
	4	5	6	7	8	9	Average gains/Months
Male Fed with DD Herbs & Commercial Pellets	14.86±0.47	18.42±0.49	23.20±0.71	25.90±1.05	33.08±1.29	38.64±0.91	4.76
Male control with Commercial Pellets	14.78±0.64	16.20±0.76	18.54±0.67	23.46±0.85	25.96±0.96	30.80±0.87	3.20
Female Fed with DD Herbs & Commercial Pellets	13.70±0.57	16.72±0.69	19.30±0.91	24.16±1.76	28.94±1.03	35.16±0.96	4.29
Female control with Commercial Pellets	13.80±0.55	14.68±054	17.00±0.79	19.74±1.09	23.64±1.31	28.63±1.19	2.97

Mean ± SD for 2 treatments are significantly different ($p<0.05$) to different sex

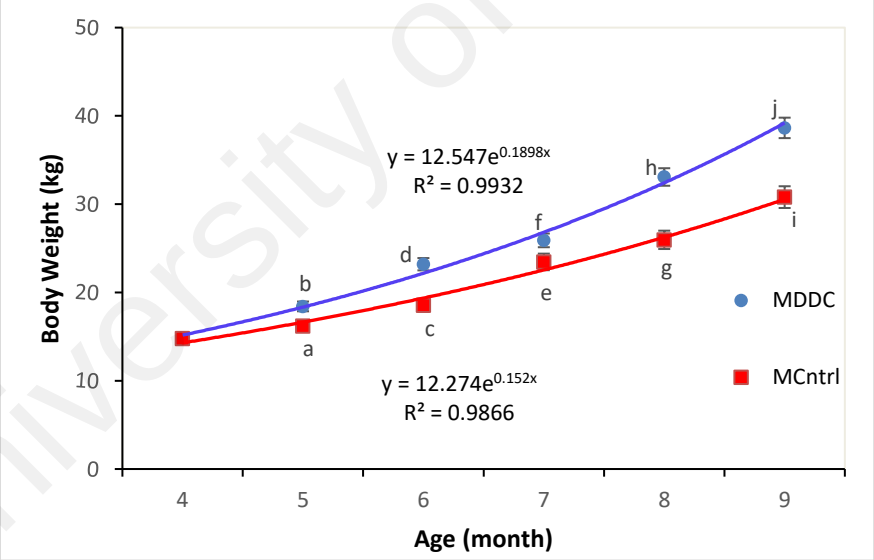
The results showed that body weight of male and female goats' kid group was significant when fed diet with commercial pellet added with 5.0% DD herbs.

4.5.2 Growth Performance of Goats' Kid Fed with 5.0% DD Herbs Added with Commercial Pellets

Supplementary feeds was provided to both the control and tested groups. Their body weights were measured every 2 weeks.

4.5.2.1 Growth of Male Goats Kid Fed with Commercial Pellets Added 5.0% DD Herbs and Male Goats Kid Fed with Commercial Pellets

The growth results were shown below :



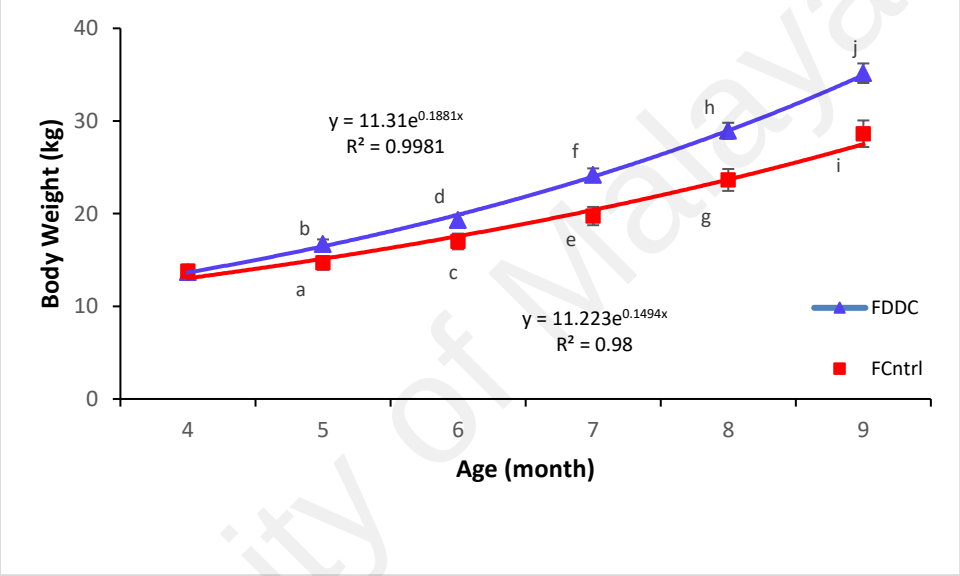
Note : Mean ± SD for 2 treatments are significantly different ($p<0.05$)
MDDC = Male Fed with Commercial Pellets Added with DD Herbs and
MCntrl = Male Fed with Commercial Pellets

Figure 4.30 Body Weight of Male Goats Kid Fed with 5.0% DD Herbs Added with Commercial Pellets and Male Goats Kid Fed with Commercial Pellets

Male goats kid fed with 5.0% DD herbs added with commercial pellets has significant body weight compared to male goats kid fed with commercial pellets from age 5 to 9 months old.

4.5.2.2 Growth of Female Goats' Kid Fed with Commercial Pellets Added 5.0% DD Herbs and Female Goats' Kid Fed with Commercial Pellets

The female goats kid fed with the treatments once a day as supplementary feed in the morning (8.00 to 9.00 a.m.). Every 2 weeks, the kids' weight were measured and recorded. The growth results were shown below :



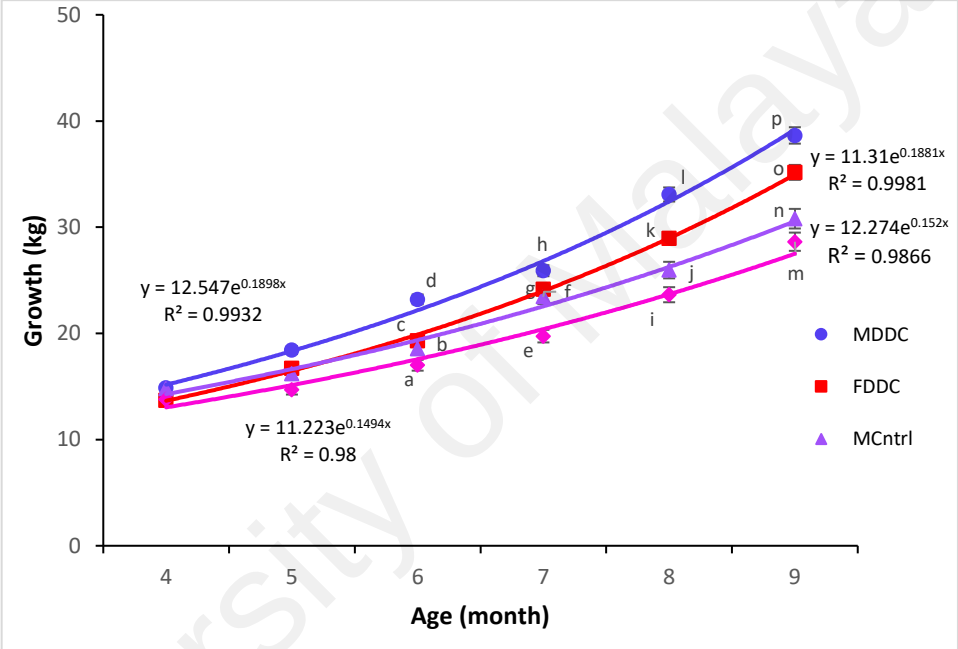
Note : Mean ± SD for 2 treatments are significantly different ($p<0.05$)
FDDC = Female Fed with Commercial Pellets Added with DD Herbs and
FCntrl = Female Fed with Commercial Pellets

Figure 4.31 Body Weight of Female Goats Kid Fed with 5.0% DD Herbs Added with Commercial Pellets and Female Goats Kid Fed with Commercial Pellets

Body weight of female goat's kid increase every month. The female kids fed with 5.0% DD herbs added with commercial pellets has significant body weight compared to female kids fed with commercial pellets from age 5 to 9 months.

4.5.3 Growth Performance of Goats' Kid Fed from 4 months to 9 months

The following figure shows the comparison of growth in four groups of goats kid fed from 4 to 9 months. One group of different sex was fed with 5.0% DD herbs added to commercial pellets as the treatment, while the other group of different sex was fed with commercial pellets as control group. The results are shown below :



Note : The *in vivo* digestibility data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between the means (n=5) was determined by the Duncan's multiple range test at 95% least significance difference ($p<0.05$)
MDDC = Male Fed with Commercial Pellets Added with DD Herbs,
MCntrl = Male Fed with Commercial Pellets,
FDDC = Female Fed with Commercial Peliets Added with DD Herbs and
FCntrl = Female Fed with Commercial Pellets

Figure 4.32 Growth Performance Comparison of Feeding Goats Kid from 4 months to 9 months

The groups fed with 5.0% DD herbs and commercial pellets had significant growth rates compared to controls from age of 5 to 9 months old. Male kids fed 5.0% DD herbs and commercial pellets have significant growth performance compared to the other kids fed with commercial pellets without added herbs. Otherwise, the female fed with 5.0% DD herbs and commercial pellets have significant growth performance compared to male and female in the control group.

CHAPTER 5: DISCUSSION

In this study, the focus was to improve digestibility in ruminants, by adding herbs which are expected to increase the digestibility of feeds in the ruminant digestive system. The purpose of adding herbs in the diet is also to reduce the quantity of food rather than merely to improve the nutritive value of the feeds. Thus the rate of digestibility and feed consumption can be reduced and the level of nutrient can be increased. This study was conducted to investigate the potential use of local herbs combined with pellets and to compare imported herbs to attain the best nutritious value of feed.

The results show that herbs added commercial pellets or PKE pellets increase the digestibility. Some medicinal herbs are not just merely to improve appetite or as digestion stimulants; they have the tendency to sustain good health and improve animal performance (Mirzaei-Aghsaghali *et al*., 2012).

5.1 Proximate Analysis and Chemical Composition Test for All Treatments

Herbs in the form of oils have dissuasive activities against a number of rumen micro-organism. Addition of herbs could improve feed fermentation and increase volatile fatty acids production in the rumen (Mazaher Hashemi, 2014). The maintenance of microbes in the rumen is mainly dependent on the ruminant host that provides necessary physiological condition for survival; required energy for their growth (Jafari Khorshidi *et al*., 2009). Carbohydrates fermentation takes place in the rumen, which produces ATP that is provided by rumen bacteria (Nocek *et al*., 1980).

Garlic one of the DD herbal formulations, had a dry matter and contained 91.79% in DD herbs on DM basis, while 88.68% in imported herbs. Crude Protein (CP) in garlic is about 15.76% Busqueat *et al.* (2005) found that garlic oil decreases acetate concentration and increases butyrate and propionate concentration. Rumen butyric acid has a specific effect on the fat content of milk. Rumen production of butyrate may have an adverse impact on production of glucose and lactose synthesis in dairy cows fed on grass silage-based diet. However, the capacity of the ketogenic in the intestinal wall and liver cannot be exceeded despite high production of rumen butyrate (Miettinen and Huhtanen, 1996).

Calorimetric studies by Graham *et al.* (Blaxter, 1962) showed that a low acetate or butyrate used by sheep with low efficiency lead to an increase in high heat in production comparison with acetate propionate. Reviews by Blaxter *et al.* (1962) revealed that a diet based on hay which was shredded and cubed, has metabolisable energy content (M/D) of about 8.5 MJ/kg of dry matter.

In another experiment, Fraser's (2007) investigation revealed cinnamon leaf oils effect on rumen microbial fermentation in two continuous culture system where by significant reduction in the number of rumen protozoa was observed in the group which received cinnamon leaf oils in comparison with the control group. Cinnamon chemical composition has a gross energy of 4155.16cal/gm in DD herbs but 4116 Cal/gm in imported herbs. Calcium content of DD herbs cinnamon is 1.26% but 0.10% in imported herbs. These differences in chemical composition may produce different effects in the digestive system of ruminants.

Chemical composition of DD herbs results contains higher gross energy (GE) (4005.33 Cal/gm) when compared with Napier grass (3898.02 Cal/gm). The highest energy value was found in commercial pellets with 4129.59 Cal/gm in GE compared with

imported herbs; 4116.01 Cal/gm and PKE pellet; 3902.43 Cal/gm. GE is the energy generated when a feed is burned (Weiss, 1999). However, the amount of the gross energy does produce the same productive effect in ruminant animals (Fox *et al*., 1990).

Ether Extract (EE) in commercial pellet; 4.87% is higher compared with Napier; 2.52%, DD herbs; 1.05% and PKE pellet; 0.48%.EE is a laboratory index to express the total fat content of food. Silva *et al.* (2011) reported that EE can estimated the impact of the energy content. Nutritive value is primarily associated with the fatty acids and their content can be as high as 80% of the EE for concentrates less than 50% for forages (Palmquist, 1988).

Neutral detergent fibre (NDF) in Napier grass is higher: 71.38% compared with a mixture of herbal formulation which is only 45.76%. NDF is an insoluble fraction containing all the components of plant cell walls after boiling food samples in neutral detergent solution. When NDF is low, it lowers the rate of digestion but it can only be broken down by microorganisms in the digestive tract (Kammes & Allen, 2012).

The NDF values can be used to predict dietary intake of ruminant animals (Linton & Allen, 2008). When NDF intake is higher in ruminants, the digestion of food can be reduced. Therefore, mixtures of local herbal formulation are good supplements as ruminant feed since they help digestion in rumen as well as reduce cost.

Napier grass contains high crude fibre (29.47%) compared to herbal formulation only (18.73%). Crude fibre is one of the chemical index of food samples. It can be calculated as the difference in weight of the sample before and after combustion. High crude fibre causes difficulty in biological digestion and affects the rate of digestion in the rumen of ruminants.

Crude protein (CP) is higher in Napier grass compared with herbal formulation. Crude protein is an estimate of total protein content of food which is determined by

analysing the nitrogen content of animal feed and multiplying the result by 6.25 (Van Soest and Fox, 1992). Crude protein, containing substances such as ammonia, amino acids and nitrate. Therefore, crude protein is an important indicator of protein in animal feed crop, and protein nitrogen budgets are important in assessing the nutritional value (Van Soest, 1994).

Herbal formulation has low dry matter compared with Napier grass. Increased feed intake of ruminants, on poor quality forages that are supplemented with bypass protein, slowly releases amino acids, peptides and branched chain fatty acids to the rumen milieu from the protected protein (Useni, 2011; Silva and Ørskov, 1988a). Even though in most studies there was no evidence of increased digestibility with such supplements in predominately fibre based diets.

Fernandez, & Rodriguez (2013) suggested when cows have high roughage, it limits physical food intake; hence cows should not eat foods that exceed the rumen contents. A physical limitation to determine the food intake is part of the cow's function and the rate of digestion; therefore, it is the rate of passage of food from the gut. If the rate of digestion can be improved, it increases the rate of circulation which in turn allows the animals to consume dried food. If the rate of digestion is slow, food intake is limited due to a full rumen. (Yambayamba and Price, 1991).

In general, a proportion of the digestible feed dry matter is converted to VFA, methane and carbon dioxide and the balance is assimilated into microbial cells. Otherwise, a number of other factors impact rumen fermentation which can be termed as amount and composition heterogonous (Abbas Pour *et al*., 2008). Ando *et al*. (2003) found that feeding 200 mg of dried herb (mint) to cows fully decreased the number of protozoa into 50%. According to Bird *et al.* (1990), protozoa have an overall negative effect in the rumen, especially in ruminants which are fed low protein forage diet.

Protozoa will engulf and digest bacteria and reduce the biomass of bacteria in the rumen (Firkins *et al*., 2007) and subsequent supply of microbial protein. Gradually moderate protein (12-20% crude protein) and mineral usually within an acceptable range even calcium and sodium should be added if feed is at high levels. Fermentation results in up to 20% of the digestible energy intake in ruminants are lost as heat and methane. One of major disadvantages is that proteins that are fermented in the rumen are not then sources of amino acids for the animal because they are hydrolysed and their constituent amino acids are deaminated by microbes.

5.2 *In Vitro* Digestibility

The *in vitro* digestibility technique is found to be easier to be employed in comparison with *in sacco* analysis, because it can avoid the need for surgical fistulation in the gastro intestinal tract of ruminants (Mabjeesh *et al*., 2000). Tilley and Terry's method (1963) has also been shown to be more accurate than digestibility predictions based on the chemical composition of the feed digestion (Van Soest, 1994).

5.2.1 *In Vitro* Digestibility of Commercial Pellets

In vitro technique simulates rumen digestion and this has been used to investigate the effects of additional commercial pellets. According to the results commercial pellets digestibility was 65.74% at 24 hours, 72.87% at 48 hours, 75.56% at 72 hours and 78.36% at 96 hours. When added to imported herbs the commercial pellets,

digestibility a slight increase showing 67.18% (24 hours), 72.92% (48 hours), 77.01% (72 hours) and 79.02% (96 hours). A significant increase was found when commercial pellets were supplemented with DD herbs, showing digestibility of 68.27% (24 hours), 76.27% (48 hours), 80.13% (72 hours) and 80.95% (96 hours).

These results indicate commercial pellets had a higher digestibility rate when were added herbs compare to PKE pellets. Digestion rate is even higher in the addition of DD herbs rather than imported herbs.

In vitro digestibility of Napier grass in was 42.47% (24 hours), 46.20% (48 hours), 55.98% (72 hours) and 62.68% (96 hours). Ruminants require grass as balanced diet. Therefore, the results of herbal supplements and Napier grass (*Pennisetum purpureum*) showed an increase in digestion. Treatment of commercial pellets with the imported herbs showed digestion rates of 62.37% (24 hours), 67.21% (48 hours), 69.83% (72 hours) and 73.91% (96 hours), while treatment consisting commercial pellets with DD herbs showed increased digestibility: 64.51% (24 hours), 71.03% (48 hours), 71.55% (72 hours) and 75.43% (96 hours). When DD herbs were added into Napier grass and commercial pellets, *in vitro* digestibility in the goat's rumen increased.

The additional number of herbs also revealed an increase in the rate of digestion. Increased addition of DD herbs to 5.0% showed higher digestibility than that recorded at 2.5%. The results with 5.0% of imported herbal added commercial pellets were 66.03% (24 hours), 72.00% (48 hours), 77.07% (72 hours) and 78.79% (96 hours). When using 5.0% DD herbs added commercial pellets, *in vitro* digestibility indicated 68.40% (24 hours), 73.50% (48 hours), 78.28% (72 hours) and 80.22% (96 hours).

Due to the need for a balanced diet for ruminant, *in vitro* digestibility tested to Napier grass, and the Napier grass added with commercial pellets and DD herbs. Napier grass added with commercial pellets and imported herbs were also conducted. The

results showed that treatment with a mixture of DD herbs, commercial pellet and Napier grass showed the increase percentage of *in vitro* digestibility than in 5.0% imported herbs mixed with commercial pellets and Napier grass.

7.5% herbs were tested to determine the appropriate number of herbs to improve *in vitro* digestibility. Commercial pellets were added DD herbs and Napier grass had a higher *in vitro* digestibility than 7.5% of imported herbs added commercial pellets and Napier grass.

However, the increase in the quantity of 7.5% DD herbs amount did not show significant percentage of *in vitro* digestibility compared to 5.0% of DD herbs. Comparisons revealed with addition of DD herbs in *in vitro* digestibility between 5.0% to 2.5%, 5.0% was found to be more significant.

The best quantity was 5.0% of herbs added which had a favourable economic impact and a high rate of *in vitro* digestibility. The results of the experiment showed that the amount 5.0% of DD herbs has higher digestive rates than the amount 2.5% DD herbs mixed with commercial pellets and Napier grass. When compared to imported herbs mixed with commercial pellets and Napier grass, 5.0% DD herbs mixed with commercial pellets and Napier grass also showed better digestibility.

According to Palic and Leeuw (2009), although only a small number of samples were used in the *in vitro* technique, the results of their study clearly indicated that the digestion of organic matter (OMD) which is a complete diet for ruminants can be successfully determined by this technique. The main problem lies in the use of nylon bags which are difficult to distinguish from gastro intestinal losses bag (Ross *et al*., 2013).

In vitro technique has been widely used in food and assessment in the study of rumen fermentation. This technique allows the selection of foods and food constituents for high efficiency of microbial protein synthesis in the rumen with high dry matter

digestion and provides the basis for the development of feeding strategies to maximise the fixing substrate into microbial cells. This can lead to an increase in protein supply to the intestine and reduce methane emissions from ruminants.

5.2.2 *In Vitro* Digestibility of Palm Kernel Expeller (PKE) Pellets

Pastures quality vary depending on soil moisture and minerals content. Pasture-based diet can reveal a lack of protein in certain times in the same year. The composition of the basic diet of grass with added supplements will determine the best option for concentrated feed.

Palm Kernel Expeller (PKE) is a by-product of the palm oil industry in Southeast Asia, especially in Malaysia. It is derived from the fruit of palm nuts after oil is extracted mechanically. This feed is dry and has a reasonable level of energy (ME) and protein, and relatively easy to introduce to ruminants in many farm systems (Alimon, 2004). PKE pellets prices also are lower than commercial pellets and it is an alternative supplement in ruminant diets.

Due to the small particle size, PKE pellets has low effective fibre (30% of NDF) so it will not cause the production to chew and drool more. PKE pellets has starch and low sugar content which can cause ruminants not to over-eat and hence no acidosis problems. However, PKE pellets taste is not very good, so it takes time to tune for ruminants. Therefore when ruminants are first introduced to PKE pellets they need to be in deficit and have access to food and water.

Supplements become essential not only to improve the quality of livestock but as a nutrition supply. However, the experimental findings obtained from *in vitro*

digestibility of PKE pellets are lower than commercial pellets, but with the addition of herbs either DD herbs or imported herbs; it increased the rate of digestion in the ruminant digestive system.

The experimental results showed that *in vitro* digestibility of PKE pellets is 65.38% (24 hours), 67.89% (48 hours), 68.29% (72 hours) and 75.69% (96 hours). 2.5% of imported herbs increased rate of *in vitro* digestibility. Another 2.5% of DD herbs, showed higher *in vitro* digestibility as 58.07% (24 hours), 65.98% (48 hours), 70.77% (72 hours) and 74.86% (96 hours).

Due to the ruminant diet consisting only of grass, the *in vitro* digestibility for 2.5% imported herbs, PKE pellets and Napier grass is found to be lower than using 2.5% DD herbs added to PKE pellets and Napier grass. After the dose is increased to 5.0% of herbs, there are significant to DD herbs in amount of 5.0%, PKE pellets and Napier grass. The findings for the 5.0% imported herbs, PKE pellets and Napier grass were lower than 5.0% DD herbs dose.

When the dose was increased to 7.5% to see the effectiveness of herbal *in vitro* digestibility, the results obtained from the experiments showed that not significant imported herbs and herbal DD when added to the PKE pellets and Napier grass compare to the results of 5.0% herbs added to the same diet treatments. Therefore, the best amount is 5.0% DD herbs, PKE pellets and Napier grass.

5.2.3 Gas Production of *In Vitro* Digestibility

The experiments conducted showed that the use of locally produced herbal mixture 5.0% gas production is higher than other mix amounts. However rumen needed for *in vitro* Tilley and Terry and the Gas Production techniques, are not always willing to laboratory analysis (Palic and Leeuw, 2009). Therefore further research, with the use of technique *in sacco* is needed to confirm the findings of this study.

Gas production method is widely used to assess the nutritional value of different classes of feed; the *in vitro* technique is more efficient than others to determine the nutritional value of foods that contain tannins.

In vitro rumen fermentation gas production method has several major advantages:

(1) it has the potential for screening a large number of food sources, for example in the breeding program for the development of varieties and cultivars value of good nutrition,

(2) it can also be a great value in the development strategy of local supplement added to existing food constituents conventional and unconventional to achieve maximum efficiency in the rumen microbes,

Consequently, supplement has become one of the most important factors in a pasture-based and supplementary have been prevent deficit (Holmes, 2007), but it increases when pasture ruminant performance alone is in adequate (Pashaei *et al*., 2010).

Microbial cells, that are synthesised from the feed resource uses ATP that is generated in the formation of VFA from the feed to provide energy for synthesis (Fellner, 2005). The microbes are lost from the rumen pool either by passage out of the rumen to

be partially digested in the intestine or by death and breakdown within the rumen and formation of VFA, CO_2 and methane (Genzebu & Tesfay, 2015). Lysis and degradation in the rumen is in efficient as it makes the protein of microbes unavailable as such to the animal (Jane *et al*., 2009a).

Microbial cells are reduced than the substrate fermented, the quantity of microbial cells leaving the rumen per unit of carbohydrate consumed is related to methane production. The efficiency of microbial growth is then a primary determinate of the quantity of methane produced (Dijkstra *et al*., 1992). The end products of rumen fermentative digestion are governed by the feed, the rate of consumption of feed, the balance of nutrients in the feed for microbial growth and the balance of micro-organisms that develop in the rumen (Moss *et al*., 2000).

5.2.4 Comparison between Commercial Pellets and PKE Pellets of In *Vitro* Digestibility

The results from the experiment showed that commercial pellets *in vitro* digestibility is better to than PKE pellets, from incubation of 24 hours, 48 hours, 72 hours and 96 hours. Commercial pellets *in vitro* digesitibility is higher than PKE pellets at measured.

Moreover, PKE pellets in the long term can lead copper (Cu) to accumulate at a faster rate than pasture-fed ruminant. Sheep fed with poor quality forage with branched chain VFA has been reported to increase the apparent flow of Microbial-N to the duodenum. The apparent stimulation of microbial growth with branched chain VFA has also been shown to increase feed intake (Hemsley and Moir, 1963).

Thus it was proposed that commercial pellets as dietary supplements be used in *in vivo* techniques at selected farms in Bidor, Perak. However, to support this finding, *in sacco* digestibility technique used the commercial and PKE pellets as treatments. The result of these techniques will support *in vitro* digestibility results. This was done with the intentions of making the research cost-effective.

5.2.5 Comparison between DD herbs and Imported Herbs of *In Vitro* Digestibility

The experimental results showed that diet with added 2.5% DD herbs had higher *in vitro* digestibility rate than 2.5% imported herbs. When the herbs amount was increased to 5.0% the rate of *in vitro* digestibility further increased compared to the diet with 5.0% imported herbs.

According to proximate analysis, DD herbs showed lower percentage in crude protein (CP), crude fibre (CF) and ether extract (EE) compared to imported herbs. When crude fibre (CF) content is higher, the energy content of the feed is lower because crude fibre is considered indigestible. Measuring crude fibre was one part of the original system of analysing the "digestible" fraction in feedstuffs (Moore and Undersander, 2002b). Higher the CF content will lower the *in vitro* digestibility.

The crude protein (CP) content of a feed sample represents the total nitrogen (N) in the diet, which includes not only true protein but also non-protein nitrogen. Crude protein in feeds for ruminants can be further fractionated according to their rate of breakdown in the rumen (National Research Council, 2001). Higher the percentage of CP will lower the *in vitro* digestibility.

Fats in feed samples are typically determined through ether extract (EE). In addition to fat, EE may solubilize some other compounds like plant pigments, esters and aldehydes (Moore and Kunkle, 1999). The higher the EE percentage will lower the *in vitro* digestibility.

5.2.6 Comparison of *In Vitro* Digestibility in Different Dose of DD Herbs

According to *in vitro* digestibility results, digestibility percentage for 2.5% DD herbs when added with commercial pellets showed was 68.27% (24 hours), 76.72% (48 hours), 80.13% (72 hours) and 80.95% (96 hours). The rate of digestibility for 5.0% DD herbs when added with commercial pellets was 70.82% (24 hours), 80.11% (48 hours), 82.34% (72 hours) and 83.51% (96 hours). Thus the rate of digestion of 2.5% DD herbs when added to the commercial pellets are significantly compared to 5.0% DD herbs when added with commercial pellets.

The percentage of digestibility for 7.5% DD herbs added with commercial pellets was 71.55% (24 hours), 81.20% (48 hours), 83.06% (72 hours) and 83.73% (96 hours). It is not significant when compared with the rate of 5.0% DD digestive herbs were added with commercial pellets.

All treatment that uses 2.5% of DD herbs when added with commercial pellets or PKE pellets and Napier grass also showed lower digestibility rate when compared to 5.0% DD herbs added with commercial pellets or PKE pellets and Napier grass as well. The rate of digestibility for 2.5% DD herbs when added with commercial pellets and Napier grass was 64.51% (24 hours), 71.03% (48 hours), 71.55% (72 hours) and 71.45% (96 hours). The rate of digestibility for 5.0% DD herbs when added with

commercial pellets and Napier grass was 68.40% (24 hours), 73.50% (48 hours), 78.29% (72 hours) and 80.22% (96 hours). The *in vitro* digestibility of 7.5% DD herbs added with commercial pellets and Napier grass was 69.18% (24 hours), 74.06% (48 hours), 78.78% (72 hours) and 80.67% (96 hours).

The rate of digestibility for 2.5% DD herbs when added with PKE pellets was 67.10% (24 hours), 69.07% (48 hours), 75.38% (72 hours) and 76.32% (96 hours). The rate of digestibility for 5.0% DD herbs when added with PKE pellets was 68.68% (24 hours), 71.01% (48 hours), 78.18% (72 hours) and 80.22% (96 hours).

Digestibility rate for 2.5% DD herbs when added with PKE pellets and Napier grass was 68.71% (24 hours), 71.38% (48 hours), 78.69% (72 hours) and 82.37% (96 hours). The rate of digestibility for 5.0% DD herbs when added with PKE pellets and Napier grass 68.71% (24 hours), 71.38% (48 hours), 78.69% (72 hours) and 82.37% (96 hours).

But when the 7.5% DD herbs added with commercial pellets and Napier grass, digestibility rate was 69.18% (24 hours), 74.06% (48 hours), 78.78% (72 hours) and 80.67% (96 hours). And when the 7.5% DD herbs added with PKE pellets, the digestibility rate were 68.71% (24 hours), 71.38% (48 hours), 78.69% (72 hours) and 82.37% (96 hours). The digestibility rate for 7.5% DD herbs when added with PKE pellets and Napier grass was 61.87% (24 hours), 68.50% (48 hours), 77.26% (72 hours) and 78.90% (96 hours).

Increasing the dose to 7.5% DD herbs is not cost effective since it has no significant effect on the percentage of digestion. However, the increase in the dose of 2.5% herbal DD herb to 5.0% of the herbs is significant because it shows a significant increase in percentage.

The experimental results showed that the 5.0% DD herbs *in vitro* digestibility rate is better than DD herbs with a dose of 2.5%. 7.5% DD herbs will increase the rate of digestion, but it is not significant if comparison is made with 5.0% doses. Thus the 5.0% dose passed the test and the next technical test is *in sacco* digestibility to gain convincing finding.

5.3 *In Sacco* Digestibility

In sacco digestibility has been used and recommended by Quin *et. al*. (1938) to evaluate the degradation of foodstuffs for DM and N in the rumen (Van Keuren and Heinemann, 1962). Nevertheless, there are various other factors that affect the rate of assessment and the ability to digest food through this method (Mehrez and Ørskov, 1977). If this method has standardised protocols then results obtained with this technique can be used to provide the rate of digestion in the rumen of ruminants (Ørskov and McDonald, 1979).

The conclusion drawn by researchers is if the size of the nylon bag used is big, it will allow free movement in the substrate. This technique is deemed to be highly useful for the assessment of digestion (Chaudhry and Webster, 2001). Although requires an estimation of the amount of protein degradation in rumen (Agricultural Research Council, 1984). Moreover, this technique is found to be a convenient and reliable technique to assess DM and protein degradation in the rumen (Thomas, 2004).

The study involves the rate of digestion in the rumen of ruminants. The materials tested were incubated in the rumen of fistulated goats for 24 to 96 hours using nylon bags that have standard sizes for all treatments. After incubation process, the nylon

bag was rinsed with tap water and dried for 48 hours at a temperature of 72ºC to gain dry matter weight. Then calculation for the rate of *in sacco* digestibility was made.

The findings from the study made a comparison of the percentage of *in sacco* digestion between two different types of pellets such as commercial pellets, PKE pellets and Napier grass. Then a comparison was made on the effects of both types of herbs to the percentage of *in sacco* digestibility. The two types of herbs are local herbs (herbs DD) and herbal import.

Besides the herbal dosage was also tested to find the most appropriate dose to improve digestion rates in *in sacco* digestibility. There are three tested doses: 2.5%, 5.0% and 7.5% of herbs. 2.5% dose is 25 gm of herbal formulation added to 975 gm of pellets. The mixed formulation was put into a nylon bag with 2 gm per nylon bag containing treatment. 5.0% dose is 50 gm of herbs and 950 gm of pellet, while 7.5% is 75 gm of herbs added with 925 gm of pellets are added.

Herbal treatments with pellets were also tested with Napier grass. As the main food for ruminants is grass, hence the test with Napier treatment will give results that are needed to examine the effectiveness of herbs and pellet to increase the rate of digestion in ruminant animals. The results of the findings are discussed further.

5.3.1 *In Sacco* Digestibility of Commercial Pellets

Commercial pellets are supplements that help the ruminant animals receive a balanced diet because it has a higher protein content compared to grass. In this study, the *in sacco* digestibility was tested with commercial pellets, PKE pellets and Napier

grass. The primary aim was to investigate which pellets has higher digestibility in *in sacco* technique.

The results obtained from the experiment shows commercial pellets has a higher rate of digestion compared to Napier grass. The percentage of *in sacco* digestibility in commercial pellets shown after post-incubation period of 24 hours is 66.40%; 48 hours is 72.36%; 72 hours is 74.88% and 96 hours is 77.35%. Napier grass digestibility after post-incubation is 24 hours is 36.82%; 48 hours is 52.06%; 72 hours is 60.05% and 96 hours is 64.71%. The difference shows better digestion rate for commercial pellets compared to Napier grass.

Commercial pellets have a high percentage of digestion because it contains the following ingredients: corn, wheat, wheat bran, rice bran, soy bean, sesame meal, molasses, limestone, dicalcium phosphate, salt and food additives that have been finely ground and subsequently formulated into pellet. The rather high fibre content in Napier grass leads to improve digestive rate in *in sacco* technique which is much lower than commercial pellets.

The lower lignin content fibres provide greater strength and are more difficult to digest in the digestive system of ruminants (Tran, 2006). Napier grass has a lignin content of 10.8% (Zawawi *et al*., 2014). Lignin is harder to digest in the digestive system of ruminants. Cellulose is the component that makes the fibres stronger (Enayati, 2009) and difficult to digest. Cellulose content (12.4%) and hemicellulose (68.2%) in Napier grass is high (Zawawi *et al*., 2014).

The high percentage of *in sacco* digestibility in ruminants is good for growth because it shows the development of microbes present in the rumen, which will increase the digestion of consumed food. The food will be digested into energy and is absorbed by the ruminants which also boost their growth.

Therefore, goats can be fed with commercial pellet without affecting their growth performance. Additionally, commercial pellets will not only reduce feed costs but also save on eating limited grass (Rahman *et al*., 2014).

5.3.2 *In Sacco* Digestibility of PKE Pellets

Palm kernel expeller (PKE) is a palm oil industrial solid waste that has been used more as a supplementary diet for livestock grazing. It is used in the animal feed industry, particularly for bovines (Bottriell and Judd, 2011) as it is low in cost compared to other commercial pellets.

Various studies have shown that mixtures containing PKE pellets are cheaper than using conventional feedstuff and besides PKE and it do not affect the quality of milk production in ruminants (Wan Zahari and Razak, 2008). A study conducted in New Zealand suggests that with the use of food supplements, production of dairy products has increased three-fold from 2000 by 500,000 mt to 1,500,000 mt in 2012 (WWF report, 2014). Due to the less intensive systems in New Zealand, feed supplements are used to cover short-term deficits feed pasture and also have been employed for purposes of lactation (Dairy NZ, 2011). Some intensive dairy farms that produce 45% dairy products have used feed supplements throughout the year as part of their daily ration to increase milk production (Holmes and Roche, 2007).

From the *in sacco* digestibility results obtained PKE has a higher percentage of digestibility compared to Napier in the incubation period of 24 to 96 hours. The study reported that PKE contains approximately 55-70% fibre, has a high phosphorus to calcium ratio, which is important for healthy bones and teeth formation and biochemical

cycles (Thin, 2001). Furthermore, fat, fibre, minerals and protein in the PKE is suitable as nutrition for livestock (Wan Zahari and Razak, 2008). Finally, due to productions using mechanical screw extraction process, PKE is free of chemicals and genetically modified organisms (WWF report, 2014).

PKE pellets digestion study shows 63.2% for DM, 59.7% for CP, 65.6% for NDF, 64.9% for GE and DE focus is 13.4 MJ/GDM (Burke *et. al*, 2011). Moss and Givens (1994) their findings where digestion of 11 samples PKE *in vivo* in sheep fed with PKE maintenance 50% and 50% hay. This had increased digestion of CP, NDF, GE and DE concentration (75.0%, 73.0%, 75.0% and 15.3 MJ/GDM, respectively) in ruminants fed experiment. However, the average contents of CP, NDF, EE and GE were also higher (17.2, 68.5 and 10.8 g/100 GDM and 20.9 MJ/GDM) than those reported here (16.3, 65.2 and 9.3 g/100GDM and 19.4 MJ/GDM). The results for PKE pellets from MARDI laboratory test in this study shows that there are similarities from previous studies as follows; 90.93% for DM, 13.62% for CP, 3902.43 Cal/gm for GE, 73.46% for NDF and 0.48% for EE. The composition shows that PKE is suitable as livestock supplement.

5.3.3 Comparison between *in Sacco* Digestibility of Commercial Pellets and PKE Pellets

In Sacco digestibility findings showed that the commercial pellets have a higher percentage of digestion compared to PKE pellets. The commercial pellets *in sacco* digestibility percentage in incubation period of 24 to 96 hours in a water bath was 24 hours (66.40%), 48 hours (72.36%), 72 hours (74.88%) and 96 hours (77.35%). The rate of *in sacco* digestibility in PKE pellet was 24 hours (36.82%), 48 hours (52.06%), 72

hours (60.05%) and 96 hours (64.71%). Thus the commercial pellets rate of *in sacco* digestibility is better than PKE pellets.

The results show that the chemical composition of PKE fibre was 73.46% compared to commercial pellets (41.98%). It is caused by higher cellulose in PKE pellets compared to commercial pellets. Higher cellulose content in fibre is more difficult to digest, thus leading to a lower rate of food digestion.

To date there have been no other studies for comparative evidence of the results relevant obtained. Mardhati *et al.* (2011) from their investigation reveal that the addition of 20% PKE does not help the growth of animals compared with other commercial pellet which are corn-soy based. Waller (2007) reports that the use of cheaper supplements will reduce the performance of the flesh and the results do not lie in the maximum margin. Thus in reality it increases the cost of animal feed although prices are cheaper than commercial PKE pellets. Unfortunately, cheaper food is not an economical at option in livestock.

5.3.4 Comparison between *in Sacco* Digestibility of DD Herbs and Imported Herbs

The chemical composition of DD herbs shows that crude fibre in DD herbs was 18.73% and imported herbs was 28.68%. Due to the crude fibre content in the chemical composition of DD herbs was lower than in the imported herbs, hence it is deemed more digestible. *In sacco* digestibility rate in the DD herbs was found to be higher than in imported herbs.

25 gm DD herbs formulation was added to 975 gm of commercial pellets or 975 gm of PKE pellets which will provide higher *in sacco* digestibility rate than 25 gm imported herbs in the incubation period of 24 to 96 hours. The results also show that commercial formulation in 975 gm or 975 gm PKE pellets when added to 25 gm DD herbs and containing Napier grass will increase the rate of digestion in *in sacco* technique compared the digestion of Napier grass. The percentage of mixed formulation in *in sacco* digestion in 975 gm commercial pellets to 25 gm DD herbs is 68.81% (24 hours), 76.57% (48 hours), 77.38% (72 hours) and 82.95% (96 hours). While the percentage of *in sacco* digestibility in commercial pellets is 66.40% (24 hours), 73.06% (48 hours), 77.38% (72 hours) and 81.40% (96 hours).

The percentage of 975 gm of commercial pellets added with 25 gm imported herbs is as follows: 67.59% (24 hours), 72.36% (48 hours), 74.88% (72 hours) and 77.35% (96 hours). These results were lower compared to the commercial formulation of 975 gm added with 25 gm of DD herbs. Thus, DD herbs help increase the rate of *in sacco* digestibility better than imported herbs.

The rate of *in sacco* digestibility with 975 gm of PKE pellets formulated to 25 gm of DD herbs and Napier grass was 63.07% (24 hours), 66.86% (48 hours), 69.95% (72 hours) and 71.89% (96 hours). Whereas 25 gm of imported herbs formulation added to 975 gm of PKE pellets and Napier grass was 55.81% (24 hours), 61.48% (48 hours), 64.07% (72 hours) and 68.56% (96 hours). The rate of *in sacco* digestibility increased when doses of DD herbs are increased in experiments. Thus it is proved that the DD herbs help increase the rate of digestion in commercial pellets, PKE pellets or Napier grass. DD herbs were selected as the preferred as herbal substances to be tested in *in vivo* techniques on a goat farm in Bidor, Perak.

5.3.5 Comparison *In Sacco* Digestibility in Different Doses of Herbs Formulated Feeds (DD Herbs)

25gm dose of herbal DD shows the percentage of *in sacco* digestion being relatively low. Therefore, action to increase the dose to 50gm of herbs was made. From the results obtained the percentage of *in sacco* digestibility of 25gm herbal DD added with 975gm commercial pellets was 67.47% (24 hours), 69.61% (48 hours), 69.83% (72 hours), 74.63% (96 hours) while 50gm DD herbs were 68.81% (24 hours), 76.57% (48 hours), 80.27% (72 hours), 82.95% (96 hours).

When increasing the dose of DD herbs to 75gm, the percentage of *in sacco* digestibility of 75gm DD herbs added commercial pellets was 68.87% (24 hours), 76.86% (48 hours), 80.53% (72 hours) and 83.01%(96 hours). The percentage difference *in sacco* digestion was evident between the two doses. A comparison was also made between 50gm dose with herbal DD 75gm dose of herbs. However, the results reveal a minimal percentage difference in *in sacco* digestion 75gm compared to 50gm DD herbs.

Then, 25gm dose of DD herbs when added with PKE pellets, the percentage of *in sacco* digestibility was 60.02% (24 hours), 66.46% (48 hours), 68.61% (72 hours) and 70.26% (96 hours). When upgraded into 50gm of DD herbs added with PKE pellets, the percentage of *in sacco* digestibility was 68.39% (24 hours), 71.27% (48 hours), 76.77% (72 hours) and 78.64% (96 hours). The dose has been upgraded into 75gm of DD herbs added with PKE pellets with the percentage of *in sacco* digestibility as 69.02% (24 hours), 71.98% (48 hours), 77.09% (72 hours) and 78.88% (96 hours).

When 25 gm of DD herbs added with commercial pellets and Napier grass, the percentage of *in sacco* digestibility was 63.13% (24 hours), 67.62% (48 hours), 67.71% (72 hours) and 72.98% (96 hours). The percentage of *in sacco* digestibility of

50gm of DD herbs added commercial pellets and Napier grass was 67.47% (24 hours), 69.61% (48 hours), 69.83% (72 hours) and 74.63% (96 hours). The percentage of *in sacco* digestibility for 75gm DD herbs added with commercial pellets and Napier grass was *in sacco* digestibility was 67.69% (24 hours), 69.73% (48 hours), 70.06% (72 hours) and 75.23% (96 hours).

Increasing the dose to 75gm of herbal DD is not cost effective since it has no significant effect on the percentage of digestion. However, the increase in the dose of 25gm herbal DD herb to 50gm of the herbs is significant because it shows a significant increase in percentage.

Based on the comparative tests of the three herbal doses, digestive percentage results *in sacco* 50gm DD herbs has the highest percentage for any period of incubation. Therefore, 50gm herbal DD dose selected for testing in *in vivo* techniques because it is significant when compared to 25gm of DD herbs *in sacco* digestibility results but not significant when compared to 75gm of DD herbs *in sacco* digestibility results.

This *in sacco* technique was done to pick herbs and doses suitable for testing in the field. In addition, *in sacco* technique also aided with the chosen pellet that provided the highest resolution for use during the course.

5.4 *In Vivo* Technique

The *in vivo* study was performed on goats. Herbs supplemented_feeds (DD herbs) found to be more effective in the digestive rates in *in vitro* and *in sacco* improving was selected for *in vivo* technique testing.

The results of *in vitro* and *in sacco* shows that commercial pellet with 5.0% DD herbs was the best treatment. The growth rate was used to demonstrate the effectiveness of DD herbs added was commercial pellets. From the results obtained, the male goats' kid fed with DD herbs had a higher rate of growth and higher body weight when compared to the control group which fed with commercial pellets.

The body weight of these male: 5 kids were as follows: 15.05 kg (4 months), 18.25 kg (5 months), 22.94 kg (6 months), 25.53 kg (7 months), 32.86 kg (8 months) and 38.25 kg (9 months). Body weight of the control male goats kid were as follows: 14.65 kg (4 months), 16.27 kg (5 months), 18.69 kg (6 months), 23.12 kg (7 months), 25.52 kg (8 months) and 31.24 kg (9 months).

The results suggest that DD herbs increases body weight. Proximate analysis showed that DD herbs have good nutritional value for growing goats' kid. This is because DD herbs contain lower crude protein (9.64%), crude fibre (18.73%) and ether ester (1.05%), compared with commercial pellets or PKE pellets and Napier grass but high in dry matter (91.79%). Reddy and Reddy (1984) reported a decrease in the digestibility of these nutrients due to an increase of roughage and fibre contents in feeds. Adding DD herbs to commercial pellets significantly increased weight gain in goat's kid. This study show that 5.0% DD herbs added commercial pellets study can improve digestion for goats' kid and gain their weight.

5.4.1 Growth of *In Vivo* Technique

Rumen degradability of *in vivo* digestibility can be used to assess the potential of a meal as a source of nutrients for goats (Ndemanisho *et al*., 2007). Digestion of food may be affected by the relative ratios of nutrients and the presence of inhibitory components in the material (Lee and Lawrence, 1997). Data on digestion is important for the formation of a suitable feed. However, *in vivo* results may be affected by environmental conditions; temperature, salinity and pH (Ezquerra *et al*., 1997; Gimenez *et al*., 2009).

The differences of goat's kid growth have been observed according to their sex, male and female because different gender has different growth rates. Male goats kid shows higher growth rates compared with female goats' kid.

Observation was made for goats' kid growth rates at the aged of 4 months until 9 months old. The growth curve, being mass or cumulative weight plotted against age, was sigmoid. This curve can be described as a function of mature mass, fractional growth rate, and age (Owen *et al*., 1993). The body weight was recorded every two weeks to dedicate growth and plotting the growth rate graph.

5.4.1.1 Male Goat Kids

The kid diet supplemented with 5.0% herbal formulations DD had higher weight than kids fed not supplemented diet. This is evident from the comparison of weight (kg). The final weight of the kids in the supplemented group was 38.25kg, while that the control group kid was 31.24kg.

Differences between initial and final weight for supplemented kids was 23.69kg with an average daily weight gained was 157.33gm/day. The difference for control kids was 16.90kg with average daily weight gain of 118.67gm/day. Thus, the daily average weight difference for the tested group was higher. Prasad *et al*. (1995), Sikosana and Maphosa (1995) reported that feeding supplement to the goats produce higher growth rates. Review by Chaudhary *et al*. (2015) concludes that concentrate supplement at 1.0% provides higher economic returns in grazing alone, while concentrated feed pellets provide a maximum net return.

5.4.1.2 Female Goat Kids

The weight of the female goats in the test group and the control group were almost same. The test group given 5.0% herbal formula DD showed a higher weight than kids fed commercial pellets. This can be seen in the comparison of weight (kg) between goats consumed 5.0% DD supplemented and unsupplemented in males. The final weight of test group was 34.8kg, while that of the control group were 24.3kg.

Difference between in initial and final weight was 23.6kg. The average daily weight gained was 126gm/day. The difference between the initial and final weight

in the control group was 16.9kg. The average daily weight gain was 94.67gm/day. Thus, the average daily weight difference was higher in test group.

In vivo results show that 5.0% herbal DD supplemented diet increased weight in goats compared with control diet for the female goats. This agrees with the favourable result in the *in vitro* and *in sacco* tests. The impact of high digestive ruminants can increase weight and body size.

CHAPTER 6: CONCLUSION

The main problem of ruminant animals is constraints of grazing areas. Moreover, quality of nutrients is the problem in the food given to ruminant animals. This problem does not only lead to nutritional deficiencies in ruminant animal feed but also leads to poor weight and poor growth.

Many research has carried out to improve the rate of digestion employing a variety of techniques. The dietary supplements are one alternative to improve ruminant nutrition. For the purposes of this research, two types of pellets were tested in *in vitro* and *in sacco* digestibility.

The *in sacco* techniques using rumen in live ruminants showed almost similar results to *in vitro* technique. Cannula was installed in the rumen of goats to allow treatments of the material to be inserted and for the analysis of research. In the experiments, two types of herbs were added to pellets at the same level with the aims of testing and determining which pellets and herbs would increase the digestion with their maximum potential.

The food that ruminants eat has high fibre. The higher the fibre content is the lower the energy value of the feedstuff is (Lee, 2008). Adding herbs to pellets as ruminant feedstuff could help to increase digestibility in the animals and would increase the absorption of nutrients to support growth and to gain body weight as well as to improve the carcass quality with less feed intake in ruminants (DePeters *et al.*, 1997; Nik Fatihah and Noraida, 2012).

A number of studies have highlighted tremendous medical concerns through the systematic investigation of herbal remedies and their adverse effects on the vital

organs of animals (Elham *et al*., 2013). However, no studies showed that the herbs can help to increase the rate of digestion of ruminant animals.

Pellets added with herbs that give the best results in the *in vitro* and *in sacco* techniques were subsequently selected for testing in *in vivo* techniques. This was done with the intentions of making the research cost-effective. The findings in *in vitro* and *in sacco* experiments showed that the different pellets give different digestion rates. Commercial pellets are better alternatives in boosting the rate of digestion in ruminant animals in *in vitro* and *in sacco* digestion. Gas production in *in vitro* technique also revealed that commercial pellets have a higher record compared to PKE pellets.

The results obtained in *in vitro* and *in sacco* techniques showed that commercial pellets provide higher rates of digestion compared to PKE pellets, Neutral Detergent Fibre (NDF) content in PKE (73.46%) was higher than that in the commercial pellets (41.98%). CF content in commercial pellets (9.93%) was lower than that in the PKE pellets (23.00%). The high value of the NDF and CF tends to lead to low digestion rates in ruminant animals (Ball *et al*., 2007; Mirzaei-Aghsaghali and Maheri-Sis, 2011). Forage high in digestible soluble carbohydrate and low in NDF are typically desired in forages crop, but NDF is an important function in animal nutrition and should not necessarily be minimised, depending on the class of animal and the ration (Van Elfen, 2014).

Therefore, commercial pellets are suitable dietary supplements for ruminant animals in order to increase the rate of digestion and provide balanced nutrition. The *in vitro* and *in sacco* experiments revealed that the addition of DD herbs improved the digestion rate in the ruminant digestive system more than imported herbs.

In this research, herbs have used to increase digestibility in ruminant animals. The mixture of DD herbs with commercial pellets showed a higher gas

production compared to the mixture of imported herbs with commercial pellets. The gas production in *in vitro* technique showed that the digestion of food was higher when higher gas production was produced (Njidda and Ikhimioya, 2010; Olivares-Palma *et al*., 2013). This revealed that the rate of digestion in the treatment of DD herbs mixture with commercial pellets was higher compared with imported herbs mixture with commercial pellets.

In vitro and *in sacco* techniques tested three different dosages of herbs. The aim was to ensure that the appropriate dose used for *in vivo* studies in order to run an effective investigation. The results showed that the mixture of DD herbs with commercial pellets had the highest rate of digestion than the mixture of imported herbswith either commercial pellets or PKE pellets.

When the comparison was made between 2.5% DD herbs supplemented with commercial pellets and 5.0% DD herb supplemented with commercial pellets showed a higher rate of digestion. It was found to be more significant with an increased dose of DD herbs.

When the dose of DD herbs was increased to 7.5%, there was an apparent increase in the rate of digestion compared with dose of 5.0 % of DD herb. However, the increase in digestion rate was much lesser than the difference in the digestion rate of 2.5% and 5.0%. Thus, increasing the dose level from 5.0 to 7.5% is not significant and not cost-effective.

The findings in the *in sacco* technique showed that a mixture of herbs with DD herbs and commercial pellets showed he highest rate of digestion than commercial pellets mixed with imported herbs or mixed with imported herbs and PKE pellets. This supports the findings of the *in vitro* technique.

The most significant findings from the *in sacco* technique which was the dose of 5.0% DD herbs supplemented with commercial pellets was selected to field test using the *in vivo* technique. The use of DD herbs to improve digestion in ruminant animals has another advantage as it can save animal nutrition cost.

Experimental findings employing *in vivo* technique showed that the treatment group fed with 5.0% DD herbs diet gained more weight compared with the control group. The treatment group was fed with 100 gm food (5.0% DD herbs supplemented with commercial pellets) every day from the age of 4 months until 9 months old. The male kids in DD herbs treatment group gained 1.97 kg average weight in two weeks (increase of 13.49% from the initial average weight). Male kids from the control group gained 1.75 kg weight in two weeks (increase of 12.68% of initial one). Female kids in the treatment group (fed as same as male) showed an average increase of 1.41 kg weight in two weeks. The increase was 10.43% of the initial weight.

Female kids in the control group showed a significant weight gain of 1.18 kg in two weeks. The average weight of the female kids was 8.37%.

The result of in *vivo* experiments suggested that the fed group with 5.0% of DD herbs diet showed better weight gain than the control group, irrespective of the sex. Therefore, DD herbs with the dose of 5.0% diet can help kids to improve their digestive system and gain better weight.

The findings showed that the addition of DD herbs with commercial pellets help the digestive system and improve animal growth. Moreover, it can help farmers to decrease the food costs and to produce good quality food for livestock. Thus, the import of meat of ruminant animals will be reduced and the needs of local meat from ruminant animals will be met.

Suggestions

This same research is also proposed to be carried out in several states in Malaysia to verify the data to include micro-climate factor, the local flora which is eaten by goats running loose and also for different goat breeds. It aims to obtain accurate data showing the effectiveness of the addition of local herbs (DD herbs) and commercial pellets in improving digestion as well as boosting the growth rate of ruminant animals.

Proposals include viewing carcass which have grown and have been fed with supplements of commercial and 5.0% DD herbs and to make comparisons with carcass of the control group. In addition, the maturity rate for reproduction can be made for future studies involving the addition of dietary supplements and 5.0% DD herbs.

Limitations

There are several limitations to this research as a survey which used only small samples in a goat farm in Bidor, Perak. A research using a larger sample and more farms are required to gain more assertive results and for informed decisions to be obtained. In addition, the same research also can be carried out on other ruminant animals such as cattle, buffalo and sheep whereby a lot of data and better analysis in connection with the addition of herbs as a nutritional supplement can be derived from various other ruminant animals. This research conducted was limited to only goats because of the limited financial allocation provided for the research.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

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Appendix 1 : *In Vitro* Digestibility of 2.5% Herbs Added with Pellets

hour	CP	CPI	CPDD	CPIN	CPDDN	PKE	PKEI	PKEDD	PKEIN	PKEDDN	N
24	65.23	66.84	69.66	63.26	66.77	64.67	66.83	67.49	50.43	58.61	41.75
24	68.86	66.27	68.49	63.46	65.32	65.61	66.52	67.32	54.45	57.42	41.96
24	67.16	64.40	68.09	62.40	64.83	65.35	64.79	67.01	53.62	59.81	42.54
24	67.52	65.44	66.82	60.38	61.12	65.91	65.94	66.56	51.38	56.44	43.62
24	67.19	65.75	68.30	62.39	64.49	65.37	66.12	67.13	52.45	58.09	42.46
48	73.42	71.93	78.23	65.85	71.12	66.29	68.07	69.47	62.35	62.47	49.63
48	74.42	74.95	77.51	68.57	70.09	68.89	67.96	70.81	63.83	68.35	47.48
48	70.52	71.96	76.49	66.03	69.88	68.74	67.57	68.06	62.28	69.37	44.53
48	73.08	72.88	74.67	68.40	73.04	67.64	68.85	67.92	60.42	63.72	43.18
48	72.90	72.96	76.69	67.18	71.00	67.88	68.08	69.1	62.2	65.97	46.19
72	76.16	78.73	79.40	68.67	69.12	69.15	73.12	76.82	66.82	73.27	59.44
72	75.13	76.74	79.48	70.40	73.04	66.91	74.48	75.67	67.08	73.09	53.79
72	76.22	75.88	80.75	69.90	71.44	68.23	75.8	74.11	67.62	68.09	53.42
72	75.56	76.34	80.18	69.86	71.05	68.27	74.89	75.35	67.43	70.74	55.97
72	74.74	77.37	80.89	70.31	73.09	68.88	76.42	74.93	68.16	68.64	57.26
96	78.90	78.23	81.84	73.46	79.25	74.88	76.32	75.82	73.56	76.88	63.15
96	78.40	79.10	80.98	73.94	75.54	75.68	75.99	76.33	73.16	74.88	62.65
96	79.45	79.27	81.89	72.05	74.42	73.06	75.69	78.61	74.41	72.08	63.33
96	77.83	78.52	81.45	76.18	73.54	75.54	75.53	76.08	73.66	73.29	62.16
96	77.26	79.99	78.62	73.93	75.88	79.29	76.24	74.77	70.92	77.19	62.09

Appendix 2 : *In Vitro* Digestibility of 5.0% Herbs Added with Pellets

hour	CP	CPI	CPDD	CPIN	CPDDN	PKE	PKEI	PKEDD	PKEIN	PKEDDN	N
24	65.23	69.95	70.68	63.59	69.60	64.67	63.80	69.78	56.19	60.09	41.75
24	68.86	69.00	72.09	63.13	68.23	65.61	64.39	69.00	58.61	62.42	41.96
24	67.16	68.48	70.12	63.34	69.51	65.35	68.15	68.88	51.88	62.85	42.54
24	67.52	68.69	70.82	63.03	68.39	65.91	65.85	68.67	54.64	61.58	43.62
24	67.19	67.31	70.43	65.07	66.24	65.37	67.07	67.07	51.78	61.00	42.46
48	73.42	76.90	80.44	70.27	72.66	66.29	67.47	70.10	66.14	64.31	49.63
48	74.42	77.57	79.98	73.34	72.78	68.89	65.34	70.66	64.29	70.54	47.48
48	70.52	74.64	80.10	71.99	73.50	68.74	68.77	71.02	63.58	68.07	44.53
48	73.08	71.26	80.56	71.37	73.21	67.64	69.01	72.00	62.65	68.23	43.18
48	72.90	72.46	79.47	73.00	75.35	67.88	73.29	71.28	60.88	69.15	46.19
72	76.16	83.14	85.12	77.76	80.57	69.15	75.82	77.70	74.37	78.70	59.44
72	75.13	81.09	82.33	77.09	78.28	66.91	76.33	78.19	76.18	76.63	53.79
72	76.22	81.48	84.48	77.98	78.00	68.23	77.89	77.40	77.16	77.29	53.42
72	75.56	82.90	79.47	77.97	78.03	68.27	73.26	78.36	74.12	74.96	55.97
72	74.74	76.95	80.29	74.57	76.55	68.88	78.32	79.26	75.30	75.61	57.26
96	78.90	77.38	87.23	80.03	80.29	74.88	80.18	77.57	74.15	78.83	63.15
96	78.40	81.77	83.50	78.80	80.21	75.68	79.08	81.66	77.17	78.99	62.65
96	79.45	82.67	79.90	79.84	80.84	73.06	80.00	83.11	76.49	81.94	63.33
96	77.83	82.65	81.66	76.88	80.12	75.54	78.32	81.07	79.62	76.64	62.16
96	77.26	84.36	85.24	78.43	79.62	79.29	77.89	84.93	78.41	77.69	62.09

Appendix 3 : *In Vitro* Digestibility of 7.5% Herbs Added with Pellets

hour	CP	CPI	CPDD	CPIN	CPDDN	PKE	PKEI	PKEDD	PKEIN	PKEDDN	N
24	65.23	70.31	70.82	67.88	69.90	64.67	64.98	70.36	54.24	61.84	41.75
24	68.86	68.85	70.56	66.33	69.23	65.61	64.86	69.02	54.92	60.36	41.96
24	67.16	69.38	71.54	66.68	69.17	65.35	66.98	69.18	54.64	61.86	42.54
24	67.52	68.86	73.25	67.61	68.93	65.91	66.88	68.20	55.81	63.21	43.62
24	67.19	69.56	71.57	64.92	68.68	65.37	69.76	69.15	53.57	62.09	42.46
48	73.42	78.86	83.49	71.12	70.87	66.29	71.47	69.86	68.16	65.81	49.63
48	74.42	75.46	80.19	72.47	74.07	68.89	73.90	74.86	64.85	68.49	47.48
48	70.52	75.46	78.65	71.92	76.57	68.74	72.83	74.47	67.46	70.43	44.53
48	73.08	73.22	78.92	74.07	72.92	67.64	69.98	73.68	61.82	69.01	43.18
48	72.90	74.36	79.73	72.81	75.88	67.88	74.20	77.44	62.05	68.76	46.19
72	76.16	84.24	84.05	78.12	81.32	69.15	76.89	79.87	73.22	79.96	59.44
72	75.13	80.57	86.08	78.65	76.46	66.91	79.87	79.76	79.82	75.88	53.79
72	76.22	81.78	83.05	77.95	78.76	68.23	79.88	78.79	76.04	77.26	53.42
72	75.56	83.82	79.08	75.69	77.69	68.27	75.24	76.69	78.45	74.07	55.97
72	74.74	78.46	83.02	79.40	79.67	68.88	77.93	78.82	72.66	79.14	57.26
96	78.90	79.57	86.54	81.02	81.45	74.88	80.17	80.21	79.80	79.67	63.15
96	78.40	81.79	83.49	80.30	80.99	75.68	79.72	82.12	77.23	79.46	62.65
96	79.45	82.32	83.71	79.65	80.65	73.06	79.28	79.41	76.19	78.89	63.33
96	77.83	82.26	80.27	80.07	80.25	75.54	79.05	82.29	78.06	78.02	62.16
96	77.26	85.65	84.62	77.26	80.04	79.29	80.07	79.32	79.06	78.44	62.09

Appendix 4 : *In Vitro* Digestibility of Gas Production of 2.5% Herbs Added with Pellets

hour	CP	CPI	CPDD	CPIN	CPDDN	PKE	PKEI	PKEDD	PKEIN	PKEDDN	N	Control
24	66.00	67.00	83.00	56.00	65.00	55.00	60.00	69.00	58.00	61.00	38.00	36.00
24	69.00	66.00	90.00	61.00	70.00	50.00	57.00	67.00	53.00	58.00	45.00	39.00
24	63.00	72.00	82.00	62.00	66.00	52.00	61.00	66.00	52.00	57.00	42.00	39.00
24	60.00	64.00	85.00	60.00	70.00	49.00	57.00	68.00	55.00	63.00	44.00	33.00
48	82.00	86.00	110.00	72.00	80.00	70.00	81.00	88.00	69.00	74.00	66.00	51.00
48	87.00	93.00	117.00	76.00	75.00	78.00	83.00	92.00	72.00	72.00	70.00	53.00
48	90.00	91.00	111.00	77.00	79.00	70.00	79.00	94.00	75.00	75.00	71.00	58.00
48	84.00	90.00	116.00	75.00	83.00	75.00	86.00	91.00	73.00	80.00	72.00	59.00
72	89.00	92.00	119.00	77.00	83.00	81.00	86.00	89.00	76.00	76.00	72.00	60.00
72	90.00	100.00	122.00	84.00	81.00	89.00	83.00	93.00	79.00	85.00	78.00	67.00
72	97.00	99.00	118.00	83.00	88.00	82.00	87.00	91.00	74.00	82.00	79.00	63.00
72	92.00	97.00	119.00	79.00	89.00	81.00	85.00	98.00	82.00	74.00	74.00	68.00
96	92.00	94.00	121.00	78.00	84.00	83.00	86.00	104.00	77.00	88.00	76.00	68.00
96	98.00	102.00	124.00	86.00	83.00	86.00	90.00	102.00	82.00	84.00	73.00	73.00
96	99.00	101.00	121.00	85.00	90.00	91.00	88.00	98.00	80.00	85.00	79.00	74.00
96	95.00	99.00	123.00	83.00	90.00	85.00	94.00	102.00	85.00	89.00	82.00	69.00

CP = Commercial Pellets, CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs, CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass,CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass, PKE = Palm Kernel Expeller (PKE), PKEI = PKE Added with Imported Herbs, PKEDD = PKE Added with DD Herbs,PKEIN = PKE Added with Imported Herbs and Napier Grass, PKEDDN = PKE Added with DD Herbs and Napier Grass and N = Napier Grass

Appendix 5 : *In Vitro* Digestibility of Gas Production of 5.0% Herbs Added with Pellets

hour	CP	CPI	CPDD	CPIN	CPDDN	PKE	PKEI	PKEDD	PKEIN	PKEDDN	N	Control
24	66.00	70.00	78.00	57.00	69.00	55.00	59.00	63.00	53.00	58.00	38.00	36.00
24	69.00	65.00	80.00	53.00	65.00	50.00	61.00	68.00	56.00	62.00	45.00	39.00
24	63.00	63.00	84.00	62.00	68.00	52.00	55.00	64.00	51.00	56.00	42.00	39.00
24	60.00	69.00	79.00	59.00	65.00	49.00	54.00	60.00	50.00	59.00	44.00	33.00
48	82.00	87.00	91.00	68.00	74.00	70.00	75.00	90.00	69.00	70.00	66.00	51.00
48	87.00	85.00	89.00	74.00	81.00	78.00	80.00	83.00	67.00	72.00	70.00	53.00
48	90.00	88.00	86.00	70.00	76.00	70.00	77.00	88.00	76.00	79.00	71.00	58.00
48	84.00	85.00	93.00	69.00	73.00	75.00	78.00	86.00	73.00	78.00	72.00	59.00
72	89.00	89.00	97.00	71.00	85.00	81.00	82.00	90.00	80.00	77.00	72.00	60.00
72	90.00	96.00	95.00	76.00	80.00	89.00	86.00	89.00	74.00	80.00	78.00	67.00
72	97.00	94.00	101.00	76.00	83.00	82.00	88.00	87.00	72.00	75.00	79.00	63.00
72	92.00	97.00	99.00	79.00	82.00	81.00	82.00	83.00	78.00	79.00	74.00	68.00
96	92.00	94.00	104.00	76.00	80.00	83.00	78.00	85.00	82.00	78.00	76.00	68.00
96	98.00	99.00	108.00	77.00	79.00	86.00	77.00	89.00	83.00	82.00	73.00	73.00
96	99.00	99.00	109.00	81.00	84.00	91.00	84.00	86.00	72.00	83.00	79.00	74.00
96	95.00	96.00	111.00	79.00	78.00	85.00	81.00	82.00	78.00	81.00	82.00	69.00

CP = Commercial Pellets, CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs, CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass,CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass, PKE = Palm Kernel Expeller (PKE), PKEI = PKE Added with Imported Herbs, PKEDD = PKE Added with DD Herbs,PKEIN = PKE Added with Imported Herbs and Napier Grass, PKEDDN = PKE Added with DD Herbs and Napier Grass and N = Napier Grass

Appendix 6 : *In Vitro* Digestibility of Gas Production of 7.5% Herbs Added with Pellets

hour	CP	CPI	CPDD	CPIN	CPDDN	PKE	PKEI	PKEDD	PKEIN	PKEDDN	N	Control
24	66.00	70.00	85.00	58.00	68.00	55.00	62.00	68.00	57.00	58.00	38.00	36.00
24	69.00	67.00	90.00	64.00	69.00	50.00	59.00	69.00	56.00	62.00	45.00	39.00
24	63.00	66.00	87.00	62.00	70.00	52.00	59.00	72.00	52.00	65.00	42.00	39.00
24	60.00	69.00	81.00	61.00	69.00	49.00	61.00	67.00	59.00	64.00	44.00	33.00
48	82.00	90.00	114.00	77.00	79.00	70.00	79.00	91.00	71.00	77.00	66.00	51.00
48	87.00	89.00	115.00	78.00	86.00	78.00	84.00	92.00	77.00	76.00	70.00	53.00
48	90.00	87.00	120.00	80.00	82.00	70.00	87.00	89.00	76.00	74.00	71.00	58.00
48	84.00	97.00	112.00	73.00	80.00	75.00	85.00	97.00	73.00	80.00	72.00	59.00
72	89.00	102.00	123.00	82.00	87.00	81.00	86.00	91.00	75.00	77.00	72.00	60.00
72	90.00	96.00	118.00	86.00	82.00	89.00	84.00	96.00	80.00	84.00	78.00	67.00
72	97.00	98.00	119.00	81.00	90.00	82.00	89.00	94.00	82.00	84.00	79.00	63.00
72	92.00	99.00	122.00	83.00	89.00	81.00	91.00	93.00	81.00	83.00	74.00	68.00
96	92.00	96.00	122.00	81.00	83.00	83.00	89.00	99.00	79.00	91.00	76.00	68.00
96	98.00	101.00	120.00	82.00	88.00	86.00	92.00	103.00	87.00	89.00	73.00	73.00
96	99.00	97.00	127.00	87.00	89.00	91.00	91.00	107.00	80.00	83.00	79.00	74.00
96	95.00	104.00	125.00	89.00	92.00	85.00	93.00	105.00	82.00	88.00	82.00	69.00

CP = Commercial Pellets, CPI = Commercial Pellets Added with Imported Herbs, CPDD = Commercial Pellets Added with DD Herbs, CPIN = Commercial Pellets Added with Imported Herbs and Napier Grass,CPDDN = Commercial Pellets Added with DD Herbs and Napier Grass, PKE = Palm Kernel Expeller (PKE), PKEI = PKE Added with Imported Herbs, PKEDD = PKE Added with DD Herbs,PKEIN = PKE Added with Imported Herbs and Napier Grass, PKEDDN = PKE Added with DD Herbs and Napier Grass and N = Napier Grass

Appendix 7 : *In Sacco* Digestibility of 2.5% Herbs Added with Pellets

hour	CP	CPI	CPDD	CPIN	CPDDN	PKE	PKEI	PKEDD	PKEIN	PKEDDN	N
24	65.34	66.27	65.31	60.39	64.51	65.13	63.22	66.13	54.95	60.82	52.72
24	66.40	66.75	67.07	61.24	63.69	61.00	62.90	64.38	54.23	60.42	54.15
24	66.59	66.03	68.89	60.65	61.50	60.09	64.16	63.38	55.50	58.98	50.37
24	67.13	66.43	67.07	60.63	63.13	63.89	62.71	64.19	54.72	60.22	51.00
24	66.40	66.67	67.04	60.24	62.82	62.53	60.57	62.85	54.18	59.85	52.06
48	74.50	71.50	74.91	66.47	68.86	66.97	68.58	66.59	61.59	65.22	58.61
48	72.07	71.26	73.67	62.62	67.62	70.66	69.00	69.11	61.75	66.46	60.88
48	72.78	70.94	76.12	66.46	66.42	72.46	69.19	69.78	65.64	67.47	60.90
48	72.07	72.00	73.31	64.57	69.33	68.80	68.46	67.38	61.95	67.68	60.09
48	70.10	70.62	70.34	67.28	65.89	65.11	69.79	72.66	61.77	65.48	59.93
72	75.67	74.51	77.13	63.39	70.86	68.15	69.80	69.98	62.22	70.26	62.42
72	73.21	75.59	74.88	61.90	64.49	69.60	72.41	73.62	62.74	68.19	66.24
72	75.27	75.41	76.95	66.20	67.72	68.93	72.69	73.00	61.09	68.62	64.68
72	75.27	76.22	77.15	62.35	66.57	72.46	73.87	72.96	60.49	68.49	60.76
72	75.35	75.31	78.66	62.85	68.93	65.52	74.67	75.42	63.21	67.51	69.29
96	76.92	77.68	78.71	70.34	71.57	74.78	73.89	77.52	66.74	71.22	67.96
96	77.31	77.65	77.82	68.31	72.98	77.52	73.75	76.82	65.64	70.26	64.69
96	78.31	76.07	76.19	70.01	72.04	75.47	72.59	78.32	65.87	70.57	66.32
96	77.01	77.37	78.12	67.29	73.67	75.09	74.66	76.54	64.30	70.43	68.75
96	78.18	79.47	78.24	65.60	74.66	74.49	73.88	74.90	66.81	68.84	63.88

Appendix 8 : *In Sacco* Digestibility of 5.0% Herbs Added with Pellets

hour	CP	CPI	CPDD	CPIN	CPDDN	PKE	PKEI	PKEDD	PKEIN	PKEDDN	N
24	65.34	67.72	65.31	60.23	67.69	65.13	62.97	70.08	55.58	62.20	52.72
24	66.40	66.93	67.07	65.61	66.99	61.00	63.24	69.95	51.74	62.89	54.15
24	66.59	66.81	72.58	68.23	66.41	60.09	66.00	63.11	56.19	62.89	50.37
24	67.13	67.59	68.81	63.90	67.47	63.89	64.23	68.39	55.81	63.07	51.00
24	66.40	68.92	70.27	61.51	68.81	62.53	64.69	70.43	59.72	64.31	52.06
48	74.50	76.34	77.23	66.68	70.92	66.97	68.13	65.59	61.59	69.21	58.61
48	72.07	71.80	78.06	67.70	67.72	70.66	68.89	73.34	61.95	67.07	60.88
48	72.78	73.06	76.57	66.67	69.61	72.46	68.93	71.27	61.48	68.86	60.90
48	72.07	72.09	77.66	64.59	68.44	68.80	68.15	73.49	61.78	70.68	60.09
48	70.10	72.02	73.34	67.71	71.37	65.11	70.54	72.66	60.62	68.48	59.93
72	75.67	76.52	80.98	66.63	72.11	68.15	76.85	75.88	64.66	74.99	62.42
72	73.21	76.01	80.64	67.73	66.91	69.60	76.25	77.36	62.88	66.15	66.24
72	75.27	77.38	80.27	69.12	69.83	68.93	76.22	76.77	64.07	69.95	64.68
72	75.27	78.40	80.41	70.88	67.31	72.46	76.20	77.31	63.44	69.15	60.76
72	75.35	78.60	79.05	71.27	73.00	65.52	75.56	76.52	65.32	69.51	69.29
96	76.92	80.27	82.34	70.62	76.61	74.78	80.14	77.73	68.74	72.48	67.96
96	77.31	81.81	83.20	68.42	77.36	77.52	78.44	80.17	67.64	72.99	64.69
96	78.31	81.40	82.95	69.88	74.63	75.47	76.62	78.64	68.56	71.89	66.32
96	77.01	82.92	83.60	72.12	70.23	75.09	75.35	77.15	68.99	71.26	68.75
96	78.18	80.60	82.67	68.35	74.34	74.49	72.57	79.49	68.88	70.82	63.88

Appendix 9 : *In Sacco* Digestibility of 7.5% Herbs Added with Pellets

hour	CP	CPI	CPDD	CPIN	CPDDN	PKE	PKEI	PKEDD	PKEIN	PKEDDN	N
24	65.34	66.67	67.62	68.51	68.21	65.13	64.34	69.11	54.95	63.09	52.72
24	66.40	68.56	68.84	64.89	67.37	61.00	65.02	68.37	52.81	65.78	54.15
24	66.59	67.44	71.06	65.09	67.85	60.09	64.02	69.20	57.86	63.56	50.37
24	67.13	68.81	67.97	68.28	67.34	63.89	65.81	69.41	58.63	65.56	51.00
24	66.40	67.87	68.87	64.08	67.69	62.53	64.80	69.02	56.06	64.50	52.06
48	74.50	74.50	78.56	64.59	71.37	66.97	69.59	68.90	69.01	68.85	58.61
48	72.07	76.61	77.45	63.39	69.13	70.66	70.64	74.28	68.33	69.12	60.88
48	72.78	73.15	76.43	64.07	68.57	72.46	69.09	71.00	68.41	68.46	60.90
48	72.07	76.85	76.85	65.69	69.73	68.80	69.81	71.98	62.31	69.64	60.09
48	70.10	71.63	74.99	66.02	69.87	65.11	69.91	73.76	69.34	72.15	59.93
72	75.67	78.18	80.41	69.59	74.07	68.15	76.41	77.11	62.09	72.77	62.42
72	73.21	79.53	81.01	66.39	69.16	69.60	77.13	76.09	60.99	68.44	66.24
72	75.27	78.38	80.54	69.27	70.60	68.93	76.96	77.09	64.57	70.10	64.68
72	75.27	80.80	80.71	66.69	68.58	72.46	77.41	78.35	64.88	69.30	60.76
72	75.35	75.01	80.00	72.02	68.41	65.52	76.90	76.82	61.27	69.88	69.29
96	76.92	80.77	84.32	69.28	77.62	74.78	78.10	82.48	65.88	73.07	67.96
96	77.31	80.35	81.94	69.87	77.08	77.52	77.04	77.06	63.09	70.47	64.69
96	78.31	83.01	83.01	70.83	75.23	75.47	76.82	78.88	68.77	72.18	66.32
96	77.01	83.58	82.81	71.18	73.31	75.09	75.69	78.99	62.48	70.29	68.75
96	78.18	81.68	82.96	72.97	72.90	74.49	76.43	77.01	66.83	74.88	63.88

Appendix 10 : Male Goat Kids Fed with Commercial Pellets Added with 50 gm of DD Herbs

	Age/Month	Tag No					Average/Mean	SD
		601	**602**	**603**	**604**	**605**		
Body Weight/kg	4	15.00	14.20	14.60	15.40	15.10	14.86	0.47
	5	19.50	15.90	18.30	19.10	19.30	18.42	0.49
	6	25.50	19.80	23.20	24.10	23.40	23.20	0.71
	7	28.20	23.90	25.70	26.20	25.50	25.90	1.05
	8	33.50	32.00	31.50	34.60	33.80	33.08	1.29
	9	38.60	37.10	39.20	39.40	38.90	38.64	0.91

Appendix 11 : Female Goat Kids Fed with Commercial Pellets Added with 50 gm of DD Herbs

	Age/Month	Tag No					Average/Mean	SD
		611	**612**	**613**	**614**	**615**		
Body Weight/kg	4	14.50	14.00	13.50	13.00	13.50	13.70	0.57
	5	17.50	16.00	17.40	16.20	16.50	16.72	0.69
	6	19.00	19.50	20.50	18.00	19.50	19.30	0.91
	7	24.00	25.30	26.50	22.40	22.60	24.16	1.76
	8	29.80	27.50	30.00	28.40	29.00	28.94	1.03
	9	35.00	34.60	36.70	34.20	35.30	35.16	0.96

Appendix 12 : Female Goat Kids Fed with Commercial Pellets

	Age/Month	Tag No					Average/Mean	SD
		616	**617**	**618**	**619**	**620**		
Body Weight/kg	4	14.00	13.50	14.50	13.00	14.00	13.80	0.57
	5	14.50	14.00	15.50	14.60	14.80	14.68	0.54
	6	17.00	16.50	18.00	17.50	16.00	17.00	0.79
	7	19.20	18.40	21.30	20.20	19.60	19.74	1.09
	8	23.10	22.50	25.50	24.50	22.60	23.64	1.31
	9	28.30	27.70	30.30	29.40	27.50	28.64	1.19

Appendix 13 : Male Goat Kids Fed with Commercial Pellets

	Age/Month	Tag No					Average/Mean	SD
		606	**607**	**608**	**609**	**610**		
Body Weight/kg	4	15.00	15.40	14.00	15.30	14.20	14.78	0.64
	5	16.50	17.00	16.00	16.50	15.00	16.20	0.76
	6	19.00	19.50	18.30	18.40	17.50	18.54	0.76
	7	23.10	23.80	22.30	24.60	23.50	23.46	0.85
	8	26.40	26.60	24.50	25.50	26.80	25.96	0.96
	9	30.00	29.60	27.80	29.00	28.50	28.98	0.87