UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF DIFFERENT FEED FORMULATION ON NUTRITIONAL RELATED BLOOD PROFILE IN GOAT

NUR HAFIZATUL AIEZZAH BINTI DAUD

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A project paper submitted to the
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CERTIFICATION

It is hereby certified that we have read this project entitled "The Effect of Different Feed Formulation on Nutritional Related Blood Profile in Goat", by Nur Hafizatul Aiezzah binti Daud and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999- Project

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DEDICATIONS

This project paper is dedicated to all who read this thesis,

To my dearest family,

Abah

Umi

Kadek, Didi, Mek and Adik

Atuk

My late beloved Umi Zie

Milo and all her babies

To my juniors,

And to all my lecturers, teachers and friends.

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Praises be to Allah for all the opportunity given to me, I'm able to breath until now and completing this project to the end.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4990-Projek

KESAN FORMULASI MAKANAN YANG BERBEZA KE ATAS PROFIL DARAH BERKAIT NUTRISI DALAM KAMBING

oleh

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2016

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Kemerosotan kesihatan haiwan dan penyakit sering kali menyebabkan penurunan produktiviti ladang dan keuntungan. Pengurusan pemakanan yang bagus perlu dilaksanakan bagi meningkatkan kesihatan gerompok dan prestasi ladang. Nutrisi yang cukup harus disediakan mengikut jenis produksi haiwan untuk memenuhi keperluan nutrisi. Pengenalpastian profil darah berkait nutrisi digunakan sebagai penunjuk aras status nutrisi dalam haiwan. Oleh itu, kajian

ini telah dijalankan untuk mengenalpasti komposisi nutrisi di dalam formulasi makanan yang berbeza dan mengenalpasti kesan perbezaan formulasi makanan ke atas profil darah berkait nutrisi.

Sebanyak 18 ekor kambing betina, *Capra aegagrus hircus* (Boer kacuk) telah dipilih dan dibahagikan kepada tiga kumpulan (n=6) yang berbeza mengikut formulasi makanan (Diet 1: diet yang sedang dipraktis di ladang, Diet 2; diet untuk keperluan dewasa dan Diet 3; diet untuk keperluan pembakaan). Sampel darah telah diambil sebelum dan selepas empat minggu ujian pemakanan dijalankan dan dianalisis untuk parameter tertentu. Semua data dianalisis menggunakan perisian SPSS. Analisis proksimat ke atas kesemua formulasi makanan telah dijalankan dan menunjukkan perbandingan nilai protein mentah dan lemak mentah. Glukosa, Jumlah Protein dan Kalsium menunjukkan perbezaan yang signifikan antara kumpulan (nilai $p < 0.05$) manakala Kolesterol and Asid Lemak tidak menunjukkan perbezaan yang signifikan (nilai $p > 0.05$). Analisis Asid Lemak dalam makanan dan serum menunjukkan Asid Lemak Poli Tidak Tepu (PUFA) lebih tinggi daripada Asid Lemak Tepu (SFA). Secara keseluruhannya, Diet 3 menunjukkan nilai yang tinggi bagi semua parameter darah yang dianalisis dalan kajian ini. Oleh itu, adalah sangat penting untuk menyediakan nutrisi mengikut jenis produksi kerana protein dan tenaga yang cukup akan meningkatkan prestasi haiwan terutamanya di dalam pembiakan dan

kualiti produk haiwan. Kajian ini menunjukkan formulasi makanan yang berbeza mempengaruhi profil darah berkait makanan.

Kata kunci: analisis proksimat, profil darah, asid lemak poli tidak tepu (PUFA)

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 - Project

THE EFFECT OF DIFFERENT FEED FORMULATION ON NUTRITIONAL RELATED BLOOD PROFILE IN GOAT

By

Nur Hafizatul Aiezzah binti Daud

2016

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Poor animal health and disease are usually related to the cause of low farm productivity level and indirectly to low profit margin. Good feeding strategies should be designed and implemented in order to improve the herd health thus increase farm performance. Adequate nutrient should be given to animal according to their production stages in order to meet the nutrient requirement. Determining the nutritionally related blood metabolite was used as an indicator of nutritional status.

Thus, this study was conducted to determine the nutrient composition of different feed formulation and the effect of different feed formulation on nutritional related blood profile in goat. .

Eighteen female adult goats, *Capra aegagrus hircus* (Boer cross) were selected and allocated into three different groups (n=6) and assigned according to their different formulation (Diet 1; current fed by the farmer, Diet 2; maintenance diet and Diet 3; flushing diet). Blood sampling were conducted before and after fourth weeks of feeding trial and analysed for selected parameters by using SPSS software. Proximate analysis of diets was done and revealed comparable value of Crude Protein and Crude Fat. Glucose, Total Protein and Calcium level showed significant difference between groups (p-value< 0.05) while Cholesterol and Fatty Acid showed no significant difference (p-value>0.05). Fatty Acid analysis of feed and plasma showed Polyunsaturated Fatty Acid is higher than Satureated Fatty Acid. Overall, Diet 3 shows the highest value of all blood parameters analysed in this study. Hence, it is very important to provide animal with nutrient according to production stages as sufficent protein and energy will improve the animal performance in terms breeding efficacy and quality of animal products. This study

indicates that different feed formulation does affect on certain nutritional related blood profile in goat.

Keywords: proximate analysis, blood profile, polyunsaturated fatty acid

1.0 INTRODUCTION

Livestock is a very important industry as it supplies the largest source of protein for Malaysian population (Fadhilah, 2015). In general, the livestock production specifically in ruminant sector is still inadequate to meet the demand, following the increase in population and consumption. However, small ruminant industry has been steadily increasing (Livestock Statistics, Department of Veterinary Services, 2009). In 2008, the total population of goats in Malaysia was 477,480 heads which was an increment of 49,217 from the previous year 2007 (Livestock Statistics, Department of Veterinary Services, 2009). Hence, small ruminant farming particularly among smallholder, should be encouraged more by the government as this industry has good potential to improve status of Malaysia as global center of halal products. In addition, a good herd health program includes vaccination practice, reproductive management and good feeding strategies should be designed and implemented in order to improve the herd health thus increase farm performance.

Adequate nutrient should be given to animal according to their production stages in order to meet the nutrient requirement. But, to majority of smallholders, balance feeding is less concerned due to time, cost and labor issues. Indeed, imbalance feeding could affect the physiological function due to inadequate

nutrient and further lead to reduction in performance and economic importance. According to Komala *et al*., 2011, it is important to make sure the animals diet is formulated to support optimal production and be economical so that it does not burden the poor farmers.

Hence, nutritional status of goat should be accurately assessed as such information is useful in relation to the formulation of the diets.

According to Waziri *et al*., (1997), examining blood for their constituent could be used to monitor and evaluate health and nutritional status of animal. Analysis of biochemical properties such as glucose, total protein, blood urea nitrogen, creatinine and cholesterol is essential in diagnosing various nutritional, pathological and metabolic disorders (Daramola *et al*., 2005) in goats.

The objectives of this study were to determine the nutrient composition of feed material used in different feed formulation and to determine the effect of different formulation on nutritional related blood profile such as Glucose, Total Protein, Calcium, Cholesterol and Fatty Acid. We hypothesized that different feed formulation may affect the nutritional related blood profile in goat.

2.0 LITERATURE REVIEW

2.1 Goat Farm Industry in Malaysia

Referring to Productivity Report 2014/2015, livestock subsector comprising of poultry, cattle and other livestock contributed about 13% to the Gross Domestic Product. Production in the livestock subsector is accounted by poultry meat contributing 1,202,000 metric ton (59.37%), pork 206,000 metric ton (10.18%), egg 510,000 metric ton, milk 62,000,000 litre (3.08%), beef 42,000 metric ton (2.08%) and mutton 22,000 metric ton (0.11%). The production of mutton in the livestock subsector reflected the small number of goats in this country (Jamaluddin et.al, 2012). The goat industry in Malaysia is characterized predominantly by small back yard type of operations (Khusahry, 1984). About 3.4 kg of the per capita consumption of the meat preferred by about 12% of the population. The present local production level is about 800 metric tonnes, approximately 5% of a total 16425 metric tonnes, and overall supply of goat meat has in fact declined at a rate of 2.7% annually (Devendra, 1983). The failure of the ruminant industry as a whole was attributed to many factors including the lack of incentives, uneconomic production systems and inadequate marketing strategies (Jalaludin and Halim, 1998).

2.2 Goat Farm Management

Goats are kept in many different types of production system depending on various factors such as investment, herd size, availability of grazing area and availability of feed supplies. The type of production system can be categorized into extensive, semi-intensive, intensive and integrated with tree crops. According to Feed and Fertilizer Technology Centre Annual Report 2008, most common practice among the smallholder in Asia are extensive and semi intensive. However, as the goat industry continues to grow, a more commercial oriented management system is needed as a method of improvement. One of another important aspect that should be focused in goat production is health management as there are a number of endemic diseases thus all animals must undergo an annual blood screening for the presence of infectious disease such as Foot and Mouth Disease (FMD), Brucellosis and Tuberculosis (TB) (Hassan and Sithambaram, 2014). Indeed, poor animal health and disease are usually related to the cause of low farm productivity level and indirectly to low profit margin (Jamaludin et al., 2012). An absolute method to prevent and control herd problem in herd/flock is advice and treatment from a veterinarian. Good herd health program includes vaccination practice, reproductive management and good feeding strategies should be designed and implemented in order to improve the herd health thus increase farm performance.

2.3 Goat Feeding and Nutrition

For profitable production, proper feeding and year-round management are essential.It has been suggested that proper nutrition is needed in order to produce a high-percentage crop, wean heavy animal and develop satisfactory flock replacement (Diego, 1994). According to Kellems and Church, 2010, the most economic productivity results from maximizing the use of forages, roughages or roughage products and when needed, combined with adequate but minimal levels of supplemental feedstuffs. All essential nutrient such as energy (e.g: fat and carbohydrate), protein, vitamins, mineral and water are present in the diet and must be provided to the animal to meet their requirement. There are various factors that affect the nutrient requirement by the animal and these include genetic and breed, age, animal biotype and productive function, thus feeding standard should be designed to meet the requirement.

2.3.1 Feedstuffs and Feeding Diet

As 45% to 64% of cost of production is feeding, a good feeding management can reduce the cost of production significantly as suggested by Solaiman, 2006. Feedstuffs can be classified as either concentrates or roughages and further classified into several categories (Cheeke, 2005) as shown in Table 1. Dietary carbohydrate can be found in roughages and will be converted into energy that eventually used for growth and production. Common grasses

resources include Guinea grass, Napier grass and *Bracharia* spp while legumes that provide protein sources include *Lucaena* spp., *Gliricidia* spp. and *Mulberry* spp. (Hassan and Sithambaram, 2013). Other than roughages, grain mix is required especially in growing and nursing goats. Concentrates is basically made of high energy or high protein feeds. Energy concentrates; corn, barley and sorghum are those that are added to the feed ration to increase the energy level. Protein concentrates can be either plant based such as cottonseed meal, soybean meal or animal based such as fishmeal and bone meal.

2.3.2 Nutritional Needs and Productive Functions

Other than determination of nutrient content in feedstuff, designing good feeding practice also concerned about nutrient requirement by animals. This aspect is influenced by several factors which include sex, age, breed, production system (dairy or meat), body size, climate and physiological stage (Rashid, 2008). As suggested by Rashid, 2008, daily feed intake is influenced by body weight, percentage of dry matter in the feeds (12-35% in forages, 86-92% in concentrates and hay), palatability, and physiological state of the goats (growths, pregnancy and lactation). For good feeding management, herd should be divided into groups such as growing kids, pregnant does, nursing does and herd sires. Average bodyweight of each animal should be fixed to determine the average maintenance requirement as suggested by (Solaiman, 2006).

2.3.2.1 Pre-Breeding Period

Breeding males are expected to lose bodyweight due to reducing in feeding time naturally (Goetsch and Merkel, 2010). Some bodyweight loss is acceptable but highly depleted state could lead to low breeding efficiency, thus, supplementation should start as early as six to seven weeks or as late as two weeks prior to breeding season. This is related to physiological reproductive system where production of fertile sperm is initiated 40 to 60 days before its deposition in the female reproductive tract (Solaiman, 2006). For the females, beginning 2 to 3 weeks prior to breeding and continuing for a week or more into breeding season, does are often flushed where the animals re fed with high quality pasture or by feeding grain, usually at a rate of 0.1 to 0.2 kg (Tisch, 2006). Flushing has been shown to result in a 10 to 20 percent improvement in lambing rate (Cole & Cupps, 1997) of ewes with moderate body condition (Gunn, 1983). Indeed, flushing thin does has been shown to improve breeding performance as measured by the ovulation rate and conception rate (Tisch, 2006).

2.3.2.1 Gestation Period

Energy needs for most species during pregnancy are more critical during the last third of the term (Pond et al., 2005). It is because the unborn fetus experiences most of its growth during this time. Thus, it is important to monitor bodyweight or body condition score of pregnant does during early stage of

pregnancy as excessively fat animal will have difficulty in kidding as it quite difficult to satisfy feed requirement during late pregnancy. Noted that does that are expected with multiple births should be monitored as they may suffered pregnancy toxaemia (Rashid, 2008). A good pasture, hay or silage as well as 0.2 to 0.5kg of 12% protein grain mix will be sufficient (Solaiman, 2006).

2.3.2.3. Lactation

Lactation also had marked a stimulating effect on metabolisable energy (ME) intake and heat production, which tended to decrease as lactation progressed (Van Niekerk & Casey, 1988). Viljoen (1985) also state that rate of energy deposited as protein and fat in lactating doe decreased relative to virgin does as lactation progressed. It is very important for animal to consume enough nutrient in order to reach target of maximum milk production and heavier weaned kids (Solaiman, 2006). It is desirable to provide high quality of forages and grain mix containing 16% protein and balanced for vitamins and minerals. In addition, water also is needed critical needed to sustain lactation as milk of most domestic species contains at most 80% of water (Pond *et al*,. 2005)

2.3.2.4 Growth and Maintenance

Growth, as measured by increase in body weight is significantly rapid during early phase and the rate will decline until puberty, followed by an even slower rate until maturity. Newly weaned kid or young animal require sufficient nutrient

to gain weight and should be provided with highest quality forages and 0.1 kg of high quality starter feed formulated for calves (Tisch, 2006). At 6 months of age up to breeding age (9-10 months), animals require about 0.5 – 0.6 kg of grain mix with at least 14% protein mix, minerals, trace minerals, and Vitamin A, D and E (Solaiman, 2006).

2.4 Effect of Dietary Intake on Goat's Performance

Nutrition is one of the most significant environmental influences on reproductive performance of sheep (Smith, 1991). Studies have concluded that the changes in follicular dynamics were not due to increased circulating levels of gonadotrophins, but to changes in local (ovarian) effects of growth factors or metabolites (Downing and Scaramuzzi, 1990; Scaramuzzi and Campbell, 1990). Increased protein and/or energy intakes have been shown to increase the circulating concentrations of insulin (Wagon et al., 1987) and insulin-like-growth I (IGF-I; Bass et al.,1984). Both insulin and IGF-I have been shown to act synergistically with FSH to increase the aromatase activity and to induce LH receptors in granulosa cells from follicles of a number of species (Adashi et al., 1985). As insulin bind to its receptor, it will facilitate the glucose transport into the cell. Glucose is the major source of energy of ovary (Rabiee et al., 1997). As suggested by Downing and Scaramuzzi (1990), the most consistent change

associated with the nutritional increase in ovulation rate was an increase in the levels of metabolic hormones, particularly insulin.

The immune system, like all system in the body, is dependent on the availability and utilization of nutrients for optimal maintenance and function of its component (Chandra, 1985). Nutrient deficiencies have demonstrated to have adverse effects on humoral immunity, phagocytosis, complement system, and on cell-mediated immunity (Baisel, 1982). Protein deficiency appears to impair the production and/or activity of interleukin 1 (IL-1) and PGE, (Hoffman-Goetz et al., 1981; Hoffman-Goetz, 1982; Bhaskaram and Sivaskumar, 1986). Protein deficiency also alters the ability of lymphocyte to respond to regulatory signal from IL-1 (Bell and Hoffman-Goetz. 1983; Bell et al., 1986)

Nutritional status of host can have an important influence on the effects of parasitic infection and it plays a role in both the resilience (ability to cope with the infection) and the resistance (the ability to resist becoming infected) of the animal (Pugh and Baird, 2012). Inadequate nutrition usually associated with inadequate immune response and higher infection level as high protein requirement is needed for food immune response (Pugh and Baird, 2012). In parasitic-infected animal, the mucosa will be damaged due to worm's activity and nutrient absorption will be compromised leading to the host's use of more body reserves.

Pugh and Baird (2012) reported if protein is less available, immune function is impaired, so the affected animal becomes more susceptible to subsequent infection.

2.5 Relationship between Different Feed Formulation and Blood Profile

Determination of the nutritional status of cattle is useful in quantifying the extent to which cattle are affected by nutrition, disease or other environmental factor (Ndlovu et al., 2007). Traditional methods that usually used to asses nutritional status of animal are bodyweights and body condition scoring though they have several limitation. According to Pambu-Gollah (2000), body mass changes are not reliable as an index of nutrient status unless it is known which animals are pregnant and which are not and, even then, is complicated by possibility of single, twin or triplet fetuses in pregnant animal. Despite the reported repeatability estimates in experienced assessors (Ferguson et al., 1994), the general subjective nature of body condition scoring makes it difficult for inexperienced herd managers, to correctly score the animals. Oulun (2005) and Agenas et al. (2006) suggested an indicator of nutritional status that would aid in management of cattle in rural area is by determining the nutritionally related blood metabolites. Studies give an immediate indication of an animal's nutritional status at that point in time (Pambu-Gollah et al., 2000).

Serum concentrations of metabolites such as glucose, cholesterol, non-esterified fatty acids, blood urea nitrogen, creatinine, total protein, albumin, globulin and minerals are commonly used to assess the nutritional status of cattle (Grunwaldt et al., 2005 and Ndlovu et al., 2007).

Blood glucose, β-hydroxy butyrate (BHB) and non-esterified fatty acid (NEFA) are the most common metabolites used to assess the energy status of cattle (Ndlovu, 2000) whereas blood urea nitrogen (BUN), creatinine, total protein and albumin are parameters that needed to assess protein status in animal. According to Ndlovu et al. (2007), albumin and total protein have low variability in blood thus they both have high diagnostic value in assessment of nutritional status as compared to creatinine which has low diagnostic value.

Determination of fatty acid in serum also important in order to evaluate the dietary changes that consist of different concentration fatty acid. Indeed, it has been reported that red meat, particularly form ruminant animals, has bad reputation due to its high saturated fatty acid (SFA) content, low ratio of polyunsaturated fatty acid (PUFA) to SFA, and high n-6 to n-3 ratio (Enser et al., 1998: Simpoulus, 2002; Cabiddu et al., 2010). Other studies also were conducted and revealed serum fatty acid changes following different nutritional formulation (Caldeira et al., 2005 and Lopes et al., 2014)

3.0 MATERIALS AND METHODS

3.1 Experimental Design

One of farm from Ladang Angkat Faculty of Veterinary Medicine, University Putra Malaysia (UPM) was selected to perform this study. The selected farm, Tok Seri Buak Agro Farm is located at Kg, Bukit Buak, Bt 13, Labu, Negeri Sembilan, Malaysia. Eighteen female adult goats, *Capra aegagrus hircus* (Boer cross) ranging from 8 to 9 months old and weighing about 20 kg were selected and kept in indoor housing system. They were then allocated into three different groups (n=6) and assigned according to their different experimental diets. The groups then were labelled as Diet 1 (Control Group), Diet 2 (Maintenance Diet) and Diet 3(Flushing Diet). The feeding trial lasted for 28 days where blood sampling were done twice; prior feeding trial implementation and after 28 days feeding trial implementation. Mineral block and free flow water were supplied during the feeding trial and Stress Pack® was provided in drinking water after blood sampling procedures to reduce stress to the animals.

3.2 Experimental Diets

Table 2 show different composition of feed ingredient in the experimental diets and all diets were analyzed for chemical composition by proximate analysis.

Diet 1 was fed with roughages, pressed soy waste and concentrate as routinely practiced by the farmer. Diet 2 then was fed with roughages and concentrate with additional 10% protein supplement while Diet 3 was fed with roughages and concentrate with additional 20% protein supplement.

Table 2 : Composition of feed ingredient

	Diet 1	Diet 2	Diet 3
Napier (g)	1500	1400	1370
Balik Angin (g)	490	460	460
Lebar Daun (g)	490	460	460
Soy Waste (g)	910	930	880
Concentrate (g)	110	250	330

3.3 Blood Sampling

Six goats from each groups were randomly chose and 10 ml of blood was collected from each animal via jugular venipuncture by using 21G Vacutainer® multiple sample needle into plain tube for serum biochemistry determination. There were two person involved in this procedure where 1 person was in charged in collecting blood and another one was in charged in restraining the animal.

3.4 Blood Analysis

Blood samples from free anticoagulant tube were centrifuged for 5 – 10 minutes at 3000 rpm and separated serum was harvested and stored in plastic tubes and immediately sent to Clinical Pathology Laboratory, Faculty of Veterinary Medicine, UPM for determination of Total Protein, Cholesterol, Calcium and Glucose. One ml of serum was kept in -20°C for fatty acid analysis**.**

3.5 Fatty Acid Analysis

3.5.1 Chemical and Glassware

Before and after fatty acid extractions all apparatus such as methylation tubes, screw caps and extraction tube stoppers were soaked for two hours in Decon 90 (Decon Laboratories Ltd., Sussex UK), scrubbed and then washed with tap water. After that they were soaked in distilled water overnight and rinsed again before oven dried at 60 °C. Other glassware such as the separating flasks, funnels, extraction tubes and round bottom flasks were washed in an automated laboratory glassware washer (Smeg Ltd., Oxon UK) using acid and alkaline washes for about three hours. All chemicals, solvents and laboratory supplies used for total lipid extraction and preparation of fatty acid methyl esters (FAME) were of analytical grade. All chemicals and solvents were free from contamination with rubber or fat derivatives. The screw caps of methylation tubes and screw caps for vials were made from Teflon. Chloroform and

chloroform-methanol solvents, (Merck KGaA, Darmstadt, Germany) had 10 mg/L of butylated hydroxytoulene (2, 6-di-tert-butyl-p-cresol) (Sigma Chemical Co., St. Louis, Missouri, USA) to prevent oxidative destruction of fatty acids during extraction, methylation and storage.

3.5.2 Total Lipid Extraction

The total fatty acids were extracted from feeds, plasma, tissues and rumen liquor using chloroform:methanol 2:1 (v/v) based on the method of Folch *et al*. (1957).

About 1g of feed samples were grinded and homogenized in 40 ml of chloroform:methanol (2:1, v/v) using an Ultra-Turrax T5 FU homogenizer (IKA Analysentechnik GmBH, Germany) without thawing (Shahidi and Wanasundara, 1998) within a 50 ml stoppered ground-glass extraction tube. The tube was gassed with nitrogen, stoppered and then vigorously shaken before allowed to stand for 12 h. The procedures that followed were similar to those described for plasma

The mixture containing the extracted fatty acids was filtered through a No. 1 Whatman paper (Whatman International Ltd., Maidstone, England) into a 250 ml separating flask using a filter funnel. The paper was washed with 10 ml of fresh chloroform-methanol (2:1, v/v). Ten ml of normal saline solution were added to facilitate phase separation. The mixture was then shaken vigorously for

one minute and was left to stand for four hours. After this washing phase, the lower phase contained 86 parts chloroform: 14 parts methanol: 1 part water (Shahidi and Wanasundara, 1998). The upper phase would contain 3: 48: 47 parts of chloroform, methanol and water respectively. The non-lipid contaminants would be retained in the aqueous upper phase (Christie, 1982). After complete separation at the end of fourth hour, the upper phase was discarded and lower phase was collected in a round bottom flask and evaporated by rotary evaporation (Heidolph GmBH, Germany) at 70 ^{0}C. The total lipid extract was then immediately transferred to a capped methylation tube by re-diluting it with five ml fresh chloroform-methanol (2:1, v/v).

3.5.3 Preparation of Fatty Acid Methyl Esters

Transmethylation of the extracted fatty acids to their fatty acid methyl esters (FAME) were carried out using 14% methanolic boron trifluoride (BF_3) according to methods in AOAC (2007). The internal standard, heneicosanoic acid (21:0) (Sigma Chemical Co., St. Louis, Missouri, USA) was added to each sample prior to transmethylation to determine the individual fatty acid concentrations within the samples.

The sample extract was then dried on a heating block (40°C) under a constant and mild flow of pure nitrogen gas. After drying the chloroform: methanol, two ml of 0.66N methanolic potassium hydroxide (R & M Chemicals,

Essex, U.K.) was added to saponify the lipid sample. The methylation tube was flushed with nitrogen, stoppered and heated in a boiling water bath for 10 min with occasional shaking. After the mixture had cooled down, two ml of 14 % BF_3 (Sigma Chemical Co., St. Louis, Missouri, USA) were added to initiate trans-esterification and the mixture was reheated for 20 min in a boiling water bath (Rajion, 1985).

After cooling, 4ml of distilled water and 4ml of petroleum ether (boiling point 40 – 60 °C) were added and the mixture was vortexed for 60sec.

The mixture was then centrifuged at 1500 G for 10 minutes to increase phase separation. The upper petroleum phase was transferred to another test tube using pasture capillary pipettes and washed with one ml of distilled water to remove residual BF_3. The upper phase from this test tube was then transferred accurately again to a second test tube and 0.5 g anhydrous sodium sulphate (R & M Chemicals, Essex, U.K.) was added to dry the sample and remove any residual water. Finally, the petroleum ether containing the FAME was transferred to a 4 ml screw-capped vial (Kimble Glass Inc., USA), flushed with nitrogen, closed tightly and stored at 4°C until analysis by gas-liquid chromatography.

3.5.3 Gas Liquid Chromatography

The methyl esters were quantified by gas chromatography (Agilent 7890N) using a 30m x 0.25mm ID (0.20 µm film thickness) Supelco SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA). One microlitre was injected by an auto sampler into the chromatograph, equipped with a split/splitless injector and a FID detector. High purity nitrogen (Malaysian Oxygen Bhd., Malaysia) was the carrier gas at 40 ml/min. High purity hydrogen (Dominick Hunter, Parker Hannifin ltd, UK) and compressed air (Malaysian Oxygen Bhd., Malaysia) were used for the flame ionization detector in the gas-liquid chromatograph. The injector temperature was programmed at 250°C and the detector temperature was 300 °C. The column temperature program initiated runs at 100°C, for 2 min, warmed to 170°C at 10°C /min, held for 2 min, warmed to 220°C at 7.5 °C /min, and then held for 20 min to facilitate optimal separation.

Identification of fatty acids was carried out by comparing relative FAME peak retention times of samples to standards obtained from Sigma (St. Louis, MO, USA). Both gravimetric calculations and normalised percentage (%) of total FA were used to determine the differences in FA composition. Peak areas were determined and calibrated using a personal computer integrator (Hewlett-Packard, Avondale, PA). Automatic expression of the peak areas as absolute and percentage amount of a detected fatty acid was obtained with a

programmed PC under Microsoft Excel 2000 (Microsoft Corp., Redmond, USA).

The amount of fatty acid is determined by their relative proportions (normalised percentages to total fatty acids) (Huerta-Leidenz *et al*., 1991; Alfaia *et al*., 2006). The normalised percentages describe the interactive and comparable relationship among fatty acids regarding lipid quality, while the gravimetric concentration can show the actual amount of fatty acids in tissues, which relates to nutritional intake.

3.6 Feed Analysis

3.6.1 Sample Collection

The local plants (e.g: Balik Angin and Lebar Daun), Napier grass, soy waste and goat concentrates were collected from Tok Seri Buak Agro Farm, Negeri Sembilan.

3.6.2 Sample Preparation

Local plants (e.g: Balik Angin and Lebar Daun) and grass samples were cut into small pieces before being dried in the oven at 65°C for two days. After drying, samples were grinded and kept in labelled air-tight plastic containers for further used in total mix ration diet. These steps were applied to the both soy waste and goat concentrate. Fifty g sample was prepared for each experimental diet

according to the composition stated in Table 2. Each ingredients was placed into the weighing boat and weighed by using analytical balance and reading was recorded for further reference. The experimental diet then kept in labelled air tight plastic container before further used for proximate and fatty acid analysis.

3.6.3 Proximate Analysis

Proximate analysis is the most common analysis performed on feed samples where it consists of a series of analyses to estimate the nutrient characteristic of feeds which includes the following: dry matter, crude protein, ether extract, crude fiber and ash. All the analysis were carried out according to certification procedures outlined by the Manual of Laboratory Techniques, University Putra Malaysia. Each experimental diet were analyzed for 3 replicates

3.6.3.1 Dry matter (DM) and moisture content

Labelled porcelain crucibles were dried in an oven for 30 minutes at 105°C. Then, the crucibles were cooled in dessicator for 20 minutes and the empty labelled crucibles were weighed. 3g of each experimental diets were weighed and placed inside respective crucible according to their label. The sample were dried in the oven at 105°C for 24 hours for drying process. After drying, the crucibles containing the samples were cooled down in dessicator for 20 minutes

and weighed. The dry matter and moisture content were calculated by using formula below:

$$\text{Moisture (\%)} = \frac{\text{Sample weight before drying} - \text{Sample weight after drying}}{\text{Sample weight before drying}}$$

$$\text{DM (\%)} = 100 - \text{Moisture content (\%)}$$

3.6.3.2 Ash

Samples from DM (%) were placed inside a muffle furnace at 550°C for 4 hours. After 4 hours, the crucibles containing ash were cooled down in dessicator for 30 minutes and later were weighed for ash determination by using formula below:

$$\text{Ash (\%)} = \frac{\text{Weight of crucible after ashing} - \text{Weight of empty crucibles}}{\text{Weight of sample}}$$

3.6.3.3 Crude Protein

Crude protein of feed samples was determined by using Kjeldahl Method which comprises of three steps; digestion, distillation and titration. In digestion step, 1g of sample was weighed and placed into Kjedahl flask together with Kjedahl catalyzer tablet (3.5g K_2SO_4 + 0.4g $CuSO_4$ x $5H_20$). Twenty ml of sulphuric acid (98%) was poured into 250 ml Kjedahl flask and shake gently. Kjedahl flask

containing sample were then fix into Kjedahl digestion set (Gerhardt Malaysia). The temperature of the heating block was gradually increased to maximum and the digestion was continued until the solution become clear (bluish/greenish) in color. The chemical reaction occurring during digestion process can be summarized as follow:

$$\text{Sample} + H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_20 + CO_2$$

Whilst the samples are being digested, acid standardization was done to determine the acid normality of a previously prepared 0.1M hydrochloric acid (HCl).

After digestion process completed, the flasks were allowed to cooled down before proceed to distillation process by using Kjedajl distillation set (Vapodest 20®, Gerhardt Malaysia). A solution made of 75ml of 2% Borid acid and 8 drops indicator (Methyl red & Bromohexdiol green) was earlier prepared in labelled Erlenmeyer flask was placed at the distillation platform. Fifteen ml of distilled water was poured slowly into digester and then transferred into distillation tube. Another fifteen ml of distilled water used to rinse the Kjedahl flask to clear any remaining of digester. The distillation then fixed to the distillation tube. During this process, 50ml of distilled water and 32% of NaOH were added as it was set up by the distillation set. This process took about 3 minutes where the entrapped sulphate salt of ammonium were released thus producing ammonia, which is

collected by the Boric acid (2%) at the distillation set via following reaction:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO + 2NH_3 \uparrow + H_2O$$

$$2NH_3 \uparrow + H_2O \rightarrow NH_4OH \text{ (collected by boric acid)}$$

The mixture in the Erlenmeyer flask was then titrated with 0.1M hydrochloric acid to determine the nitrogen content by using formula below:

$$\text{Nitrogen (\%)} = \frac{(\text{Vol. of titrant} - \text{Blank Value}) \times \text{Acid Normality} \times 14.0067 \times 100}{\text{Weight of sample}}$$

Crude Protein (%) = % Nitrogen x Protein factor

Where; Blank Value = 0.2 ml

Acid Normality = 0.0955

Protein Factor = 6.25

3.6.3.4 Crude Fiber

The crude fiber content of samples were determined by using Gerhardt Fiberbag System. The fiberbags were labelled and dried in the oven at 105°C for 1 hour to remove moisture and further allowed to cool down in the dessicator for 20 minutes. Each fiberbags were weighed and inserted with a fiber glass before 3g of sample was added into each labelled fiberbags. A beaker containing 360mL

of 0.13mol/L sulphuric acid that was earlier prepared then heated on the heating plate. The fiberbags were then loaded on the carousel and inserted into the beaker for 30 minutes for boiling process. After that, the fiberbags were rinsed with boiling distilled water while another beaker containing 360mL of 0.32mol/L NaOH and placed on the heating plate. The rinsed fiberbags were inserted into the boiling NaOH for 30 minutes before being rinsed with running water to settle down samples to the bottom of the fiberbags. Then, the fiberbags were dried by using paper towel and left overnight in oven at 105°C. After drying process, the dried labelled fiberbags were cooled down in dessicator for about 30 minutes before being put inside a previously weighed porcelain crucible and kept in muffle furnace at 600°C for 4 hours to produce ash. After 4 hours, the crucibles were left to cool down inside a dessicator before being weighed and the amount of crude fiber was calculated via the following formula:

$$\text{Crude fiber (\%)} = \frac{\text{Wa (g) - Wb (g) x 100}}{\text{Sample of weight (g)}}$$

Where ; Wa = Weight of crucible and sample after drying

Wb = Weight of empty crucible and sample after ashing

3.6.3.5 Ether extract

Empty labelled round bottom flask were dried in the oven at 105°C for 1 hour. After that, the empty round bottom flask were weigh before being poured with 250mL of petroleum benzene. Three g of sample was weighed and recorded before being transferred into extraction thimble and covered with cotton wool. The thimble and flask containing petroleum benzene were then fixed into Soxhlet apparatus. Water flow was allowed so that condensation process would be occur. The temperature of heating plate was increased gradually and boiling was allowed for 4 hours. After boiling completed, the flask was detached and dried in the oven at 80°C overnight. After drying, the flask were allowed to be cooled inside dessicator before weigh. Crude fat determination was carried as follow:

$$\text{Crude Fat (\%)} = \frac{\text{Dried flask after evaporation weight - Empty flask weight} \times 100}{\text{Weight of sample}}$$

3.7 Statistical Analysis

All data were analysed by using SPSS Version 22. The mean of each parameter were tested using analysis of variance (ANOVA) and mean comparison using Tukey test.

4.0 RESULTS

4.1 Proximate Analysis

Table 3 : Proximate Analysis of all Feed Formulation

Nutrient Composition (% DM)	Diet 1	Diet 2	Diet 3
Dry Matter	92.98	92.57	93.06
Ash	10.31	12.98	11.23
Crude Fiber	20.44	20.44	20.35
Crude Protein	4.55	14.81	20.01
Ether Extract	2.1	2.27	2.36

The results for proximate analysis was tabulated in Table 3. Result obtained revealed that Dry Matter for Diet 3 was the highest followed by Diet 1 and Diet 2. For the ash, Diet 2 showed the highest percentage and followed by Diet 3 and Diet 1. Crude fiber for all diets showed more or less a similar. Both Crude protein and Ether extract showed comparable value. For crude protein, Diet 3 showed the highest value and followed by Diet 2 and lowest in Diet 1. Similar to ether extract, Diet 3 contain the highest percentage of ether extract and followed by Diet 2 and Diet 1.

4.2 Nutritional Related Blood Profile

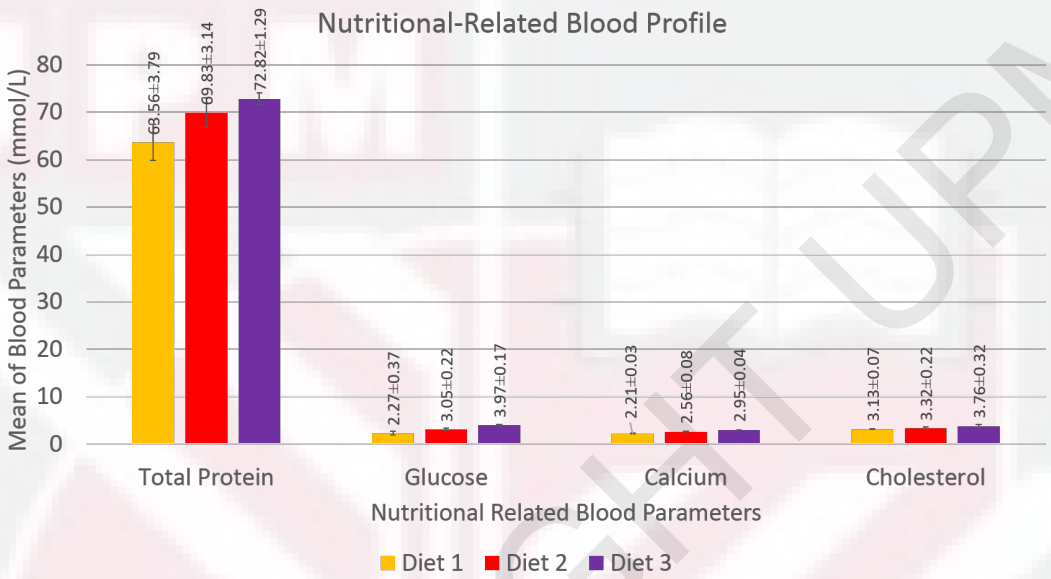

Figure 1: Nutritional-Related Blood Profiles in Goat

The result of nutritional-related blood profile of goat fed with different feed formulation was presented in Figure 2. Total Protein, Glucose and Calcium value for Diet 3 is significantly higher (p-value<0.05) compared to Diet 1.

There was no statistical difference observed in Cholesterol value between feed formulations.

4.3 Fatty Acid Analysis of Feed Formulation

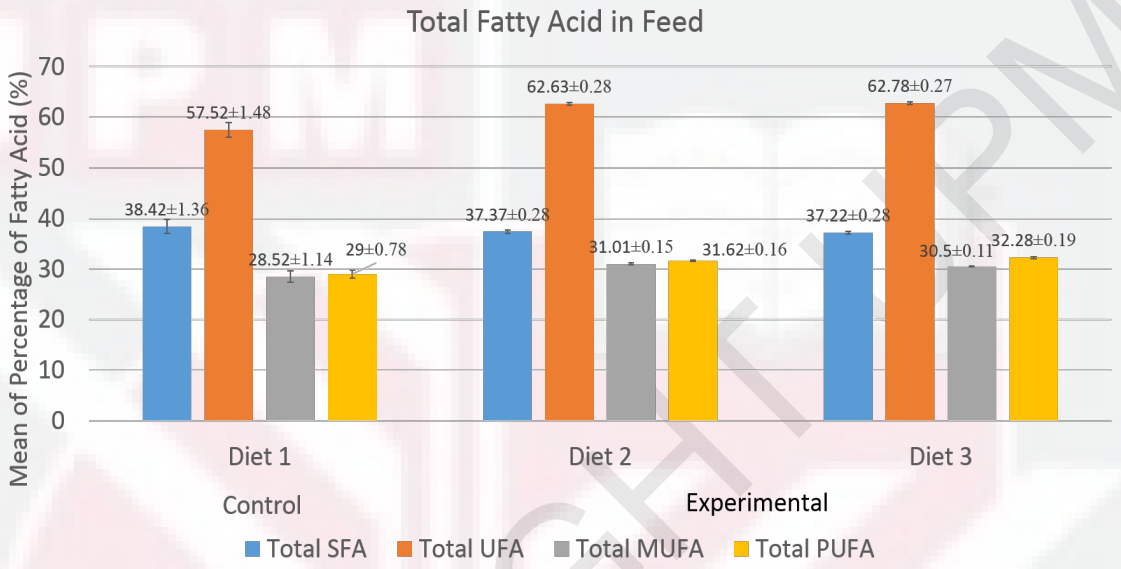

Figure 3: Fatty Acid Analysis on Feed

Fatty Acid Analysis of all feed formulation was depicted in Figure 3. All feed formulation shows high Unsaturated Fatty Acid (UFA) compared to Saturated Fatty Acid (SFA). This high UFA was contributed high PUFA which mainly consist of C18:2, n-6 (Linoleic acid) and C18:3, n-3 (α-Linolenic acid) in which Diet 3 shows the highest PUFA among the other diets.

4.4 Fatty Acid Analysis of Serum

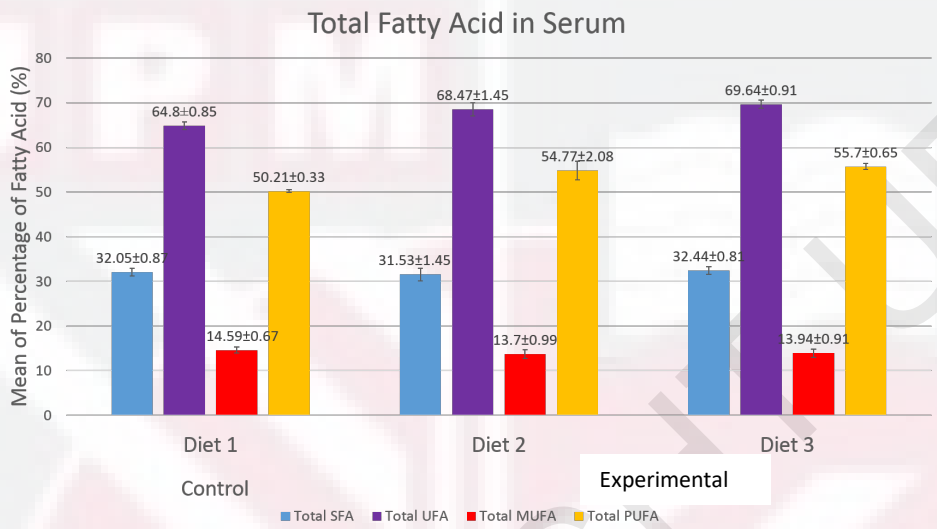

Figure 4: Fatty Acid Analysis of Serum

Serum Fatty Acid Analysis of goat fed with different feed formulation was presented in Figure 4. UFA was the highest value in the fatty acid profile for all diets and this also contributed by PUFA which mainly includes C18:2, n-6 (Linoleic acid), C18:3, n-3 (α-Linolenic acid), C20:4, n-6 (Arachidonic acid), C20:5, n-3 (Eicosapentaenoic acid; EPA), C22:5, n-3 (Docosapentaenoic acid; DPA) and C22:6, n-3 (Docosahexanoic acid; DHA).

5.0 DISCUSSION

Dry matter determination is very important as the results of nutrient composition are often expressed on a dry matter basis. It also allows comparing the nutrient

content of two or more diets that must be done on dry matter basis. The result revealed that Diet 3 had the highest DM content, suggesting that it has lower moisture content compared to other diets. Moisture content would determine the successful storage of feed and high moisture content might favor toxin production and bacterial action (Ball et al,. 2001)

Ash component mainly consists of inorganic matter such as mineral and soil after incineration process at a high temperature of 600°C. The values obtained give only the total ash and no information on individual minerals. Variability of total ash between diets might be due to volatilization which causes loss of certain minerals such as selenium, lead and chromium. After combustion, some metals can appear as carbonates (e.g. calcium carbonates) and as such, increasing the weight of the ash (Skoog and West, 1982).

Crude fiber revealed noncomparable value between diets which indicates that the fiber content might be similar. Crude fiber is primarily measured to define the indigestible parts in feed such as lignin, pentosan, cellulose and etc. However, crude fiber underestimates the total fiber content as part of lignin and hemicellulose are dissolved during the boiling procedure, thus is not constant.

As expected, as the amount of concentrate which is energy based feedstuff is increased in Diet 2 and Diet 3, the crude fat value for both diets are affected and higher compared to Diet 1 as depicted in Table 4. Crude fat value provides an

approximation of the total fat content which includes triglycerides, oil-soluble dyes (chlorophyll and carotenoids), waxes and others. Crude value for Diet 2 and Diet 3 is acceptable and meet the animal's requirement and this results were supported by Molly, 1997.

As the dietary protein intake is increased in Diet 2 and Diet 3, it is clearly reflected by the crude protein value in both diets. Crude protein in both diets show high value and meet the requirement according to the physiological status of the animal. Crude protein determination was done by calculating the nitrogen content by multiplying with protein factor = 6.25 as it is assumed that crude protein contains 16% nitrogen.

Fatty acid analysis of feed sample revealed high polyunsaturated fatty acid especially in Diet 3 which attributed by high concentrate that rich in C18:2, n-6 (linoleic acid). Linoleic acid is very important and needs to be provided in diet as it one of essential fatty acid. According to Palmquist (2010), linoleic acid able to improve pregnancy rate by enhancing progesterone secretion by granulosa cell.

From the nutritional related blood profile result of all feed formulation, Diet 1 shows the lower value of total protein and this might be due to a low quality of grass in the feed and less availability of protein as supported. Low quality of grass specifically low crude protein can be as a result of soil condition in terms

of its type and fertility (Mueller and Orloff, 1994) . A minimum 6 – 7% of crude protein (Bactawar, 2015) should be included in the diet for normal rumen function as microbes need nitrogen sources from protein to enhance fermentation process. Crude protein value should be add up to meet the animal's requirement following the production stages or physiological status (NRC, 1981)

Glucose determination in this study indicates that high concentrate especially in Diet 2 and Diet 3 increased the rate of carbohydrate metabolism. Increase in blood glucose is due thyroid hormone which stimulated by the increase of carbohydrate metabolism. It is very important to provide sufficient nutrient as glucose is a major source of energy and in relation to Diet 3 which is flushing diet, glucose is believed to improve the breeding performance through conception and ovulation rate (Tisch, 2006)

Calcium is used as indicator as it is one of important minerals that need to be provided in diet sufficiently. All calcium value were within normal range but Diet 3 shows the highest value compared to others. Calcium is very important as it serve various function such as skeletal formation, excitation of nerve impulse and cardiac muscle contraction. Calcium also is needed for good strength of skeleton so that animal will be fit for breeding and also to prevent from lameness or bowed leg (Gasparotto, 2105)

Short duration of experimental period might cause non-significant finding in Cholesterol value thus, 2-3 months of feeding trial is needed to produce significance results. Low to normal blood cholesterol indicates that provision of healthy diets is believed to prevent artherosclerosis (Daley et al., 2010)

As dietary energy in Diet 3 much higher than the other diets, it is reflected by the level of PUFA in serum which also show the highest value especially in Diet 3. PUFA mainly linoleic acid and α-linolenic acid will undergo biohydrogenation process for the conversion into saturated fatty acid which is stearic acid. Along this process, production of intermediates; conjugated linoleic acid and conjugated α-linolenic acid will be further incorporated into meat and milk thus improve the quality of animal products. Both linoleic and α-linolenic acid will also undergo elongation whereby they will be further elongated into other polyunsaturated fatty acid such as eicosopentaenoic acid, docosapentaenoic acid, docosahexanoic acid and arachidonic acid that serve various important function in physiological environment of animals.

6.0 CONCLUSION

From this study, it can be concluded that different feed formulation does affect certain blood nutritional related blood profile in goat such as Total Protein, Glucose and Calcium. Thus, it is very important to implement proper feeding

management in order to improve the productivity level and herd performance by optimizing the feed availability.

7.0 RECOMMENDATION

For future study, the author would like to recommend to increase the duration of experimental period to produce more significant result. Other indicators that be used to assess nutritional status of goat upon different feeding formulation include Albumin, Globulin, High Density Lipoprotein and Low Density Lipoprotein for definite and comprehensive understanding.

8.0 APPENDICES

Classification	
I	Concentrate (Energy sources) - Cereal grains, e.g: corn, sorghum, barley, rye, triticale - Other grains, e.g: buckwheat - Grain milling by-products, e.g: wheat bran, corn gluten meal - Roots and tubers, e.g: cassava, potatoes - Food processing by-products, e.g: molasses, bakery waste, citrus pulp, distillers and brewers by-products - Industrial by-products, e.g: wood molasses
II	Concentrates (Protein sources) - Oilseed meals, e.g: soybean, cottonseed, rapeseed, canola, linseed, peanut, safflower, sunflower meals - Grain legumes, e.g: beans, peas, lupins -Animal protein, e.g: meat meal, tankage, fishmeal, whey, feather meal - Nitrogen sources for ruminants - Nonprotein nitrogen source, e.g: urea, biuret, dried poultry waste - Bypass proteins, e.g: corn gluten meal
III	- Roughage, e.g: pasture, grasses, legumes - Green chop - Silage -Dry forage, e.g: hay, straw, stover,chaff - Agricultural by-products, e.g: corncob, hulls, baggasse
IV	Feed additives - Mineral supplements, e.g: salt, limestone - Vitamin supplements -Synthetic amino acids - Drugs, e.g: antibiotics, ionophores - Preservatives, e.g: antioxidants, mold inhibitors

Table 1 Classification of Feeds

Source: Cheeke (2005)

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