DETERMINATION OF OXALIC ACID AND SILICA CONTENTS IN THE FEED INGREDIENTS COMMONLY USED IN RUMINANT DIETS

Abirramy a/p Jagatheswaran

F16A0001

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Faculty of Agro Based Industry

University Malaysia Kelantan

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DECLARATION

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

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Student

Name:

Date:

I certify that the report of this final year project entitled "**Determination of oxalic acid and silica contents in the feed ingredients commonly used in ruminant diets**" by Abirramy a/p Jagatheswaran, matric number F16A0001 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry) with Honours, Faculty of Agro-Based Industry, University Malaysia

Kelantan.

Approved by:

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Supervisor

Name:

Date:

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Determination of oxalic acid and silica contents in the feed ingredients commonly used in ruminant diets

ABSTRACT

Although forage grasses are used as feed due to its nutritive values, but also these grasses contain anti-nutrients. Oxalic acid and silica contents are examples of anti-nutrients that are usually present in different feed ingredients. The soluble form of oxalate salts is the anti-nutrients as it can be combined with blood calcium or magnesium to form an insoluble oxalate crystal that excreted in the faeces. On the other hand, silica is the hairy part on the leaves that will reduce palatability and caused some physical damages. The aim of this study was to determine the contents of oxalic acid and silica contents in different feed ingredients. The studied feed ingredients included oil palm frond (OPF) (*Elaeis guineensis*), humidicola (*Brachiaria dictyoneura*), mulberry leaf (*Morus nigra*), banana leaf (*Musa acuminate*), napier grass (Kobe spp.) (*Pennisetum purpureum*), leucaena leaf (LL) (*Leucaena leucocephala*), cassava leaf (*Manihot esculenta*), bracharia grass (*Brachiaria mutica*), pineapple waste (*Ananas comosus*) and rumput Nyonya (*Asystasia gangetica*). Total oxalate and soluble oxalate contents in samples were determined by the high performance liquid chromatography (HPLC) method while silica content was measured by conventional method. Results showed that cassava leaf had the highest soluble oxalate content in the leaf part while leucaena leaf showed the lowest content. In the leaf part, the soluble oxalate content of OPF and LL were significantly different ($p<0.05$). No significant ($p<0.05$) differences were observed on insoluble oxalate content in the feed ingredients except for OPF and LL. For the silica, the LL showed the highest content compared to other feed ingredients. There were no significant differences on the ash and silica contents among all the different ingredients. In conclusion, sources of feed showed a great variation in oxalate content. Evaluation of different feed ingredients is helpful in balanced ration formulation for animals for better utilization of these commonly available feed resources.Therefore, in my study oil palm frond is the best feed ingredient with lowest oxalate content and silica content.

Keywords: *Feed ingredients, concentrates, anti-nutrient, oxalate, silica*

Penentuan asid oksalat dan kandungan silika dalam bahan makanan yang biasa digunakan dalam diet ruminans

ABSTRAK

Walaupun rumput ternakan digunakan sebagai makanan kerana nilai nutrisi, tetapi juga rumput ini mengandungi anti-nutrien. Asid oksalat dan kandungan silika adalah contoh anti-nutrien yang biasanya terdapat dalam ramuan makanan yang berbeza. Bentuk garam oksalat yang larut adalah anti-nutrien kerana ia dapat digabungkan dengan kalsium atau magnesium darah untuk membentuk kristal oksalat yang tidak larut yang diekskresikan dalam najis. Sebaliknya, silika adalah bahagian berbulu pada daun yang akan menyebabkan beberapa kerosakan fizikal. Tujuan kajian ini adalah untuk menentukan kandungan asid oksalik dan kandungan silika dalam bahan makanan yang berbeza. Bahan-bahan makanan yang dipelajari termasuk daun kelapa sawit (OPF) (*Elaeis guineensis*), Humidicola (*Brachiaria dictyoneura*), daun Mulberry (*Morus nigra*), daun pisang (*Musa acuminate*), rumput Napier (Kobe spp.) (*Pennisetum purpureum*) (*Leucaena leucocephala*), daun singkong (*Manihot esculenta*), rumput Bracharia (*Brachiaria mutica*), sisa nanas (*Ananas comosus*) dan Rumput Nyonya (*Asystasia gangetica*). Kandungan oxalate dan oksalat yang larut dalam sampel ditentukan oleh kaedah kromatografi cecair prestasi tinggi (HPLC) manakala kandungan silika diukur dengan kaedah konvensional. Keputusan menunjukkan bahawa daun Cassava mempunyai kandungan oxalate terlarut tertinggi di bahagian daun manakala daun Leucaena menunjukkan kandungan yang paling rendah. Di bahagian daun, kandungan oksalat yang larut OPF dan LL adalah sangat berbeza ($p < 0.05$). Tidak terdapat perbezaan terhadap kandungan oksalat yang tidak larut dalam bahan makanan kecuali OPF dan LL. Untuk silika, LL menunjukkan kandungan tertinggi berbanding bahan makanan lain. Tidak terdapat perbezaan yang signifikan terhadap kandungan abu dan silika di antara semua ramuan yang berbeza. Sebagai kesimpulan, sumber makanan menunjukkan variasi yang besar dalam kandungan oksalat. Penilaian bahan suapan yang berbeza membantu dalam perumusan ransum yang seimbang untuk haiwan untuk penggunaan yang lebih baik dari sumber suapan yang lazim disediakan. Oleh itu, dalam kajian saya, sawit kelapa sawit adalah bahan makanan terbaik dengan kandungan oksalat yang terendah dan kandungan silika.

Kata kunci: *Bahan makanan, pekat, anti-nutrien, oksalat, silika*

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List of Abbreviations

HPLC	High Performance Liquid Chromatography
DM	Dry Matter
HCl	Hydrochloric acid
NaOH	Sodium Hydroxide
Ca^{2+}	Calcium
Mg^{2+}	Magnesium
K^{+}	Potassium

List of Symbols

g	Gram
mm	Millimetre
mL	Millilitre
˚C	Celsius

CHAPTER 1

INTRODUCTION

1.1 Research background

Plants obtain the source of energy from sunlight. And these plants that undergo photosynthesis will actually store or produce the naturally-occurring nutrients in the plant. They are not edible by humans but the energy from the plant which is consumed by ruminants can be converted to available nutrients for human use. Most plants have fibrous material whereby humans cannot consume them directly. Ruminants are the special creation of nature which can efficiently consume and convert these energy to meat and milk which later on can be safely consumed by humans.

However, plants does not only contain nutrients but they also have anti-nutrients that can cause toxic poisoning to the animal health. The plants that have anti nutrients for their own protection from the pests around their plantation. For example, plants with high concentrations of oxalate include rice straw, the leaves of *Accacia spp*. and some forage grasses such as Setaria (*Setaria sphacelata*), buffelgrass (*Cenchrus Ciliaris*), Kikiyu grass (*Pennisetum clandestinum*) and Napier grass (*Pennisetum purpureum*). Based on the previous research studies, oxalic acid usually exists in the plant as soluble and insoluble salts. Studies have also shown that oxalate may play various roles in plants including calcium regulation, ion balance (e.g. N and K), plant protection, tissue support and heavy metal detoxification. (Libert and Franceschi 1987; Franceschi and Nakata 2005).

Despite these functional roles in plants, excess levels of oxalate adversely affect their nutritional quality as animal feed. Soluble oxalate can bind with calcium (Ca) in the gut with blood, Ca and magnesium (Mg) to form insoluble oxalate. It will eventually precipitate in the kidneys or it will be excreted into the faeces. This can eventually lead to chronic Ca deficiency called hypocalcaemia (a condition in which there are lower-than-average calcium levels in the body) or form kidney stones (urolithiasis; the formation of stony concretions in the bladder or urinary tract).

Plants are made up of a huge number of substances that maybe poisonous to the livestock (Cheeke and Shull, 1985). Oxalates are widely distributed in plants either in a soluble form which consists of potassium (K), sodium (Na) and ammonium salts or in an insoluble form which consists of Ca and Mg salts (Holloway et. al. 1989). Grazing animals such as ruminants are prone to Ca deficiency through eating forages with high concentration of oxalate salts (Jones et. al. 1972). Therefore, the bioavailability of Ca is reduced due to the oxalate salts.

Most mineral elements, whether essential or nonessential, can adversely affect an animal if included in the diet at excessively high levels (Gough et al. 1979). Silicon receives major significance in this context because its role in forage and animal nutrition has not been throughly investigated. Rice straw is commonly used as substitute for a part of forage ration and becoming a major energy source rather than nutrient source. This is because of the low nutrients content of rice straw as a barrier to be directly feed to animal. The rice straw also contains high amount of silica, oxalate and lignin (Van Soest, 1968). Lignin limits the quality of rice straw having a large by degree of ester bonding between lignin and hemicelluloses that can be broken with chemical treatment such as concentrated alkaline agents (Yamagishi et al. 2011). Silica is the main barrier on ruminal system where degradation process will replace to silicic acid and silicon dioxide. The high performance liquid chromatography (HPLC) method has been shown to be the most accurate and reliable to determine the oxalate content in plant materials (Holloway et al. 1989). The conventional method has been used to investigate the silica content in the plant substances.

1.2 Problem statement

Ruminants (e.g. cattle and goats) tend to be more tolerant of oxalate than non-ruminants (e.g. horses), because rumen bacteria can degrade oxalate into harmless formic acid and carbon dioxide. Allison et al. (1977) mentioned that adapted cattle and sheep gradually to increasing amounts of oxalate and demonstrated an enhanced degradation by rumen bacteria. Later work with horses indicated that oxalate-degrading bacteria were also found in the intestines, but were not always present (Allison and Cook, 1981). Although rumen bacteria can readily metabolize soluble oxalate (Davies, 1979), most of the ingested Ca oxalate appear to pass intact through the ruminant digestive tract (McKenzie and Schultz, 1983) as oxalate cannot be degraded by most rumen or intestinal bacteria. The gut bacterium that appears capable of degrading oxalate is *Oxalobacter formigenes;* this is a slow-growing bacterium that cannot utilize other substrates. The ability of other intestinal organisms to degrade oxalate has been less well studied, but *Enterococcus faecalis* as well as some lactic acid bacteria can degrade oxalate (McKenzie and Schultz, 1983) and their contribution to oxalate elimination in the gut may be significant. There are certain factors that affect oxalic acid and silica contents such as soil fertility, pH of soil, climatic condition and plant maturity. Therefore, this study was designed to investigate the contents of oxalate and silica in different varieties of feed ingredients to compare and contrast which feed ingredient has the highest oxalate and silica contents However, in Malaysia very limited studies and researches have been conducted in this regard.

1.3 Hypothesis

H_O: Different types of feed ingredients will not have significant different levels of oxalic acid and silica contents

H_A: Different types of feed ingredients will have significant different levels of oxalic acid and silica contents

1.4 Objectives

The objectives of this study were:

1. To investigate the oxalate and silica contents in different feed ingredients usually used in ruminant diets;
2. To identify the best and proper feed ingredients with low oxalate and silica contents.

1.5 Scope of study

The study was conducted in 3 different farms in Kelantan and different feed ingredients were collected. Different feed ingredients included Humidicola (*Brachiaria dictyoneura*), Mulberry leaf *(Morus nigra*), Banana leaf (*Musa acuminate*), Napier grass (Kobe spp.)(*Pennisetum purpureum*), Cassava leaf (*Manihot esculenta*), Bracharia grass (*Brachiaria mutica*), Pineapple waste (*Ananas comosus*), Rumput Nyonya (*Asystasia gangetica*), Oil palm frond (*Elaeis guineensis*) and Leucaena leaf *(Leucaena leucocephala*) commonly used in ruminant diets. A total of 30 samples was collected, three (3) replications for each feed ingredients. The samples were dried and grounded for determination of oxalic acid and silica contents research was carried out in the Faculty of Agro-Based Industry (FIAT)'s laboratory. The oxalic acid was determined by HPLC method and the silica content was measured using conventional method. All the data and results were recorded.

1.6 Limitation of Study

In this study, there were some limitations due to some factors. First, some of the feed ingredients did not have much information about the content of anti-nutrients present in it. The oxalic acid and silica contents of the different feed ingredients were determined by the research. However, based on the literatures, only some of the feed ingredients had information on the anti-nutrients. Most of the published articles are not the latest articles but aged more than 10 years.

CHAPTER 2

LITERATURE REVIEW

2.1 Forages

Forages can be grasses or legumes that is given to the livestock as a major feed source. A good quality of forages that has been given to the livestock will improve the livestock performance on the body weight gain, high milk production yield, efficient in reproduction and also generate profit to the farmers. Different types of forages will have different forage quality. The forage quality usually depends on the species, seasons, temperature, maturity stage, leaf to stem ratio, fertilization, grass-legume mixtures, harvesting and storage effects. (Ball, 2001)

2.1.1 Oil palm frond (*Elaeis guineensis jacq*.)

Oil palm is a plant that produces numerous results utilized by the animal feed industry, including palm oil. The territory under development for palm oil is quickly expanding in South East Asian nations. Malaysia, with 4.6 million ha under palm oil development presently creates more palm oil than Nigeria, Congo and Indonesia, the traditional sources of palm oil.

In recent times, OPF has been distinguished as a potential feed for ruminants and different herbivores (Dahlan, 1989).

2.1.2 Humidicola *(Brachiaria humidicola*)

Brachiaria humidicola originated from South Africa, and was introduced into Australia, Fiji and Papua New Guinea before spreading into most countries in South East Asia (Cook et al., 2005). It is favored by many smallholders because it establishes readily and reliably and is very easily propagated by way of the stem cutting method, usually planted at 1 m x1 m spacings (Cook et al. 2005). In terms of production, its volume is inferior to other species, as it is a creeping crop. Often uses the cut and carry system. Using this cut and carry system, it would be more convenient and more productive for the farmers as they can just cut and harvest the grass and feed it to their livestock in the shed. This cut-and-carry system is said to be more efficient in using forages as it avoids wastage of feed due to trampling by animals as when they are let to graze.

2.1.3 Mulberry leaf (*Morus nigra)*

Mulberry belongs to the genus *Morus* of the family Moraceae. It is an economically important plant being used for sericulture, as it is the sole food plant for the domesticated silkworm, *Bombyx mori*. The genus *Morus*, is widely distributed in Asia, Europe, North America, South America, and Africa, and is cultivated extensively in the eastern, central, and southern Asia for silk production. Mulberry is found from temperate and sub-tropical regions of the northern hemisphere, as well as in the tropics of the southern hemisphere, because it can grow in a wide variety of climatic, topographical, and soil conditions. They spread throughout all regions from the tropics to the sub-arctic and from sea level to altitudes as high as 4000 m. (Elmacı and Altuğ, 2002).

Among the most promising resources is the mulberry, with great adaptability to tropical conditions and easy to integrate into livestock production systems; also it offers security elements in feed supply, and sustainability in environmental aspects (Benavides, Lachaux and Fuentes, 1994). Mulberry contains crude protein concentrations between 15 and 28% in the dry matter (DM); 15% crude fibre (CF); from 33 to 46% neutral-detergent fibre (NDF); 28 to 35% of acid-detergent fibre (ADF); 5% lignin; 2.42–4.71% Ca; and 0.23–0.97% P. (Singh and Makkar, 2002). Total contents of tannins and phenols reported are very low (1.8% as tannic acid equivalent). These characteristics of the mulberry suggest its possible utilization as supplement in diets for animal species, and particularly for ruminants.

2.1.4 Banana leaf *(Musa acuminate)*

Banana plants are perennial, parthenocarpic polyploids of the genus Musa (Price, 1995). The plant itself is large and herbaceous, with a pseudostem composed of tightly packed leaves arranged in sheaths (Jones, 2000). Plants grow collectively in clumps, producing bunches of fruit then dying back to be replaced by clonal suckers from the rhizome. Banana has high nutritional value, it is a rich source of carbohydrates, mainly pectin (10 to 21%) (Mohapatra et al., 2010), as well as high soluble carbohydrate content, which can reach 32.4% dry matter, depending on the cultivar (Emaga et al., 2007). It has 2 to 10.9% ether extract

(Mohapatra et al., 2010), mainly composed of linoleic and α-linolenic acids (Emaga et al., 2007) and presents a high content of flavonoid compounds, especially gallocatechin, which has anti-inflammatory, antimicrobial and antioxidant activities (Someya et al., 2002).

2.1.5 Napier grass (Kobe spp.) *(Pennisetum purpureum)*

Napier grass *(Pennisetum purpureum),* also known as Elephant grass, has been the most promising high-yielding fodder, giving DM yields that surpass most other tropical grasses (Ansah *et al.,* 2010). It originates from sub-Saharan tropical Africa (Clayton et al., 2013) and has been introduced in most tropical and subtropical regions worldwide as forage. According to Mdziniso (2012), Napier grass is a tall perennial grass that grows to 2-5 metres (m) tall, rarely up to 7.5 m, with leaves about 30-120 centimetres (cm) long and 1 -5 cm broad.

The grass has been used by smallholder farmers in cut-and-carry feeding systems. Research consistently indicates that as Napier grass matures, the DM yield increases, while CP decreases (Ansah et al., 2010). Hence, the maturity stage of pasture grass at harvesting or grazing is considered a crucial management practice because this determines nutritional value of the grass (Jusoh et al., 2014). Napier grass is the most popular fodder used in dairy and feedlot production (Halim et al., 2013).

2.1.6 Leucaena leaf (*Leucaena leucocephala*)

L. leucocephala is one the highest quality and most palatable fodder trees of the tropics, often being described as the 'alfalfa of the tropics'. It was developed as forage in Hawaii. Leucaena provides nutritious and high protein forage for ruminants such as cattle, water buffalo, sheep and goats which increases milk production and is a protein supplements fed for dairy cows (Ter Meulen et. al, 1979).

2.1.7 Brachiaria grass (*Brachiaria decumbens*)

A grass is a highly productive tropical grass that is widespread through South America, Australia, Indonesia, Vanuatu and Malaysia due to its adaptation to a wide range of soil types and environments. The grass is of intermediate to high category in digestibility (50–80%), chemical composition and intake. Its CP ranges from 9% to 20%, but can decline rapidly with the age of leaf, from 10% at 30 days to 5% at 90 days. In Malaysia, Brachiaria species have been planted on more than 80% of improved farming pastures with *B. decumbens* as the most favoured species (Chin, 1989). While some other reports claimed that based on estimated nutritive values, animal production from *B. decumbens* pastures would be expected to be comparable to production from other commonly used tropical grass species and from medium quality temperate pastures.

2.1.8 Pineapple waste (*Ananas comosus)*

Pineapple (*Ananus comosus*, Bromeliaceae) is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavour and immense health benefits. Pineapple contains considerable calcium, potassium, fibre, and vitamin C. It is low in fat and cholesterol. Vitamin C is the body's primary water soluble antioxidant, against free radicals that attack and damage normal cells. It is also a good source of vitamin B1, vitamin B6, copper and dietary fibre. Pineapple is a digestive aid and a natural Anti-Inflammatory fruit. A group of sulfur-containing proteolytic (protein digesting) enzymes (bromelain) in pineapple aid digestion. Fresh pineapples are rich in bromelain used for tenderizing meat. Bromelain has demonstrated significant anti-inflammatory effects, reducing swelling in inflammatory conditions such as acute sinusitis, sore throat, arthritis and gout and speeding recovery from injuries and surgery. (Chin, 1989). Pineapple enzymes have been used with success to treat rheumatoid arthritis and to speed tissue repair as a result of injuries, diabetic ulcers and general surgery. Pineapple reduces blood clotting and helps remove plaque from arterial walls.

2.1.9 Rumput nyonya (*Asystasia gangetica*)

Asystasia gangetica is a perennial herbaceous plant with opposite entire leaves. The lamina is oval, 5 to 10 cm long and 3 to 6 cm wide. The leaves are covered on both sides with short sparse hairs. The stem 30 to 60 cm high, is often bent at its base. At the nodes, it thickens and becomes purplish. The stem and petioles are covered with tiny hairs. The flowers are in spikes at the top of the plant. The corolla is white but can also have yellowish shades and sometimes red. It measures 10 to 15 mm long. The corolla is formed of a tube having at the top 5 irregular rounded lobes, spread with the lower lobe stained or streaked in purple. The fruit is a capsule, 2 cm long, with a bulge on the upper part and a top corner.

This plant, commonly referred to as a weed in the plantations, has been shown to be excellent feed for ruminant animals; highly digestible (DM digestibility = 62%) and containing up to 20% crude protein (Mokhtar and Wong 1988). *Asystasia* thus serves as a good source of protein supplement to improve the poor nutritional status of ruminant animals' feeding solely on native pastures. Although the amount of *Asystasia* used as cut forage and its effect on animal production under farmers' conditions is not known, field observations have shown that grazing cattle and buffaloes consume *Asystasia* readily. However, excessive consumption of *Asystasia* results in watery faeces and may occasionally cause digestive disorders such as bloat.

2.2 Utilization of forages (Ruminant vs. non-ruminants)

Forage is a plant material that includes legumes, grasses, crops and also crops by product. Forages are a major feed resource for ruminant livestock to be consumed as fodder or pasture. Forages can be successfully digested by ruminant compared to non-ruminant animal. The constituent in forages like cell wall can be digested well in most ruminant digestive system (Wilkins, 2000). Ruminants like goat, sheep and cattle fed on forages as they can efficiently digest the fibre in their rumen with the help of microbial fermentation. Non-ruminant cannot utilize the forages very well as the absence of enzymes that used to catabolise the complex β-linked polymers that form plant cell walls (Beever, 1993).

2.3 Concentrates

Concentrates are low-fiber, high-energy feeds. They may be low, medium, or high protein. Most often they are fed to raise the energy level of the ration for dairy cattle and to compensate for any other deficiencies that remain beyond those provided by the forage portion of the ration which includes energy—nonfiber carbohydrates (NFC) and fat, Protein—crude protein, degradable intake protein (DIP), soluble protein (SP), and undegradable intake protein (UIP), Fiber—neutral detergent fiber (NDF) and acid detergent fiber (ADF), macro minerals—Ca, P, Mg, K, Na, sulfur, chloride, micro minerals—manganese, copper, zinc, iron, selenium, cobalt, iodine and fat-soluble vitamins—vitamins A, D, E and K. Concentrates may serve as carriers for various feed ingredients such as vitamins and minerals (macro and micro), as well as a variety of feed additives. (Mokhtar and Wong 1988) Proper preparation and processing of grains, feed ingredients, or a concentrate mix are essential. Concentrates must be palatable to attain required levels of feed intake. Proper processing and attention to particle size are important for palatability and ruminal degradation of nutrients. Concentrates should meet nutritional and performance needs at a reasonable cost. Purchased feed, including limited amounts of forage, may represent 40 to 55% of the total expenses on farms.

2.3.1 Molasses

Molasses is a dark brown, viscous liquid produced as a co-product of the production of sugar. After dissolving sugars out at high temperature, the crystals of sugar settle out as the liquid cools leaving the molasses, much of which was traditionally mixed back with the pulped fibers to produce molasses sugar beet feed. Molasses is suitable for inclusion in the diets of all ruminant livestock and can offer a very cost effective way to increase the palatability of feeds whilst contributing good levels of energy and protein (Mokhtar and Wong 1988). In dairy cows, ideal for complete diets added up to 3kg of molasses per head per day. Whereas, in beef cattle up to 10 per cent of molasses can be included in beef diets depending on the nature of other feeds in the mix and subsequent storage facilities for the finished ration. Similarly, up to 10 per cent of molasses can be safely included in young stock diets from four weeks of age. In sheep, molasses can be used at up to 10 per cent of dry sheep diets, but is not generally the preferred molasses product for ball or lick feeders (Senthilkumar et al., 2016).

2.3.2 Soybean

Soybean (*Glycine max, L*) is not only a source of high quality edible oil for humans, but also a high quality vegetable protein in animal feed worldwide. Its universal acceptability in animal feed has been due to favourable attributes such as relatively high protein content and suitable amino acid profile except methionine, minimal variation in nutrient content, ready availability year-round, and relative freedom from intractable anti-nutritive factors if properly process. Also, attention has been focused on soybean utilisation as an alternate protein source in animal diets due to the changing availability or allowed uses of animal proteins coupled with relatively low cost.(Gu, 2010)

2.3.3 Goat pellet

The most common processing method in animal feed production is the pelletising process, which involves mechanical action, in combination with moisture, pressure and temperature elements, to agglomerate particles of ingredients (Muramatsu et al., 2015) into a pellet form. Pelleted feed is not only convenient for feed handling, but also improves feeding efficiency in animals. And therefore, goat pellets can be utilised in livestock grazing system.

2.4 Oxalic acid content

Oxalic acid is an organic compound with a molecular formulae of $C_2H_2O_4$ or $(COOH)_2$ or HOOCCOOH. It is produced in the body by metabolism of glyoxylic acid or ascorbic acid. It is not metabolized but excreted in the urine. It is used as an analytical reagent and general reducing agent. On the basis of the published literature, it is difficult to find any agreement regarding a safe level of oxalate content in plants for grazing ruminants. McKenzie et al. (1988) suggested that 2% or more soluble oxalate can lead to acute toxicosis in ruminants, while Moir (1953) considered that grasses containing oxalate levels greater than 4% might be considered toxic to cattle. The soluble oxalate can combine with calcium or magnesium to form an insoluble oxalate crystal that excreted by feces due to the block of urine flow. (Rahman et al., 2013) said that "Oxalates are known to interrupt the calcium body and internal health of the animal. Insoluble calcium oxalate is formed from the combination of the soluble oxalate which will then react with the calcium in the blood. The calcium oxalate that has been formed reduces the calcium bioavailability (Rahman et al., 2013).

2.4.1 Factors affecting the oxalic acid

There are certain factors that affects the content of oxalic acid. According to Rahman et al. (2011), the soluble oxalate level in the plant can be increased when the K fertilizer was applied. Internal factors include the leaves and stem parts while external factors are climatic changes which will also affect the leaf and stem part. Internal factors also include the plant part where the young shot contains more oxalate followed by leaves and stem parts. Pre-treatment can be done like ensilage, which will eventually reduce the oxalic acid content.

2.4.2 Methods to determine the oxalic acid

Total oxalate and soluble oxalate can be determined using HPLC. Giving the strong acid solution like 1 M HCl make it washed away of crystalline oxalate includes the soluble and insoluble oxalates (Rahman, 2009). Rahman (2009) stated that "several methods also had been established to determine the oxalic acid like the amperometric techniques such aspolarographic, chemiluminescence, fluorometric, spectrophotometric and gas chromatography. The HPLC is the latest equipment that had been used by researcher to analyse the oxalic acid. (Rahman, 2009)

2.4.3 Oxalate tolerance of animals

It is generally accepted that ruminants can degrade certain levels of oxalate salts into harmless formic acid and carbon dioxide (Allison et al. 1977). Most studies have been conducted on oxalate degradation in the rumen and blood Ca level in response to different levels of dietary oxalate (Allison et al. 1985; Rahman et al. 2013). A study with sheep and goats showed that the administration of free oxalic acid led to changes in the rate of oxalic acid breakdown in the rumen, particularly of goats, thus protecting the host animal from toxic symptoms (Duncan et al. 1997).Duncan et al. (1997) also reported that goat's rumen is a special place for the microbial population which degrades oxalic acid more rapidly. Frutos et al. (1998) observed that goats were adapted to oxalic acid by daily oral administration of 3.4 g oxalic acid/animal/d of free oxalic acid, which successfully generated an active oxalic acid-degrading rumen microbial population, though goats showed a chronic mild hypocalcaemia.Oxalic acid is typically a corrosive substance and acidic, which can produce a sour taste in the mouth. Further, needle-like oxalate crystals cause pain and swelling when they contact lips, tongue and oral mucosa. Rahman et al. (2013) reported that some oxalate-rich plants contain abundant Ca (e.g. Medicago sativa), which are safe to feed despite their high oxalate content.

2.5 Silica content

The second most abundant element on Earth's crust is silicon (Si). In the constituents of plants, silicon is represented as silica (SiO_2). Silica is one of the constituents that can be found in the family of Gramineae. Silica can be seen as a hairy structure on the leaves of forages which is assembled as the rough surface which eventually reduces the palatability for grazing land livestock animals as it can cause physical damage on to them. There are three different forms of silica that we can find in the silica bodies within the cell (phytoliths), silica associated with cell walls and free silica (Bailey 1981). There are two types of silica when we narrow it down. One is soluble and another is insoluble. Insoluble silica is silica bodies and cell wall silica that has been polymerized with silicic acid. Soluble silica is the free-moving silica that has not been polymerized within silicic acid (Bailey, 1981).

2.5.1 Factors affecting the silica content

There are several factors that affect the silica content. They are biotic and abiotic factors. Abiotic factors include soil type, climate change and the moisture content that influences the silica content. Also, the rate of grazing affects the content of silica. Biotic factors include the species of plants and the genotype. And to determine the silica content in plants, there are several methods that has been introduced. Perry (2013) stated that the colorimetric method that uses molybdenum yellow and blue, atomic absorption and inductively coupled plasma analysis to study the silicon concentration.

2.5.2 Methods to determine the silica

There are several methods to determine the silica content in plant. According to Perry (2013), "a titrimetic method is used to measure the percentage of amorphous silica in (Rice Husk Ash) RHA. The titrimetric method advantage is time saving which would provide faster accurate result compared to standard method" According to Perry (2013), the colorimetric method uses molybdenum yellow and blue, atomic absorption and inductively coupled plasma analysis to determine Silicon (Si) concentration in solution (as cited in Currie & Perry, 2007).

2.5.3 Silica tolerance in animals

Silicon is usually found in combination with oxygen as silica. Silicon compounds play an especially significant part in some lower organisms (Carlisle, 1974). At least traces of silicon are found widespread in tissues and fluids of higher animals. The normal human blood level is less than 5 ppm (Carlisle, 1974). Silicic acid is readily absorbed across the intestinal wall and is excreted in the urine. Evidence was obtained that silica was not absorbed in sheep and cattle to a significant extent and appeared to be useful as an inert indicator to determine digestibility (Gallup et al. 1945). The amount of silicon in blood and intestinal tissues in rats is affected by age, sex, castration, adrenalectomy and thyroidectomy (Carlisle, 1974). Silica can enter in the respiratory tract, go to the lung as silicic acid and is eventually eliminated. The quantity of silicon present in the active growth areas of the bone appears to be related to the maturity of the bone (Carlisle, 1974). In the early stages of mineralization, silicon is present in small quantities. As mineralization progresses, silicon increases concomitantly with calcium. Later, the amount of silicon drops, and as the calcium reaches the level in apatite, silicon level is at the detection limit.

CHAPTER 3

METHODOLOGY

3.1 Experimental section

Samples are collected from three different places around Kelantan which is AgroTechnoPark of UMK Jeli, Ayer Lanas and Jeli Town. The period of collection was around June to July. 13 samples were collected that includes 10 roughages and 3 concentrates. 3 replication for each samples was used. Feed ingredients included oil palm frond (OPF) (*Elaeis guineensis*), humidicola (*Brachiaria dictyoneura*), mulberry leaf (*Morus nigra*), banana leaf (*Musa acuminate*), napier grass (Kobe spp.) (*Pennisetum purpureum*), leucaena leaf (LL) (*Leucaena leucocephala*), cassava leaf (*Manihot esculenta*), bracharia grass (*Brachiaria mutica*), pineapple waste (*Ananas comosus*) and rumput Nyonya (*Asystasia gangetica*). Concentrates include soybean, molasses and goat pellet.

3.1.1 Sample preparation

The leaf parts of the plant were cut and chopped separately into small size (2-3cm) manually which is under the sunlight and then it is dried in an oven for 48 hours at 70^{o}C. (AOAC, 1942)The concentrates were also placed separately. Dried samples were then grinded in a grinder and passed through a 1-mm laboratory test sieve and stored in a zipper bag.

Figure 3.1.1 (a): Leaves were cut from 2cm to 3cm

Figure 3.1.1 (b): Leaves were dried under the sun to remove excess moisture

3.1.2 Extraction and determination of total and soluble oxalates

Around 0.5g of sample was mixed with 15ml of distilled water for soluble oxalate extraction. For total oxalate extraction, 15ml of 1 mol HCL is extracted. Samples must be heated for 18 minutes on stirring hot plate. Mixtures must be allowed to cool down to room temperature filtered using filter paper. Mixtures were then washed with distilled water and made upto 50 ml in a volumetric flask. The filtrate for the total oxalate extraction will be adjusted to pH 3.0 using 5 mol NaOH. The solutions were filtered using 5ml syringe using hydrophilic membrane filter paper and kept in the HPLC via 1.5ml for HPLC analysis.(AOAC 1995)

Figure 3.1.2 (a): 0.5g of each sample is weighed and placed on aluminum foil

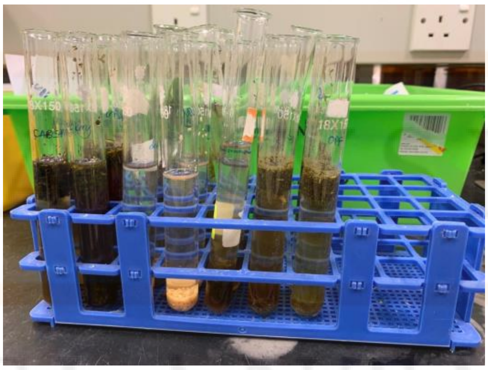

Figure 3.1.2 (b): Leaf part extraction of total oxalate with 1 mol HCl

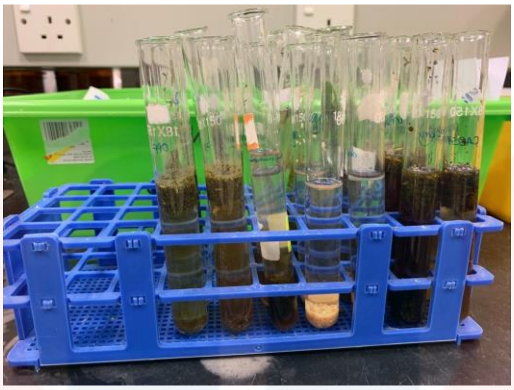

Figure 3.1.2 (c): Leaf part extraction of soluble oxalate with distilled water

Figure 3.1.2 (d): Samples were heated for 18 minutes on stirring hot plate

3.1.3 Standard calibration

Four sets of standard solution were prepared by adding 1.41g of commercial oxalic acid to 100 mL of volumetric flask and making up to volume with distilled water. Serial dilutions of standard solution were prepared by diluting the standard oxalic acid to 100 mL with 0.5mL, 1.0 mL, 2.0 mL and 3.0 mL, which was taken from the stock solution and then made 100 mL with distilled water in the volumetric flask respectively. (AOAC, 1995)

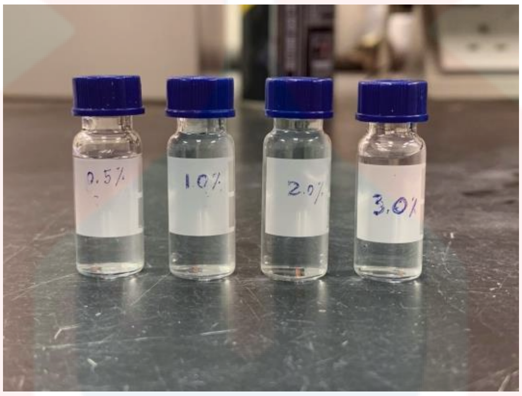

Figure 3.1.3 (a): Four sets of stock solution were prepared

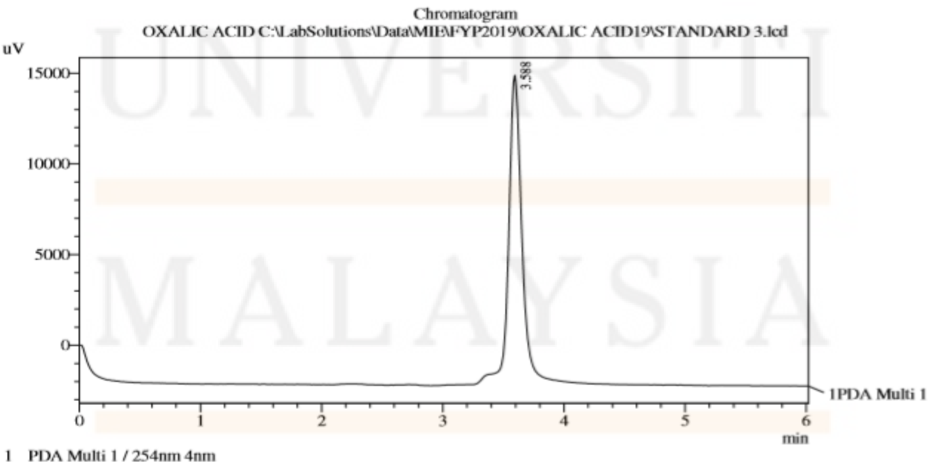

Figure 3.1.3(b): Chromatogram of an authentic oxalic acid (2%) by high performance liquid chromatography

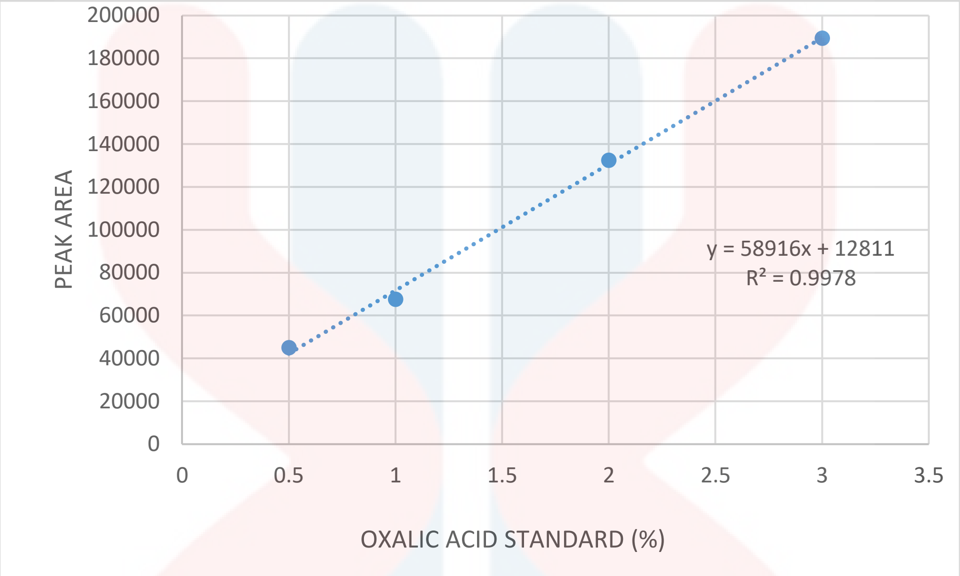

Figure 3.1.3(c): Calibration curve of authentic oxalic acid

3.1.4 HPLC analysis

The analysis of oxalate was conducted on a column Synergi 4µm Hydro- RP 80 Å, LC Column 250 x 4.6m, Ea (Phenomenex,USA). A sample of 1.5ml was chromatographed at 30ºC using 20mM Potassium phosphate buffer with pH 2.9 as eluent A at a flow rate of 0.7 mL $min^{-1.}$ Oxalate was detected at 220 nm using detector UV- Vis Ans- Variable Wave. The amount of oxalate was determined with a reference to an original of commercial oxalate. The insoluble oxalate was estimated by subtracting the soluble oxalate from total oxalate.(AOAC, 1995)

Figure 3.1.4(a): Oxalate extraction in the vial tray ready for HPLC analysis

Figure 3.1.4(b): The HPLC machine

3.1.5 Sample preparation (silica)

2g of dried samples were mixed, weighed and then kept in the crucible. Then, crucibles were labelled and was kept in the muffle furnace at 600^0C for 2 hours (AOAC, 1942). The remaining ash was then used to determine the silica content.

Figure 3.1.5 (a): 2g of dried sample were mixed, weighed and then kept in the crucible

Figure 3.1.5 (b): Crucibles are labelled and kept in the muffle furnace at 600^0C for 2 hours

Figure 3.1.5 (c): The remained ash of the sample after taken out from muffle furnace

3.1.6 Silica determination

Silica content was determined as described by Widyastuti and Abe (1989). 10 ML HCL (50%) solution were added twice to the remained ash in the crucible. Then, let it to be dehydrated on the hot plate for 12 hours at 155^0C. After the sample has been dehydrated and the dried silica is transferred on a filter paper and manifold it to wash the silica with water about three to four times. The filter paper was placed on the same crucible and dried in the forced air oven for 3 hours at 105^0C. The dried samples were incinerated in the furnace for 600^0 C for 8 hours.

Figure 3.1.6 (a): Dehydration process after HCl were added to the remained ash

Figure 3.1.6 (b): Washing of dried silica for almost three to four times


Figure 3.1.6 (b): Sample after the ashing method

3.1.7 Statistical analysis

The data for oxalate and silica contents were analyzed statistically using SPSS software. DUNCAN Multiple Range test (DMRT) was used to separate the treatment means at the 95% confidence level ($p<0.05$). The data were presented as mean ± standard deviation. The silica content was calculated with the formula below:

$$\text{Silica content (\%)} = (W1-W0)/S \times 100 \qquad (3.2.5)$$

Where, W0: Empty crucible weight (g)

W1: Crucible weight after incineration + ash (g)

S : Sample weight (g) in dry matter

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Types of oxalate

Oxalate is a type of salt that present in the forages in a form of soluble and insoluble. Both forms of oxalate will affect differently towards the consumer, animal and human. Oxalates that are found in plant has different content basis depending on the internal and external factors such as plant species, seasons, plant maturity, the quantity and type of fertiliser applied and much more. In this research carried out, the oxalate content of different feed ingredients and concentrates showed different values.

4.1.1 Soluble oxalate

From Table 4.1.1, we can conclude that cassava leaf had the highest soluble oxalate content in the leaf part while leucaena leaf has the lowest content (1%). In the leaf part, the soluble oxalate content of oil palm frond and leucaena leaf were significantly different ($p<0.05$). Meanwhile, humidicola, mulberry leaf, banana leaf, napier grass (*Kobe spp*.), cassava leaf, bracharia grass, pineapple waste and rumput nyonya had no significant differences on soluble oxalate content. In this study, the oil palm frond and leucaena leaf were significantly different ($p<0.05$) on soluble oxalate content compared to the other eight different feed ingredients. All the details of soluble oxalate are shown in Table 4.1.1.

Table 4.1.1: Soluble oxalate (mean ± standard deviation) of different feed ingredients as affected by different locations in Kelantan

Feed ingredients	Location name (mean ± standard deviation)			Overall	P-value
	ATP, UMK	Ayer Lanas	Jeli		
Oil palm frond (*Elaeis guineensis*)	$0.52^{a} \pm 0.19$	$0.39^{b} \pm 0.22$	$0.34^{b} \pm 0.01$	0.39 ± 0.14	0.013
Humidicola (*Brachiaria dictyoneura)*	0.39 ± 0.13	0.40 ± 0.01	0.39 ± 0.14	0.39 ± 0.12	0.515
Mulberry leaf (*Morus nigra)*	0.37 ± 0.15	0.37 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.711
Banana leaf (*Musa acuminate*)	0.40 ± 0.00	0.40 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	0.295
Napier grass (Kobe spp.) (*Pennisetum purpureum*)	0.55 ± 0.04	0.53 ± 0.03	0.53 ± 0.02	0.54 ± 0.03	0.265
Leucaena leaf *(Leucaena leucocephala*)	$0.35^{a} \pm 0.01$	$0.35^{b} \pm 0.01$	$0.33^{b} \pm 0.01$	0.34 ± 0.01	0.010
Cassava leaf (*Manihot esculenta*)	0.38 ± 0.03	0.38 ± 0.01	0.39 ± 0.02	0.38 ± 0.02	0.845
Brachiaria grass (*Brachiaria mutica*)	0.33 ± 0.01	0.33 ± 0.00	-	0.33 ± 0.01	0.448
Pineapple waste (*Ananas comosus*)	0.36 ± 0.02	-	0.32 ± 0.02	0.35 ± 0.02	0.110
Rumput Nyonya (*Asystasia gangetica*)	-	0.40 ± 0.02	0.42 ± 0.02	0.41 ± 0.02	0.408

^{ab} means with different superscripts differ significantly ($p \leq 0.05$) DM, dry matter

From Table 4.1.1, the soluble oxalate content of the leaf part in the oil palm frond and Leucaena leaf were differed significantly ($p<0.05$). Most of the oxalates are usually present in the leaf tissue (Rahman et al. 2006). Thus, the oil palm frond and leucaena leaf contained lower soluble oxalate content compared to the other feed ingredients. The nutrient requirements of plantation tree crops are usually calculated based on the nutrient balance concept (Chew et al., 1994b; Kee et al., 1994). These feed differed significantly might be due to the nutrient imbalance of the soil in growing the oil palm plantation. The soluble oxalate in the forages is a salt in the form of Na oxalate, K oxalate and ammonium (NH_4^+) oxalate. The other eight feed ingredients did not differ significantly ($p<0.05$).

4.1.2 Insoluble oxalate

The insoluble oxalate was calculated by the difference of the total oxalate and the soluble oxalate value. The insoluble oxalate usually does not give any negative effect to the animal body. From Table 4.1.2, cassava leaf contained the higher insoluble oxalate content (2.13%) followed by mulberry leaf (1.25%), rumput nyonya (1.04%), banana leaf (0.98%), pineapple waste (0.57%), leuceana leaf (0.35%), humidicola (0.33%), bracharia grass (0.32%) and oil palm frond (0.25%).

Table 4.1.2: Insoluble oxalate (mean ± standard Deviation) of different feed ingredients as affected by different locations in Kelantan

Feed ingredients	Location name (mean ± standard deviation)			Overall	P-value
	ATP, UMK	Ayer Lanas	Jeli		
Oil palm frond (*Elaeis guineensis*)	$0.15^{a} \pm 0.16$	$0.31^{b} \pm 0.02$	$0.29^{b} \pm 0.01$	0.25 ± 0.12	0.023
Humidicola (*Brachiaria dictyoneura)*	0.32 ± 0.02	0.32 ± 0.03	0.35 ± 0.02	0.33 ± 0.03	0.230
Mulberry leaf (*Morus nigra)*	1.24 ± 0.02	1.26 ± 0.03	1.24 ± 0.03	1.25 ± 0.03	0.284
Banana leaf (*Musa acuminate*)	1.02 ± 0.02	0.97 ± 0.06	0.96 ± 0.05	0.98 ± 0.05	0.099
Napier grass (Kobe spp.) (*Pennisetum purpureum*)	0.24 ± 0.04	0.24 ± 0.06	0.26 ± 0.02	0.24 ± 0.04	0.645
Leucaena leaf (*Leucaena leucocephala*)	$0.37^{a} \pm 0.01$	$0.31^{ab} \pm 0.05$	$0.35^{b} \pm 0.05$	0.35 ± 0.05	0.087
Cassava leaf (*Manihot esculenta*)	2.12 ± 0.01	2.12 ± 0.06	2.11 ± 0.08	2.13 ± 0.08	0.447
Brachiaria grass (*Brachiaria mutica*)	0.32 ± 0.04	0.33 ± 0.03	-	0.32 ± 0.03	0.497
Pineapple waste (*Ananas comosus*)	0.57 ± 0.04	-	0.58 ± 0.07	0.57 ± 0.05	0.796
Rumput Nyonya (*Asystasia gangetica)*	-	1.06 ± 0.04	1.02 ± 0.08	1.04 ± 0.06	0.226

^{ab} means with different superscripts differ significantly ($p \leq 0.05$).

From Table 4.1.2, there were no significant (p>0.05) differences for insoluble oxalate of content different feed ingredients except oil palm frond among all the locations. Insoluble oxalate is available in plants in a form with Ca, Mg, and salts (Mason, 2000). The Ca oxalate or Mg oxalate commonly exists in all plant tissues as an insoluble salt. Therefore, it cannot be absorbed and no further utilisation of it in the animal body and it will pass through the digestive tract. The insoluble oxalate seems not to be harmful but it is related to the mineral content. (Rahman & Kawamura, 2011).

4.1.3 Total oxalate

Plants like spinach, buckwheat grin, rhubarb, *Setaria*, and nuts have a high amount of oxalate content as it all includes in the *Oxalis* genus (Penniston, 2014). The total oxalate content is the total of soluble and insoluble oxalates content that found in the different feed ingredients. Total oxalate content also determines the safety level of a fodder that can be consumed by the animal. Table 4.1.3 shows the total oxalate content in different feed ingredients.

According to Table 4.1.3, cassava leaf showed the highest value of total oxalate (2.51%) while oil palm frond showed the lowest total oxalate content (0.61%). No significant (p >0.05) differences were observed on the total oxalate content among the all locations for all feed inngredients.

Table 4.1.3: Total oxalate (mean ± standard deviation) of different feed ingredients as affected by different locations in Kelantan

Feed ingredients	Location name (mean ± standard deviation)			Overall	P-value
	ATP, UMK	Ayer Lanas	Jeli		
Oil palm frond (*Elaeis guineensis*)	$0.58^a \pm 0.12$	$0.62^b \pm 0.00$	$0.63^b \pm 0.01$	0.61 ± 0.07	0.367
Humidicola (*Brachiaria dictyoneura)*	0.71 ± 0.01	0.72 ± 0.26	0.73 ± 0.03	0.72 ± 0.02	0.281
Mulberry leaf (*Morus nigra)*	1.61 ± 0.02	1.63 ± 0.03	1.61 ± 0.03	1.62 ± 0.02	0.224
Banana leaf (*Musa acuminate*)	1.41 ± 0.02	1.37 ± 0.05	1.36 ± 0.05	1.38 ± 0.05	0.100
Napier grass (Kobe spp.) (*Pennisetum purpureum*)	0.79 ± 0.02	0.78 ± 0.04	0.78 ± 0.01	0.78 ± 0.03	0.374
Leucaena leaf *(Leucaena leucocephala*)	$0.72^a \pm 0.01$	$0.65^{ab} \pm 0.04$	$0.68^b \pm 0.05$	0.68 ± 0.05	0.059
Cassava leaf (*Manihot esculenta*)	2.55 ± 0.10	2.50 ± 0.07	2.51 ± 0.07	2.51 ± 0.08	0.491
Bracharia grass (*Brachiaria mutica*)	0.64 ± 0.04	0.66 ± 0.03	-	0.65 ± 0.03	0.382
Pineapple waste (*Ananas comosus*)	0.93 ± 0.04	-	0.92 ± 0.06	0.92 ± 0.05	0.710
Rumput Nyonya (*Asystasia gangetica*)	-	1.47 ± 0.03	1.43 ± 0.07	1.45 ± 0.06	0.286

^{ab} means with different superscripts differ significantly ($p \leq 0.05$)

Table 4.1.4: Soluble, insoluble and total oxalate concentration in various forage grasses

Feed ingredients	Soluble	Insoluble	Total
Oil palm frond	0.39 ± 0.14	0.25± 0.11	0.63±0.01
(*Elaeis guineensis*)	(9)	(9)	(9)
Humidicola	0.39 ± 0.01	0.33 ± 0.03	0.72 ± 0.02
(*Brachiaria dictyoneura)*	(9)	(9)	(9)
Mulberry leaf	0.37 ± 0.01	1.25± 0.03	1.62 ± 0.02
(*Morus nigra)*	(9)	(9)	(9)
Banana leaf	0.40 ± 0.01	0.98 ± 0.05	1.38 ± 0.05
(*Musa acuminate*)	(9)	(9)	(9)
Napier grass (Kobe spp.)	0.54 ± 0.03	0.24 ± 0.04	0.78 ± 0.03
(*Pennisetum purpureum*)	(9)	(9)	(9)
Leucaena leaf	0.34 ± 0.01	0.35 ± 0.05	0.68 ± 0.05
(Leucaena leucocephala)	(9)	(9)	(9)
Cassava leaf	0.38 ± 0.02	2.13 ± 0.08	2.51 ± 0.08
(*Manihot esculenta*)	(9)	(9)	(9)
Bracharia grass	0.34 ± 0.02	0.39 ± 0.12	0.73 ±0.014
(*Brachiaria mutica*)	(6)	(6)	(6)
Pineapple waste	0.37 ± 0.04	0.81 ± 0.26	1.19 ± 0.29
(*Ananas comosus*)	(6)	(6)	(6)
Rumput Nyonya	0.41 ± 0.03	0.99 ± 0.09	1.40 ± 0.07
(*Asystasia gangetica)*	(6)	(6)	(6)
P-value	0.00	0.00	0.00

*Figures in parenthesis indicate the number of replication

Based on Table 4.1.4, the soluble oxalate had the highest in Napier grass (*Pennisetum purpureum*), cassava leaf *(Manihot esculenta*) had the highest insoluble oxalate and total oxalate contents.

4.2 Concentrates

Animal feeds can be classified as concentrates, high in energy value, including fat, cereal grains and their by-products (barley, corn, oats, rye, wheat), high-protein oil meals or cakes (soybean, canola, cottonseed, peanut [groundnut]), and by-products from processing of sugar beets, sugarcane, animals, and fish. Increasing the concentrate feed input in diets based on grass silage (Agnew et al., 1996) and maize silage (Fitzgerald and Murphy, 1999) has a positive effect on milk production and BCS loss (Delaby et al., 2009), otherwise known as a response to concentrate (Bargo et al., 2003). However, animals respond differently to concentrate supplementation due to variation within the herd, which is caused by differences in stage of lactation, parity, and genotype (Horan et al., 2005).

4.2.1 Types of concentrates

Molasses is a concentrated appetizer and dust settler. It is sticky, sweet, and smells good. It contains 54 percent of TDN, very little minerals, no fibre, and no digestible protein. Unit cost of TDN is usually as high as or higher than the cost of the same amount of energy as grain. However, either cane or sugar beet molasses is nearly always included at levels of 5 to 15 percent in commercially mixed rations. Soybean, cottonseed oil meal, peanut oil meal, and linseed oil meal are called "protein supplements." Although these protein supplements are high in energy value also, feeding excessive amounts is useless, expensive, and causes digestive upsets. Quality goat feed ensures quality meat or milk production. Providing adequate feed according to the demand of your goats help them to grow faster and produce more milk or meat. This type of food habit helps them to meet up the nutritional demands.

Table 4.2.1: Soluble, insoluble and total oxalate concentration in various concentrates

Concentrates	Soluble	Insoluble	Total
Mollases	$0.31^{b} \pm 0.02$	$0.46^{a} \pm 0.02$	0.77 ± 0.00
Soybean	$0.33^{ab} \pm 0.01$	$0.45^{ab} \pm 0.01$	0.77 ± 0.00
Goat Pellet	$0.34^{a} \pm 0.01$	$0.44^{b} \pm 0.01$	0.77 ± 0.00
P-Value	0.044	0.044	0.000

^{ab} means with different superscripts differ significantly ($p \leq 0.05$)

Based on Table 4.2.1, it was found that soluble and insoluble oxalate has significant difference ($p<0.05$) among the various concentrates. For total oxalate, there was no significant difference ($p<0.05$) among the concentrates.

4.3 Oxalate toxicity

Soluble oxalate is the main concern in the forages. Soluble oxalate is soluble and easy to be absorbed in the body. Soluble oxalate will give negative impact to the animals' health and performance as it can combine with the blood calcium and formed calcium oxalate. Calcium oxalate can contribute to form kidney stones and also cause hypocalcaemia as the calcium level in the body was decreased. Oxalate can cause acute poisoning and death when the level ranges 7% to 16.6% as reported by El-khodery, El-boshy & Gaafar (2008). El-Khodery et. al (2008) reported that ewes that fed with the beet tops (*Beta vulgaris*) is a forage that associated high oxalate content and it showed the positive result about the deposition of calcium oxalate crystal formation under the microscope. To prevent the calcium oxalate formation excess, the safety level for ruminant to consume forages containing soluble oxalate is less than 2.0% DM. (Nayananjalie, 2015) Moreover, several cases on oxalate toxicity have been reported a long time ago, Jones et. al (1970) reported that oxalate poisoning in cattle was found when fed on Setaria *(Setaria sphacelata*) pastures. Sheep poisoning was also reported by McKenzie et al. (1988) ,when sheep was fed with Buffel grass (*Cenchrus ciliaris*) that is high in oxalate content. A case study on feeding Napier grass *Pennisetum purpureum* cv. Pusa giant caused high mortality in cattle and buffalo calves due to 3.01% of oxalic acid present and low in calcium level as reported by Dhillon et al (1971) and Sidhu et. al (1996).

4.3.1 Ruminant vs. Non-Ruminant

Forages can be successfully digested by ruminant compared to the non-ruminants. The presence of rumen bacteria in the ruminant digestive system helps the ruminant to digest the forage better than non-ruminant as the forages contains lignin. The oxalate will be degraded by the rumen bacteria in the ruminant but the non-ruminant cannot tolerate with the oxalate as it cannot be degraded without the rumen bacteria. (Rahman et al., 2013). Rahman et al. (2013) stated that ruminant can prevent the oxalate poisoning, if soluble oxalate content is at <2.0%, while the non-ruminants toleration towards the soluble oxalate content is at <0.5%.

4.3.2 Prevention

An economic loss will occur when the animal died after fed with forages that high in oxalate content. In order to reduce the oxalate toxicity in the animal, the animal should not be fed with the forages that have high oxalate content such as Setaria grass as it contains up to 5.6% soluble oxalate compared with Napier grass contains about 3.8% soluble oxalate (Rahman et. al., 2011). Moreover, the grazing animal should put more attention as the animal might consume the oxalate-rich containing forage that would interrupt the calcium level in the animals' body. Thus, the animal that only depends on forages diet also should be concerned more as they might they might not consume enough calcium like the concentrate feeds can provide.

Besides that, we should give some supplement like dicalcium phosphate in the diet before and during the feeding. (Rahman et al., 2013). Urea Molasses Mineral Block (UMMB) is one of the suitable supplement that can be supplied to the animal under mineral deficiency as the UMMB consisted of molasses, urea, dicalcium phosphate, trace minerals and salts (Wadhwa et al., 2015). Oxalates cause the loss of calcium absorption thus, supplementing the UMMB may be one of the ways to solve or reduce this problem. Therefore, further research should be carried out in order to prove it. According to Rood et al., 2014, recommendations for avoiding oxalate toxicity is providing plenty fresh water at the animal grazing area and calcium –enriched trace mineral salt is available.

4.4 The silica content

From the Table 4.4.1(a), the ash content was the highest in pineapple waste (*Ananas comosus*) (18.63%). For the silica, the leucaena leaf *(Leucaena leucocephala*)has showed the highest content compared to other feed ingredients.. Based on Table 4.4.2, the highest ash content and silica content had been containing in the goat pellet.

Table 4.4.1(a): Ash and silica content (DM%) in different feed ingredients

Feed	Ash (Mean ± Standard Deviation)	Silica (Mean ± Standard Deviation)
Oil palm frond (*Elaeis guineensis*)	11.08 ± 4.43	2.44 ± 0.47
Humidicola (*Brachiaria dictyoneura)*	14.41 ± 3.94	3.09 ± 0.47
Mulberry leaf (*Morus nigra)*	14.70 ± 0.44	3.54 ± 0.12
Banana leaf (*Musa acuminate*)	14.27 ± 1.67	4.35 ± 0.54
Napier grass (Kobe spp.) (*Pennisetum purpureum*)	16.24 ± 0.00	2.74 ± 0.18
Leucaena leaf *(Leucaena leucocephala*)	12.73 ± 0.01	4.18 ± 0.27
Cassava leaf (*Manihot esculenta*)	18.45 ± 1.20	3.54 ± 0.22
Bracharia grass (*Brachiaria mutica*)	16.45 ± 1.28	2.92 ± 0.29
Pineapple waste (*Ananas comosus*)	18.62 ± 1.13	4.11 ± 0.32
Rumput Nyonya (*Asystasia gangetica)*	12.62 ± 0.44	3.21 ± 0.07

Table 4.4.1(b): Ash and silica content (DM%) in different concentrates

Feed	Ash (mean ± standard deviation)	Silica (mean ± standard deviation)
Mollases	2.66 ± 0.4	0.46 ± 0.01
Soybean	1.39 ± 5.6	0.32 ± 0.01
Goat Pellet	5.53 ± 6.0	0.54 ± 0.03

4.4.2 Silica in forage

Massey and Hartley (2006) stated that the grasses are considered as silica accumulating plants that accumulate the silica phytoliths in their tissue as it absorbed the silicon in silicic acid form in the soil. Quigley and Anderson (2014) reported that bunch grasses like T.Triandra and lawn-grass D. macroblephara had silica content of 3.7% and 2.7% respectively. Silica in the leaves of grasses can constitute 2–5% dry matter, 10–20 times higher than levels found typically in dicotyledonous plants (Russel 1961). Silica is stored primarily as opaline phytoliths in the epidermis (Parry & Smithson 1964; Kaufman et al. 1985). Accumulation of such high levels of a single mineral element within this plant family suggests that it has a functional significance. Suggested functions include silica as a waste product resulting from uncontrolled uptake at the roots (Ellis 1979), or that silica acts a mechanism of structural rigidity forming a metabolically inexpensive alternative to carbon-based support (Iler 1979; McNaughton et al. 1985). Cell wall silification and increases in silica deposition around damage sites have also been proposed as a mechanism to reduce fungal attack (Jones & Handreck 1967).

4.4.3 Silica effects on animal performance

The main concern about the silica in the forages is it can cause silica urolithiasis in sheep and cattle as reported by Jones & Handreck (1967). This is related to the silica excretion where the absorbed silica by the kidney will pass through the urine (Jadaosingh, 2009). Through the physical observation, the abundance of the hairy structure of silica on the leaf surface will cause refusal of forages when fed to the animal in huge amount. Moreover, there is lacking current studies on the effect of silica toward ruminant performance as there is no safety level of silica content of animals' tolerance as the published literatures.

CHAPTER 5

CONCLUSION

5.1 Conclusion

In conclusion, oxalate content was significantly different among the all different feed ingredients. However, there were no significance differences on silica content among all the different feed ingredients. Mulberry leaf had the highest oxalate content and Banana leaf had the highest silica content. On the other side, the oil palm frond had the lowest oxalate content and silica content. Anti-nutrients contents in the feed ingredients received little attention from farmers as they tend to focus more on the nutritional value of forages. Therefore, to select the best feed ingredients that has high nutrient quality and low anti-nutrient needs the whole study on the nutritional and anti-nutritional content of all feed ingredients.

5.2 Recommendations

This is the initial studies focused on the anti-nutritional composition, the oxalic acid and the silica contents in different feed ingredients such as Humidicola , Mulberry leaf, Banana leaf, Napier grass (Kobe spp.), Cassava leaf, Bracharia grass, Pineapple waste, Rumput Nyonya, Oil palm frond, Leucaena leaf and concentrates such as molasses, soybean and goat pellet, which were taken from three different locations in Kelantan which is AgroTechnoPark, UMK, Ayer Lanas and Jeli. The findings of oxalate and silica also rely on the mineral availability in that particular location. Therefore, different locations of the study will come out with a different result in the future. To improve this research, the feeding trials experiment must be done to know the recommended and safety level of consuming the forages and concentrates that is rich in oxalate and silica content as the available studies are limited.

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APPENDIX A

Table A.1: Soluble oxalate content in different feed ingredients

Descriptive Statistics

Dependent Variable: SOLUBLE

TREATMENT	Mean	Std. Deviation	N
OPF	.3906	.13897	18
HUMIDICOLA	.3906	.01211	18
MULBERRY	.3683	.01150	18
BANANA	.3972	.00752	18
KOBE SPP	.5356	.02955	18
LEUCEANA	.3389	.01491	18
CASSAVA	.3833	.01879	18
BRACHARIA	.3363	.01708	16
PINEAPPLE	.3744	.04195	16
ASYTASIA	.4075	.02630	4
Total	.3919	.07397	162

Table A.2 Soluble oxalate content in concentrates

Descriptive Statistics

Dependent Variable: SOLUBLE

TREATMENT	Mean	Std. Deviation	N
Molasses	.3117	.01835	6
Soybean	.3250	.01378	6
Goat Pellet	.3350	.01049	6
Total	.3239	.01685	18

Table A.3 Insoluble oxalate content in feed ingredients

Descriptive Statistics

Dependent Variable: INSOLUBLE

TREATMENT	Mean	Std. Deviation	N
OPF	0.25	0.11	18
HUMIDICOLA	0.33	0.03	18
MULBERRY	1.25	0.03	18
BANANA	0.98	0.05	18
KOBE SPP	0.24	0.04	18
LEUCEANA	0.35	0.05	18
CASSAVA	2.13	0.08	18
BRACHARIA	0.39	0.12	16
PINEAPPLE	0.81	0.26	16
ASYTASIA	0.99	0.09	4
Total	7.72	0.86	162

Table A.4 Insoluble oxalate content in concentrates

Descriptive Statistics

Dependent Variable: INSOLUBLE

TREATMENT	Mean	Std. Deviation	N
Molasses	0.46	0.02	6
Soybean	0.45	0.01	6
Goat Pellet	0.44	0.01	6
Total	1.35	0.04	18

Table A.5 Total oxalate content in feed ingredients

Descriptive Statistics

Dependent Variable: TOTAL OXALATE

TREATMENT	Mean	Std. Deviation	N
OPF	0.61	0.07	18
HUMIDICOLA	0.72	0.02	18
MULBERRY	1.62	0.02	18
BANANA	1.38	0.05	18
KOBE SPP	0.78	0.03	18
LEUCEANA	0.68	0.05	18
CASSAVA	2.51	0.08	18
BRACHARIA	0.65	0.03	16
PINEAPPLE	0.92	0.05	16
ASYTASIA	1.45	0.06	4
Total	11.32	0.46	162

Table A.6 Total oxalate in concentrates

Descriptive Statistics

Dependent Variable: TOTAL OXALATE

TREATMENT	Mean	Std. Deviation	N
Molasses	0.77	0.00	6
Soybean	0.77	0.00	6
Goat Pellet	0.77	0.00	6
Total	2.31	0.00	18

Table A.7 Silica content in feed ingredients

Descriptive Statistics

Dependent Variable: SILICA

FEED	Mean	Std. Deviation	N
DRIED	0.46	0.01	3
SOY	0.32	0.01	3
GOAT	0.55	0.03	3
Total	1.33	0.05	9

Table A.8 Silica content in concentrates

Descriptive Statistics

Dependent Variable: SILICA

TREATMENT	Mean	Std. Deviation	N
OPF	2.44	0.48	18
HUMIDICOLA	3.10	0.47	18
MULBERRY	3.54	0.12	18
BANANA	4.36	0.54	18
KOBE SPP	2.75	0.18	18
LEUCEANA	4.18	0.27	18
CASSAVA	3.54	0.22	18
BRACHARIA	2.92	0.30	16
PINEAPPLE	4.11	0.39	16
ASYTASIA	3.21	0.07	4
Total	34.15	3.04	162

APPENDIX B

Figure B.1: Brachiaria Grass sample

Figure B.2: Leucaena leaf sample

Figure B.3: Mulberry leaf sample and banana leaf sample

Figure B.4: Humidicola grass and rumput nyoya sample

Figure B.5: Napier grass and cassava leaf sample

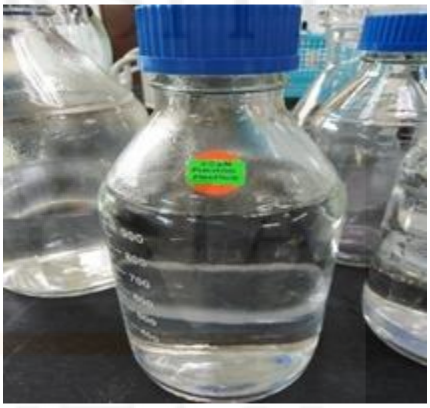

Figure B.6: 20mM of potassium phosphate as eluent a for HPLC analysis

Figure B.7:20mM of potassium phosphate pH 2.90

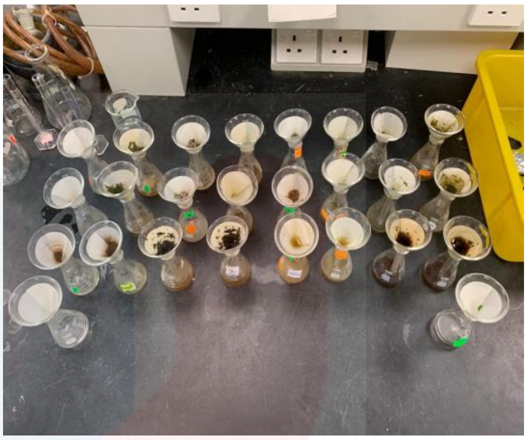

Figure B.8 Collection of residues of samples for HPLC analysis