Determination of Oxalic Acid by HPLC and Silica Contents by Conventional Method in Different Varieties of Napier Grass (*Pennisetum purpureum*)

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Faculty of Agro Based Industry

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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

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I certify that the report of this final year project entitled "Determination of oxalic acid by HPLC and silica contents by conventional method in different varieties of Napier grass (*Pennisetum purpureum*)" by Norshazwani binti Muhamad Shariman, matric number F15A0122 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, Universiti Malaysia Kelantan.

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Supervisor

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Determination of oxalic acid by HPLC and silica contents by conventional method in different varieties of Napier grass (*Pennisetum purpureum*)

ABSTRACT

Napier grass (*Pennisetum purpureum*) is used as a common feed source for livestock especially ruminant. Although it is used as a fodder due to its moderate nutritive values, it contains the anti-nutrients. Oxalic acid and silica contents are examples of anti-nutritive factors that present in Napier grass. 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. Therefore, the aim of this study was to determine the contents of oxalic acid and silica in the Napier grass. Seven varieties of Napier grass including Taiwan, Zanzibar, Australian Dwarf, Pakchong, Purple, Kobe and Indian were planted under standard level (300 kg NPK/ha/year) of fertiliser application. Plants were harvested at 2 months of plant maturity. Total oxalate and soluble oxalate contents in samples were determined by high-performance liquid chromatography (HPLC), while silica content was measured by conventional method using ashing method. Results showed that Dwarf Napier contained the highest total oxalate content (3.23%) followed by Kobe (2.61%), Zanzibar (2.60%), Purple (2.44%), Taiwan (2.43%), Indian (2.15%) and Pakchong (1.95%) varieties. Similarly, Dwarf Napier contained the highest soluble oxalate content (3.00%), while Pakchong showed the lowest soluble oxalate content (1.80%). No significant ($p>0.05$) differences were observed on insoluble oxalate content among Napier grass varieties. There were no significant ($p>0.05$) differences on silica content among Napier grass varieties. Dwarf Napier, however, showed numerically the highest silica content (4.19%), while Pakchong showed the lowest silica content (3.14%). In conclusion, Pakchong variety is safer than Dwarf variety for ruminant feeding. However, careful attention should be needed for grazing animals since leaf contains more soluble oxalate than stem parts. Further feeding experiments using Napier grass varieties are needed on oxalate toxicity in ruminants.

Keywords: Napier grass, anti-nutritive factors, oxalate, silica.

Penentuan asid oksalat menggunakan HPLC dan kandungan silika dengan kaedah konvensional dalam pelbagai variasi rumput Napier (*Pennisetum purpureum*)

ABSTRAK

Rumput Napier (*Pennisetum purpureum*) digunakan sebagai sumber makanan bagi ternakan terutama ruminan. Walaupun ia digunakan sebagai makanan ternakan kerana nilai nutrisi pemakanannya, ia juga mengandungi nilai anti-nutrisi. Asid oksalat dan silika adalah contoh anti-nutrisi yang terdapat di dalam rumput Napier. Asid oksalat yang larut adalah anti-nutrisi kerana dapat digabungkan dengan kalsium atau magnesium untuk membentuk kristal oksalat yang tidak dapat larut yang akan dirembeskan bersama najis. Silika adalah bahagian yang berbulu pada daun yang akan menyebabkan beberapa kecederaan fizikal pada lidah haiwan ternakan dan menyebabkan haiwan memilih makanan. Oleh itu, kepekatan kedua-dua kandungan anti-nutrisi dalam rumput Napier perlu ditentukan. Dalam kajian ini, tujuh jenis rumput Napier termasuk Taiwan, Zanzibar, 'Dwarf', Pakchong, Ungu, Kobe dan India ditanam di Universiti Universiti Malaysia Kelantan kampus Jeli di bawah tahap penggunaan baja yang normal. Rumput Napier dituai apabila tanaman mencapai tahap kematangan 2 bulan dan pertumbuhan semula akan dituai pada selang 45 hari. Sampel akan dikumpulkan untuk menentukan kandungan asid oksalat dan silika. Kandungan asid oksalik ditentukan oleh kromatografi cecair prestasi tinggi (HPLC), manakala kandungan silika diukur dengan kaedah konvensional yang merangkumi kaedah pengabuan. Hasil kajian menunjukkan bahawa jumlah kandungan oksalat dalam jenis 'Dwarf' adalah yang paling tertinggi (3.23%), diikuti oleh Kobe (2.61%), Zanzibar (2.60%), Ungu (2.44%), Taiwan (2.43%), India (2.15%) dan yang terendah adalah di dalam Napier Pakchong (1.95%). Pakchong juga mempunyai kepekatan oksalat yang paling larut terendah iaitu 1.80% manakala Napier 'Dwarf' mempunyai kepekatan oksalat yang paling larut tertinggi sebanyak 3.00%. Tiada perbezaan ketara di antara variasi rumput Napier untuk kandungan asid oksalat tidak larut. Untuk kandungan silika, jenis 'Dwarf' secara numerik menunjukkan kepekatan tertinggi iaitu 4.19%, manakala Pakchong menunjukkan kepekatan silika terendah sebanyak 3.14%. Sebagai kesimpulan, variasi Pakchong adalah lebih selamat untuk pemakanan ruminan berbanding variasi 'Dwarf'. Walau bagaimanapun, perhatian perlu diberikan kepada haiwan peragut kerana daun mengandungi lebih oksalat larut daripada bahagian batang. Eksperimen pemakanan perlu dilakukan kepada haiwan ruminan. untuk mengetahui ketoksikan asid oksalat dengan menggunakan pelbagai variasi rumput Napier

Kata kunci: Rumput Napier, faktor anti-nutrisi, asid oksalat, silika.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

List of Aberrations

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

Malaysia population had increased year to year and subsequently this will increase the demand for food. Food security policy in Malaysia was established to ensure the availability and accessibility of the food supply for Malaysian. Malaysia is focusing on agricultural products as an essential source of food. Ruminant, poultry and aquaculture industry are the industries that produce animal protein food for Malaysian. However, ruminant industry self-sufficiency level is still lower compared to poultry industry that achieved more than 100% self-sufficiency level. The demand for animal protein source from beef and mutton has increased as well as for the demand for cheap animal protein from poultry. About 60% of the Malaysian population consumed beef as a source of animal protein where the total consumption rose from 138,980 tonnes in 2005 to 201,556 tonnes in 2013 (Ariff, Sharifah & Hafidz, 2015). To fulfil the demand, the country is depended on the importation of animal protein from beef, mutton and also the dairy product. To reduce the dependency on the importation of food, more studies need to be done to achieve the self-sufficiency, especially in ruminant industry. Huda (2015) stated that the policies of food securities to improve the livestock industries are to increase the ruminant industry efficiency, animal feeds production and also toward free-disease nation.

The ruminant industry is the industry that has low feed cost as it is depended much on the locally available feedstuffs as a major feed constituent mixed with some supplement from imported ingredient (Loh, 2002). Most of the farmers in Malaysia is a smallholder. They are lacking of money to run a big farming business. Thus, the farmers usually reared the livestock with low facilities and reduced the feed cost by providing local feedstuff such as rice, palm kernel cake, banana, sweet potato, sago, rice bran and cassava to replace maize (Wan Zahari & Wong, 2009). Besides, most of the smallholder farmers prefer to have a free-range system, semi-intensive system or integrated system for their livestock. The pasture and fodder are the most preferable feed that the smallholder farmers usually provide to their livestock. Their livestock will be set free to graze on pasture and also will be fed by giving some fodder.

Pasture is known as grass that available on the pasture field or grassland. In the oil palm plantation in Malaysia, the cover crops like *Centrosema pubescens, Desmodium audifolium, Pueraria phaseoloides, Calopogonium caeruleum,* were found in the inter-rows in the plantation are considered as pasture for livestock in Malaysia. Meanwhile, fodder is the crop that has been cultivated and given to the livestock by chopping it at first. Fodder has usually been fed to the feedlot livestock. In Malaysia, the type of fodder given usually is a grass. Grass contains high crude fibre compared to crude protein which is high percentage in legumes. Examples of common grasses in Malaysia provided by smallholder farmers that is suitable for livestock are *Setaria sphacelata*, *Brachiaria humidicola*, *Panicum maximum*, *Brachiaria decumbens* and *Pennisetum purpureum*

(Wan Zahari et al., 2009). The most popular grass used by the farmer as a fodder is Napier grass (*Pennisetum purpureum*) due to its high biomass yield and ease of propagation.

Napier grass is also known as Uganda grass or elephant grass. It is under the Poaceae family. The lifetime of the Napier grass is more than two years known as the perennial grass. Napier grass also can grow by seed or by the vegetative propagation. The nodes from creeping stolon produce roots that help the growth more rapidly. Moreover, Napier grass can produce seed but not consistent and rarely developed. Napier grass can survive well under temperature ranges from 25 ˚C to 40 ˚C, but it will less survive under lower temperature and in the saturated area. Napier grass grows in a clump with a height of 4 to 7 meter according to its varieties. The colour of Napier grass commonly in green colour, but it also resembles in purple colour which may be termed as ‘Purple Napier’ variety. In the foreign country, sometime Napier grass is planted as a windbreak. In Malaysia, we can see the Napier grass which is growing wild on roadsides. Farmers also cultivate the Napier grass as it is one of the highly productive tropical forage grasses and has its nutritional content that is suitable to be served as feed for the livestock.

The morphological characteristics of Napier grass provides different feed quality such as stem and leaf parts (Kebede, Assefa, Megistu & Feyessa, 2014). Usually, the leaf part is more preferable to the livestock and also has high nutritive value compared to the stem part. According to Moran (2011), in order to increase the utilisation of forages, the forages need to be chopped and wilted under the sun for several hours resulting in less moisture, increase the livestock appetite and facilitates rumination. Napier grass can be served to the livestock as a fresh cutting, pasture, hay and also in high quality of silage due to its variation in morphological characteristics (Getnet & Ledin, 2001).

The advantage of Napier grass is widely used as a fodder for livestock as it claims for its nutritive value, easy to grow, short harvest interval, cheapest fodder source for ruminant and non-ruminant like rabbit and also can be kept in longer time in form of silage or pellet. However, there is also the disadvantage of Napier grass such as it can become a weed without proper management as it invades the crop fields. The older Napier grass will be less palatable and reduce the feed intake for livestock. Moreover, the feed intake also decreases when the Napier grass contains high amount of oxalate and silica.

Oxalate is one of the plant constituents that can be an anti-nutrient as well as a toxin (Rahman & Kawamura, 2011). On the other hand, silica deposits on leaf cell-wall that can provide the physical support and protect the cell wall carbohydrates from digested by the digestive microorganism (Jones & Handreck, 1967).

1.2 Problem Statement

Napier grass is widely used as a fodder around the world including Malaysia for its nutritional value and high biomass yield. Despite its nutritional value, there are also several anti-nutrients that can be found in the Napier grass such as oxalates, phytates, saponins, cyanogenic glycosides and tannins (Okaraonye & Ikewuchi, 2009). Oxalates found as a constituent in the Napier grass, while the silica deposits on leaf cell-wall that can provide physical support and protect the cell wall carbohydrates from digested by the digestive microorganism (Jones & Handreck, 1967).

Both oxalates and silica classified as anti-nutrients as it reduces the feed intake and reduces the digestibility of the forages. Oxalic acid in the plant resembles in the form of oxalate salts, which can act as anti-nutrient. For example, soluble oxalate can be combined with blood calcium (Ca^{2+}) or magnesium (Mg^{2+}) to form an insoluble oxalate crystal that excreted with faeces, because it can block the urine flow (Rahman, Abdullah & Wan Khadijah, 2013). Meanwhile, silica is the constituent on the leaves and also within plant cell wall that will cause low feed intake due to the low palatability and can cause some physical damages because of its hairy structure. There are seven varieties of Napier grass focused in this study which were Taiwan, Zanzibar, Kobe, Pakchong, Purple, Indian and Dwarf. Thus, this study was designed to find out the contents of oxalate and silica in different Napier grass varieties as to compare which varieties contain high oxalate and silica contents that would give the negative effect to the animal.

1.3 Hypothesis

H0: Different types of Napier grass will not have significant different levels of oxalic acid and silica contents.

HA: Different types of Napier grass will have significant different levels of oxalic acid and silica contents.

1.4 Objectives

i. To evaluate the content of oxalic acid using HPLC and silica content using conventional method in seven varieties of Napier grass.

ii. To determine the best varieties of Napier grass to be served as an excellent fodder with low level of oxalic acid and silica contents.

1.5 Scope of Study

This study was conducted at Universiti Malaysia Kelantan (UMK) Jeli Campus. Seven varieties of Napier grass including Taiwan, Zanzibar, Australian Dwarf, Pakchong, Purple, Kobe and Indian were planted in Agro Techno Park, UMK Jeli Campus. The varieties of Napier grass were purchased from the supplier at Johor and also collected some of the varieties like Kobe and Purple Napier in UMK Jeli campus. The land that I used for this study in the Agro Techno Park were cleared and prepared for the planting purpose. A standard level of fertiliser was used for this experiment which was NPK 15-15-15 fertiliser. Napier grass planting was done in early of July and it was grown for 2 months before the harvesting time. After harvesting, the samples of Napier grass were dried and grounded for determination of oxalate and silica contents that was carried out in the Fakulti Industri Asas Tani (FIAT)'s laboratory. The oxalate was determined using the HPLC, while the silica was measured by conventional method. All the data and results were recorded.

1.6 Significance of Study

This study will help to generate the information for the anti-nutrients like oxalate and silica contents in Napier grass. The simple experimental method was used to determine the oxalate and silica contents in the Napier grass. This study was focused on many varieties of Napier grass that will acknowledge people on which Napier grass type will have low anti-nutrients as it will be the best fodder for the livestock. This study can be applied as the reference to the farmers or people in choosing the best Napier grass to be an excellent fodder.

1.7 Limitation of Study

In this study, there were some limitations due to some factors. First, the some varieties of Napier grass did not have much information about the content of anti-nutrients present in it. The oxalate and silica contents for seven varieties of Napier grass were determined by this research. However, based on the literatures, only some of the Napier grass varieties had information on oxalate and silica contents that had been published. The available published articles are not the latest articles, which was more than 10 years aged.

CHAPTER 2

LITERATURE REVIEW

2.1 Forages

Forages can be grasses or legumes that is been 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 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, fertilisation, grass-legume mixtures, and harvesting and storage effects (Ball, 2001).

Napier grass with a scientific name, *Pennisetum purpureum* is classified under Poaceae family. From Purseglove (1972) described that perennial grass like Napier grass can be harvested around four to six cuts per year and about 50 to 150 tonnes per hectare produce a biomass yield produced (as cited in Farrell et al., 2002a). Van der Wouw (1999) reported that Napier grass can re-grow in the short interval by producing a palatable leaf and high biomass yield (as cited in Farrell et al., 2002b). Rengsirikul et al. (2013) studied about dry matter yield on several types of Napier grass including Bana (BN), Taiwan A148 (TW), Common (CM), Wruk wona (WW), Tifton (TT) and Kampheng San (KS), representing tall types, and Dwarf (DW) and Muaklek (ML) as shown in the Table 2.1(a) below.

Table 2.1(a): Yield distribution and annual dry matter (DM) yields of 8 Napier grass cultivars.

Cultivars	Dry matter yield (tonnes/ha)				Total
	1st harvest	2nd harvest	3rd harvest	4th harvest	
DW	6.6^{a}	6.9^{d}	4.1^{cd}	9.5^{c}	27.1^{a}
ML	7.3^{a}	9.5^{c}	3.4^{d}	15.1^{b}	35.1^{d}
BN	13.6^{c}	15.5^{a}	4.5^{cd}	15.5^{b}	49.1^{bc}
TW	14.6^{bc}	15.4^{a}	6.3^{ab}	15.2^{b}	51.5^{b}
CM	16.0^{ab}	13.3^{b}	6.7^{a}	18.8^{a}	58.4^{a}
WW	13.6^{c}	15.8^{a}	4.9^{bcd}	17.8^{ab}	52.1^{b}
TT	17.2^{a}	15.6^{a}	6.7^{a}	18.8^{a}	58.4^{a}
KS	10.9^{d}	13.3^{b}	5.1^{bc}	16.8^{ab}	46.3^{c}
Average	12.5	13.2	5.2	15.5	46.4

^{abcd} Means with common superscripts are not significantly different ($P<0.05$).

Source: Rengsirikul et al. (2013)

Table 2.1 (b): Nutritive quality of nine Napier grass varieties.

Variety	Crude protein (%)	Neutral detergent fibre (NDF) (%)	Acid detergent fibre (ADF) (%)	Acid detergent lignin (ADL) (%)
King grass	10.11^{c}	70.10^{ab}	38.10^{ab}	6.58^{a}
Common Napier	9.79^{c}	70.90^{ab}	38.80^{ab}	9.24^{a}
Red Napier	10.36^{c}	69.30^{bc}	38.20^{ab}	7.45^{a}
Taiwan Napier	10.09^{c}	70.00^{ab}	39.90^{a}	7.99^{a}
Uganda	10.36^{c}	71.80^{a}	39.80^{a}	8.22^{a}
Indian Napier	10.64^{bc}	70.00^{ab}	38.80^{ab}	8.65^{a}
Dwarf Napier	11.56^{ab}	69.10^{bc}	37.00^{bc}	8.77^{a}
Dwarf 'Mott'	11.61^{ab}	67.80^{cd}	36.90^{bc}	8.96^{a}
Australian Dwarf	12.08^{a}	66.10^{d}	35.70^{c}	8.19^{a}

^{abcd} Means with common superscripts are not significantly different ($P<0.05$).

Source: Halim, Shampazuraini & Idris (2013).

Besides of the nutritive value of Napier grass, there are also the anti-nutrients in the Napier grass. In a research of anti-nutritional components in Napier grass, a phytochemical screening was carried out with the findings of phytates, saponins, tannins, oxalates, alkaloids, flavonoids and cyanogenic glycosides (Okaraonye & Ikewuchi, 2009). Five components are classified as anti-nutritional contents.

Table 2.1(c): The anti- nutritional content of Napier grass

Anti-nutrient	Composition (%)
Cyanogenic glycosides	2.830±0.04
Oxalates	0.159±0.01
Phytates	0.006±0.00
Saponins	0.850±0.03
Tannins	28.640±0.00

Values are Mean ± SD of triplicate determinations

Source: Okaraonye et al. (2009)

2.2 Utilisation of forages (Ruminant vs non-ruminant)

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 utilise 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). Chesson (1988) stated that in the rumen, not all carbohydrate can be degraded as lignin that present in the forages is one of the major problem for microbial enzymes to digest (as cited in Beever, 1993).

2.3 Oxalic acid

2.3.1 Oxalic acid in plant

Oxalic acid is the organic compound with chemical formula, $C_2H_2O_4$. The oxalate is the conjugate based for oxalic acids. Oxalic acid is present ii all plant foods with different contents according to the plant maturity (Davis, 1981) or the genotypes of the plant (Smitha Patel et al., 2013). According to Libert and Franceschi stated that ”oxalate in plants provide a tissue support, protection to plant, detoxification of heavy metal, ion balancing and calcium regulation” (as cited in Rahman & Kawamura, 2011). The young plant tends to have slightly high content of oxalates as it declined according to the increasing plant maturity level (Rahman et al., 2011b). Another alternative to reduce the oxalate content in forages plants to acceptable levels is to increase the harvest interval as the level of oxalates decline (Rahman et al., 2011c). In a plant tissue, oxalate presents as a salt such as sodium oxalate and potassium oxalate.

2.3.2 Oxalic acid mechanism

The soluble oxalate can combine with calcium (Ca^{2+}) or magnesium (Mg^{2+}) to form an insoluble oxalate crystal that excreted with faeces due to the block of urine flow (Rahman et al., 2013). “Oxalates are found to disturb the calcium body in the animal. Insoluble calcium oxalate is formed from the combination of the soluble oxalate reacts with the calcium in the blood. The calcium oxalate reduces the calcium bioavailability.

Thus, the hypocalcaemia occurs as the calcium level in the body is disturbed. Prolonged mobilisation of bone mineral results in nutritional secondary hyperparathyroidism or osteodystrophy fibrosa" (Rahman et al., 2011d). Ruminant in the tropical and subtropical areas is mostly fed on fodder like Napier grass that contains up to 3.8% of soluble oxalate (Rahman, Ishii, Niimi, & Kawamura, 2010; Rahman et al., 2006;. Rahman et al., 2011e). Different morphology of forages has different oxalate concentration in it. Rahman et al. (2006) stated that leaf of Napier grass contained about 2.78% DM of soluble oxalate, while stem part contained 2.05% DM of soluble oxalate (as cited in Smitha Patel et al., 2013).

2.3.3 Factors affecting the oxalic acid

The oxalic acid or oxalates is affected by many factors include the internal and external factors. According to Rahman et al. (2011f), the soluble oxalate level in the plant increased when the potassium [K^+] fertiliser was applied. Moreover, the climate (summer) as an external factor can usually be increased the soluble oxalate content in the tissue of the stem and the leaf part. The different plant species also have a different level of oxalate accumulation. For example, leaves of Setaria, Kikuyu and Napier grass contained 2.90%, 1.33% and 2.78%, respectively. Internal factors also include the plant part where the young shoot contains more oxalate followed by leaves and stem parts. Pre-treatment can be done like ensilage, which can reduce the oxalate content.

2.3.4 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 by Leon, Rois Valcarcel & Luque (1990), polarographic by Rodrigous & Barros (1993), chemiluminescence by Perez-Ruiz et al. (1999), fluorometric by Perez-Ruiz et al. (1994), spectrophotometric by Infantes et al. (1991) and gas chromatography by Yanagawa et al. (1983)". The HPLC is the latest equipment that had been used by researcher to analyse the oxalic acid (Rahman, 2009).

2.4 Silica

2.4.1 Silica in Plant

The second most abundant element in the earth's crust is silicon (Si). In the plant constituents, silicon represents as silica (SiO_2). Silica is one of the constituents that can be found in family Gramineae. The percentage of silica content in the plant is about 0.1% to 10% of dry weight (Jusdado, 2011). Silica can be seen as the hairy part of the forages leaf which assemble as rough surface that reduce the palatability for grazing animal as it causes a physical damage to them. According to Perry and Fraser (1991), "characterisation of the silica ultra- structures found in plant hairs from an example of the Poaceae and nettle stinging hairs (Hughes, 1989) revealed similar microstructural forms, including globular, fibrous and sheet-like structures with the distribution of these ultrastructural motifs being dependent on the anatomical region studied. The silica was formed in biological systems with a narrow particle size distribution for specific structural motifs" (as cited in Currie & Perry, 2007). Silicon also protects the leaf from insect and fungal attack (Jones and Handrek 1967; Shewmaker et al. 1989; Mayland & Shewmaker, 2001).

2.4.2 Silica mechanism

There are three forms of silica that we can find like silica bodies within cells (phytoliths), silica associated with cell walls and free silica (Blackman 1968; Blackman and Bailey 1971). There are insoluble and soluble silica. Insoluble silica is silica bodies and cell wall silica that has polymerized silicic acid while soluble silica is the free silica that has unpolymerized silicic acid (Bailey, 1981). The dissolved silica can be found in the alimentary tract known as monosilicic acid. The dissolved silica excretes through urine as it absorbed from alimentary tract and carried to the kidney. The dissolved silica may cause silica urolithiasis in sheep and cattle which is dangerous for the animal and economic loss for the farmer (Jones & Handreck, 1967). Moreover, silica acts as a defences in grass, which gives impact to the feeding behaviour more on small herbivores compared to large herbivores species like mammals. Silica causes the wearing of teeth and mouthparts of insects and larger mammalians herbivore (Hartley & Degabriel, 2016). Herbivores that fed on high silica grass grew more slowly and ate less as silica reduce digestibility and palatability (Massey and Hartley 2006 ; Hunt, Dean & Johnson 2008).

2.4.2 Factors affecting the silica

Abiotic and biotic factors affect the silica content. Abiotic factors like soil type, climate and water availability influence the silica content. Jones and Handreck (1965) had established the relevance of monosilic acid in the soil solution to the uptake of silica by plant (Jones et al., 1967). The iron and aluminium oxides contents are related with the silica uptake (Jones et al., 1967b). Uptake of silicon also depends on the pH and soil type (Hartley & Degabriel, 2016). Another abiotic factor is grazing level which may affect the silica content. “Heavily grazed area tend to have high silica accumulation compared to the plant species in the lab (ungrazed one) as reviewed by Hartley & Degabriel (2016). The biotic factors are the plant species and genotype, which may affect on the silica content. According to Russel, “grass from Gramineae family tends to has high concentration of silica compared to the legumes” (Jones et al., 1967c) as the grasses is known as the Si-accumulating plant.

2.4.3 Methods to determine the silica

There are several methods to determine the silica content in plant. According to the Payá, Monzó, Borrachero, Mellado and Ordoñez, (2001), “a titrimetric method is used to measure the percentage of amorphous silica in (Rice Husk Ash) RHA. The titrimetric method advantage is time saving which could provide faster accurate result compared to standard method”. According to Perry (2003), the colorimetric method uses molybdenum yellow and blue, atomic absorption and inductively coupled plasma analysis to determine the silicon (Si) concentration in solution (as cited in Currie & Perry, 2007).

CHAPTER 3

METHODOLOGY

3.1 Land preparation and plant management

3.1.1 Study Site

Napier grass was planted at Agro Techno Park, UMK Jeli campus (N5°44’45.79”, E101°52’31”; average altitude 62 m above sea level). This study was conducted from July to September 2018. The recorded temperatures were ranged from 24°C to 32°C. According to the data stated by the Local Weather from AccuWeather.com, Jeli area received monthly rainfall for July to September with a range of 115 mm to 174 mm. Table 3.1 shows the recorded temperatures.

Table 3.1.1: Temperature recorded at Jeli during the experimental period from July to September 2018.

Month	Temperature (°C)	Rainfall (mm)
July	24 to 32	115
August	24 to 31	142
September	24 to 31	174

Source: Local Weather from AccuWeather.com

3.1.2 Experimental design

Before plantation, required land was measured for plantation of seven different varieties of Napier grass. After that it was cleared and ploughed using tractor to soften the soil. Three varieties of Napier grass (Taiwan Napier, Pakchong Napier and Indian Napier) were purchased from supplier from Johor in April 2018, Zanzibar Napier was from Kampung Tandak Machang, Dwarf Napier was from Kampung Sungai Satan and also two varieties of Napier grass (Kobe and Purple Napier) were collected from UMK Jeli campus. About 45 Napier stems for each variety were planted for three plots. Each plot (area: 2 m × 2 m) consisted of 15 Napier stems. The experiment was set up as a Completely Randomized Design (CRD) with three replicates of each treatment (variety). Stem cuttings of Napier grass were planted in rows with spacing 0.5 m x 0.5 m, laying them horizontally on the ground and covering with soil.

Before planting, the goat manure was applied to the land at a rate of 1 kg per m^2 as a basal fertiliser. The lime [$Ca(OH)_2$] was also applied at a rate of 30 g per m^2. During plantation, the NPK (15-15-15) fertiliser was applied at the rate of 33.33 g per m^2. During the first five days, water was given to the Napier grass and also was given during the hot days. The continuous management was weeding. The hand pulled weeding method was done every weekend. Napier grass was harvested at two months of plant maturity. Napier grass was cut 10 cm above the ground.

Figure 3.1.2: The land preparation at Agro Techno Park

3.1.3 Soil nutrient analysis

The soil samples were collected by taking the soil sample in a range of 0 – 20 cm depth before plantation and after harvesting the grass. The soil samples were air dried, crushed and sieved to pass through a 2-mm sieve. Afterwards, the soil samples were kept in zipper bags. The pH of the soil was determined using pH meter. The soil salinity and soil texture were determined using standard method while the soil mineral contents were measured using atomic absorption spectrophotometer. The soil sample analysis result is shown in Table 3.1.3.

Table 3.1.3: The soil sample analysis

Parameter	**(Mean ± Standard Deviation)**	
	Before Planting	**After Harvest**
Calcium $_{\text{exchangeable,}}$ cmol/kg	11.45 ± 0.04	4.25 ± 0.34
Magnesium $_{\text{exchangeable}}$, cmol/kg	12.38 ± 0.02	3.66 ± 2.92
Potassium $_{\text{exchangeable}}$, cmol/kg	5.00 ± 0.01	1.33 ± 0.26
Sodium $_{\text{exchangeable}}$, cmol/kg	2.23 ± 0.02	0.90 ± 0.20
Phosphorus, mg/kg	10.81 ± 4.71	7.02 ± 0.50
Copper, mg/kg	2.38 ± 0.02	2.96 ± 0.06
Zinc, mg/kg	6.15 ± 0.04	4.31 ± 0.10
Manganese, mg/kg	55.35 ± 0.01	44.97 ± 18.00
Iron, mg/kg	8.02 ± 0.03	9.42 ± 2.81

3.2 Experimental Section

3.2.1 Sample Preparation

The stem and leaf parts of Napier grass were cut and chopped separately into small size (2-3 cm) manually, then it was dried in an oven for 48 hours at 70°C. Dried samples were ground using grinder and passed through a 1-mm laboratory test sieve and stored in the zipper bag.

Figure 3.2.1(a): Stems were cut into 2 to 3 cm.

Figure 3.2.1(b): Leaves were cut into 2 to 3 cm.

3.2.2 Extraction and Determination of Total and Soluble Oxalates

Oxalate was determined as described by Rahman, Niimi, and Kawamura (2007). Briefly, about 0.5 g of sample was mixed with 15 ml of distilled water for soluble oxalate extraction. For the total oxalate extraction, it was extracted with 15 ml of 1 mol HCl. In a boiling water bath, the samples were heated for 18 min. Then, the mixtures were cooled in room temperature and filtered through filter paper. Next, the mixtures were washed with distilled water and made up to 50 ml in the volumetric flask. The filtrate for the total oxalate extraction was adjusted until pH 3.0 using 5 mol NaOH. Then, the solutions in the 5 ml syringe were filtered using the hydrophilic membrane (0.45 µm) filter paper and were kept in the HPLC vial 1.5 ml for HPLC analysis.

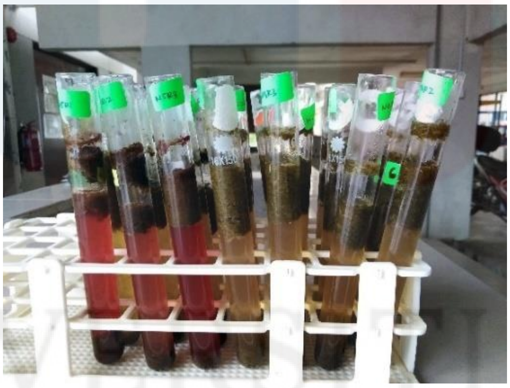

Figure 3.2.2 (a): Leaf part extraction of total oxalate with 1 mol HCl.

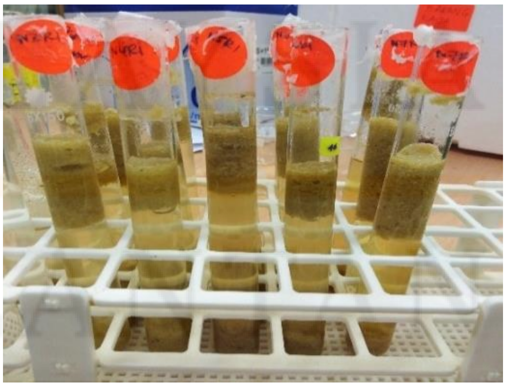

Figure 3.2.2 (b): Stem part extraction of soluble oxalate with distilled water.

3.2.3 Standard Calibration

Three 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 1 mL, 2 mL and 3 mL, which was taken from the stock solution and made 100 mL with distilled water in the volumetric flask respectively.

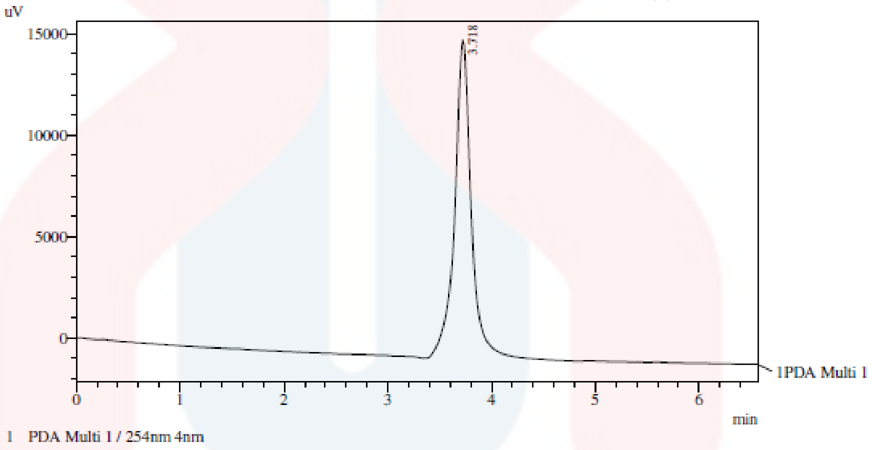

Figure 3.2.3 (a): 1 Chromatogram of an authentic oxalic acid (1%) by high performance liquid chromatography (HPLC).

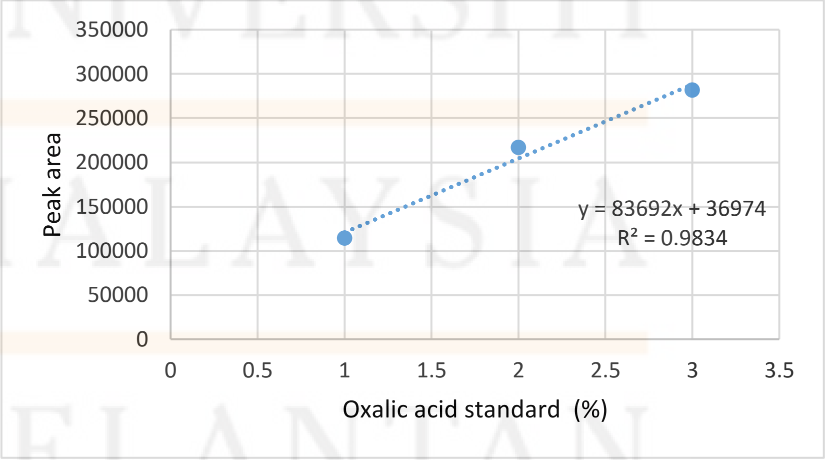

Figure 3.2.3 (b): Calibration curve of authentic oxalic acid.

3.2.4 HPLC analysis

The analysis of oxalate was conducted on a column Synergi 4 µm Hydro-RP 80 Å, LC Column 250 x 4.6 m, Ea (Phenomenex, USA). A sample of 1.5 ml was chromatographed at 30°C using 20 mM 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.

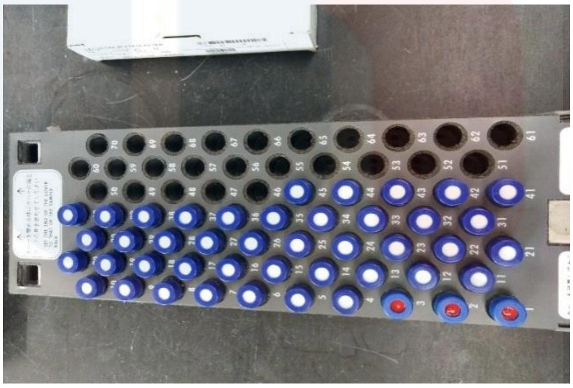

Figure 3.2.4 (a): Oxalate extraction in the vial tray ready for HPLC analysis.

Figure 3.2.4 (b): The HPLC machine.

3.2.5 Silica Determination

About 2 g dried ground samples (stem and leaf parts) were mixed, weighed and then kept in the crucible. After that, all of the crucibles with right labelled were placed in the muffle furnace at 600˚C for 2 hours (AOAC, 1942). The remained ash was used to determine the silica content.

Figure 3.2.5 (a): Sample after the ashing method.

Silica content was determined as described by Widyastuti and Abe (1989). Briefly, 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 ˚C. After the sample being dehydrated and the dried silica were transferred on the filter paper and manifold it to wash the silica with water about three to four times. Again, the remained silica in the filter paper were placed in the same crucible and dried in the forced air oven for three hours at 105˚C. Lastly, the dried samples were incinerated in the furnace for 600˚C for 8 hours.

Figure 3.2.5 (b): Dehydration process after adding HCl to the remained ash.

Figure 3.2.5 (c): Washing the dried silica for three to four times.

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)

3.3 Statistical Analysis

The data for oxalate and silica contents were analysed statistically using one-way ANOVA, whereas 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.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Type 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 found in the plant has different content based on the internal and external factors like plant species, seasons, plant maturity, the effect of fertiliser applied and others. In this study, the oxalate content in different varieties of Napier grass showed different values.

4.1.1 Soluble oxalate

The botanical fraction of the plant exhibited different contents of soluble oxalate. From the Table 4.1.1, Dwarf variety had the highest soluble oxalate content in leaf part (3.45%) and whole plant (3.00 %), while Pakchong had the lowest content in the stem part (1.21%) and whole plant (1.80%). In the leaf part, the soluble oxalate content in

Dwarf variety was significantly ($p<0.05$) different with the Taiwan variety. Meanwhile, Taiwan variety (2.73%) had no significant ($p>0.05$) difference on soluble oxalate content in the leaf with Zanzibar (2.09%), Kobe (2.22%), Pakchong (2.64%), Purple (2.44%) and Indian variety (2.48%). For the soluble oxalate content in the stem part, there was no significant ($p>0.05$) difference among all varieties. The animal will be provided forages that consists of stem and leaf part so we would consider it as a whole plant. Then, the soluble oxalate for the whole plant is important to know. To calculate the oxalate content in the whole plant, we need to know the stem and leaf ratio. Then, we proceed to the calculation of the whole oxalate content in the Napier grass. In this study, for the whole plant part, the level of soluble oxalate content in ascending order was lowest in Pakchong (1.80%), numerically followed by Purple (1.97%), Kobe (2.00%), Zanzibar (2.04%), Indian (2.13%), Taiwan (2.29%), and Dwarf (3.00%). The Dwarf variety was significantly ($p<0.05$) differed on soluble oxalate content with the other six varieties for the whole plant. All the details of soluble oxalate are shown in Table 4.11.

Table 4.1.1: Effect of botanical fractions on soluble oxalate content (DM %) in different varieties of Napier grass.

Botanical fractions	**Napier Varieties (Mean ± Standard deviation)**							**Level of Significance**
	Taiwan	**Zanzibar**	**Kobe**	**Pakchong**	**Purple**	**Indian**	**Dwarf**	
Leaf	2.73 ^{ab} ± 0.30	2.09 ^{a} ± 0.62	2.22 ^{a} ± 0.37	2.64 ^{a} ± 0.52	2.44 ^{a} ± 0.20	2.48 ^{a} ± 0.49	3.45^{b} ± 0.37	*
Stem	1.85 ^{a} ± 0.22	2.11 ^{a} ± 0.58	1.86 ^{a} ± 0.38	1.21 ^{a} ± 0.33	1.60 ^{a} ± 0.59	1.84 ^{a} ± 0.79	1.66 ^{a} ± 0.9	NS
Whole ‡	2.29 ^{a} ± 0.21	2.04 ^{a} ± 0.19	2.00 ^{a} ± 0.27	1.80 ^{a} ± 0.30	1.97 ^{a} ± 0.46	2.13 ^{a} ± 0.62	3.00 ^{b} ± 0.32	*

DM, dry matter; *$p < 0.05$; NS, not significant. Means with different superscript in a row differ at 5% level of probability.‡ Oxalate content (%) of whole plant = oxalate content (%) in leaf × weight ratio of leaf + Oxalate content (%) in stem × weight ratio of stem.

From Table 4.1.1, the soluble oxalate content of whole plant and leaf part in the Dwarf variety were differed significantly ($p<0.05$) with Taiwan and Pakchong varieties respectively. Taiwan and Pakchong is a non-dwarf variety which is taller, has stem and leaf fraction more evenly compared to the dwarf variety that has more leaf. Most of the oxalates are usually present in the leaf tissue (Rahman et al, 2006). Thus, the Dwarf variety contained higher soluble oxalate content compared to other varieties in this study.

The soluble oxalate in the forages is a salt in the form of sodium (Na^+) oxalate, potassium (K^+) oxalate and ammonium (NH_4^+) oxalate. Moreover, from this study, the soluble oxalate of Napier grass in Table 4.1.1 ranged from 2.09% to 3.45%, which is in line with the published article of Rahman & Kawamura (2011) who reported that the soluble oxalate content ranged from 1.8 - 3.8% DM. One of the factors that affect the oxalate accumulation is the maturity of the plant. Early harvesting leads to higher oxalate content compared to the late harvesting (the optimum maturity). In this study, the Napier grass was harvested at 60 days which is the optimum maturity of the plant to be harvested. Thus, the amount of soluble oxalate in different varieties of Napier grass that present in Table 4.1.1 is acceptable.

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 a negative effect to the animal body. From Table 4.1.2, Kobe contained numerically the highest concentration of insoluble oxalate (0.61%) followed by Zanzibar (0.56%), Purple (0.47%), Dwarf (0.23%), Pakchong (0.15%) and Taiwan (0.13%).

Table 4.1.2: Effect of botanical fractions on insoluble oxalate content (DM %) in different varieties of Napier grass.

Botanical fractions	**Napier Varieties (Mean ± Standard deviation)**							**Level of significance**
	Taiwan	**Zanzibar**	**Kobe**	**Pakchong**	**Purple**	**Indian**	**Dwarf**	
Leaf	0.34 ^{ab} ± 0.47	0.57 ^{b} ± 0.54	0.29 ^{ab} ± 0.22	0	0.08 ^{ab} ± 0.19	0	0	*
Stem	0	0.25 ^{a} ± 0.91	0.82 ^{a} ± 0.54	0.37 ^{a} ± 0.53	0.78 ^{a} ± 0.47	0.17 ^{a} ± 0.97	0.92 ^{a} ± 0.62	NS
Whole ‡	0.13 ^{a} ± 0.23	0.56 ^{a} ± 0.34	0.61 ^{a} ± 0.25	0.15 ^{a} ± 0.25	0.47 ^{a} ± 0.37	0.02 ^{a} ± 0.60	0.23 ^{a} ± 0.21	NS

DM, dry matter; *$p < 0.05$; NS, not significant. Means with different superscript in a row differ at 5% level of probability.‡ Oxalate content (%) of whole plant = oxalate content (%) in leaf × weight ratio of leaf + Oxalate content (%) in stem × weight ratio of stem.

From Table 4.1.2, there were no significant (p>0.05) difference for insoluble oxalate among all varieties of Napier grass. Insoluble oxalate is available in plants in a form with calcium (Ca^{2+}), magnesium (Mg^{2+}) and iron (Fe^{2+}) ions (Mason, 2000). The calcium oxalate or magnesium 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 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 Napier grass varieties. 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 varieties of Napier grass. Stem and leaf showed a different content of total oxalate.

Table 4.1.3: Effect of botanical fractions on total oxalate content (DM %) in different varieties of Napier grass.

Botanical fractions	Napier Varieties (Mean ± Standard deviation)							Level of Significance
	Taiwan	**Zanzibar**	**Kobe**	**Pak Chong**	**Purple**	**Indian**	**Dwarf**	
Leaf	3.07^{bc} ± 0.26	2.66^{ab} ± 0.38	2.51^{a} ± 0.16	2.46^{a} ± 0.09	2.52^{a} ± 0.05	2.41^{a} ± 0.50	3.45^{c} ± 0.08	*
Stem	1.81^{ab} ± 0.63	2.36^{bc} ± 0.33	2.68^{c} ± 0.32	1.58^{a} ± 0.06	2.38^{bc} ± 0.44	2.01^{abc} ± 0.32	2.59^{c} ± 0.36	*
Whole ‡	2.43^{bc} ± 0.23	2.60^{bc} ± 0.34	2.61^{c} ± 0.25	1.95^{a} ± 0.05	2.44^{bc} ± 0.26	2.15^{ab} ± 0.29	3.23^{d} ± 0.13	*

DM, dry matter; *p< 0.05; NS, not significant. Means with different superscript in a row differ at 5% level of probability.‡ Oxalate content (%) of whole plant = oxalate content (%) in leaf × weight ratio of leaf + Oxalate content (%) in stem × weight ratio of stem.

In the leaf fraction, Dwarf variety showed the highest value of total oxalate content (3.45%) while Indian variety showed the lowest total oxalate content (2.41%). For the stem fraction, Kobe had the highest content of total oxalate (2.68%) while Pakchong had the lowest content of total oxalate (1.58%). For the whole plant, Dwarf variety contained the highest content of total oxalate (3.23%) while Pakchong variety had the lowest content of total oxalate (1.95%).

4.2 Factor affecting oxalate accumulation

4.2.1 Effect of varieties

From Table 4.1.3, Dwarf had the highest content of total oxalate in the leaf part and also for the whole plant and it is significantly ($p<0.05$) different from the other non-dwarf varieties of Napier grass (Kobe> Zanzibar> Purple> Taiwan> Indian> Pakchong). Rahman et al. (2006) stated that the dwarf varieties (dwarf-early) had higher oxalate content compared to the non-dwarf (Wruk wona and Merkeron) varieties that had lower oxalate content. This finding is also related to the previous study of Mukthar et al. (2003) as cited by Rahman et al. (2006). They reported that Dwarf variety had more leaf than the stem, which contributed to have higher oxalates present in the leaf tissue. In contrast, Pakchong showed the lowest content of total oxalates and it also had more tiller numbers, taller plants, big basal circumference and more dry matter in the stem (Wangchuk, Division, Rai, Nirola, & Thukten, 2015). Same goes with the Table 4.1.1, showed that the soluble oxalate was higher in the dwarf variety (Dwarf) and lowest in the non-dwarf variety (Pakchong).

4.3 Botanical fractions

Figure 4.3 (a) shows the effect of botanical factions on soluble, insoluble and total oxalate contents in Napier grass without comparing the varieties. Each botanical fraction had different contents of oxalate. Leaf part had the highest oxalate content for soluble (2.58%) and total oxalate (2.72%), while insoluble oxalate was higher in the stem part (0.47%) compared to the leaf part (0.15%).

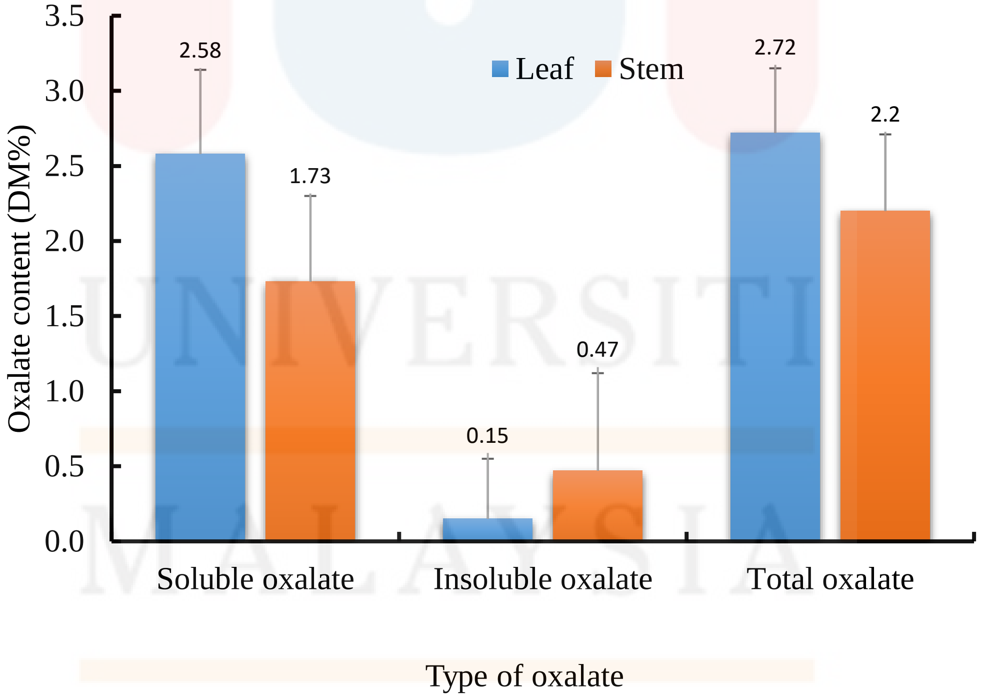

Figure 4.3 (a): Effect of botanical fractions on soluble insoluble and total oxalate content in Napier grass (irrespective of varieties).

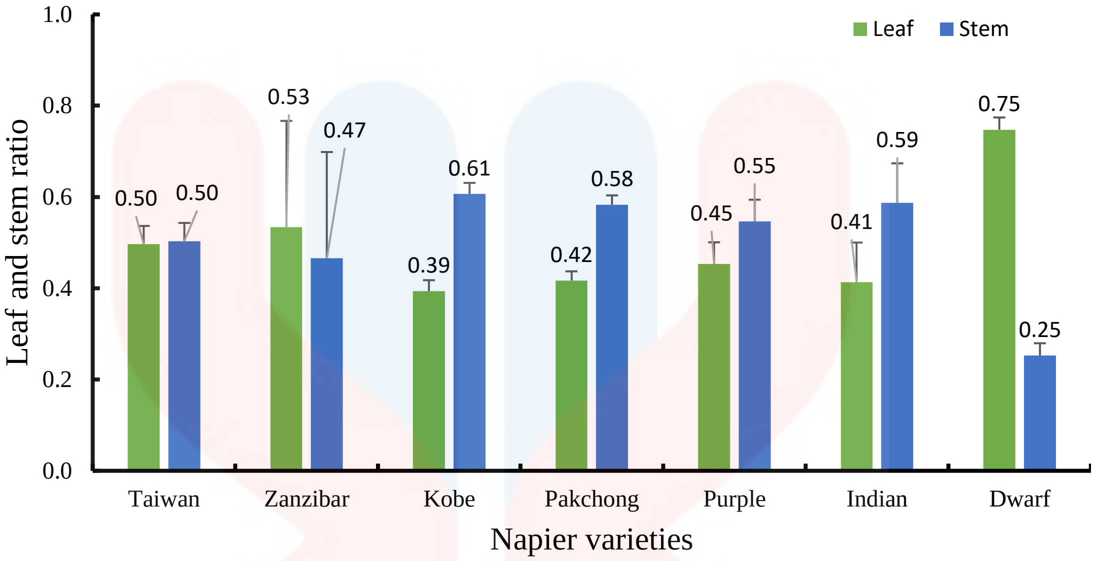

Figure 4.3 (b): The botanical ratio of Napier grass varieties.

From Figure 4.3 (b), obviously, the Dwarf variety contained the highest leaf amount than the stem amount. To compare, the Dwarf variety to the other non-dwarf variety, stem proportion was higher than the leaf proportion for all varieties except the Zanzibar variety.

The relationship of the botanical fraction on oxalate content with the botanical ratio is about the animal preferences on consuming the leaf part more compared to the stem part. The total oxalate and soluble oxalate were higher in the leaf fraction compared to stem (Figure 4.3 a) and this will give negative impact towards the animal performance.

4.3.1 Leaf fraction

From the physical observation, I observed that dwarf was short in size and leafy while non-dwarf was tall in size. This is in line with the Figure 4.3 (b) of this study. It was observed that the leaf proportion was higher than the stem proportion for Dwarf variety. From Table 4.1.1 and Table 4.1.3, Dwarf variety had the highest content of soluble oxalate and total oxalate, which might be contributed significantly by leaf proportion. This can be confirmed with the published article of Rahman et al. (2006) who found that leaf tissue contained higher oxalate content than the stem tissue. The plant part containing oxalate in ascending order is stem < leaf sheaths < leaf blades found in the *Setaria sphacelata* (Jones & Ford, 1972). Nayananjalie (2015) also found that the soluble oxalate content was higher in the leaf tissue compared to stem tissue in Guinea grass. Even though the Dwarf variety is a hybrid Napier grass, it is small in size, which makes it easy to manage but it consists more leaf proportion. Higher oxalate content in Dwarf variety may cause animal toxicity in prolong period of exposure, if animals graze on this variety.

4.3.2 Stem fraction

The stem is less preferable for the animal, but it also contains the nutrients that required for the animal optimum diet. The different morphological part in the Napier grass provides different feed quality (Kebede et al., 2014). The stem is important to give to the animal together with the leaf for economical utilisation of forages. From Figure 4.3 (b), only Zanzibar and Dwarf varieties showed higher leaf proportion, but the other varieties (Kobe, Taiwan, Pakchong, Purple and Indian) exhibited higher stem proportion than the leaf proportion. The importance of a stem is to give physical support to the Napier grass and consists of xylem and phloem as a pathway for nutrient uptake from the root to the all the part of a plant. If a variety has stem ratio higher than leaf ratio, it will cause less economic for the farmers as their animal will prefer leaf part more. Thus, Dwarf is one of the hybrid variety that has equivalent stem and leaf ratio and from this study, Dwarf was leafy. The stem for Dwarf variety was slightly soft as it holds more water compared to the other tall varieties stem was slightly hard to cut.

4.4 Oxalate toxicity

Soluble oxalate is the main concern in the forages. Soluble oxalate is soluble and easy to absorb in the body. Soluble oxalate will give negative impact on animal performance as it can combine with the blood calcium and formed a calcium oxalate. Calcium oxalate can contribute to form a kidney stone and also cause the hypocalcemia 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 the 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 on Buffel grass (*Cenchrus ciliaris*) that 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).

4.4.1 Ruminant vs non-ruminant

Forages can be successfully digested by the ruminant compared to the non-ruminant. The presence of rumen bacteria in the ruminant digestive system helps the ruminant to digest the forage better than non-ruminant as the forages contain lignin. The oxalate will be degraded by the rumen bacteria in the ruminant but 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%.

4.4.2 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 be put more attention as the animal might consume the oxalate-rich containing plant that will disturb the calcium level in the animal body. Thus, the animal that only depends on forages diet also should be concerned more as they might not consume enough calcium like the concentrate feeds can provide.

Other than 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 salt (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, Panter, Gardner, Stegelmeier, and Hall (2014), recommendations for avoiding oxalate toxicity is providing plenty of fresh water at the animal grazing area and calcium-enriched trace mineral salt is available.

4.5 The silica content

From Table 4.5, the ash content was the highest in Kobe (16.43%) and the lowest in Indian (12.33%). For the silica, Dwarf variety showed the higher content (4.19%) followed by Taiwan (3.92%), Zanzibar (3.89%), Indian (3.49%), Purple (3.42%), Kobe (3.25%) and Pakchong (3.14%). There were no significant differences on ash and silica contents among the varieties.

Table 4.5: Ash and silica content (DM %) in different varieties of Napier grass.

Varieties	**Ash (Mean ± SD)**	**Silica (Mean ± SD)**
Taiwan	$14.23^{ab} \pm 0.31$	$3.92^{a} \pm 0.50$
Zanzibar	$13.50^{a} \pm 0.61$	$3.89^{a} \pm 1.35$
Kobe	$16.43^{b} \pm 0.55$	$3.25^{a} \pm 0.80$
Pakchong	$12.87^{a} \pm 0.67$	$3.14^{a} \pm 0.95$
Purple	$13.50^{a} \pm 1.61$	$3.42^{a} \pm 1.89$
Indian	$12.33^{a} \pm 0.60$	$3.49^{a} \pm 1.04$
Dwarf	$16.33^{b} \pm 2.54$	$4.19^{a} \pm 0.11$
Level of significance	*	NS

DM, dry matter; *$p < 0.05$; NS, not significant. Means with different superscript in a row differ at 5% level of probability.

4.5.1 Silica in the grass

Massey and Hartley (2006) stated that the grasses are considered as Si-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. Pathan, Tumbare and Kamble (2014) reported that pearl millet × Napier hybrid contained the lowest silica content (2.43%) at 45 days cutting interval. Silica content in Napier grass in this study ranged from 3.14 to 4.19% in respect to its variety, but there were no significant differences among the varieties. Silica content in this study is in line with the findings of Jusdado (2011) who reported that the silica content among the plant species was varied with ranges of 0.1 to 10.0% of dry weight.

4.5.2 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 (Jugdaohsingh, 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 a 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 for animal tolerance’s in the published literatures.

CHAPTER 5

CONCLUSION

5.1 Conclusion

Based on results of this experiment, it is concluded that oxalate content was significantly differed among the Napier grass varieties, while there were no significant differences on silica content among the Napier grass varieties. Dwarf variety contained the highest oxalate and silica content, while Pakchong variety contained the lowest amount of oxalate and silica. For botanical fractionations, leaf part contained more oxalate compared to stem part. Anti-nutrient content in Napier grass received little attention as people tend to focus more on the nutritional value of the forages. Therefore, to select the best varieties of Napier grass that is high in nutrient quality and low in anti-nutrient need the whole study on the nutritional and anti-nutritional content of different Napier grass varieties.

5.2 Recommendation

This is the initial studies focused on the anti-nutrient composition, the oxalic acid and silica contents in different varieties of Napier grass (Taiwan, Zanzibar, Kobe, Pakchong, Purple, Indian and Dwarf) which were planted at Agro Techno Park, UMK Jeli campus. The findings of oxalate and silica also rely on the mineral availability in the soil at the certain area. Thus, different location of the study area 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 on consuming the forages that rich in oxalate and silica content as the available studies are limited.

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APPENDIX A

Table A.1: Soluble oxalate content of leaf fraction in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
Zanzibar	3	2.0889	
Kobe	3	2.2181	
Purple	3	2.4419	
Indian	3	2.4846	
Pakchong	3	2.6417	
Taiwan	3	2.7286	2.7286
Dwarf	3		3.4452
Sig.		.126	.062

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.2: Soluble oxalate content of stem fraction in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05
		1
Pakchong	3	1.2138
Purple	3	1.5961
Dwarf	3	1.6614
Indian	3	1.8396
Taiwan	3	1.8481
Kobe	3	1.8582
Zanzibar	3	2.1085
Sig.		.124

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.3: Soluble oxalate content of whole plant in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
Pakchong	3	1.8019	
Purple	3	1.9680	
Kobe	3	1.9971	
Zanzibar	3	2.0391	
Indian	3	2.1286	
Taiwan	3	2.2921	
Dwarf	3		2.9993
Sig.		.169	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.4: Total oxalate content of leaf fraction in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Indian	3	2.4122		
Pakchong	3	2.4553		
Kobe	3	2.5064		
Purple	3	2.5217		
Zanzibar	3	2.6623	2.6623	
Taiwan	3		3.0698	3.0698
Dwarf	3			3.4451
Sig.		.313	.082	.106

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.5: Total oxalate content of stem fraction in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Pakchong	3	1.5849		
Taiwan	3	1.8098	1.8098	
Indian	3	2.0140	2.0140	2.0140
Zanzibar	3		2.3595	2.3595
Purple	3		2.3796	2.3796
Dwarf	3			2.5859
Kobe	3			2.6788
Sig.		.216	.116	.075

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.6: Total oxalate content of whole plant in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
Pakchong	3	1.9481			
Indian	3	2.1459	2.1459		
Taiwan	3		2.4260	2.4260	
Purple	3		2.4394	2.4394	
Zanzibar	3		2.5997	2.5997	
Kobe	3			2.6118	
Dwarf	3				3.2322
Sig.		.333	.051	.399	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.7: Insoluble oxalate content of leaf fraction in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
Pakchong	3	-.1863	
Indian	3	-.0724	-.0724
Dwarf	3	-.0002	-.0002
Purple	3	.0798	.0798
Kobe	3	.2883	.2883
Taiwan	3	.3412	.3412
Zanzibar	3		.5734
Sig.		.137	.074

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.8: Insoluble oxalate content of stem fraction in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05
		1
Taiwan	3	-.0383
Indian	3	.1745
Zanzibar	3	.2509
Pakchong	3	.3711
Purple	3	.7835
Kobe	3	.8206
Dwarf	3	.9246
Sig.		.128

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.9: Insoluble oxalate content of whole plant in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05
		1
Indian	3	.0174
Taiwan	3	.1338
Pakchong	3	.1463
Dwarf	3	.2329
Purple	3	.4714
Zanzibar	3	.5606
Kobe	3	.6147
Sig.		.077

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.10: The ash content in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
Indian	3	12.3333	
Pakchong	3	12.8667	
Zanzibar	3	13.5000	
Purple	3	13.5000	
Taiwan	3	14.2333	14.2333
Dwarf	3		16.3333
Kobe	3		16.4333
Sig.		.108	.056

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.11: Silica content in different varieties of Napier grass

Duncan

Treatment	N	Subset for alpha = 0.05
		1
Pakchong	3	3.1396
Kobe	3	3.2540
Purple	3	3.4197
Indian	3	3.4898
Zanzibar	3	3.8891
Taiwan	3	3.9213
Dwarf	3	4.1936
Sig.		.237

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX B

 Figure B.1: N1R1 (Taiwan Napier)	 Figure B.2: N1R2 (Taiwan Napier)
 Figure B.3: N1R3 (Taiwan Napier)	 Figure B.4: N2R1 (Zanzibar Napier)
 Figure B.5: N2R2 (Zanzibar Napier)	 Figure B.6: N2R3 (Zanzibar Napier)

Figure B.7: N3R1 (Kobe Napier)

Figure B.8: N3R2 (Kobe Napier)

Figure B.9: N3R3 (Kobe Napier)

Figure B.10: N4R1 (Pakchong Napier)

Figure B.11: N4R2 (Pakchong Napier)

Figure B.12: N4R3 (Pakchong Napier)

Figure B.13: N5R1 (Purple Napier)

Figure B.14: N5R2 (Purple Napier)

Figure B.15: N5R3 (Purple Napier)

Figure B.16: N6R1 (Indian Napier)

Figure B.17: N6R2 (Indian Napier)

Figure B.18: N6R3 (Indian Napier)

Figure B.19: N7R1 (Dwarf Napier)

Figure B.20: N7R2 (Dwarf Napier)

Figure B.21: N7R3 (Dwarf Napier)

Figure B.22: Preparation of 20mM of potassium phosphate

Figure B.23: 20mM of potassium phosphate pH 2.90

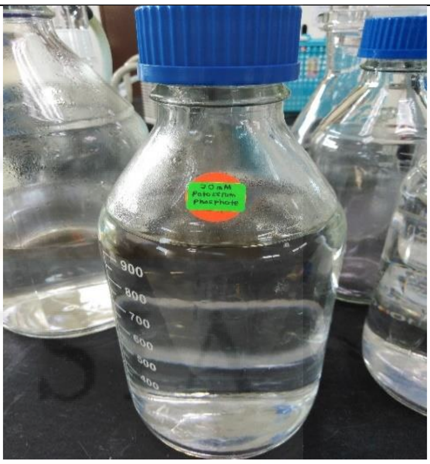

Figure B.24: 20mM of potassium phosphate as Eluent A for HPLC analysis