UNIVERSITI PUTRA MALAYSIA

CHEMICAL COMPOSITION OF TAIWAN NAPIER GRASS (Pennisetum purpureum Schumach) AT DIFFERENT GROWTH STAGES

NUR ZAHIRAH ABD JALIL

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FSPM 2019 25

CHEMICAL COMPOSITION OF TAIWAN NAPIER GRASS (*Pennisetum purpureum* Schumach) AT DIFFERENT GROWTH STAGES

By

NUR ZAHIRAH BINTI ABD JALIL

A Project Report Submitted in Partial Fulfillment of the Requirement
for the Degree of Bachelor of Bioindustrial Science in the
Faculty of Agriculture and Food Science
Universiti Putra Malaysia Bintulu Sarawak Campus

2019

Dedicated to,

My loving family and relatives

Abd Jalil Abdullah, Hasimah Hasan

Muhammad Fauzi, Muhammad Fadzli, Nur Zulaikha, Nur Haslina, Shafinaz Amalina, Mohd Firdaus, Sarah Alia, Muhammad Al-Fateh, Ramlah

My Supervisor

Dr. Noorasmah Saupi

My Co-Supervisor

Dr. Masnindah Malahubban

My close friends

Sam Nureszuan, Maisarah, Wan Nura Aisya, Nabilah, Syafiqah, Norfaziey aini, Kristal, Anis, Shahirah, Emilia, Nurain, Ain

And all my loved ones

ABSTRACT

Taiwan Napier (*Pennisetum purpureum*) is a perennial forage crop with good nutritive value, high productivity, and growth rate and currently used for cutting and carry system over the tropical and sub-tropical area of the world. The aim of this study is to evaluate the chemical composition content in different growth stages of Taiwan Napier. The different harvesting days of growth stages were 15, 30, 45, 60, 75 and 90 days. The plant's samples were collected at Ladang Kongsi 1, Pasture Unit (TPU), Universiti Putra Malaysia Bintulu Sarawak Campus. This study was analyzed using proximate analysis methods ie dry matter, moisture, crude protein (CP), crude fibre (CF), ether extract (EE), and acid detergent fibre (ADF). Results showed that it has a significant effect at different growth stages on the percentages in leaves and stems content for all parameters that have been measured in Napier grass. The percentage of dry matter increased as the days increased, respectively. The moisture content, crude protein stems, and both crude fats decreased when days increased. Crude fibre amount in leaves and stems were highest at days 60 (36.50±1.80%) and days 90 (40.33±0.76%), while lowest amount at days 15 (28.17±1.04%) and (29.67±1.61%). For crude protein leaves, both part ash and ADF result were increased from early to intermediate and then decreased at maturity stages. The different harvesting days after given an effect on the chemical compositions of Napier grass. Hence, the best Napier grass can be harvesting is within two months of the cutting period which is providing the high nutritive amount of the animal fodder.

ABSTRAK

Napier Taiwan (*Pennisetum purpureum*) merupakan tanaman foraj saka yang mempunyai nilai pemakanan yang baik, produktiviti dan kadar pertumbuhan yang tinggi dan digunakan di dalam sistem potong dan angkut untuk kawasan tropika dan sub-tropika. Objektif kajian ini dijalankan adalah untuk menilai kandungan komposisi rumput Taiwan Napier pada peringkat pertumbuhan yang berbeza. Perbezaan hari penuaian peringkat pertumbuhan adalah 15, 30, 45, 60, 75 dan 90 hari. Sampel tumbuhan ini telah diambil di Ladang Kongsi 1, Unit pastura, Universiti Putra Malaysia Kampus Bintulu, Sarawak. Kajian ini dianalisis dengan menggunakan kaedah analisis proksimat untuk bahan kering, lembapan, protein mentah (CP), serat mentah (CF), ekstrak ether (EE), dan serat detergen asid (ADF). Hasil kajian menunjukkan bahawa pada peringkat pertumbuhan yang berbeza, peratusan untuk semua parameter yang diukur pada daun dan batang rumput Napier adalah menunjukkan perbezaan yang ketara. Peratusan bahan kering bertambah apabila jumlah hari meningkat. Kandungan lembapan dan jumlah EE untuk kedua-dua bahagian tumbuhan adalah menurun apabila jumlah hari meningkat. Kandungan protein mentah juga menunjukkan corak yang sama dari awal pertumbuhan sehingga pertengahan pertumbuhan. Jumlah serat mentah tertinggi adalah bahagian daun dan batang pada hari 60 (36.50±1.80%) dan hari 90 (40.33±0.76%), manakala jumlah terendah adalah daun pada hari 15 (28.17±1.04%) dan batang juga pada hari 15 (29.67±1.61%). Untuk protein mentah di dalam daun, serta kandungan abu dan ADF dalam daun dan batang meningkat dan kemudian menurun pada peringkat kematangan. Bilangan hari penuaian yang berbeza memberi kesan kepada komposisi kimia rumput Napier. Oleh itu, pemotongan rumput Napier yang terbaik dapat dituai dalam tempoh dua bulan dimana komposisi makanan adalah yang tertinggi.

ACKNOWLEDGEMENTS

Alhamdulillah, praises to Allah for giving me the strength and guidance to complete this thesis. Gratitude to both of my supervisor, Dr. Noorasmah binti Saupi and my co-supervisor Dr. Masnindah binti Malahubban, for their encouragement, patience, guidance, and close supervision during the period in completing final year project. There are also few people that I respect in helping me all the techniques in laboratory and for all the help they gave me with my experiments especially Puan Anna Anak Gutoh @ Kundat. She really spent a lot of time instructing me, and I thank her for her patience and for giving me the confidence in being comfortable in the laboratory environment. Thank you also to graduate student, Saipul Izzuwan bin Dahari that always help, guidance and support in doing the project. Moreover, many thanks to my loving family especially my dad and mom then my siblings who always pray for me and being supportive for encouraging me to do this project with patient and happiness. Lastly but not least, thank you so much to my friends Sam Nureszuan bin Sam Sabtu, Maisarah binti Matali and Norfaziey Aini binti Sukarno that always be a supportive, helpful hand along this journey. Besides, many thanks also go to those who may, directly and indirectly, helped me in any mean, conditions and behaviors that I challenged during doing the project.

APPROVAL SHEET

I certify that this research project report entitled "Chemical Composition Of Taiwan Napier Grass (*Pennisetum purpureum* Schumach) at Different Growth Stages" has been examined and approved as a partial fulfillment of the requirement for the Bachelor of Bioindustrial Science in the Faculty of Agriculture and Food Science, Universiti Putra Malaysia Bintulu Campus, Sarawak.

Dr. Noorasmah Binti Saupi
Faculty of Agriculture and Food Science
Universiti Putra Malaysia Bintulu Sarawak Campus
(Supervisor)

Dr. Masninqah Binti Malanubban
Faculty of Agriculture and Food Science
Universiti Putra Malaysia Bintulu Sarawak Campus
(Co-Supervisor)

Prof. Madya. Dr Shahrulrazid Bin Sarbini
Dean
Faculty of Agriculture and Food Science
Universiti Putra Malaysia Bintulu Sarawak Campus

Date:

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LIST OF ABBREVIATIONS

FAO	Federation Agriculture Organization
USDA	United States Department of Agriculture
AOAC	Association of Official Analytical
CP	Crude protein
CRD	Completely randomized design
CF	Crude fibre
DM	Dry matter
ADF	Acid detergent fibre
EE	Ether extract

CHAPTER 1

INTRODUCTION

Napier grass (*Pennisetum purpureum* Schumach) is a monocot belonging to the family Poaceae (grass family). It is also as known as elephant grass, or Uganda grass which is a tropical grass native to Africa and could be found in Tropical Africa and sub-Saharan region (Clayton *et al.* 2013). It is mainly used as a feed crop (fodder grass) for livestock and insect repellant for food crops, such as maize. Napier grass is currently the most popular fodder grass in dairy and feedlot production systems due to high productivity and good nutritive value (Wadi *et al.* 2014). Architecturally, Napier grass is a stout, tall and deep-rooted perennial bunch grass.

The Napier grass is easy to grow, highly adaptable and productive grass which is very suitable for the Malaysia climate (Halim *et al.* 2013). It can be propagated from cuttings or from root slips. Napier grass is best suited to be grown in high rainfall areas as well as drier areas, due to its drought-tolerant ability. Optimum growth condition for Napier grass is in the range of 30 – 35°C (Ferrais 1978). Studies identified, Napier grass produced high biomass yield when planted in tropical and sub-tropical climates and used for livestock fodder (Ansah *et al.* 2010; Halim *et al.* 2013; Kebede *et al.* 2016; Rusdy 2016). Napier grass has been introduced to Malaysia and used as a feed for livestock since the 1920s. (Wong *et al.* 1982; Halim *et al.* 2013; Zailan *et al.* 2016). Recently, Napier grass is gaining much more attention to feed ruminant in ruminant production systems due to its high nutritive value and productivity.

The nutritive value of grass for livestock is depended upon the amount of dry matter consumed, the chemical composition and the coefficient of digestibility of the dry

matter (Halim *et al.* 2013; Wangchuk 2015). Understanding the chemical requirement is important to provide diets that meet the animal's needs, especially for growth and milk production. To improve the nutritive values of the animal's diet, selecting the right and good fodder grass is important. Selection of a good fodder grass is based on consideration the yield, chemical composition, and digestibility of the grass.

The chemical compositions such as crude protein (CP), crude fibre (CF), calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg) and nitrogen (N) are the most mineral needed for the animal growth, which can obtained from the animal feed such as a grass or forage (Gaal *et al.* 2004; Gomes *et al.* 2011; Halim *et al.* 2013; Kebede *et al.* 2016). Many studies showed Napier grass has a good nutritive value as well as the chemical compositions (Gomes *et al.* 2011; Halim *et al.* 2013; Lounglawan *et al.* 2014; Zailan *et al.* 2016). However, the chemical composition of the grass shown to be varied with the stage of maturity, soil conditions and climate (Lounglawan *et al.* 2014). Then very few information of different growth stages of Napier grass, especially Taiwan Napier grass. The aim of this research work is to evaluate the chemical composition content in different growth stages (15, 30, 45, 60, 75, and 90 days) of Taiwan Napier grass.

CHAPTER 2

LITERATURE REVIEW

2.0 Background Taiwan Napier Grass

Taiwan Napier grass is also known as Elephant grass is a species of perennial grass which is native to subtropical Africa (Kebede *et al.* 2016). Anindo and Potter (1986) stated that Napier grass can be propagated vegetatively from a stem cutting technique due to seed is not available in commercial quantities (Inanaga *et al.* 1990), and interestingly it could withstand several cuttings and regenerates rapidly, hence produce a high yield fodder.

Napier grass produces high-protein forage and it is important for fodder grass. Napier grass has many desirable features such as tolerance to intermittent drought and high-water use efficiency, making it forage of choice, high yield per unit area because it could withstand repeated cutting and rapidly regenerate, producing palatable leafy shoots (Alemayehu *et al.* 2017).

2.1 Taxonomy of Napier Grass

According to United States Department of Agriculture (n.d.), they recognized Napier grass has 21 species and 16 accepted taxa. Napier grass used for this study was classified as follows:

Kingdom:	Plantae
Clade:	Angiosperms
Class:	Monocots
Order:	Poales
Family:	Poaceae
Genus:	*Pennisetum*
Species:	*P. purpureum*
Botanical name:	*Pennisetum purpureum* Schumach

2.2 Varieties of Napier Grass

Napier grass has many varieties and difference morphology between the varieties such as nutritive quality and agronomic characteristics. Napier grass has been introduced since the 1950s in Malaysia for two types of cultivars which are tall cultivars (Common, Red, Taiwan, Indian, Uganda, King grass, Zanzibar and Kobe) has the grass height of more than 130 cm and short cultivars (Dwarf, Dwarf "Mott" and Australian Dwarf Napier) is in the range of 100 cm (Halim *et al.* 2013). Normally, tall cultivars are normally grown due to its high yield and using the cut-and-carry planting system in Malaysia. Halim *et al.* (2013) stated Common Napier is among the highest yielding crops and has a better nutritive value compared to Uganda Napier. According to Kamaruddin *et al.* (2018), there are several species of Napier grass has the potential to be used as fodder, for example, Taiwan Napier, Red Napier, and India Napier. This is due to the morphological study done by, Bach *et al.* (1995), variety of Taiwan A-144 has a high dry matter, whereas, Red Napier is high in metabolizable energy and this

crucial parameter reflected the actual level of energy available for absorption (Haryani *et al.* 2012).

2.3 Chemical Composition

Various studies have been conducted on dry matter productivity and nutritional content of Napier grass cultivars (Halim *et al.* 2013; Ansah *et al.* 2010; Manyawu *et al.* 2003). According to Kanjanapruthipong *et al.* (2001) and Mertens (1997), increase of roughage neutral detergent fibre (NDF) proportion in a diet would reduce energy intake, density, and productivity of dairy cows. Hence, utilizing Napier grass is live feedstock diet is reverent due it has dry matter digestibility ranging from 53 - 80% (Budiman *et al.* 2012 and Wijitphan *et al.* 2009), high leaf-steam ratio (LSR) in Napier grass (Zewdu 2005) as shown in Table (2.1) and range of nutritive qualities in different varieties (Table 2.2).

Table 2.1 Morphological characteristics and dry matter yield of nine Napier grass varieties (Zewdu 2005)

Variety	Plant Height (cm)	Number of Tillers/Plants	Leaf to Stem Ratio	Dry Matter Yield at Harvest 2 (kg/ha)	Cumulative Dry Matter yield (t/ha)
King grass	145	12.6	0.80	15,840	61.6
Common Napier	139	14.8	0.87	14,420	65.1
Red Napier	139	13.5	0.92	12,640	59.8
Taiwan Napier	146	11.9	0.68	11,120	60.4
Uganda	147	13.7	1.01	11,640	65.9
Indian Napier	144	14.2	0.92	11,440	56.7
Dwarf Napier	95	18.1	1.63	11,580	51.0
Dwarf 'Mott'	79	19.6	1.22	8,720	55.9
Australian Dwarf	71	18.7	1.15	8,000	43.7

Table 2.2 Nutritive quality of nine Napier grass varieties (Halim *et al.* 2013)

Variety	Crude Protein %	Neutral Detergent Fibre (NDF) %	Acid Detergent Fibre (ADF) %	Acid Detergent Lignin (ADL) %
King grass	10.11	70.10	38.10	6.85
Common Napier	9.79	70.90	38.80	9.24
Red Napier	10.36	69.30	38.20	7.45
Taiwan Napier	10.09	70.00	39.90	7.99
Uganda	10.36	71.80	39.80	8.22
Indian Napier	10.64	70.00	38.80	8.65
Dwarf Napier	11.56	69.10	37.00	8.77
Dwarf 'Mott'	11.61	67.80	36.90	8.96
Australian Dwarf	12.08	66.10	35.70	8.19

2.4 Proximate Composition

According to Dublecz (2011), a proximate composition is referring to the determination of the major constituents of food and it is used to determine if the food is within its normal compositional parameters or somehow have been adulterated. This method partitioned nutrient in food into five components which are moisture, ash, crude protein, crude fat, and crude fibre.

2.4.1 Moisture

According to Yoana *et al.* (2000), generally moisture content is the basis from dry matter and wet. Wet basis indicated on how fresh forage would be required to meet the DM condition of the animals. The dry-matter base is considered if the forage had no moisture. Ambo *et al.* (1999) stated Napier grass has the flexible receptive capability in dry matter productivity to high rates of nitrogen application.

2.4.2 Ash

Ash is a method to determine the quantity of the total mineral content to identify the residue remaining after burning a sample. Above 10% of values for grasses usually, show soil contamination of forage. Besides, ash, ADF-ash, and NDF-ash have different values because of ADF and NDF procedures that eliminate some minerals.

2.4.3 Crude Protein (CP)

Protein is one of the main important nutrients for livestock and the source of energy. This is because rumen microbes in the stomach degrade forage rue proteins and produce 60 - 80% of the total plant nitrogen (N), with soluble protein and a small percentage of fibre-bound N making up the balance (Yoana *et al.* 2000). Proteins are organic compounds composed of amino acids. Protein is a key component of vital organs, muscle, tissue, skin, hair, milk, and enzymes (Glossary of Nutrient Terms 2018). Besides, protein is essential daily for maintenance, growth, lactation, and reproduction (Glossary of Nutrient Terms 2018).

The crude total protein content of a feed sample can be precisely determined by laboratory analysis. Total crude protein is ca by determined by measuring the amount of nitrogen in the feed transformed to protein by multiplying the amount with 6.25 and the basis for this is that protein contains 16% nitrogen or 1 similarity nitrogen to 6.25 parts protein (Schroeder 1994). Ambo *et al.* (1997) have stated the application of good fertilizer use by increasing the crude protein and lowering the dry matter digestibility.

2.4.4 Crude Fat / Ether Extract (EE)

Ether extraction is used to determine the crude fat. When fat is introduced to the ether extraction method, it will solubilize fat to becoming esters, plant pigments, and aldehydes. Therefore, the amount is called crude fat. Furthermore, fat has 2.25 times more of the energy found in carbohydrates (Glossary of Nutrient Terms 2018). Fat is added to the ratio to increase energy levels when ingestion may be limiting.

2.4.5 Crude Fibre (CF)

Fibre analysis is used to divided carbohydrates into digestible and indigestible fractions. This is because crude fibre accounts for most of the cellulose and only a portion of the lignin (Glossary of Nutrient Terms 2018). This method is not the most precise method for measuring fibre, particularly for forages, however, given the number of grains are low in lignin, it is an accurate approximation of fibre in grains and is still used nowadays as the permissible measurement of fibre in grains and finished feeds (Glossary of Nutrient Terms 2018).

2.4.6 Acid Neutral Fibre (ADF)

Energy derived from a feed used by the animal is calculated by the acid neutral fibre calculation (Carpenter 2017). The calculation is important to know how much feed must be given to an animal. For example, a milk cow and a beef cow have dissimilar energy requirements. A milk cow needs more energy from its feed to meet the demands of producing milk.

2.5 Factor Affecting Chemical Composition

Climate condition for the plantation of the Napier grass is one of the factors affecting the chemical composition of Napier grass. Chemical composition of Napier grass such as structural carbohydrate content is different when planted in two different conditions in tropical and temperate climate (Zailan *et al.* 2016). Humid and hot features of the tropical region are supposed to influence the value of the grass due to high temperature, which usually improves the rate of plant growth and reduces digestibility (Buxton 1996). According to Zailan *et al.* (2015) and Lounglawan *et al.* (2014), the digestibility of Napier grass can be reproduced by several factors based on cultivar selection and management practices such as cutting interval, harvesting age and cutting height. Table 2.3 showed the chemical composition of fresh Napier cultivars in 6 - 8 weeks old at first cutting, while Table 2.4 showed the variation of the composition of proximate in the different cutting period.

Table 2.3 Mean chemical composition of fresh Napier cultivars within 6 to 8 weeks old at first cutting (Zailan *et al.* 2016)

Napier Cultivar	CP%	NDF%	ADF%	ADL%	Hemi-Cellulose%	Cellulose %	GE (MJ/kg DM)
Common	8.71	72.77	44.99	11.15	27.78	31.84	16.93
Silver	10.83	71.87	39.63	8.74	32.24	30.89	17.08
Red	10.44	67.76	40.70	6.63	27.06	34.07	17.09
Dwarf	15.90	64.29	31.33	5.32	32.96	24.59	17.31
SEM1	0.56	0.70	1.00	1.00	0.92	1.22	0.08

Table 2.4 Nutritive values for six types of Napier at the different cutting period (Haryani *et al*. 2012)

Types of Napier	35 Days				42 Days			
	Dry Matter (%)	Crude Protein (%)	Crude Fibre (%)	Energy/ ME (MJ/kg)	Dry Matter (%)	Crude Protein (%)	Crude Fibre (%)	Energy ME (MJ/kg)
Napier 3rd Generation	12.87	17.5	35.5	8.52	14.4	15.03	34.23	8.82
Napier India	9.47	17.77	29.6	8.76	12.47	14.93	33.00	8.71
Napier Kobe	11.97	18.73	31.93	8.89	13.00	15.27	34.6	9.06
Napier Merah (Red)	12.17	17.07	31.47	9.59	14.33	13.47	33.2	9.00
Napier Taiwan	10.67	17.23	32.23	8.83	12.47	13.97	33.1	8.75
Napier Zanzibar	12.93	19.43	31.33	9.09	12.07	13.97	35.00	8.57

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of The Study Site

This experiment was conducted at the pasture field located at Ladang Kongsi 1, Pasture Unit (TPU), Universiti Putra Malaysia Bintulu Sarawak Campus. The average temperature ranges in the study area are between 29 – 36°C.

3.2 Planting Methods

In this study, the variety of Taiwan Napier grass was used. The Napier grass stem at 20 cm length was placed at 45° from ground level with two nodes were buried in the soil and the other nodes were being left exposed. The planted plot size was at 11 m x 1 m with 0.3 m planting distance between plants and 0.6 m between rows (Figure 3.1). This study was conducted from October 2018 until January 2019 in three months.

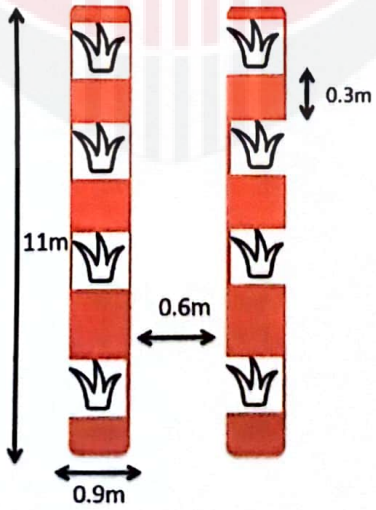

Figure 3.1 Planting distances of Napier grass

3.3 Harvesting of Napier Grass

Napier grass with different growth stages (15, 30, 45, 60, 75 and 90 days) were harvested manually using sickle and a stem of 10 - 15 cm was left behind from the ground after harvesting. The Napier grass was harvested randomly at three locations on each bed. Two replication sample for stem and leaves were taken from each location. Furthermore, the plant samples had been chopped into smaller pieces (2 - 5 cm). Then, the plant samples were placed into plastic bags and brought to the Nutrition Laboratory, UPMKB.

Figure 3.2 Planting beds of Napier grass.

3.4 Sample Preparation for Proximate Analysis

The fresh Napier grass samples from the field were brought to the laboratory to be dried. All harvested were dried using the drying oven for 4 to 7 days until consistent

weight. After that, the dry Napier grass samples were grinded into powder form. Next, the samples were sieved using 2 mm steel mesh sieve. After that, the samples were kept in an airtight bottle for further analysis.

3.5 Proximate Analysis

3.5.1 Determination of Moisture Content (MC)

Moisture content was determined by drying an accurately weighed sample of Taiwan Napier grass in the oven (AOAC 1990). Firstly, a blank tray was dried in a drying oven at 105°C for 24 hours. After 24 hours, the tray was cooled in a desiccator for 15 minutes and weighed. About 100 - 500 g Napier grass was added to the tray which has been weighed and the weight was recorded exactly within 0.001 g. The tray was kept in the oven at 60°C until a constant weight was obtained. Then, they were cooled in a desiccator and re-weighed. Each sample weight was determined in triplicate. The moisture and dry matter were calculated by using the following formula.

$$\text{Dry Matter Content (\%)} = (W3 - WI) \times 100 / (W2-W1)$$

Where, W1 = weight of the empty tray (g), W2 = weight of tray and sample (g), and W3 = weight of tray and sample after drying (g).

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

3.5.2 Determination of Ash

Ash content was determined by combusting the plant material in crucibles in a muffle furnace according to (method 930.05, AOAC 1990) method. Ash is considered as the

total mineral or inorganic contents of the samples. Firstly, the weight of the blank crucible was weighted. Then 2.0 - 3.0 g of the dry samples (Napier grass) were placed inside the crucible and total weight of the samples and the blank crucible was determined. The samples and crucible were placed inside a furnace and heated at 550°C for three hours, then left to cool for one or two hours and transferred to a desiccator for 15 minutes. After that, the samples and crucible were re-weighed. All the steps were conducted in triplicate. the process was repeated for dried leaves and steam. The percentage of ash content as in formulation below.

$$\text{Ash Content (\%)} = (W3-W1) \times 100/(W2-W1)$$

Where, W1 = the weight of empty crucible and lid (g), W2 = the weight of crucible, lid and sample (g), and W3 = the weight of crucible, lid and residue after incineration (g).

3.5.3 Determination of Crude Protein (CP)

3.5.3.1 Preparing Samples Before Digestion

0.2 g of samples Napier grass with 5 mL of 98% concentrated sulphuric acid (H_2SO_4) and place in digestion tubes. One Kjeldahl catalyst tablet was added for each tube to facilitate the digestion. The tubes were placed in the tube rack and the rack placed onto preheated digester. The digester was set at 320°C for five hours. If the samples digested completely, the samples were left overnight to cool them.

3.5.3.2 Procedure for Using Distillation

30 mL of distilled water was added into the digestion tube containing the samples that were left overnight. Then, the samples were vortex and filtered using filter paper. After that, the filtered samples were further diluted with distilled water up to 100 mL. Then, the samples were transferred into Kjeldahl distillation unit, boiled the water at 250°C. After the samples were boiled, 10 mL of samples were taken out and placed into a new conical flask, and 10 mL of 40% sodium hydroxide (NaOH) was added. Then, 10 mL of 2% boric acid was added in the plastic vial at the pump, Then, by opening the steam valve and delivering the steam with boric acid which eventually changes the colour of boric acid from purple to green.

3.5.3.3 Procedure for Using Titration

The sample solution was titrated against 0.01 M of hydrochloric acid (HCl) of predetermined molarity to the endpoint (green to purple) so that no green colour. The percentage of crude protein was calculated by multiplying with 6.25 using the following equation:

$$\%\text{Nitrogen (N)} = [14.01] \times (\text{ml HCL for sample} - \text{ml HCL for blank}) \times (\text{molarity of HCl}) 10 \times \text{sample (g)}$$

$$\text{Crude Protein (\%)} = \%\text{N} \times \text{Conversation factor}$$

Where; Conversation factor = 6.25

3.5.4 Determination of Crude Fat / Ether Extract (EE)

Fat in the samples was determined by extracting a known weight of powdered plant sample with petroleum ether, using Soxtec system-tecator according to (method

930.09, AOAC 2000) method. The fats were extracted from the sample with petroleum ether and evaluated as a percentage of the weight before the solvent is evaporated. The extraction cups were cleaned, dried and cooled in the desiccator and weight. About 3 g of dry samples were weighed in a thimble, handling it with tongs and were placed in the extraction unit. About 70 mL of petroleum ether was used for extraction. The extraction cups containing petroleum ether was connected to the extractor and condenser which then will be brought to boil and while the heat was adjusted to obtain about 10 refluxes per hour. The length time of the extraction for Napier grass was boiled for 15 minutes, rinsed for 30 minutes, recovered time at 10 minutes and 5 minutes rest before the stop. Fats materials to be processed normally within an hour. After evaporating the solvent, the extraction cups were released and dried in the oven for four hours at 103°C. The extraction cups were then cooled in the desiccator. After being cooled, the weight with the extract is taken. Fat of Napier grass was measured on three replicated for stem and three replicated for leaves at every sample and values were recorded. The percentages of lipid were calculated using the following equation.

$$\text{Crude Fat (\%)} = (W3-W2) \times 100/(W1)$$

Where, W1 = the weight of the sample (g), W2 = weight of extraction cup (g), W3 = the weight of extraction cup with residue weight (g).

3.5.5 Determination of Crude Fibre (CF)

The crude fibre was determined according to Van Soest (1991), using the fibertec system. About 0.2 g of sample was weighed into the crucible. Crude fibre is defined to be the residue after sequential treatment with hot 1.25% sulphuric acid (H_2SO_4) and hot 1.25% sodium hydroxide (NaOH). The crucibles were inserted using the holder

and locked into position in front of the radiator in the Fibertec Hot Extraction Unit. The reflector was placed in front of the crucible and put all valves to the closed position. The cold tap water was run (1-2 min) for the reflux system and sulphuric acid was added into each column from the top using a funnel. About two to four drops of n-Octanol were added to prevent foaming and turned 'HEATER' control fully clockwise. The reagents were measured the boiling time until reached the boiling point. At the end of extraction, the heater was turned off and placed the valves in 'VACUUM' position. The cold tap water was opened to full flow rate for the water suction pump and started the filtration. After filtration, the residues were removed from the filter surface by using 'PRESSURE' button. The valves were put to pressure position and back to vacuum position. The valves were washed using deionized water. The methods were repeated and replaced sulphuric acid with sodium hydroxide. The crucibles were released and dried at 130 ± 2°C for two hours. The crucibles then were cooled at room temperature in a desiccator and weighed. Then, the samples were ash in the crucibles at least three hours at 525 ± 10°C. The crucibles were cooled slowly to room temperature in a desiccator and weighed again. The percentage of fibre was calculated using the following equation:

$$\text{Crude Fibre (\%)} = [(W2-W3)/W1] \times 100$$

Where, W1 = sample weight (g), W2 = crucible + sample weight (after drying in the oven) (g), W3 = crucible + ash (g).

3.5.6 Determination of Acid Detergent Fibre (ADF)

3.5.6.1 Prepare of Acid Detergent Solution

The solution that needs for an acid detergent solution was about 20 g cetyltrimethylammonium bromide (CTAB) dissolved in 1 L of 0.5 M sulfuric acid.

3.5.6.2 Procedure for Analysis Acid Detergent Fibre

Acid detergent fibre (ADF) was determined by using the Van Soest (1991) method. About 1 g of sample was placed into fibre cap capsules and 100 mL of a cold solution of cetyltrimethylammonium bromide (CTAB) in sulphuric acid (H_2SO_4) 2 M was added. The mixture was rapidly brought to boiling and allowed to boil gently for 1 hour. The contents of the beaker were filtered through a pre-weigh sintered glass crucible and rinsed twice the residue with acetone and one time with warm distilled water to wash out the detergent, then finally dry under vacuum. The fibre cap capsules were placed in an oven and allowed to dry at 105°C overnight. Weight the crucible then put the fibre cap capsules that have dry into the crucibles then weight it again. After that, put the crucible and fibre cap capsules into the furnace with 550°C for 4 hours. The crucibles were placed in desiccators to cool and weighed. The following formula was used to determine the ADF content.

$$A.D.F\ (\%) = [(W2-W1)/W3] \times 100$$

Where, W1 = crucible weight (g), W2 = crucible + fibre weight after furnace (g), W3 = dry sample weight (g)

3.6 Statistical Analyses

The experimental design consisted of an analysis of one variety of Napier grass. The analysis was carried out in triplicates of each different stage's growth (15, 30, 45, 60, 75, 90) days after planting for each analysis in proximate analyses and data was inserted in electronic sheets to calculate mean and standard deviation for the sample Napier grass. All data were statistically analyzed using ANOVA (LSD) to compare the vegetative growth and chemical compositions of Napier grass at different stages of growth by using SAS 9.4 statistical software. For comparison by different part of grass (leaves and stem) T-test was used in the same growth stages. The p-value ($p<0.05$) is considered a significant difference.

CHAPTER 4

RESULTS

4.1 Proximate Compositions of Napier Grass Leaves

As shown in Table 4.1, the proximate composition of Napier grass leaves at different growth stages was varied. The highest value of dry matter content on day 90 (18.00±0.25%). At day 15, 45, and 60, the dry matter content is similar within the range of 13.82 - 14.40%. The lowest value among growth stages on days 30 (12.02±0.17%).

Moisture content in leaves a relatively high moisture content when compared to ash, crude protein, crude fat, and crude fibre (Table 4.1). The young leaves of Napier grass in early stages planting at day 30 (87.98±0.17%) was significantly higher than in another day. The amount of ash in this grass growth at 30, 45, and 60 days is comparatively higher than in other growth stages. At the growth stages of 75 days, the ash content is significantly the lowest when compared to other growth stages with value 5.07±1.55%. In this study, crude fibre at days 15 was comparatively lower than other growth stages with the value 28.17±1.04%. The result showed that crude protein has no significant difference between 15 to 90 days. The value was similar with each stage of growth of Napier grass.

The ether extract was a significant difference from 15 to 90 days. The higher value in EE was on days 15 (3.28±0.21%) and the lowest was on days 90 (1.00±0.59%). Besides, the ADF value showed a higher significant difference on days 30 (32.64±5.05%) and the lowest on days 75 (22.54±0.55%). There was no significant difference between days 15 and 60 with the value of 27.55±2.26% and 26.96±0.13%.

Table 4.1 Proximate compositions of Napier grass leaves at different stages of growth.

Parameters	Stage of growth (Days)					
	15	30	45	60	75	90
DM (%)	$13.82^{b}\pm0.30$	$12.02^{c}\pm0.17$	$14.40^{b}\pm0.40$	$14.10^{b}\pm0.44$	$17.58^{a}\pm0.38$	$18.00^{a}\pm0.25$
M.C (%)	$86.18^{b}\pm0.30$	$87.98^{a}\pm0.17$	$85.60^{b}\pm0.40$	$85.90^{b}\pm0.44$	$82.42^{c}\pm0.38$	$82.00^{c}\pm0.25$
Ash (%)	$6.13^{ab}\pm0.06$	$6.42^{a}\pm0.46$	$6.82^{a}\pm0.21$	$6.32^{a}\pm0.20$	$5.07^{b}\pm1.55$	$5.70^{ab}\pm0.13$
CF (%)	$28.17^{b}\pm1.04$	$34.50^{a}\pm0.50$	$34.83^{a}\pm0.29$	$36.50^{a}\pm1.80$	$34.17^{a}\pm3.01$	$35.00^{a}\pm1.32$
EE (%)	$3.28^{a}\pm0.21$	$2.72^{b}\pm0.20$	$1.79^{c}\pm0.13$	$2.27^{bc}\pm0.20$	$2.01^{c}\pm0.12$	$1.00^{d}\pm0.59$
CP (%)	$14.59^{a}\pm2.53$	$18.24^{a}\pm4.56$	$20.43^{a}\pm9.11$	$21.89^{a}\pm4.38$	$17.51^{a}\pm4.38$	$15.32^{a}\pm2.19$
ADF (%)	$27.55^{b}\pm2.26$	$32.64^{a}\pm5.05$	$29.32^{ab}\pm0.34$	$26.96^{b}\pm0.13$	$22.54^{c}\pm0.55$	$25.92^{bc}\pm0.50$

DM = dry matter, M.C = moisture content, CF = crude fibre, EE = ether extract, CP = crude protein, ADF = acid detergent fibre. Different letters in superscript (a, b, c, d) within the same row indicate a significant difference (LSD's test, $p<0.05$) among different growth stages. Value is the mean ± standard deviation.

4.2 Proximate Compositions of Napier Grass Stems

The percentage of proximate composition of Napier grass stems at a different stage, are presented in Table 4.2. Dry matter content was no significant difference on days 15, 30, 45, 60, and 75. The value was similar with each stage of growth, respectively. For stages growth days 90 (13.57±0.62%), the value has higher significantly different than the other stages of growth.

Moisture content was the higher value among the other composition. The highest value in on days 60 (91.80±1.07%) and the lowest on days 90 (86.43±0.62%). There was a significant difference between days 90 and other days stages growth (15, 30, 45, 60, and 75). Ash content has the lowest amount on days 75 which is the value 6.77±0.12%. The value was significantly differenced with days 15, 30, 45 and 60 meanwhile no significant difference with days 90 (7.30±0.69%).

In this study, the highest for crude fibre content was on days 90 (40.33±0.76%) (Table 4.2). There was no significant difference between 15, 30, and 45 days. While the lowest value was on early stage at days 15 with the value 29.67±1.61%. The amount of EE was significantly higher on days 30 (2.03±0.40%) when compared to other stages. While the lowest was on days 90 (0.10±0.10%). There was no significant difference between 45, 75, and 90 days.

Table 4.2 showed that the highest significant difference in crude protein was on days 15 with value 21.89±4.38%. There was no significant difference on days 30, 45, 60 and 90. The lowest value was on days 75 (11.68±6.69%). For ADF, there was a significant difference between each day. The highest value was on day 90 (25.51±0.50%). The lowest value was on days 75 (19.97±0.48%).

Table 4.2 Proximate compositions of Napier grass stems at different stages of growth.

Parameters	Stage of growth (Day)					
	15	30	45	60	75	90
DM (%)	$9.22^{b}\pm0.74$	$9.14^{b}\pm0.97$	$9.06^{b}\pm1.44$	$8.20^{b}\pm1.07$	$9.90^{b}\pm1.04$	$13.57^{a}\pm0.62$
M.C (%)	$90.78^{a}\pm0.74$	$90.86^{a}\pm0.97$	$90.94^{a}\pm1.44$	$91.80^{a}\pm1.07$	$90.10^{a}\pm1.04$	$86.43^{b}\pm0.62$
Ash (%)	$10.97^{b}\pm0.34$	$13.03^{a}\pm0.75$	$12.50^{a}\pm1.14$	$11.03^{b}\pm0.61$	$6.77^{c}\pm0.12$	$7.30^{c}\pm0.69$
CF (%)	$29.67^{c}\pm1.61$	$32.50^{c}\pm1.73$	$30.83^{c}\pm1.44$	$36.67^{b}\pm1.61$	$39.50^{ab}\pm2.65$	$40.33^{a}\pm0.76$
EE (%)	$0.73^{b}\pm0.15$	$2.03^{a}\pm0.40$	$0.13^{c}\pm0.15$	$0.37^{bc}\pm0.21$	$0.23^{c}\pm0.06$	$0.10^{c}\pm0.10$
CP (%)	$21.89^{a}\pm4.38$	$19.70^{ab}\pm3.79$	$18.97^{ab}\pm2.53$	$13.86^{bc}\pm1.26$	$11.68^{c}\pm6.69$	$14.59^{bc}\pm2.53$
ADF (%)	$21.56^{d}\pm0.90$	$22.68^{bc}\pm0.51$	$22.44^{cd}\pm0.13$	$23.39^{b}\pm0.22$	$19.97^{e}\pm0.48$	$25.51^{a}\pm0.50$

DM = dry matter, M.C = moisture content, CF = crude fibre, EE = ether extract, CP = crude protein, ADF=acid detergent fibre.
Different letters in superscript (a, b, c, d, e) within the same row indicate a significant difference (LSD's test, $p<0.05$) among different growth stages. Value is the mean ± standard deviation.

4.3 Comparison Between Nutrient Content in Leaves and Stems of Napier Grass

In Figure 4.1, showed comparison nutrient content in leaves and stems of Napier grass i.e. crude fibre, crude protein, and ADF at different growth stages. The study was carried out according to interval 15, 30, 45, 60, 75, and 90 days.

In days 15, there was no significant difference between crude fibre and crude protein, while has a significant difference at ADF in leaves and stem. The mean value of leaves and stems for crude fibre were 28.17±1.04% and 29.67±1.61% respectively. At days 30, both stem and leaves had a similar amount of CP, CF, and ADF. The average higher part days 30 in the graph were leaves because of the result of crude fibre and ADF. ADF leaves on days 30 were the highest value among other stages growth.

The leaves have more fibre than in stems with the value was 34.83±0.29% on days 45. However, ADF also has a higher value for leaves than the stem. Crude protein was comparable for leaves and stems respectively. Leave and stems crude fibre was not significantly different on days 60. There was a significant difference in leaves and stems part on days 60 for crude protein and ADF because leaves have more protein and ADF than in stems, respectively.

Besides, crude fibre and crude protein were similar for leaves and stem on days 75. However, there was a significant difference between leaves and stems part on days 75 for ADF. The leaves were the highest amount ADF than in leaves with the value was 22.54±0.55%. The crude protein and ADF for leaves and stems were comparable on days 90 respectively. Crude fibre has a significant difference on days 90 which is the stems were the highest amount than in leaves (Figure 4.1).

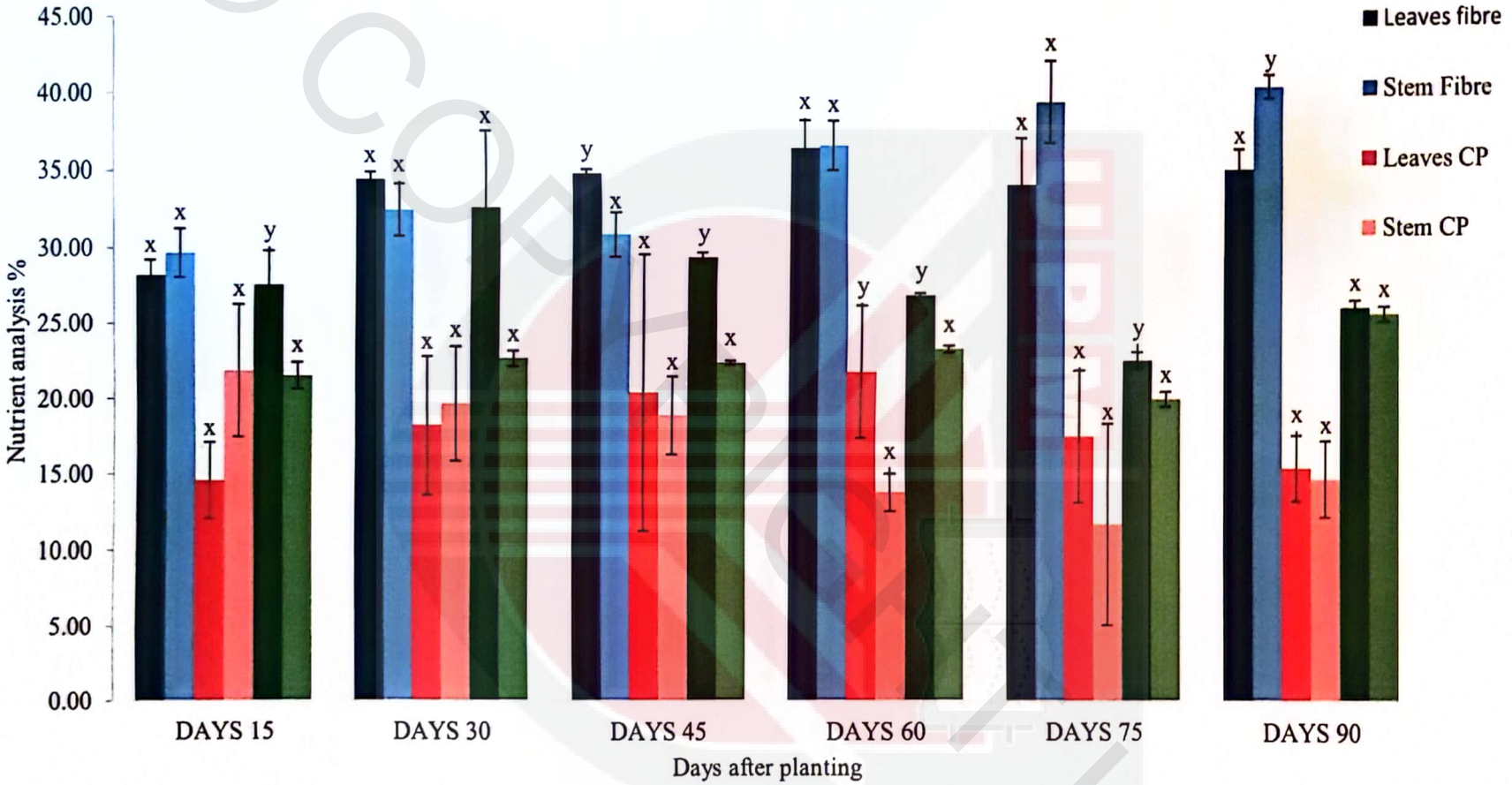

Figure 4.1 Percentage of nutrient analysis in leaves and stems Napier grass in different days after planting. CF = crude fibre, CP = crude protein, ADF=acid detergent fibre. a) x-x= no significant different. b) y-x = significant difference. Comparison is between leaves and stems in different parameter (crude fibre, crude protein, ADF) based on days after planting. The bar sharing a common letter at same days are not statistically significant between days after planting according to t-Test ($p \leq 0.05$) i.e. x=x and have significant i.e. y>x.

CHAPTER 5

DISCUSSION

Dry matter intake (DMI) generally expressed for feed intake or feed consumption. Feed intakes refer to the amount of feed consumed by an animal or group of animals in a given period during which they have free access to that feed (Forbes 2007). Animal production is basically reliant on the daily rate of feed consumption (Illius *et al.* 2000). At week 8 to 12 the result showed higher percentages of dry matter; it is approximately the same finding by Zailan *et al.* (2016).

Generally, as grass matures, herbage yield is increased due to the fast increase in the tissues of the plant (Minson 1990). Napier grass might play an important part in providing a significant quantity of high-quality forage to the livestock (Tessema 2005) both under the smallholder farmers and intensive livestock production systems with appropriate management practices (Seyoum *et al.* 1998). Water supply is highly connected with nutrient uptake and growth of biomass because of an enhanced maturation process when other factors such as soil fertility, temperature, and light intensity are not limiting for forage growing (Van Soest 1982).

However, Napier grass can endure considerable periods of drought (Butt *et al.* 1993), produces better DM yield than other tropical grasses (Boonman 1997), and is of high nutritive value for dairy animals mainly when supplemented with high quality feeds such as legumes (Nyambati *et al.* 2003). In the present study, the DM content increased as the grass matured, and higher DM was observed at the late stage of maturity. Kramberger and Klemencic (2003), Bayble (2007) and Ansah *et al.* (2010) reported that the DM content increased as Napier grass maturity increased.

For the moisture content, the Napier grass with different stages of growth also showed it is significantly different at level $p > 0.05$ for leaves and stems. The result of moisture analysis obtained from this study, nearly like the findings done by Lounglawan *et al.* (2014). Besides, the water content in stems is higher than the leaves. This is because more water is being stored at stems than leaves. Moisture content was higher in the early stages of growth at the first and second month and decreased in the third month which is at days 90. The moisture content represents the quantity of water contained in the plants. This could be explained by a high-water content of stem that resulted in an increase in moisture content (Mohammed *et al.* 2015).

The increase in dry matter and ADF with an increase in harvesting day agrees with the report of Bayble (2007) who recorded a similar trend when Napier grass was harvested at 15, 30, 45, 60, 75 and 90 days and should be expected with increasing grass maturity. According to Pinkerton (2005), crude protein is often used as indicators of forage quality. This is due to crude protein is the most essential component in animal nutrition. This component is often the critical limiting factor to animal production. It has been recommended that, on a practical level, maximum benefit in terms of protein nutrition comes from grazing or cutting grass at early maturity of even less than 4 weeks (Halim *et al.* 2013).

The CP decreased with increment of harvesting day. This result is like Bayble (2007), Kranberger and Klemencic (2003) and Peiretti (2009). Thus, it was observed that CP in stems decreased started from days 30 (19.70±3.79%) to the 75 days (11.68±6.69%). For days 90, the percentages are increased (14.59±2.53%) slightly but much less with days 75 due to the harvesting handling. While CP amount in leaves is increased from days 15 (14.59±2.53%) to days 60 (21.89±4.38%) and started decreased from days 75

(17.51±4.38%) to days 90 (15.32±2.19%). Plant maturity is measured as the main factor that affects the nutritive value of forages. A few factors affect the amount of change in nutrient composition with evolving plant growth and maturity stages. These factors include climate, soil moisture, plant type, season, soil type, weather and fertility, leaf stem ratio, physiological and morphological characteristics, and may contrast with annuals against perennials and grasses against legumes (Kilcher 1981). Halim *et al.* (2013) also stated that low crude protein content of the Napier grass could be due to several factors including maturity, soil, pasture management, species variety, and climate during the study, the weather fluctuates.

This could be accredited mostly to dilution of the CP contents of the forage crops by the fast buildup of cell wall carbohydrates at the later stages of growth (Van Soest 1994). CP content helps as an important pointer of fodder quality. In other studies, the CP content of Napier grass also decreased with maturity (Kramberger and Klemencic 2003; Bayble, 2007; Sultan *et al.* 2007; Ansah *et al.* 2010; Jusoh *et al.* 2014). In the present study, despite the decline in CP content with increasing stage of maturity, the final concentration exceeded the minimum CP level (7.5%) required for rumen function (Jusoh *et al.* 2014).

This designated the opportunity of improving the feeding of animals in tropical regions by planting Napier grass, thus enhancing the quality of nutrients supplied to animals. In the study, fat content was increased at early stages and decreased at day 90. The crude fat in leaves and stems at day 90 are 1.00±0.59% and 0.10±0.10% respectively. They have a significant difference in growth stages. It was like the results observed by Karabulut *et al.* (2006) that the fat content decreased when maturity stages.

Conversely, a study by Kamalak *et al.* (2005) and Kanak *et al.* (2012) showed increased fat content with progressing stages of maturity.

Generally, ash content gives an indication of the minerals present in the sample. Stems have higher mineral than leaves. The percentages of ash in leaves and stems were increased at early stages and decreased at maturity stages. The indifference with the findings of the previous study, conducted by Aganga *et al.* (2005) and Kanak *et al.* (2012) that reported increased of ash content with the stage of maturity.

Moreover, crude fibre has significant implications on the nutritive quality of the grass as leaves contain higher levels of nutrients and less fibre than stems. The study revealed that the stem had significantly low in fibre at early stages but higher in the maturity stage. Therefore, stems have more fibre than leaves in maturity stages.

In the present study, ADF contents increased from premature to intermediate and then decreased for stems at the days 75 while for leaves the ADF was varied from early stages to maturity. ADF on days 15 increased and then decreased at day 75 after that increased again at days 90. They were significantly difference of ADF content between leaves and stems.

CHAPTER 6

CONCLUSION

In this study, it can conclude that cutting period or different harvesting days gave an effect on the chemical compositions of Napier grass in three months cropping season. The composition of dry matter in both leaves and stems were decreased with growth stages. However, crude fibre only increased for stems and decreased in leaves, whereas moisture content, ash, crude protein, and crude fat and ADF all showed a decreased. Therefore, the performance and nutritive quality of Napier grass from leaf and stem parts at Napier grass over vary in different growth stages. Thus, it can be concluded that the Napier grass should be harvested within two months of the cutting period depends on the number of animals, species of animal and environmental factors.

Moreover, further research can be done on the Napier grass (*Pennisetum purpureum*) in order to develop more growth efficiency and cost-effective strategies to the farmers.

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Chemical Composition of Taiwan Napier Grass (*Pennisetum purpureum* Schumach) at Different Growth Stages" by the supervisor in a join authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the faculty.

Nur Zahirah binti Abd Jalil
Date: