KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,
KUMASI- COLLEGE OF AGRICULTURE AND NATURAL RESOURCES,
FACULTY OF AGRICULTURE

DEPARTMENT OF ANIMAL SCIENCE

EFFECT OF VARIETY, HARVEST DATE AND FRACTION ON THE HERBAGE YIELD, CHEMICAL COMPOSITION AND *IN VITRO* GAS PRODUCTION (ANKOM^{RF} GAS PRODUCTION SYSTEM) OF NAPIER GRASS (*PENNISETUM PUEPUREUM*) IN THE HUMID ZONE OF GHANA

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

M.SC. (ANIMAL NUTRITION AND MANAGEMENT) DEGREE

BY

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B.SC. (HONS) AGRICULTURAL TECHNOLOGY

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DATE: 27th January, 2010

CERTIFICATION

I certify that this thesis is the candidates own account of his research.

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DECLARATION

I do hereby declare that the work presented in this thesis is the result of my own effort and that in no previous application for degree in this University or elsewhere has such work been presented.

All sources of information have been specifically acknowledged by reference to authors.

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Terry Ansah (Student)

Date: 27/01/2010

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to the almighty God for giving me life and grace to complete this work. My thanks also goes to Dr. E.L.K Osafo and Dr. Hanne H. Hansen my project supervisors for their direction and constructive criticism in the planning and execution of this study. I also thank, Dr. Ida K. A. Hindrichsen for her selfless support during my visit to the *in vitro* Gas Laboratory of the Copenhagen University, Denmark. My sincere thanks goes to my colleagues especially Mr. Solomon K. Pigangsoa for their assistance at all times.

I will like to thank the Danish Development Research Network (DDRN) for funding my trip to Denmark and also the Department of Large Animal Science, Copenhagen University for baring the cost of my accommodation, meals and internal transport whilst in Denmark.

Finally I will like to thank my parents Mr. and Mrs. Ansah, my siblings Stanley Ansah, Stephen Ansah, Gladys Ansah and Naomi Ansah and my wife Mrs. Janet Ansah for their encouragement and prayer support.

ABSTRACT

Two Experiments were conducted to assess the effect of variety, harvest date and fraction on the herbage yield, chemical composition and *In vitro* gas production of Napier grass. The varieties were Local, 16798, 16786 and 16840. Except for the Local variety, all other varieties were improved varieties from the International Livestock research Institute (ILRI). The harvest dates were 60, 90 and 120 and the fractions were leaf and stem. In Experiment I, a 4X3 factorial in a randomized design was used for the herbage yield evaluation. The main factors were varieties (4) and fraction (3). A simple randomized design was used for the evaluation of the chemical composition with plant material from Experiment I. The chemical composition parameters measured were crude protein (CP), neutral detergent fiber (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), organic matter (OM), ash, cellulose, hemicellulose and dry matter.

In Experiment II, a 4X3X2 factorial in a randomized design was used to evaluate the *in vitro* gas production. The main factors were varieties (4), harvest date (3) and fraction (2). The Napier grass varieties harvested in Experiment I was used in Experiment II. The Ankom^{RF} gas production system was used for *In vitro* gas studies. Measurement from the herbage yield for the four varieties indicated that, dry matter herbage yield was significantly ($P<0.05$) higher for variety 16798 (44,994 kg/ha) with variety 16840 giving the lowest dry matter herbage yield (24,863 kg/ha).

Measurement from the herbage yield for the three harvesting dates indicated that harvesting at 120 days gave a significantly ($P<0.05$) higher dry matter herbage yield (46,013 kg/ha) with the 60 days giving the lowest (22,489 kg/ha).

Measurement from the chemical composition for the four varieties indicated that the Local variety gave the highest CP (96.77g/kg) with variety 16786 giving the lowest (85.35g/kg). The cellulose content was high for the Local variety (420.8g/kg) with variety 16840 producing the lowest (360.3). The ADL content was higher for variety 16840 (138.2g/kg DM) and lower for variety 16798 (89.0g/kg DM)

Measurements from the chemical composition for the three harvesting dates indicated that the 60 days harvest gave the highest CP (109.88g/kg), lowest NDF (686g/kg) lowest ADF (488.63gkg DM) and lowest ADL (84.13g/kg DM), and the lowest DM (478.5g/kg). The cellulose fraction was highest for the 60 days harvest (427.6g/kg DM) with 120 days harvest recording the lowest (354.6g/kg DM).

Measurements from the chemical composition for the two fractions indicates that leaf fraction had a higher CP (122.24g/kg DM), lower NDF (708.61g/kg), lower ADF (468.53g/kg DM) and a lower ADL (105.83g/kg DM). The stem fraction however recorded a higher cellulose fraction (406.9g/kg DM) compared to the leaf fraction.

The results from the *in vitro* gas production evaluation showed multi-phasic curves. In view of this, the results were separated into three phases based on the mean rate of gas production for the statistical analysis. In phase I and II, there was no significant difference ($P>0.05$) among the 4 varieties for the mean gas and mean rate of gas production. However, there was significant difference ($P<0.05$) among the 4 varieties for mean gas and mean rate of gas production in phase III. The Local variety yielded the lowest mean gas and the slowest mean rate of gas production whilst variety 16798 yielded the highest mean gas and the fastest mean rate of gas production.

There was no significant difference ($P>0.05$) among the 3 harvest dates in phases I and II for the mean gas production. There was however a significant difference ($P<0.05$) among the 3 harvest date for mean gas production in phase III. The 60 days harvest yielded the highest mean gas with the 120 days harvest yielding the lowest. The mean rate of gas production for the 3 harvest dates was significantly different ($P<0.05$) for phases I, II and III. In phase I, the 120 days harvest produced gas at most fastest rate with the 60 days harvest producing gas at the slowest rate. However in phases II and III, the 60 days harvest yielded a higher mean rate of gas with 120 days harvest yielding the lowest.

There was a significant difference ($P<0.05$) between the 2 fractions for mean gas production in phases I and II. The stem fraction yielded a higher mean gas compared to the leaf fraction. The mean rate of gas production was significantly different ($P<0.05$) for fractions in phases I, II and III. In phases I and II, the stem fraction produced gas at a faster rate compared to the leaf fraction. However, in phase III, the leaf fraction produced gas at a faster rate compared to the leaf fraction.

There was an interaction between the variety and harvest date in phases II and III, variety by fraction in phase III and fraction by harvest date in phases I and II.

Variety 16786 harvested at 90 days produced a significantly higher gas than the 60 and 120 days harvest in phase II, however in phase III there was no significant difference among the 3 harvest dates. The stem fraction of variety 16798 produced more gas in phase II but in phase III, the leaf fraction produced a significantly higher gas. The leaf fraction of the 3 harvest date did not show any significant difference in phase II, however in phase III, the leaf fraction of the 90 days harvest produced a significantly higher gas than the other 3 harvest dates.

In conclusion, variety 16798 produced the highest herbage yield and gas at 48 hours of incubation. The 120 days harvest produced the most herbage yield but produced the lowest gas at 48 hours of incubation. The leaf fraction produced the highest gas compared to the stem fraction at 48 hours but was not significantly different.

Based on the results of this study, variety 16798 is recommended for cultivation and use for livestock feeding. It is also recommended that Napier grass be harvested at 90 days even though the herbage yield was not as high as the 120 days and the gas production lower than the 60 days harvest. This is because harvesting at 60 days will give a very low herbage yield with high moisture content and harvesting at 120 days will give only one time harvest within the 8 month major rainy season in the humid zone where as with the 90 days, harvesting can be done twice. The whole plant is recommended for feeding livestock without separating into leaves and stem fractions since there was no significant difference between them after 48 hours of incubation.

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CHAPTER ONE

1.0.0 INTRODUCTION

Forages continue to represent the single most important feed resource for livestock in developed and developing countries. Despite its important position in ruminant production, little effort is made to cultivate and conserve forage in Ghana. Most livestock producers depend largely on natural pastures and crop residue as a main source of feed. Natural pastures are usually affected by changes in the rainfall pattern. Ghana has two major seasons, the dry and wet season. In the dry season, most forages drop in quality (crude protein content and digestibility). After start of the rainy season, forage growth is stimulated and this early growth has a relatively high protein and carbohydrate content. This forage is useful for animal production, but is generally unavailable to free-grazing animals because of intensive cultivation of crops during this time. This calls for a conscious effort to cultivate forges particularly in developing countries. Selecting forage species for cultivation must take into consideration the yield, digestibility and nutrient composition.

Napier grass, (*Pennisetum purpureum*) also known as "elephant grass" is native to Africa and has been used extensively in Kenya to improve upon small holder dairy production. Napier grass is the main fodder grown by over 70% of small holder farmers in Kenya. A yield of 85.4 tons/hectare without fertilizer application and a record high yield of 130 tons/hectare with 1320 kg/ha of nitrogen fertilizer application have been recorded.

Napier grass can grow as an intercrop within the same row or within alternate rows with legumes. Herbage yield of Napier grass may be affected by the harvesting date. As

Napier grass ages, herbage yield is increased due to the rapid increase in the tissues of the plant.

In the early stages of growth, the herbage of most forages consists mainly of leaves. As grass ages, stems comprise a greater percentage of the bulk of the forage. The nutrient composition and digestibility of tropical grass is influenced by stage of harvesting, species, soil fertility and climate. Plants usually produce fibrous tissues as they age. There is a relative increase in the production of fibrous tissues there by leading to an increase in structural carbohydrate (cellulose and hemicellose) and lignin. Protein concentration in grasses decrease with increase in age. There is therefore a reciprocal relationship between the protein and fiber contents as the grass ages.

Quantifying the nutritive composition and digestibility is an important aspect of identifying beneficial forage to ruminant particularly in the light of the reciprocal relation of nutrient value and maturity and herbage yield digestibility. The individual composition of a feed can be evaluated in the laboratory and each component related to the nutrient value of the feed, but the interaction between components and or digestibility of the feed for a given animal may differ. In order to assess the digestibility of a given feedstuff, the productivity of an animal fed the particular feedstuff is the best measure. However feeding trials are quite expensive, laborious and time consuming. Alternatively, there are other less costly but reliable methods that can be used in assessing the nutritive value of feedstuff. One of these is the *in vitro* gas production system. This system can be used to measure the potential digestibility, intake, and anti-nutritive factors present in forages. A relatively large number of samples can be assessed with this system at a relatively cheaper cost and within a shorter time interval.

The Ankom^{RF} gas production system is an automated gas measuring device that is designed to monitor pressure of numerous vessels and remotely record the data in a computer spreadsheet. The system is designed to investigate the kinetics of fermentation and metabolism.

The objective of the study was:

- To compare the dry matter herbage yield of the four Varieties of Napier grass cultivated and harvested at different dates in the humid zone of Ghana.
- To determine the chemical composition of Napier grass cultivated in the humid zone and harvested at three different cutting dates
- To determine the digestibility of Napier grass cultivated in the humid zone, harvested at three different cutting dates (60, 90, and 120 days) and separated into 2 fractions (leaf and Stem) using the *in vitro* gas production technique (Ankom^{RF} gas production system)

CHAPTER TWO

2.0.0 LITERATURE REVIEW

2.1.0 Factors affecting the growth and development of grass

2.1.1 Soil moisture

Rainfall is a major factor that affects plant growth and herbage dry matter production in most of the tropics and subtropics. Its seasonal nature, variability and erratic nature and the high evaporation potential in many areas results in periods of short water stress and prolonged droughts. This drought affects the growth and development of grass in the tropics.

According to Crowder and Chheda (1982), the plant itself increases the effectiveness of water availability. Leaves of many grasses tend to grow upright and flex upward from the midrib, thus forming a leaf catchment. Leaf cuticles have also been reported to have minute wax platelets.

A well developed platelet formation increases the contact angle of water droplets falling on the leaf surface and promotes runoff. As the droplets merge, they move to the plant base. This results in water penetration of the soil and wetting around the plant roots. Species have been reported to differ in their water utilization. This Crowder and Chheda (1982) attributed to root extension and to the extraction of water from the soil.

2.1.2 Temperature

The dry matter yield of plant tops and roots of tropical grasses increases markedly with an increase in temperature to an optimum which lies between 30^0C and 35^0C (Chudleigh

et al., 1977). Most species grow vigorously at 35^{0}C and some up to 38^{0}C. Subjecting tropical and subtropical species to colder temperatures, such as occur at night in the higher elevations and upper latitudes, adversely affects plant growth. Burt (1968) reported that growth rate declined at low temperatures. This was attributed to the altering of the distribution of photosynthetic product at low temperatures. At low temperature, photosynthetic material is used more for stem development with less going into root development thereby affecting growth of the plant.

2.1.3 Plant competition

As the sward of perennial grasses ages, there is increased competition among plants for soil nutrients, soil moisture and solar radiation. This competition could lead to a decline in plant growth and herbage production. This decline is frequently attributed to overcrowding of plants or excessive root mass accumulation (Crowder and Chheda 1982).

2.1.4 Light intensity

Cooper and Tainton (1968) observed that plant growth increases as light intensity increases until the leaves in a canopy are saturated with the light. Burt (1968) also reported that production of plant parts are enhanced by higher solar radiation. This implies that shading has a negative effect on the growth and development of plants. Burton *et al.*(1959) reported that reduced light decreased herbage yields, production of roots and rhizomes, nutrient reserves for regrowth and total available carbohydrates in the herbage of *Cynodon dactylon* cv.

When the amount of sunlight reaching the leaves canopy of grass was less than 50 percent, available carbohydrates in the herbage yield for the animals is drastically reduced there by affecting the growth of these animals. The lower energy value of grass growing under reduced light conditions could limit rumen flora activity and affect animal output. Shade significantly increased the lignin content of the herbage which goes a long way to decrease digestibility (Crowder and Chheda 1982).

2.2.0 Cutting management

The interval between harvests of grasses profoundly affects herbage production, nutritive value, regrowth potential, botanical composition and species survival. The following effects were observed by Crowder and Chheda (1982), when the harvesting period was prolonged:

1. There is an increase in the percentage content of dry matter, crude protein, lignin and cell wall with increase in age. With increase in plant maturity, older leaves yield more cell wall constituents and thus reduce intercellular space as well as condensed cellular inclusions. In addition, midribs and leaf sheaths attain a greater percentage of fibre and lignin, older leaves senesce and lose water; stems elongate and are less succulent.

2. There is also a fluctuation in total dry matter production and nitrogen free extract. An initial increase in yield occurs due to greater accumulation of total photosynthate, but as leaves mature and senesce, leaching from them occurs along with translocation of photosynthate from actively photosynthesizing leaves to the senesced leaves.

3. There is a decrease in leaf to stem ratio, percentage crude protein, minerals (P, K, Ca and Mg) and soluble carbohydrate. As stem elongates, the percentage of leaves declines. This brings about a relative decrease in cellular inclusions since cell walls of older leaves and of stems thickens. The decrease in crude protein and mineral is brought about by a dilution effect because of increased dry matter yield.

4. There is a fluctuation in the amount of nitrogen uptake by the plant and nitrogen recovery. More frequent cutting stimulates plant development and sustains biological processes, thus a greater demand for nitrogen. As plants mature, these activities decline. There is also the issue of dilution effects of nitrogen with increased forage production.

5. There is a rapid decline in animal intake and digestibility of matured grass. More mature herbage is less nutritive and thus less appealing to the grazing animal than juvenile and nutritious material. Digestibility diminishes as cell wall structure increases so that passage through the animal is slowed down.

Increased dry matter yields with extended cutting intervals are consequences of additional tiller and leaf formation, leaf elongation and stem development (Robertson *et al.*, 1976). The period of maximum forage production varies with different grass species (Haggar, 1970). Mackenzie and Chheda (1970) reported that cutting *Cynodon dactylon* at six weeks interval is better than four weeks. This according to Mackenzie and Chheda will allow the plant to develop more roots and achieve deeper soil penetration.

It has also been reported that cutting forage frequently reduces total forage yields, depletes carbohydrate reserves, causes a decline in root development, favors weed invasion and adversely affect regrowth potential.

2.3.0 Cutting Height

Cutting near ground level increases total and seasonal forage production over a short period as compared to more elevated cutting heights. A cutting height of 15-18cm above ground level helped in maintaining stands of *Andropogon gayanus*) and *Cynodon dactylon* (Chheda, 1974).

2.4.0 Factors affecting chemical composition of grass

Chemical composition of forages can be affected by a number of factors. Some of these factors are soil and climate conditions, stage of growth and genotype.

2.4.1 Soil and climatic conditions

Tropical countries experience changes in climate conditions, which go a long way to alter the soil conditions. Wide seasonal fluctuations in chemical compositions are common in tropical forages (Crowder and Chheda, 1977).

Nutrient absorption is hampered under high moisture stress, and high heat intensity induces rapid physiological maturation accompanied by the formation of highly lignified tissues (French, 1957). Yields of dry matter, percent crude protein, silica,

free ash and nitrogen free extract are directly related to the amount of precipitation (Oyenuga, 1960).

The physical, chemical and biological properties of soil, rates at which nutrients are supplied and renewed in the rooting zone and fertilizer application affect forage chemical composition. Tropical soils are generally low in nitrogen. In highly weathered tropical soils, forages have a greater tendency to absorb large quantities of silica (D'Hoore and Coulter, 1972) which significantly depress digestibility of the herbage.

2.4.2 Stage of growth

Advancing maturity is accompanied by increase in dry matter which is reflected in increases in cell wall contents, and a decrease in cell contents. In most tropical grasses, crude fibre and nitrogen free extract continue to increase with age, and in the case of crude fibre most of the increase occurs during the first month of growth. The percentage increase of nitrogen free extract is more pronounced during later stages of growth (Crowder and Chheda 1982).

Cell contents of herbage decrease with maturity. This is due to the rate of dry matter accumulation through photosynthetic activity which is greater than mineral absorption (Crowder and Chheda, 1982). This situation causes a dilution of mineral contents in proportion to the increase in bulk and results in lower crude protein, phosphorus and potassium percentage.

Frequent defoliation, however, also produces a cumulative destructive effect on forage yield because of rapid exhaustion of carbohydrate reserves in the stubble and roots.

Chemical compositions also vary vertically in a sward due to endogenous variation in maturity along the axis. The top of the sword, therefore, could remain young and contain low amounts of cell wall component. Percent dry matter, ADF and lignin increased downwards along the axis while crude protein decreased in Bermuda grass (Wilkinson *et al.* 1970). Remarkable changes in chemical composition have been reported to occur only after extensive lower differentiation has taken place between 30 and 60 days of growth. The overall quality of forage depends on the relative properties of high quality fractions. Leaves and stems are nutritionally of equal value in the beginning of growth. The different dietary components in them decline, however, at different rates with age. The cell wall contends increase at a faster rate in stems as compared to leaves (Reid *et al.*, 1973).

2.4.3 Genotype

Differences in chemical composition also arise as a result of genetic diversity of forage plants. When different species of forage plants and different genotypes within a species are grown in a common environment and under uniform management, estimations of their chemical composition and feeding value often reveal significant differences (Klock *et al.*, 1975).

When the chemical composition of 107 grass species out of about 450 that occur in Kenya was studied by Dougall and Bogdan (1958) at early flowering stage, crude

protein varied from 5 – 20 percent and in the majority of species it ranged from 8 – 16 percent. Crude fibre varied between 14 and 43 percent.

2.4.4 Sampling and processing

Obtaining a representative sample from a field for chemical composition can affect the reliability of the nutritive value estimated. Animals tend to graze pastures selectively and laboratory estimates of herbage nutritive value may be only of limited value. Hand-picked samples in the field reportedly narrow the error margin as compared to mowed samples, since they simulate grazing conditions more closely (Miller and Cowlishaw, 1976).

The pre-treatment of samples for chemical analysis can affect the results usually reported. A typical example is drying which is a common pre-treatment method in chemical analysis. Improper drying can bring about irreversible changes in the sample and obscure the actual herbage quality.

Soluble carbohydrates are underestimated in oven dried samples as compared to freeze–dried samples. Significant increases in lignin and fibre have been reported in samples dried at high temperatures, possibly due to non-enzymatic browning reactions and by reactions causing hemicelluloses to be estimated as part of lignin (Van Soest, 1965). Drying *Cynodon dactylon* at 57^0C for 48hours gives more reliable results (Wilkinson *et al.*, 1969).

2.5.0 Factors affecting digestibility of grass

It is generally accepted that tropical forages have a lower digestibility, with wider variations among species, than their temperate counterparts at all stages of growth. (Milford and Minson, 1966). A mean difference of 12.8% units in digestibly was calculated when tropical and temperate forages were compared (Minson and McLeod, 1970). This difference was associated with high temperature and transpiration rates.

The primary cause of large variations in the digestibility of various forages is the amount of lignin present. A unit increase in lignin can result in three to four unit decrease in digestibility of forage (Bula *et al*., 1977).

2.5.1 Stage of growth and genotype

Forages are highly digestible at young and immature stages of growth. High *in vitro* digestibility values ranging from 75 – 85 percent after 1 week's regrowth have been reported in several improved varieties of tropical pastures (Reid *at al*., 1973). Digestibility declines with advance in maturity. Digestibility and the rate of its decline with age vary considerably between genera, species and varieties.

In tropical grasses, Milford and Minson (1966) observed the rate of decline of digestibility to be 0.1 – 0.2 percent daily. However, Crowder and Chheda (1977) and Wilson and Mannetje, (1978) reported a daily rate of digestibility decline of 0.5 – 0.6 percent in East Africa, West Africa, Trinidad and Queensland respectively.

2.5.2 Plant fractions

The leaf and stem fractions of a forage plant are equally highly digestible because of high level of cell contents and high digestibility of the cell wall fractions at young stage of growth.

With advancing maturity however, considerable differences in the digestibility between the two fractions arise. As the stem matures, the cell contents decrease rapidly. The cell wall contents increase and the cell wall fraction is also now less digestible because of increased lignifications.

The cell contents of leaves remain at higher level and rate of lignifications is much slower with increase in age than in stems. This result in a rapid decline in digestibility of the stem fraction compared to the leaf fraction with advancing maturity (Raymond, 1969).

Samples of leaf and stem plus sheath did not differ in digestibility until the 16th week of sampling, and there after marked decrease *in vitro* digestibility was observed in stem plus sheath fraction (Reid *et al.*, 1973). Haggar and Ahmed (1970) reported the daily *in vitro* digestibility decline in leaves and stems of *Andropogon gayanus* to be 0.5 and 0.4% respectively in the vegetative phase, but after seed-head emergence the decline in digestibility of stems was 0.75% daily.

Vertical differences in digestibility are also encountered in tropical pastures. Within the mass of herbage, digestibility decreases from the top downwards. Rapid maturation of stems and senescence of leaves in the basal layers can reduce the overall forage digestibility. Leaf to stem ratio at the stage of forage utilization and

date of flowering are therefore important factors associated with digestibility. Species with short internodes may have an advantage since they would contain higher proportions of leaf (Crowder and Chheda 1982). Minson (1971), however, observed that in *Chloris gayana*, *Panicum maximum* and *Pennisetum coloratum*, digestibility was not related to leafiness or floral development. This suggested that morphological characteristics of forage may not always be indicative of its digestibility.

2.5.3 Climate

Reduced digestibility and poor nutritive value of tropical forages can be attributed to high heat intensity that causes rapid growth, enhanced maturity with decreasing leaf to stem ratio and increased crude fibre content, particularly higher lignifications, as well as increased levels of acid detergent fibre (ADF) and cell wall contents.

Climate variations, particularly temperature changes during the growing season can cause differences in the digestibility of forage. In regions that experience wet equatorial or wet monsoonal climates, and where daily temperatures are comparable during the wet and dry seasons, forage digestibility during the two seasons does not differ markedly. Miller and Cowlishaw (1976) recorded similar patterns of digestibility in *Digitaria spp* during the two growing seasons in Trinidad.

In another study, Olubajo and Oyenuga (1970), who studied digestibility of various grass-legume mixtures in Nigeria found consistent 8-10% differences in digestibility between wet and dry seasons. The average decline in digestibility with 1^0C increase in temperature for a large group of forages was estimated to be 1.4% (Minson and McLeod 1970).

2.5.4 Protein content

In many parts of the tropics under natural fertility conditions, and up to about two months of growth after the onset of rains, the forages rapidly become matured and crude protein content droped drastically, reaching values of 4-6% after 3-5months. During dry months, crude protein content often drops below 4% and in later parts of the dry season values of 1-2% have been recorded (Crowder and Chheda, 1977). Crude protein content in forages can have a significant effect on digestibility. When it exceeds 7% in the herbage, digestibility does not appear to be affected (Milford and Minson, 1966).

If herbage with a crude protein percentage of below 7 is fed to animals, microbial activity in the rumen is depressed by lack of nitrogen. This causes an incomplete utilization of structural carbohydrate in the ingested forage (Milford and Minson 1966). Therefore, forage digestibility and voluntary intake are significantly reduced. Glover and Dougall (1960) observed marked decline in total carbohydrate digestibility when crude protein content in the forage fell below 6%. It is therefore important to maintain crude protein level of animal diet above 6-7 % for maintenance and production.

2.6.0 Napier grass a quality tropical forage

Napier grass *(Pennisetum purpureum)* is also known as "elephant grass". It was named after colonel Napier of Bulawayo in Zimbabwe who early in the last century urged Rhodesia's (now Zimbabwe) Department of Agriculture to explore the possibility of using it for commercial livestock production (Boonman, 1993). Napier

grass (*Pennisetum purpureum*) has been used extensively in Kenya to make quality forage available to smallholder dairy producers. Animals in Kenya are confined in stalls and fed mainly on Napier grass under zero grazing. In central Kenya over 80 percent of dairy animals are kept under zero grazing (Staal *et al.*, 1998) and Napier grass is the main fodder grown by over 70 percent of smallholder farmers in the region and normally provides over 40 percent of feed (Bayer, 1990 and Staal *et al.*, 1998). Napier grass has been the most promising and high yielding fodder giving dry matter yields that surpass most tropical grasses (Skerman & Riveros, 1990; Humphreys, 1994).

Wouters, (1987) reported on-farm dry matter yields of about 16 tones/ha/year from different regions of the country with little or no fertilizer, Schreuder *et al.* (1993) reported an on station of about 10-40 tones dry matter per hectare depending on soil fertility, climate and management factors. These yields surpass those of *Panicum maximum* and *Andropogon gayanus* which are popular pasture grasses in Ghana and yields 8.7-15 tones/ha/annum and 1.0-1.2 tones/ha/annum respectively (James Duke 1983). High yields of up to 85 tones DM/ha have been cited when high rates of fertilizers were applied (Skerman & Riveros, 1990), for example under natural rainfall of 2000 mm per year where 897 kg of N fertilizer were applied per hectare per year and the grass was cut every 90 days the yield was 84, 800 kg DM/year (Vicente-Chandler *et al.*, 1959). Watkins and Lewy-Van Severen, (1951) recorded a yield of 85.4 tons/hectare without fertilizer application. Dry matter yield alone, however, is of limited value if it is not closely related to the DM intake of the animals. At farm level, the combination of DM yield and observed DM intake can

form the basis for estimating the number of livestock that can be supported by available forage. As Napier grass tolerates frequent defoliation, under good weather conditions it can be cut every 6-8 weeks giving up to 8 cuts in a year, depending on fertilizer application, rainfall amount and distribution.

It is the main fodder crop in Central Kenya, and is fed to livestock by cut-and-carry; by 1983 approximately 240,000 ha or 4 percent of the arable land of Kenya is under Napier grass. About 90 percent of farmers in Central Kenya grow Napier grass and the proportion may be higher now. Napier grass can grow in mixture with legumes. Although in Kenya it is generally grown and managed as a pure stand, it can grow as an intercrop within the same row or within alternate rows with legumes such as *Pueraria phaseoloides, Centrosema pubescens, Neonotonia wightii, Desmodium uncinatum, Desmodium intortum and Stylosanthes guianensis.* When intercropped with herbaceous legumes, cutting or grazing management is adjusted to favour the legumes in order to maintain a satisfactory mixed sward. Napier grass can also be grown as an alley crop with fodder legumes such as leucaena, (*Leucaena leucocephala*), calliandra (*Calliandra calothyrsus*) sesbania (*Sesbania sesban*) and gliricidia (*Gliricidia sepium*). Legumes improve the quality of Napier grass-based feed and also increase the overall yield. Napier grass can withstand heavy grazing and provide a considerable bulk of feed to livestock, especially if well fertilized and irrigated (Harrison & Snook, 1971). Hay and silage can be made for dry season use. It makes good hay if cut when young but is too coarse if cut late. It is more usually made into silage of high quality without additives. In Taiwan Napier grass is widely used for the production of dehydrated grass pellets used as supplementary stock feed.

In a study to compare the *in vitro* microbial fermentation of tropical grasses at and advanced maturity stage, it was revealed that napier grass compared to *Cynodon dactylon, Cynodon plectostachyus and Brachiaria humidicola* was less affected by age and for that matter a very promising grass for species for ruminant feeding after 100days of age in the dry season conditions. (Nogueira Filho *et al.*, 1999).

Feeding accounts for up to about 60% of the cost of livestock production and in most developed and developing countries forages continue to represent the single most important feed resource (Jung and Allen, 1995). Availability of quality forage at the right time at the right place has the potential of reducing the feeding cost in livestock production. Increased biomass yield is one way of improving upon the availability of forage to livestock. Herbage yield in grasses can be separated into stem weight, leaves, leaf sheath, and inflorescences. (Crowder and Chhede, 1982)

Yield of tropical grasses vary with varying climate condition, weather conditions, water supply, soil fertility, fertilizer application and management. Specie differences also have an effect on the yield of forages in the tropics. (Bogdan,1977). McDonalds *et al.* (1995) reported that the rate at which grass grows is dependent upon the environment, the nutrients available and the amount of leaf within the sward which is intercepting light.

Despite the numerous benefits derived from Napier grass, little study has been carried out on it in Ghana. It appears as a natural pasture in the humid zone of Ghana. Little or no effort is made to cultivate it on commercial basis for livestock production.

2.7.0 Digestibility and nutritive value of tropical grass as affected by stage of harvesting and fraction

The nutrient composition and digestibility of tropical grass is influenced by stage of harvesting, species, soil fertility and climate (Minson, 1990). Plants usually produce fibrous tissues as they age. The increase in production of fibrous tissues leads to an increase in the structural carbohydrates (Cellulose and hemicelluloses) and lignin. Protein concentration decrease with increase in age. There is therefore a reciprocal relationship between the protein and fiber contents as the grass ages. (McDonald, 1995). Leaves have been generally reported to contain more crude protein and cell contents than the stem. Early in the life of the plant, leafiness is high thereby leading to increase in the nutritive value and digestibility.

When Napier grass was harvested at 60, 90 and 120 days, Taye Bayble *et al.*, (2007) recorded a decrease in the crude protein content. Taye Bayble., (2007) recorded a crude protein of 14.3%, 10.4% and 7.77% at a harvesting *et al* date of 60, 90 and 120 days respectively.

Kidunda *et al.* (1990), Van Soest (1994) and Seyoum *et al.* (1998) attributed the lowering of crude protein with age to the rapid accumulation of cell wall carbohydrate at the latter stages of growth. Taye Bayble *et al.*, (2007) observed that when Napier grass was harvested at 60, 90 and 120 days, it yielded an NDF of 68.3%, 71.7% and 73.6% respectively. Taye Bayble *et al.*, (2007) also reported that ADF and ADL increased with increase in age when Napier grass was harvested at 60, 90 and 120 days. An ADF of 42.1%, 43.1% and 44.1% and an ADL of 4.6%, 5.5% and 6.3% was recorded for the

Napier grass at 60, 90 and 120 days respectively. Van Soest (1994) observed that digestibility of forage in the rumen is related to the proportion and extent of lignifications. The overall digestibility of plants as they mature decreases, this is attributed to the spatial arrangements of lignin within the plant. Increased synthesis of lignin reduces the digestibility of cellulose and hemicelluloses. (France and Forbes, 1996)

Differences have been reported in the digestibility and nutrient composition of different fractions (leaf, stem and whole) of grass with age. Woodard and Prine (1991) and Williams and Hanna (1995) reported that the rate of decline in crude protein content is more rapid in stems than in leaves. Dzimale (2000) showed that crude protein content of leaf fraction was higher than stem and whole fractions of Napier grass. This shows that the nutritive value of stem declines faster than that of leaf with age.

When sheep and goat were fed different fractions of grass, leaf, stem and whole, it was observed that the leaf fraction was digested faster than the stem and whole fraction (Larbi *et al.*, 1991). Poppi *et al.* (1981) attributed the high intake and digestibility of leaves to the high crude protein (9.9%) content. Leaf and stem harvested in the early stages of plant growth gave about the same digestibility and nutritive value. This means that feeding leaf and stem separately when harvested at a young stage is not necessary as their digestibility is almost the same (Fianu and Winch, 1984).

According to Tang *et al.* (2008), morphological fractions affected the chemical composition of maize. Leaf blade was found to have a high crude protein (125g/kg DM), a lower neutral detergent fibre (572g/kg DM) and a lower acid detergent fibre (342g/kg

DM) compared to other fraction. This result is consistent with the findings of Tolera and Sundstøl (1999).

Tang *et al.* (2008) reported that the volume of *in vitro* gas produced from leaf fraction of maize was higher than that of stem. This is consistent with the report of Tolera and Sundstøl (1999).

2.8.0 The *in vitro* gas technique

While the Tilley and Terry method measures the residual matter after digestion out of the organism, gas techniques measure the amount of gas produced during a digestive process. When feedstuff is incubated with buffered rumen fluid *in vitro*, the carbohydrates are fermented to short chain fatty acids (SCFA) gases (mainly CO_2 and CH_4) and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Beuvink and Spoelstra, 1992, Blummel and Ørskov, 1993).

Fermentation of substrate to acetate and butyrate is the main source of the gas. Substrate fermentation to propionate yields gas only from buffering of the acid and, therefore, relatively lower gas production is associated with propionate production (Van Soest, 1994). Kinetics of gas production is dependent on the relative proportions of soluble, insoluble but degradable and undegradable particles of the feed.

Much of the earlier work on gas measurement centered on investigations of rumen microbial activity using manometric measurements. (El-Shazy and Hungate, 1965). McBee (1953) developed a manometric method of gas measurements for the evaluation of rumen microbial activity with respect to cellulose and hemicelluloses fermentation and

concluded that the rate of substrate fermentation is not constant but subject to fluctuation due to changes in the diet of the animal.

Czerkawaski and Breckenridge (1975) developed a syringe system with the capacity of ten units for studying rumen fermentation. Although the manometric method permits a quantitative determination of acids and gases evolved during fermentation, and allows incubation of a large amount of sample by increasing the volume of the manometric vessel (Hungate *et al.*, 1955), a large number of samples cannot be handled easily.

Wilkins (1974) developed an automated pressure transducer method for measuring gas production by microorganisms.

There are basically two approaches for measuring gas volumes:

1. Measuring gas collected at atmospheric pressure and its volume determined directly
2. Measuring gas accumulated in a fixed volume container and the volume is calculated from pressure changes.

Within these broad groups, different techniques have been developed. These include

a. Hohenheim gas method or Menkes method (Menke *et al.*, 1979)
b. Liquid displacement system (Beuvink *et al.*, 1992)
c. Manometric method (Waghorn and Stafford., 1993)
d. Pressure transducer system: manual (Theodorou *et al.*, 1994)
e. Computerized (Pell and Schofield, 1993)
f. Combination of pressure transducer and gas release system (, Cone *et al.* 1996, Davies *et al.*1995)

The *in vitro* gas production technique generates kinetic data but rather than measuring the disappearance of dietary components, it measures the appearance of fermentation gases notably CO_2, CH_4, H_2.

Compared to the in situ degradability technique, gas production methods are less animal dependent, more appropriate for characterizing soluble or small particulate feeds and they can be automated thus reducing the labor input. They can also be used to generate information on rates and extent of digestion, proportions of volatile fermentation products and microbial protein production (Schofield 2000)

2.9.0 Brief background of the Ankom^{RF} Gas Production

The ANKOM gas instrument is designed to investigate the kinetics of fermentation and metabolism. It has application in studying the kinetics of gas production in the rumen and brewing operations associated with the production of alcohol. It can also study anabolic activity through a measurement of gas uptake by the decrease in gas pressure similar to the Warburg apparatus.

The equipment is a fully automated metabolic gas measurement device that is designed to monitor pressure of numerous vessels and remotely record the data in a computer spread sheet. Pell and Schofield (1993) reported that the fully automated gas production system reduces labour, cost and complexities associated with the manual and semi-automated systems. The system includes up to 50 individual gas modules that communicate information to a computer, using radio frequency (RF) transmission. From the computer interface, the operator can control numerous aspects of the module performance such as

recording interval and the release of pressure through internal values (Ankom Technology, 2008).

2.10.0 Factors affecting the accuracy of *in vitro* fermentation gas productions technique.

2.10.1 Sample form

Allowing samples to wilt increases the rate of fermentation. Freeze drying and milling increases gas production relative to chopped / unchopped fresh forage (Sanderson *et al.*, 1997).

2.10.2 Oven drying samples

Deaville and Givens (1998) observed that oven drying of samples eliminates volatile constituents from fermented substrates thus reducing the indirect gas produced from their reaction with the buffer.

2.10.3 Buffer Composition

High phosphate buffers reduce gas production utilizing protons that would have been used for CO_2 production (Schofield 2000).

2.10.4 Prevailing PH and temperature

Russell and Dombrowsk, (1980) reported that if the pH and temperature falls below the optimum requirement for cellulolytic bacteria growth, gas production is decreased.

2.10.5 Atmospheric Pressure

The atmospheric pressure determines the actual gas volumes. Williams (2000) noted that in the course of *in vitro* induction atmospheric pressure can change. These factors call for the need to standardize the gas production system being used in a particular study.

CHAPTER THREE

3.0.0 MATERIALS AND METHODS

3.1.0 Introduction

Two separate experiments were conducted between March, 2008 and June, 2009 at the Department of Animal Science Department of the Kwame Nkrumah University of Science and Technology (K.N.U.S.T) Kumasi Ghana and the Department of Large Animal Sciences of the University of Copenhagen, Denmark.

Experiment I which was conducted at the K.N.U.S.T Ghana involved the assessment of the herbage yield of four varieties of Napier grass (*Pennisetum purpureum*) and the chemical composition of these grasses harvested at three different harvesting dates and separated into leaves and stem fractions. The varieties tested were 16840, 16798, 16786 all improved varieties from ILRI and a Local variety common in the Ashanti Region of Ghana. The harvest dates were 60, 90 and 120 days after planting and the fractions were Leaf and stem.

Experiment II was conducted at the Copenhagen University, Denmark, to evaluate the *in vitro* gas production of Napier grass cultivated in Experiment I using the Ankom^{RF} gas production equipment. The effect of variety, harvest date and fraction on the *in vitro* gas production was tested.

3.2.0 Experiment I

In Experiment 1, data was collected on the herbage yield of four varieties of elephant grass also known as Napier grass (*Pennisetum purpureum*) harvested at three different dates. The chemical composition of the Napier grass was also determined.

3.2.1 Location and climate of study area

The Department of Animal Science farm of the Kwame Nkrumah University for Science and Technology (K.N.U.S.T) was the site used to investigate the herbage yield of the Napier grass. The animal nutrition laboratory of the Department of Animal Science K.N.U.S.T was used for the chemical analysis. The area for the study falls within the latitude 06^0 41N, longitude 01^0 33W and altitudes 261.4 above mid sea level (MSL).

The site for the herbage yield data collection study falls within the moist semi-deciduous forest belt of Ghana and with a bimodal rainfall pattern. The annual rainfall averages about 1194mm. The major wet season extends from March to July with the peak rainfall in April and May. The minor wet seasons begins in September and ends in November after a short dry season in August. The major dry season is from December through January to February (Osafo, 1976). The climate variables at the Department of Animal Science of the K.N.U.S.T during the periods of April to August, 2008 are shown in the Appendix Table 1.

The mean maximum and minimum temperature for the site were 34.0°C and 21.4°C respectively.

3.2.2 Soil Description

The soils of the experimental site consist mainly of the Asuansi Series, belonging to the Kumasi-Asuansi/Nta-Ofin Compound association developed over Cape Coast granite under moist semi-deciduous forest. The soils consist of yellowish red moderately sandy loams and clays and occur on gentle to moderately steep upper to middle slopes. The diagnostic properties include ustic moisture regime, Ochric A horizon, low base saturation and low cation exchange capacity. The soil reaction is extremely acid.

3.2.3 Source of planting material

The planting materials used for this study were made up of three improved varieties obtained from International livestock Center for Africa (ILCA) now International Livestock Research Institute (ILRI) which had been maintained in a herbarium and a Local Variety from Ghana. The four varieties were selected based on the total herbage yield in the study conducted by Dzimale (2000). Four varieties, including the Local variety, were selected for this experiment from the original nine varieties tested previously, because of their high total herbage yield.

3.2.4 Experimental land preparation, planting and maintenance

The plot was ploughed and harrowed with a tractor. The field was divided into 16 plots with each plot measuring $36m^2$. The intra and inter row spacing were 0.5m and 1.0m respectively. Each plot had 12 rows. The parent plant was cut into stems with a minimum

of three nodes per cutting for planting and were planted 15-20cm deep at angle of about 30°- 45°. Weeding was carried out 40 days after planting.

3.2.5 Experimental layout, design and treatments

A 4×3 factorial in a completely randomized design (CRD) was used in the herbage yield study. The main factors were four varieties; comprising; 16786, 16798 and16840 from ILRI and the Local variety and harvesting date (3); 60, 90, and 120 days after planting. There were four replicate plots for each treatment.

In all, a total of 16 plots with an area of $36m^2$ for each replicate were used for the planting. There were 12 rows in each replicate. Each treatment had four replicates. The intra and inter row spacing were 0.5m and 1m respectively

3.2.6 Harvesting procedure and data collection

At each harvesting day (60, 90 and 120days), three rows of each variety occupying an area of $9m^2$ (1.5m×6m) was randomly selected and harvested with a machete. A stump (stubble height) of 15cm was left behind after harvesting. Herbage yield data were collected after harvest.

3.2.7 Herbage yield (DM) of Napier grass

For each variety at each harvesting time, the total harvest per plot of fresh forage was weighed and sub samples taken from each variety and chopped into short lengths (2-5cm) for dry matter determination using the AOAC (1990) procedure. This involves drying in an oven at 60°C for 48hours. Total herbage yield of each variety was calculated on dry matter basis by multiplying the percentage dry weight of the sub samples from the whole fraction to the fresh weight of the varieties per $9m^2$ and converted to hectares.

3.2.8 Chemical analysis

Data on the chemical composition was collected on the four varieties harvested at three different dates and separated into leaves and stem. The chemical composition included CP, ADF, NDF and ADL. Other components also determined were dry matter (DM), Ash, organic matter (OM), hemicellulose and cellulose.

3.2.9 Sample preparation for chemical analysis

The four varieties, three harvesting dates and two fractions of Napier grass samples were dried at 60°C for 48hours and ground using a laboratory mill (Wiley mill) to pass through 1mm sieve screens for laboratory analysis. 500 grams of each sample was packaged in transparent polythene bags and stored for the chemical analysis.

3.2.10 Procedure for chemical analysis

The AOAC (1990) procedure was used in the determination of DM, CP, ash and OM. In determining the DM, 200g of each sample of Napier grass was taken and chopped into short lengths (2-5cm). They were then placed in an oven at 60^{0}C for 48hours. The weight after drying is the dry matter (DM).

The CP was calculated as 6.25×N (Nitrogen) which was obtained using the micro-Kjeldahl technique. The ash component was determined by igniting 2grams of Napier grass sample in a muffle furnace at 600°C for 4hours. The residue after burning in the furnace is the ash. The OM was determined by subtracting the ash component from the initial weight of the sample before ashing.

The detergent method was used to determine the structural carbohydrates or cell wall constituents (neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Hemi cellulose was calculated by subtracting the ADF from the NDF whist the cellulose was determined by subtracting the ADL from the ADF.

3.3.0 Experiment II

In Experiment II, data on the *in vitro* gas production of the four varieties of Napier grass cultivated in Experiment I was investigated.

3.3.1 Sample preparation, packaging and transportation for *in vitro* gas analysis.

The Napier grass samples described in 3.1 were dried at 60^0C for 48hours and ground using a laboratory mill (Wiley mill) to pass through 1mm sieve screens for laboratory analysis. Fifty grams of each sample was packaged in transparent polythene bags and transported by air to the Copenhagen University Denmark for the *in vitro* gas analysis.

3.3.2 Experimental layout and design.

A 4×3×2 factorial in a completely randomised design was used for the gas production studies. The main factors were the varieties (4), harvesting date (3) and fraction (2). The study was carried out using the ANKOM^{RF} Gas Production system version 3. Approximately 500mg of milled Napier grass sample was weighed into the glass jar and placed into the incubator at 39.5^0C overnight. The actual sample weight was used to calculate all results in units of gas production (psi) per gram sample.

The samples were separated into their respective varieties, harvesting dates and fractions (leaf and stem). A total of five 5 trials were conducted using 36 gas production modules at each trial. During each trial, 12 samples leaf fraction and 12 samples of stem fraction for the various harvesting days and varieties were weighed into the glass jars. An extra twelve samples of leaves was weighed into glass jars for the first trial giving a total of 24 samples of leaves and 12 samples of stems for the various harvesting days and varieties. In the second trial, the extra 12 sample of leaves weighed in the first trial were replaced with stems sample to give a total of twenty 24 stems and 12 sample of leaves for the

various harvesting days and varieties. The extra twelve samples were alternated in each trial for the entire experiment. This was done to give equal replication to all the samples.

A mixture of 60ml of rumen fluid and media was injected into the glass jars containing the grass samples. After that the grass sample, rumen fluid and media mixture were flushed with carbon dioxide for about 30 seconds. The glass jars were then closed and put into the incubator.

3.3.3 Source of rumen mixture

Two rumen fistulated Jerseys heifers were used. They were fed low quality hay with a minimal amount of concentrate. The concentrate was fed at least 4 hours before the rumen fluid was collected. Roughage and water were withheld two hours and 30minutes respectively before rumen fluid was taken. The fluid was collected in thermos-flask that had been heated with warm water. A fist full of rumen content was taken, placed in a pre warmed thermo-flask (40^0C) and rumen fluid was taken with a cup and added to the content in the thermo-flask. The mixture of rumen content and fluid was immediately transported from the farm to the laboratory by bus. When the bus arrived at the *In Vitro* Gas Laboratory of the Copenhagen University, the rumen mixture was quickly taken and a double layered cheese cloth was used to sieve the mixture to separate the rumen content from the rumen fluid.

3.3.4 Procedure for gas measurement

The ANKOM^{RF} Gas production equipment was used because of its automated nature. It has modules that are connected to a computer by a wireless system which records gas production by substrate in modules at any time interval selected by the researcher. It reduces the problem of manually recording volume of gas produced when using gas production equipment that is not automated.

A module consists of a glass jar and lid in which a computer chip, battery and vent valve are found (Ankom technology, 2008). Each module was tested before each trial to make sure it can hold gas correctly.

On the day of the experiment 4 of the 5 component solutions (Appendix Table 2) for the media were mixed together at least 2 hours before inoculation according to the procedure of Menke and Steingass (1988) to a temperature of 39.5°C and a pH of approximately 7. During preparation process, the media was continuously flushed with CO_2 in order to maintain the pH and also provide an anaerobic condition for the rumen microbes. The fifth and final component solution (reduction agent), which must be freshly prepared, was mixed with the others as soon as the rumen fluid arrived in the laboratory. A 1000ml of the sieved rumen fluid was measured and added to the media to form the inoculum.

The inoculum (media and rumen fluid) was flushed with carbon dioxide for 15minutes after which 60ml of the inoculum was poured in the glass jar containing sample.

Each unit was flushed with CO_2 until the lid, containing vent valve, computer chip and battery, was placed firmly on top and the unit to form the module was put back in the

incubator. This was done as fast as possible to prevent the temperature of the unit, sample and inoculum from dropping. Vent valves were closed as soon as the incubation period began. The computer was set to do measurements in psi once every minute for the first 30 minutes after which it was changed to measure pressure once every 30 minutes for a period of 48 hours. No shaking of the samples took place during the incubation period.

Due to limited number of modules, no blank measurement containing only rumen fluid and buffer was carried out. A unit measuring the atmospheric pressure at all times was also included.

The incubation period lasted for 48 hours. After each experiment, the average gas production in psi per gram sample for each sample at 30 minutes interval was computed and subjected to statistical analysis

3.3.5 Data Correction

All readings from varieties, harvesting dates and fractions were corrected for abnormalities that were observed.

Three different abnormalities were identified in the readings.

The causes and resultant necessary corrections were as follows:

1. Gas (carbon dioxide) absorption by rumen microbes due to an increase in the pH of the inoculumn. In the beginning of the incubation when readily fermentable substrates are available, microorganisms multiply at a rapid rate. However when readily available fermentable substrate becomes limited, some of the micro

organisms do not survive in the media. The death of these microbes led to an increased protein content in the media thereby resulting in an increased pH. The increased pH led to re-absorption of carbon dioxide from the inoculumn by the microbes. This led to a negative rate of gas production.

2. Leaky Valves: It was observed that some of the modules had their valves leaking there by resulting in a decline in the pressure reading after reaching a maximum psi level.
3. Loss in radio transmission: It was also observed at some times in the readings that there was a break in communication between the modules and the computer. This led to a "jump" or a drop in the readings.

The procedure used in correcting the abnormalities is as follows:

1. Gas absorption: To correct this abnormality, the difference between every value and the previous value was calculated. The absolute values for the differences were computed. These absolute values were then added cumulatively to the original readings. This correction assumes that gas absorbed is equal to gas produced.
2. Leaky valves: This was corrected by stopping the value at its maximum reading.
3. Loss in radio transmission: This was corrected by placing the "dropped" or "jumped" value to the level of the previous value.

In addition to data correction, 34 samples out of the 162 samples were not used in the statistical analysis. If the cumulative pressure reading for a sample did not increase above 3.5psi for the entire period of observation, it was deleted. This was based on the assumption that for those samples, normal fermentation pattern was in some way

impaired. The distribution of the successful and unsuccessful samples can be seen in Appendix (Table 3) and are randomly distributed.

3.3.6 Phase selection for statistical analysis

All the curves generated from the data collected showed a similar multi-phasic shape. There were three different phases in the curves within the 48 hour digestion period.

Phase I covered the period from 0-7 hours.

Phase II covered the period from 07:30-24.30 hours.

Phase III covered the period from 25-48 hours.

The rate of gas production within the 48 hours was the basis for the phase selection. Phase I covered a period of a rapid rate of gas production. The rapid rate in this phase was attributed to the concentrate fed to the two fistulated cows from which the rumen fluid was taken. The rate in this phase started declining after the first half hour period and continued till the 7^{th} hour.

Phase II covered a period of a constant rate of gas production. At this phase, the microbes had finished the concentrate and some highly soluble carbohydrate in the sample so they were now multiplying to attack the insoluble but digestible carbohydrates in the sample. This phase saw a low rate with some samples even reading a zero rate of gas production.

Phase III covered the period of digesting the insoluble but digestible carbohydrate. In this phase, the rate of gas production started rising again.

3.3.7 Statistical analysis

The General linear module from SAS version 8 was used in the analysis of variance for the herbage yield data collected. The cumulative gas curves were divided into three phases as described in 3.2.6. The Mixed MODEL from SAS was used to analyze for variation in the various Phases for the varieties, harvest dates and fraction. The various fermentation sessions (run) were included as a random variable in the statistical analysis. The following interactions were also analyzed for variation: variety by harvest date (VXHD), variety by fraction (VXF) and harvest date by fraction (HDXF). The standard deviation for the chemical composition was calculated using Microsoft EXCEL (MS EXCEL 2007).

CHAPTER FOUR

4.0.0 RESULTS

The results cover the herbage yield, chemical composition and *in vitro* gas production. There was a regular rainfall during the herbage yield experiment. No disease was observed during the herbage yield experiment. The *in vitro* gas experiment was without a blank due to the inability of some modules to function properly so there was a shortage in the number of modules used for the study. There was a constant supply of electricity during the entire period of experiment. The results of the 2 experiments are as follows.

4.1.0 Effect of Variety on the chemical composition of Napier grass.

Table 4.1 below shows the chemical composition of the four varieties of Napier grass. Variety 16786 had the highest dry matter of 521.5g/kg DM with Variety 16798 having the lowest of 482.52g/kg DM. The organic matter content was highest for variety 16840 (939.93g/kg DM) compared with variety 16786 which had the lowest of 930.77g/kg DM. The Local variety produced the highest crude protein (CP) of 96.77g/kg DM while Variety 16786 had the lowest crude protein of 85.35g/kg DM. Hemicellulose and cellulose composition ranged between 252.33 to 195.5g/kg DM and 420.83 to 360.33g/kg DM respectively.

Table 4.1 Effect of variety on the chemical composition of Napier grass

	Variety				
Chemical composition	**Local**	**16786**	**16798**	**16840**	**s.d.**
DM (g/kg)	483.8	521.5	482.6	512.1	45.9
OM (g/kg DM)	939.8	930.8	931.8	939.9	24.6
Ash (g/kg)	60.2	69.2	68.2	60.1	24.6
CP (g/kg DM)	96.8	85.4	92.9	93.1	34.8
NDF (g/kg DM)	728.2	745.0	723.7	743.0	45.3
ADF (g/kg DM)	532.7	505.5	471.3	498.5	60.7
Hemi cellulose (g/kg DM)	195.5	239.5	252.3	245.2	48.5
Cellulose (g/kg DM)	420.8	375.8	382.3	360.3	63.9
Cellulose + Hemi cellulose	616.3	615.3	634.6	605.5	12.1
ADL (g/kg DM)	111.8	129.7	89.0	138.2	37.4
ADL:Hemi cellulose	1:1.7	1:1.8	1:1.28	1:1.8	

CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, OM=Organic matter, DM=Dry matter ADL=Acid detergent lignin, s.d=Standard deviation.

4.2.0 Effect of harvest date on the chemical composition of Napier grass

Table 4.2 below shows the chemical composition of the Varieties harvested at three different harvest dates. The dry matter, organic matter, NDF, ADF,ADL and hemicellulose increased with increase in harvesting days (60<90<120days) whilst the crude protein and cellulose showed a decreasing trend with increase in harvesting days (60>90>120days).

Table 4.2 Effect of harvest date on the chemical composition of Napier grass

	Harvest date			
Chemical composition	**60**	**90**	**120**	**s.d.**
DM (g/kg)	478.5	506.3	511.1	45.9
OM (g/kg DM)	944.9	933.2	928.6	24.6
Ash (g(kg)	71.4	66.8	55.0	24.6
CP (g/kg DM)	109.9	86.3	79.9	34.8
NDF (g/kg DM)	686.0	720.3	765.1	45.3
ADF (g/kg DM)	488.6	505.6	511.8	60.7
Hemi cellulose (g/kg DM)	208.3	231.7	259.5	48.5
Cellulose (g/kg DM)	427.6	372.3	354.6	63.9
Cellulose + Hemi cellulose	635.9	604.0	614.1	16.3
ADL (g/kg DM)	84.1	116.4	151.0	37.4
ADL: Hemi cellulose	1:2.5	1:1.9	1:1.7	

CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, OM=Organic matter, DM=Dry matter ADL=Acid detergent lignin, s.d=Standard deviation.

4.3.0 Effect of Fraction on the chemical composition of Napier grass

Table 4.3 below shows the chemical composition for the leaf and stem fractions of Napier grass. The leaf fraction yielded lower values for dry matter, organic matter, NDF, ADF, cellulose and ADL compositions whilst the stems recorded higher values. The leaf fraction also yielded higher values for crude protein and hemicellulose compositions with the stems producing the lowest.

Table 4.3 Effect of Fraction (Leaf and Stem) on the chemical composition of Napier grass

	Fraction		
Chemical composition	**Leaf**	**Stem**	**s.d.**
DM (g/kg)	499.9	500.0	45.9
OM (g/kg DM)	922.4	948.8	24.6
Ash (g/kg)	77.6	51.2	24.6
CP (g/kg DM)	122.2	61.8	34.8
NDF (g/kg DM)	708.6	761.7	45.3
ADF (g/kg DM)	468.5	535.4	60.7
Hemi cellulose (g/kg DM)	240.0	226.3	48.5
Cellulose (g/kg DM)	362.8	406.9	63.9
Cellulose+ Hemi cellulose	602.8	633.2	21.5
ADL (g/kg DM)	105.8	128.5	37.4
ADL: Hemi cellulose	1:2.7	1:1.8	

CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, OM=Organic matter, DM=Dry matter, ADL=Acid detergent lignin, s.d=Standard deviation.

4.4.0 Effect of variety on the herbage yield of Napier grass

The results on the effect of variety on herbage yield are shown in Table 4.4 below.

From Table 4.4, the Local and 16798 varieties had significantly ($P<0.05$) higher herbage yield compared to the 16840 and 16786. The highest herbage yield was produced for the variety 16798 (44,994kg/ha DM) and the lowest produced for variety 16840 (24,863kg/ha DM).

Table 4.4 Effect of variety on herbage yield of Napier grass (*Pennisetum purpureum)*

	Variety				
Parameter	**Local**	**16786**	**16798**	**16840**	**s.e.d**
Herbage yield(kg DM/ha)	41050[a]	28589[b]	44994[a]	24863[b]	3693.4

Means with different superscripts in the same row are significantly different ($P<0.05$).

4.5.0 Effect of harvest date on the herbage yield of Napier grass

Table 4.5 below shows the effect of harvest date on herbage yield. Herbage yield was significantly different ($P<0.05$) for all the harvesting dates. Herbage yield increased with increase in harvesting date (60 days < 90days < 120days) for all the varieties. Harvesting at 60 days gave the lowest (22,489kg/ha DM) dry matter yield with 120 days recording the highest yield (46,013kg/ha DM).

Table 4.5 Effect of harvest date on the herbage yield of Napier grass (*Pennisetum purpureum)*

	Harvest date			
Parameter	**60**	**90**	**120**	**s.e.d**
Herbage yield (kg DM/ha)	22489[a]	36121[b]	46013[c]	3198.6

Means with different superscripts in the same row are significantly different (P<0.05)

s.e.d = standard error of difference

4.6.0 Effect of variety on mean gas and mean rate of gas production for three different Phases

Table 4.6 below shows that there was no significant difference (P>0.05) among the 4 varieties of Napier grass for the mean total gas production in Phases I. In Phase II and III, there was a significant (P<0.05) interaction between the Variety and Harvest Date.

Table 4.7, below shows the interaction between Variety and harvest date for mean gas production. The results showed that the varieties behaved differently at the 3 different harvest dates in the 3 Phases but the interaction was significant only in Phases II and III. In Phase II of Table 4.7, variety 16786 harvested at 90 recorded a significantly (P<0.05) higher mean total gas production whilst variety 16786 harvested at 60 recorded a significantly (P<0.05) lower mean gas production among the 4 varieties and 3 harvest date. In Phase II of Table 4.7, the Local Variety, 16840 and 16798 did not show any significant difference (P>0.05) at the 3 different harvesting dates for mean gas production. However, variety 16786 recorded a significant difference (P<0.05) among the 3 harvesting dates in Phase II of Table 4.7.

Table 4.6 Effect of variety, harvest date and fraction on the mean gas and mean rate of gas production for three different time phases

		Phase					
		I (LS Means)		II (LS Means)		III (LS Means)	
Treatments		Average accumulated gas production (psi)	Rate of gas production (per hour)	Average accumulated gas production (psi)	Rate of gas production (per hour)	Average accumulated gas production (psi)	Rate of gas production (per hour)
Variety (V)	Local	2.35	0.56	5.23	0.15	8.07^{a}	0.16^{a}
	16840	2.49	0.59	5.57	0.17	8.71^{a}	0.17^{a}
	16786	2.51	0.60	5.57	0.16	8.60^{a}	0.17^{a}
	16798	2.61	0.60	5.50	0.15	9.20^{b}	0.20^{b}
Harvest date (HD)	60	2.32	0.55^{a}	5.21	0.17^{a}	9.21^{a}	0.21^{a}
	90	2.55	0.60^{b}	5.58	0.15^{b}	8.44^{b}	0.16^{b}
	120	2.59	0.61^{b}	5.61	0.15^{b}	8.29^{b}	0.15^{b}
Fraction (F)	Leaf	2.32^{a}	0.55^{a}	5.17^{a}	0.15^{a}	8.46	0.20^{a}
	Stem	2.59^{b}	0.62^{b}	5.77^{b}	0.17^{b}	8.29	0.15^{b}
V						**	
HD			*		**	***	**
F		***	***	***	***		***
V X HD				*		*	
V X F						*	
F X HD		***		***			

*Means with different superscript in the same column within the same row heading are significantly different (P<0.05). *significant at 0.05, **significant at 0.01, ***significant at 0.001*

The 90 days harvest for variety 16786 recorded a significantly higher mean gas than the 60 and 120 days in Phase II (Table 4.7). In addition, the 120 days harvest was also significantly higher in mean gas production compared to the 60 days harvest in Phase II (Table 4.7).

In Phase III of Table 4.7, variety 16798 harvested at 60 had a significantly ($P<0.05$) higher mean gas with variety Local harvested at 90 days producing the significantly lowest mean gas among the 4 varieties and 3 harvest date.

There was no significant difference ($P>0.05$) in mean gas production for varieties 16840 and 16786 in Phase III of Table 4.6.

The Local variety in Phase III of Table 4.7 shows that the 60 days harvest had a significantly ($P<0.05$) highest mean gas among the 3 harvest dates. However, there was no significant difference ($P>0.05$) between the 90 and 120 days harvest (Table 4.7).

Variety 16798 in Phase III of Table 4.7 shows that the 60 days harvest was significantly higher ($P<0.05$) in terms of mean gas production compared to the 90 and 120 days. There was however no significant difference ($P>0.05$) between the 90 and 120 days harvest for variety 16798 (Table 4.7).

There was a significant difference ($P<0.05$) in the rate of gas production among the 4 varieties in phase III. Variety 16798 was significantly faster in the rate of gas production compared to the other 3 varieties. The Local variety produced gas at the slowest rate in Phase III of Table 4.7.

Table 4.7 Average accumulation of gas between variety and harvest day (Appendix Figure 2a-2c)

Phase				
Variety (V)	**Harvest date (HD)**	**I (LS Means)**	**II (LS Means)**	**III (LS Means)**
Local	60	2.32	5.32^{a}	8.95^{b}
	90	2.55	4.98^{a}	7.41^{a}
	120	2.45	5.40^{a}	7.84^{a}
16840	60	2.22	5.23^{a}	9.31^{b}
	90	2.59	5.73^{a}	8.60^{b}
	120	2.66	5.75^{a}	8.22^{b}
16786	60	2.13	4.71^{c}	8.08^{b}
	90	2.80	6.21^{b}	9.13^{b}
	120	2.58	5.79^{a}	8.60^{b}
16798	60	2.52	5.58^{a}	10.49^{c}
	90	2.63	5.41^{a}	8.61^{b}
	120	2.68	5.51^{a}	8.50^{b}

Means with different superscript in the same column are significantly different ($P<0.05$)

Table 4.8 Average accumulation of gas between variety and fraction (Appendix Figure 3a-3c)

Phase				
Variety (V)	**Fraction (F)**	**I (LS Means)**	**II (LS Means)**	**III (LS Means)**
Local	Leaf	2.17	4.94	7.66[a]
	Stem	2.52	5.53	8.47[c]
16840	Leaf	2.32	5.23	8.44[c]
	Stem	2.66	5.86	8.99[b]
16786	Leaf	2.25	5.13	8.12[c]
	Stem	2.77	6.01	9.09[b]
16798	Leaf	2.52	5.34	9.61[b]
	Stem	2.70	5.67	8.80[b]

Means with different superscript in the same column are significantly different (P<0.05)

Table 4.9 Average accumulation of gas between fraction and harvest day (Appendix Figure 4a-4c)

Phase				
Fraction (F)	**Harvest date (HD)**	**I (LS Means)**	**II (LS Means)**	**III (LS Means)**
Leaf	60	2.27[a]	5.11[a]	8.94
	90	2.49[a]	5.45[c]	8.35
	120	2.19[a]	4.95[b]	8.08
Stem	60	2.38[a]	5.32[a]	9.48
	90	2.61[b]	5.71[c]	8.53
	120	3.00[c]	6.27[d]	8.51

Means with different superscript in the same column are significantly different (P<0.05)

Table 4.8 Average accumulation of gas between variety and fraction (Appendix Figure 3a-3c)

Phase				
Variety (V)	**Fraction (F)**	**I (LS Means)**	**II (LS Means)**	**III (LS Means)**
Local	Leaf	2.17	4.94	7.66^{a}
	Stem	2.52	5.53	8.47^{c}
16840	Leaf	2.32	5.23	8.44^{c}
	Stem	2.66	5.86	8.99^{b}
16786	Leaf	2.25	5.13	8.12^{c}
	Stem	2.77	6.01	9.09^{b}
16798	Leaf	2.52	5.34	9.61^{b}
	Stem	2.70	5.67	8.80^{b}

Means with different superscript in the same column are significantly different (P<0.05)

Table 4.9 Average accumulation of gas between fraction and harvest day (Appendix Figure 4a-4c)

Phase				
Fraction (F)	**Harvest date (HD)**	**I (LS Means)**	**II (LS Means)**	**III (LS Means)**
Leaf	60	2.27^{a}	5.11^{a}	8.94
	90	2.49^{a}	5.45^{c}	8.35
	120	2.19^{a}	4.95^{b}	8.08
Stem	60	2.38^{a}	5.32^{a}	9.48
	90	2.61^{b}	5.71^{c}	8.53
	120	3.00^{c}	6.27^{d}	8.51

Means with different superscript in the same column are significantly different (P<0.05)

4.7.0 Effect of harvest date on mean gas and mean rate of gas production for three different Phases

Table 4.6 shows that there was a highly significant interaction ($P<0.05$) between harvest date and fraction in Phases I and II and also a significant interaction ($P<0.05$) between harvest date and variety in Phases II and III. The results for the harvest date and variety interaction have been presented.

In Phase I of Table 4.9, 120 days harvest for the stem fraction was significantly higher in mean gas production with the 120 days harvest of the leaf fraction recording the significantly lowest mean gas among the 2 fraction for the 3 harvest dates. There was no significant difference ($P>0.05$) for the leaf fraction harvested at 60, 90 and 120 days in Phase I of table 4.9. The Stem fraction however showed a significant difference ($P<0.05$) among the 3 harvest dates. The stem fraction harvested at 120 days recorded a significantly ($P<0.05$) higher mean gas compared to the 60 and 90 days harvest. The stem fraction harvested at 90 days was also significantly higher than the 60 days harvest.

In Phase II of Table 4.9, there was a significant difference ($P<0.05$) in the mean gas production. The 120 days harvest of the stem fraction had a significantly ($P<0.05$) higher mean gas with 120 days harvest of the leaf fraction producing a significantly lower mean gas production among the 3 harvest dates for the leaf and stem fraction. The 90 days harvest of the leaf fraction was significantly higher than 60 and 120 days whilst the 60 days harvest was also significantly higher than the 120 days harvest.

The 120 days harvest of the stem fraction recorded a significantly higher mean total gas compared to the 60 and 90 days whilst the 90 days harvest was also significantly higher than the 60 days harvest.

The mean rate of gas production in Table 4.7 below showed a significant difference ($P<0.05$) among the 3 harvest dates in the 3 Phases. In Phase I, the 120 day harvest produced gas at a significantly faster rate compared to the 60 and 120 days harvest. The 60 day harvest recorded the lowest mean rate of gas production in Phase I.

4.8.0 Effect of Fraction on mean gas and mean rate of gas production for three different Phases

Table 4.6 below shows that, there was a significant interaction between Fraction and harvest date in Phases I and II and also a significant interaction between Fraction and variety in Phase III. The result of the interaction between fraction and harvest date can be found in Table 4.9 below.

In Phase III of Table 4.8, the leaf fraction of variety 16798 recorded a significantly higher mean gas with leaf fraction of the Local variety recording the lowest mean gas between the 2 fractions for the 4 different varieties. The stem fractions of varieties Local, 16840 and 16786 all recorded a significantly higher mean total gas compared to the leaf fraction (Table 4.8). The leaf and stem fractions of variety 16798 however, did not show any significant difference in the mean total gas.

The mean rate of gas production was significantly different ($P<0.05$) for the 2 fractions in Phases I, II and III. In Phase I and II, the stem fraction produced gas at a significantly faster rate than the leaf fraction. In Phase III, the leaf fraction produced gas at a significantly faster rate than the stem fraction.

CHAPTER FIVE

5.0.0 DISCUSSION

5.1.0 Effect of variety on the chemical composition and herbage yield of Napier grass *(Pennisetum purpureum)*

The high dry matter yield for varieties 16786 and 16840 suggest that more nutrients will be available to the ruminant for feeding and digestion. This is because the passage rate will be reduced thereby giving the rumen microbes enough time to degrade the sample. However, it was realized that these same varieties had the highest lignin content implying that though they had a high dry matter, the high lignin content could bind the cellulose and hemicellulose and prevent them from being digested and utilized efficiently by the rumen microbes.

The difference in the chemical composition recorded for the 4 varieties agrees with Dzimale (2000) who reported similar results for the same varieties. The Local variety and variety 16798 which had a lower dry matter content yielded a lower lignin and high cellulose compared to the varieties 16786 and 16840. The low lignin content and high cellulose could lead to a better intake and digestion for varieties Local and 16798. All the 4 varieties had a CP level equal or higher than the 80g/kg minimum CP requirement for dairy cows reported by Muia *et al.* (1999).

Though varieties 16786 and 16840 recorded the highest dry matter, it yielded the lowest herbage yield. Varieties Local and 16798 recorded about twice the herbage yield recorded for 16786 and 16840.

5.2.0 Effect of harvest date on the chemical composition and herbage yield of Napier grass *(Pennisetum purpureum)*

The increase in dry matter, with an increase in harvest day implies more nutrients availability as a result of the slow passage rate. The high dry matter could also lead to more chewing time which will bring about more saliva production to keep the rumen environment at a neutral pH. ADF, NDF and ADL all increased with increase in harvest date. This could have an influence on intake and digestibility of the grass. The increase in ADF, NDF and ADL could be attributed to the rapid multiplication of tissues as grass ages. The results obtained for the dry matter, ADF, NDF and ADL agrees with the report of Taye Bayble *et al.* (2007) who observed a similar trend when Napier grass was harvested at 60, 90 and 120 days and should be expected with increasing grass maturity.

Barnes, Hetta and Martinsson (2007) reported an increase in DM, ADF, NDF and ADL with an increase in maturity date in timothy grass (*Phleum pratense*).

Kramberger and Klemencic (2003) and Peiretti (2009) reported similar result in *Cerastiem holosteoides* and sunflower (*Carthamus tinetories L.*) respectively when harvested at increasing maturity dates.

The low dry matter recorded in the early harvesting date could negatively affect the storage ability of the Napier grass since the moisture present can lead to a rapid deterioration of the forage. The decrease in CP with increase in harvest date observed in this study is consistent with what has been reported in literature (Taye Bayble *et al.*, 2007, Kranberger and Klemencic 2003 and Peiretti 2009). It was observed that the C.P levels decreased by 27% from the 60 day harvest to the 120 days. The CP results for all

the harvest dates were equal or above the 80g/kg minimum CP requirement for dairy cows (Muia *et al.,* 1999). Even though the 60 day harvest yielded the highest cellulose and CP, it produced the lowest herbage yield. The increase in herbage yield with an increase in harvesting date could be attributed to the increase in tiller number, leaf formation, leaf elongation as well as stem development (Robertson *et al.* 1976).

5.3.0 Effect of Fraction on the chemical composition and herbage yield of Napier grass *(Pennisetum purpureum)*

The relatively higher cellulose fraction recorded for the stem fraction could account for the high digestibility recorded for the stem fraction in the early hours of digestion.

The CP in the leaf fraction is almost twice that of the stem fraction. A similar trend was observed by Tang *et al.* (2008). The CP in leaf was higher than the minimum CP of 80g/kg required for dairy cows however the stem fractions produced a CP which was lower than the minimum requirement for dairy cows (Muia, *et al.,* 1999)

5.4.0 Effect of Variety on the mean *In vitro* gas and rate of gas production of Napier grass in different Phases

The difference observed among the varieties in mean gas and mean rate of gas production in table 4.6 in Phase III could be attributed to the low lignin content of the variety 16798 Table 4.1. This result supports the conclusion from France and Forbes (1996) that shows that more gas was produced in low lignin content forage and also agrees with Dzimale (2000) who reported no significant difference among the same varieties after 25 hours of

incubation. Dzimale (2000) recorded a significant difference after 72 hours of incubation among the same varieties.

This is not in agreement with the result in Phase III of Table 4.7 which shows significant difference after 48 hours of digestion for the varieties.

The rate of gas production was significantly higher for variety 16798 than the other 3 varieties which were not significantly different from each other. The Local varieties produced gas at the most slow rate.

There was a significant effect of variety and harvest date interaction on the mean gas production in phase II and III of Table 4.6.

Rumen microbes degraded variety 16786 harvested at 90 more for Phase II than any other variety harvested at any date. Though at 90 days Napier grass recorded higher lignin content and also increased in cell wall contents compared to the 60 day harvest, it recorded a higher digestibility.

This could be due to late microbial adaptation to the less fibrous plant materials. The fistulated cows from which the rumen fluid was collected were fed low quality hay which was high in fiber. The rumen fluid was filled with more fiber degrading microbes and therefore did not need much time to degrade it.

In Phase III of Table 4.7, variety 16798 harvested at 60 days recorded the highest digestibility after 48 hours with the Local variety harvested at 90 days recording the least digestibility. Even though the Local variety had similar yield and chemical composition

as variety 16798, the 60 days harvest for variety 16798 had a significantly higher digestibility than the Local variety.

The significant difference reported in Phase III between the Local and variety 16798 suggests that nutrients in the Local Variety were probably not utilized by the rumen microbes as much as they used that of Variety 16798. Probably the rumen microbes attached themselves more to Variety 16798 than the Local.

5.5.0 Effect of harvest date on the mean *in vitro* gas and rate of gas production of Napier grass

The higher gas production recorded for the 120 days harvest in Phases I and II suggest that the rumen fluid was probably filled with more fiber digesting microbes.

The highest gas production obtained for the 120 harvest date in Phases I and II disagree with most studies that reported that digestibility reduces with age (Ogwang and Mugerwa 1976, Crowder and Chheda, 1977 Wilson and Mannetje 1978). However there was a significant effect of Harvest date and Fraction interaction in both Phases I and II. In Phase I, the high mean gas recorded for the 120 day harvest was due to the effect of the stem fraction. It is the same for phase II of Table 4.9 where the high mean gas from the 120 day harvest was due to the effect of the stem. The influence of the stem fraction on the 120 days harvest could be due to the high cellulose present in the stem fraction compared to the leaf fraction (Table 4.3). Richard and Reid (1953) and Sulivan (1966) both reported that rumen microbes degrade cellulose more than hemicellulose. This result could also be attributed to the low quality hay offered to the fistulated cows from which

the rumen fluid was collected. The microbes from these cows used in this research were probably already accustomed to highly fibrous forages and therefore did not need much time to adhere to the fibrous samples.

In Phase III of Table 4.7, the 60 days harvest recorded a significantly higher gas and higher rate of digestion compared to the 120 days harvest.

The 90 days harvest did not differ significantly from the 120 days harvest. The significantly higher gas and rate of digestion for the 60 days harvest agrees with earlier studies conducted by Ogwang and Mugerwa (1976), Crowder and Chheda (1977) Wilson and Mannetje (1978). There was however a variety and harvest date interaction in Phase III. The high mean gas recorded for the 60 days harvest was due to the effect of the Variety 16798. The high mean gas production and rate of gas production could be attributed to the low lignin reported for the 60 days harvest in Table 4.2. The 60 day harvest recorded the lowest NDF meaning that there will be more soluble carbohydrate for the rumen microbes to degrade. The low hemicellulose and high cellulose in Table 4.2 could also be attributed to the high gas production for 60 day harvest. Sulivan, (1966) reported that hemicellulose is less digestible in ruminants compared to cellulose and since there was high cellulose in the 60 day harvest a high gas production was expected. The results of the higher gas for the 120 days in Phase I of Table 4.7 confirm the earlier accession that the microbes needed time to get used to the less fibrous sample hence the significantly high gas production of the 60 day harvest in Phase III of Table 4.7.

5.6.0 Effect of Fraction on the mean *in vitro* gas and rate of gas production of Napier grass

Mean gas and rate of gas production were significantly higher for stem fraction in Phases I and II of Table 4.6 than the leaf fraction. However there was an interaction between fraction and harvesting date in Phases I and II where the stems harvested at 90 and 120 days produced most mean gas (Table 4.9). These findings disagree with the report of Raymond (1969) and Tolera and Sundstøl (1999). It however agrees with Tang *et al,* (2008) who reported a higher gas production for maize stems than leaf blade.

The high gas production for the stem fraction could be attributed to the high cellulose and low hemicellulose. Rumen microbes digest cellulose more than they will digest hemicellulose (Sulivan, 1966). The non significance of the final gas for leaf and stem at 48 hours suggest that microbes degrade leaf and stem differently in the early hours of incubation with higher digestion for the stem.

In phase III, there was no significant difference between the 2 Fractions; however the leaf fraction produced more gas than the stem fraction. The high mean gas obtained in Phase III of Table 4.6 was due to the effect of Variety 16798.

CHAPTER SIX

6.0.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Based on the results of this study, it can be concluded that Variety 16798 yielded the highest CP, cellulose + Hemi cellulose and the best ADL: Hemi cellulose ratio. However they it had the lowest dry matter. The 60 days harvest recorded the highest CP, cellulose + Hemi cellulose and the best ADL: Hemi cellulose ratio. The Leaf fraction yielded the highest CP and the best ADL: Hemi cellulose ratio. It however gave the lowest cellulose + hemi cellulose.

Varieties 16798 and Local produced the highest herbage yield among the four varieties with the 120 days harvest also having the highest herbage yield among the three harvest dates.

Varieties 16840 and 16786 produced the highest gas in Phases I and II even though they both contained a relatively high amount of lignin. In Phase III, Variety 16798 produced the highest gas with the Local Variety producing the lowest.

In phase I, variety 16798 gave the highest mean gas and mean rate of gas production. The 120 days harvest produced the most gas at the fastest rate in phase I. The stem fraction in phase I gave the highest mean gas at the fastest rate.

In phase II, varieties 16840 and 16876 produced the most gas and at the fastest rate. The 120 days harvest and the stem fraction both produced the most gas and at the fastest rate among the three harvest days and two fractions respectively.

In phase III, variety 16798 gave the highest mean gas production at the fastest rate. The 60 days harvest and the leaf fraction produced the most gas and at the fastest rate in phase III.

6.2 RECOMMENDATIONS

It is recommended that:

1. Variety 16798 should be selected for production since it had the highest herbage yield, less lignin and highest digestibility after 48 hours.
2. Napier grass be harvested at 90 days even though it had a lower herbage yield compared to the 120 days and a lower digestibility compared to the 60 days after 48 hours. This is because harvesting at 90 days will offer the farmer the opportunity to harvest twice in the 5 months major rainy season in the humid zone compared to the one time harvest for the 120 days. Though the digestibility was high for the 60 days, the herbage yield was relatively lower.
3. Further studies should be carried out to investigate the arrangement of cells in the lignin fraction of Napier grass.

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Appendices

Table 1 Average monthly rainfall, temperature and humidity during the period of planting (April-August, 2008)

Month	Rainfall (mm)	Temperature (°C)	Humidity	
			0.9:00	15:00
April	117.1	33.3	83	59
May	185.8	33.0	82	59
June	279.8	31.4	85	64
July	145.0	29.8	88	68
August	114.5	29.5	88	59
Average for the study period	168.44	31.4	85.2	61.8

Source: Ghana Meteorological Agency K.N.U.S.T station (2008)

Table 2 Media components used for inoculumn preparation

Components	Reagents
Micromineral (MiM) (Menke and Steingass, 1988)	***Dissolve in 100 ml distilled water (enough for 250 run):*** 13, 0 g $CaCl_2$ * 2H2O (Calciumchloride dehydrate) 10, 0 g $MnCl_2$ * 4 H_2O (Mangan (II) chloride tetrahydrate) 1, 0 g $CoCl_2$ *6 H_2O (Cobaltchloride hexahydrate) 8, 0 g $FeCl_2$ * 6 H_2O (Iron (III) chloride hexahydrate)
Macrominerals (MaM) (Menke & Steingass, 1988)	***Dissolve in 1 L distilled water (enough for one run):*** 5, 70 g Na_2HPO_4 * 12 H_2O (di-Natriumhydrogenphosphate dihydrate) 6, 20 g KH_2PO_4 (Kaliumdihydrogenphosphate) 0, 60 g MgSO4 * 7 H_2O (Magnesiumsulfate heptahydrate)
Buffer solution (BS) (Menke and Steingass, 1988)	***Dissolve in 1 L distilled water (enough for one run):*** 35, 0 g $NaHCO_3$ (Natriumhydrogencarbonate) 4, 0 g $(NH_4)_2HCO_3$ (Ammoniumhydrogencarbonate) ***Adesogan et al., 2005 adds:*** 2, 6 g trypticase peptone
Redox indicator (RI) (Menke and Steingass, 1988)	***Dissolve in 100 ml distilled water:*** 100 mg resazurin
Reduction agent (RA) (Menke and Steingass, 1988)	This must be prepared shortly before rumen fluid is collected: For 40 glass jars of 60 ml per jar, 89.8ml deionized H_2O, 3.7ml 1-n-NaOH, 538 Na_2S(7-9 H_2O) was used.

Table 3 Distribution of successful and unsuccessful gas readings

Parameter	Sub parameter	Total Number of sample	Successful	Unsuccessful
Variety	Local	36	28	8
	16798	42	34	8
	16786	42	35	7
	16840	42	31	11
Total		**162**	**128**	**34**
Fraction	Leaf	81	65	16
	Stem	81	63	18
Total		**162**	**128**	**34**
Harvest date	60 days	54	40	14
	90 days	54	43	11
	120 days	54	45	9
Total		**162**	**128**	**34**

Table 4 Raw data for Experiment I

Variety	Plot No	Harvest date	Replication	Herbage yield kg/ha DM
Local	13	60	1	36208.3
Local	10	60	2	27111.1
Local	15	60	3	23250.0
Local	16	60	4	34472.2
Local	13	90	1	37350.0
Local	10	90	2	42366.6
Local	15	90	3	34333.3
Local	16	90	4	49200.0
Local	13	120	1	44644.4
Local	10	120	2	50222.2
Local	15	120	3	47666.6
Local	16	120	4	65777.7
16840	5	60	1	14222.2
16840	2	60	2	23250.0
16840	7	60	3	11111.1
16840	4	60	4	28333.3

16840	5	90	1	19111.1
16840	2	90	2	20277.7
16840	7	90	3	18888.8
16840	4	90	4	29944.4
16840	5	120	1	32111.1
16840	2	120	2	21777.7
16840	7	120	3	21777.7
16840	4	120	4	57555.5
16786	1	60	1	6888.8
16786	6	60	2	10333.3
16786	3	60	3	8333.3
16786	8	60	4	15069.4
16786	1	90	1	26000.0
16786	6	90	2	26444.4
16786	3	90	3	39333.3
16786	8	90	4	43555.5
16786	1	120	1	32666.6
16786	6	120	2	32666.6
16786	3	120	3	49333.3
16786	8	120	4	52444.4
16798	9	60	1	27555.5
16798	14	60	2	32722.2
16798	11	60	3	32083.3
16798	10	60	4	28875.0
16798	9	90	1	56888.8
16798	14	90	2	40055.5
16798	11	90	3	56077.7
16798	10	90	4	38111.1
16798	9	120	1	65777.7
16798	14	120	2	42666.6
16798	11	120	3	53333.3
16798	10	120	4	65777.7

Table 4 ANOVA Table for Herbage yield DM kg/ha

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	9339123109	667080222	11.06	<.0001
Error	33	1990985012	60332879		
Corrected Total	47	11330108121			

Table 5 Raw data for Experiment II

Run	Variety	Harvest date	Fraction	Phase I (0-7)	Phase II (7.5-24.5)	Phase III (25-48)
3	Local	60	Stem	2.3476	7.2306	12.4423
4	Local	60	Stem	3.1541	5.2329	9.0321
5	Local	60	Stem	3.9773	6.4715	10.8533
5	Local	60	Stem	3.9270	7.0686	12.1381
6	Local	60	Stem	4.4456	6.7037	10.0908
3	Local	90	Stem	1.3941	3.1309	5.4848
4	Local	90	Stem	4.6621	10.0098	12.7523
5	Local	90	Stem	1.9262	3.1218	3.9189
5	Local	90	Stem	4.6228	7.2542	10.0990
6	Local	90	Stem	4.0544	6.0092	12.0184
3	Local	120	Stem	5.3394	7.9033	12.4901
3	Local	120	Stem	4.9396	7.3153	8.8207
5	Local	120	Stem	3.9006	6.0219	8.0749
5	Local	120	Stem	4.3063	6.5268	10.0257
6	Local	120	Stem	4.2631	6.6475	9.1764
4	Local	60	Leaf	4.8785	7.3531	12.7266
4	Local	60	Leaf	4.6085	7.2801	12.4897
5	Local	60	Leaf	3.8436	5.5208	10.6224
6	Local	60	Leaf	3.0809	5.4615	8.6124
6	Local	60	Leaf	3.2334	5.4827	8.2241
4	Local	90	Leaf	3.6802	6.0891	10.6392
4	Local	90	Leaf	4.0618	6.2709	10.1902
5	Local	90	Leaf	4.4456	6.3509	10.8671
6	Local	90	Leaf	2.6815	4.8267	7.1059
4	Local	120	Leaf	4.2155	6.4237	10.3046
4	Local	120	Leaf	3.5099	5.9186	10.6673
5	Local	120	Leaf	1.5981	4.3079	7.5040
6	Local	120	Leaf	2.9625	5.3469	8.0926
1	16840	60	Stem	4.2671	6.0696	10.7760

5	16840	60	Stem	3.8632	6.6534	11.3751
5	16840	60	Stem	3.2970	5.8539	11.3714
6	16840	60	Stem	3.8372	6.3712	10.7876
4	16840	90	Stem	5.4929	9.2651	12.3755
5	16840	90	Stem	3.8859	6.4083	8.9307
5	16840	90	Stem	4.9169	7.4823	11.1878
6	16840	90	Stem	3.4004	6.2341	11.5472
1	16840	120	Stem	6.0512	8.4133	10.7091
3	16840	120	Stem	4.3095	6.8272	8.6644
4	16840	120	Stem	4.5867	7.4799	10.5142
5	16840	120	Stem	5.1015	7.6873	10.1332
5	16840	120	Stem	3.9540	5.6584	6.6128
6	16840	120	Stem	4.0380	6.6591	9.7761
1	16840	60	Leaf	4.2874	6.8716	12.5531
3	16840	60	Leaf	2.3545	7.3804	13.6967
4	16840	60	Leaf	3.9540	6.4765	11.7940
6	16840	60	Leaf	3.0988	4.6482	9.1556
1	16840	90	Leaf	4.8052	6.6353	10.7295
3	16840	90	Leaf	3.5450	7.2945	10.0442
4	16840	90	Leaf	5.0317	7.0583	11.3212
5	16840	90	Leaf	4.0069	5.7340	9.8790
6	16840	90	Leaf	3.3304	5.5024	8.2536
6	16840	90	Leaf	2.2277	4.1073	7.9362
1	16840	120	Leaf	4.6218	7.2182	10.4369
3	16840	120	Leaf	3.3595	6.6275	8.5244
4	16840	120	Leaf	3.3415	5.6388	10.0246
4	16840	120	Leaf	5.2433	7.6135	11.9948
5	16840	120	Leaf	3.7546	5.8090	10.0595
6	16840	120	Leaf	3.2715	5.0495	9.4589
6	16840	120	Leaf	2.9448	4.6687	9.05
1	16786	60	Stem	5.3360	7.7840	12.2556
3	16786	60	Stem	2.4896	4.7100	8.0743
3	16786	60	Stem	3.1731	5.5641	9.2958
4	16786	60	Stem	3.6766	6.7875	13.2215
5	16786	60	Stem	4.2422	7.0703	12.3730
5	16786	60	Stem	3.0610	5.9890	11.3125
6	16786	60	Stem	2.4507	3.9911	6.3017
1	16786	90	Stem	4.9932	8.0475	11.9339
3	16786	90	Stem	3.9832	5.9982	8.9270
4	16786	90	Stem	4.8690	7.7622	12.2784
5	16786	90	Stem	5.4805	8.2207	11.6100

5	16786	90	Stem	5.6371	8.1501	11.8856
6	16786	90	Stem	4.2716	6.8780	10.1360
1	16786	120	Stem	4.7287	7.0615	9.6456
4	16786	120	Stem	5.1015	8.3162	11.2514
5	16786	120	Stem	5.2747	8.5261	11.7054
5	16786	120	Stem	4.4409	6.9978	10.3621
6	16786	120	Stem	4.4620	6.9809	10.4354
1	16786	60	Leaf	3.3474	5.8280	11.0753
4	16786	60	Leaf	4.1609	7.3510	11.1651
5	16786	60	Leaf	3.0824	4.5877	5.5913
6	16786	60	Leaf	2.9684	4.6336	9.4844
1	16786	90	Leaf	4.2961	6.4829	11.2211
3	16786	90	Leaf	4.1553	9.3200	12.8414
4	16786	90	Leaf	4.1088	6.6591	10.9096
4	16786	90	Leaf	5.4681	8.5136	12.1128
5	16786	90	Leaf	4.1822	5.9648	10.2841
6	16786	90	Leaf	2.8597	4.7430	8.5792
6	16786	90	Leaf	3.9440	5.7751	9.5782
1	16786	120	Leaf	5.0612	8.2622	12.9927
3	16786	120	Leaf	3.9825	6.7964	10.2781
4	16786	120	Leaf	3.6470	6.0784	11.1437
4	16786	120	Leaf	3.6486	6.2957	10.8028
5	16786	120	Leaf	3.2651	5.3235	10.0082
6	16786	120	Leaf	2.4421	3.8786	8.1163
1	16798	60	Stem	4.4105	6.3609	11.1754
3	16798	60	Stem	2.6466	7.7155	13.0311
4	16798	60	Stem	4.0463	6.6475	12.4279
5	16798	60	Stem	4.7912	7.6526	12.3772
6	16798	60	Stem	4.5968	7.4698	10.7738
1	16798	90	Stem	4.5981	6.9782	11.1261
3	16798	90	Stem	2.2793	5.4077	10.5919
3	16798	90	Stem	2.3163	2.9781	3.5957
4	16798	90	Stem	5.2024	8.5984	11.5609
5	16798	90	Stem	3.9992	6.7573	11.3082
6	16798	90	Stem	4.0222	5.2959	7.7763
1	16798	120	Stem	4.1668	5.2213	7.8368
3	16798	120	Stem	4.0618	6.5559	8.0049
5	16798	120	Stem	5.4870	7.9098	10.4752
5	16798	120	Stem	5.1138	7.8052	10.4294
6	16798	120	Stem	4.0066	6.6777	9.7002
1	16798	60	Leaf	4.7749	6.7743	13.5311

3	16798	60	Leaf	2.8969	7.2309	13.1388
4	16798	60	Leaf	4.1878	6.7431	13.8412
4	16798	60	Leaf	5.3010	7.9514	15.0659
6	16798	60	Leaf	2.6920	5.3840	11.5472
6	16798	60	Leaf	3.3101	5.8455	12.6066
1	16798	90	Leaf	5.0253	7.1311	11.5795
3	16798	90	Leaf	4.1487	6.8986	12.0904
4	16798	90	Leaf	4.1198	6.3485	11.8866
4	16798	90	Leaf	4.3727	6.7382	12.1145
5	16798	90	Leaf	4.9302	7.1590	12.9672
1	16798	120	Leaf	4.5755	6.6789	13.0575
4	16798	120	Leaf	3.5988	5.6452	10.3025
4	16798	120	Leaf	3.7737	5.8703	11.3911
5	16798	120	Leaf	5.2225	7.8696	13.0206
5	16798	120	Leaf	3.7202	5.2941	11.8759
6	16798	120	Leaf	3.6924	5.7920	11.1496
6	16798	120	Leaf	2.0912	3.3171	8.2928

Table 6 ANOVA Tables for in vitro gas analysis.

Table 6.1 ANOVA table for phase I (0-7hours)

Effect	DF	F Value	Pr > F
Variety	3	1.18	0.3218
Fraction	1	11.70	0.0009
Harvest date	2	2.88	0.0606
V X HD	6	1.52	0.1781
V X F	3	0.57	0.6342
HD X F	2	5.98	0.0035

Table 6.2 ANOVA table for phase II (7.5-24.5hours)

Effect	DF	F Value	Pr > F
Variety	3	0.82	0.4840
Fraction	1	11.97	0.0008
Harvest date	2	2.32	0.1034
VXHD	6	2.26	0.0433
V X F	3	0.49	0.6923
HDX F	2	4.86	0.0096

Table 6.3 ANOVA table for phase III (25-48hours)

Effect	DF	F Value	Pr > F
Variety	3	3.39	0.0206
Fraction	1	2.22	0.1392
Harvest date	2	5.21	0.0070
V X HD	6	2.86	0.0126
V X F	3	2.89	0.0390
HD X F	2	0.18	0.8334

Figure 1a Accumulated *in vitro* gas production for the four Varieties and three Harvest Day in Phase I

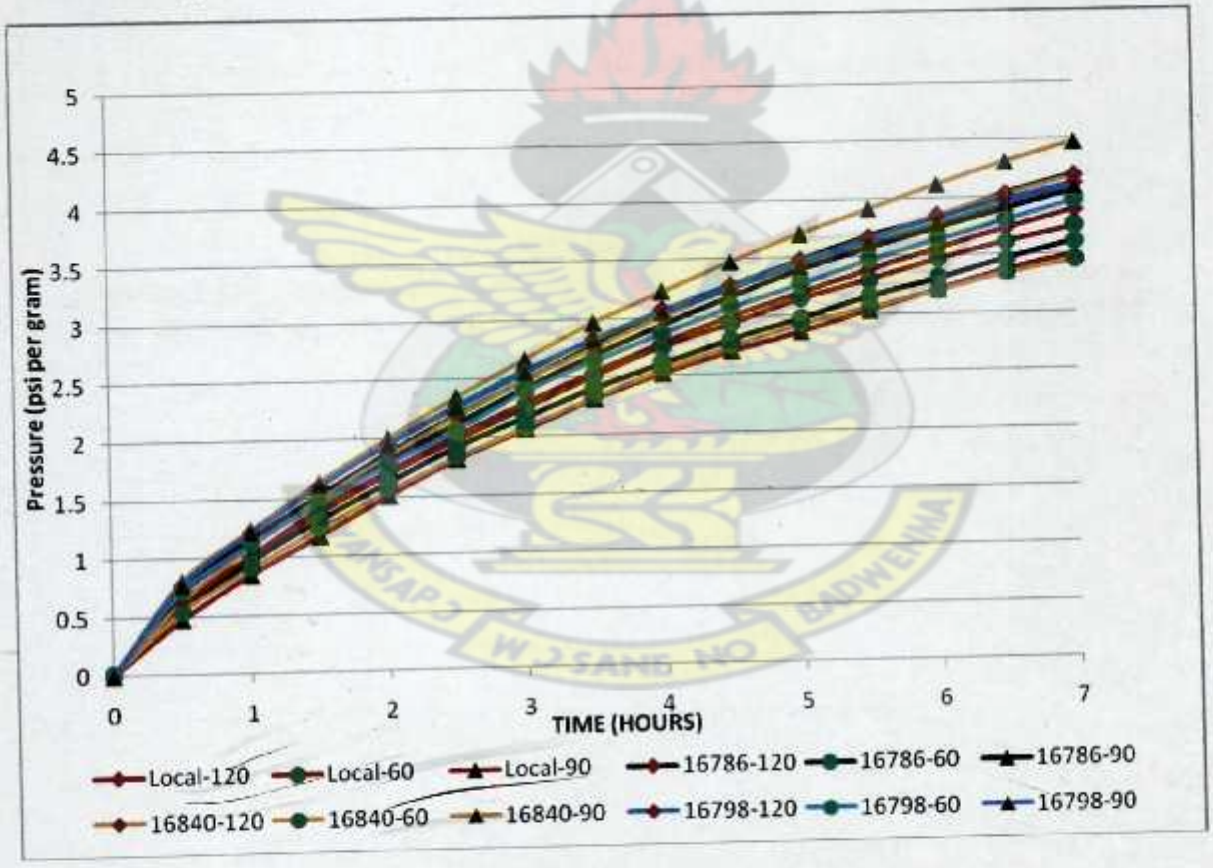


Figure 1b Accumulated *in vitro* gas production for the four Varieties and three Harvest Day in Phase II

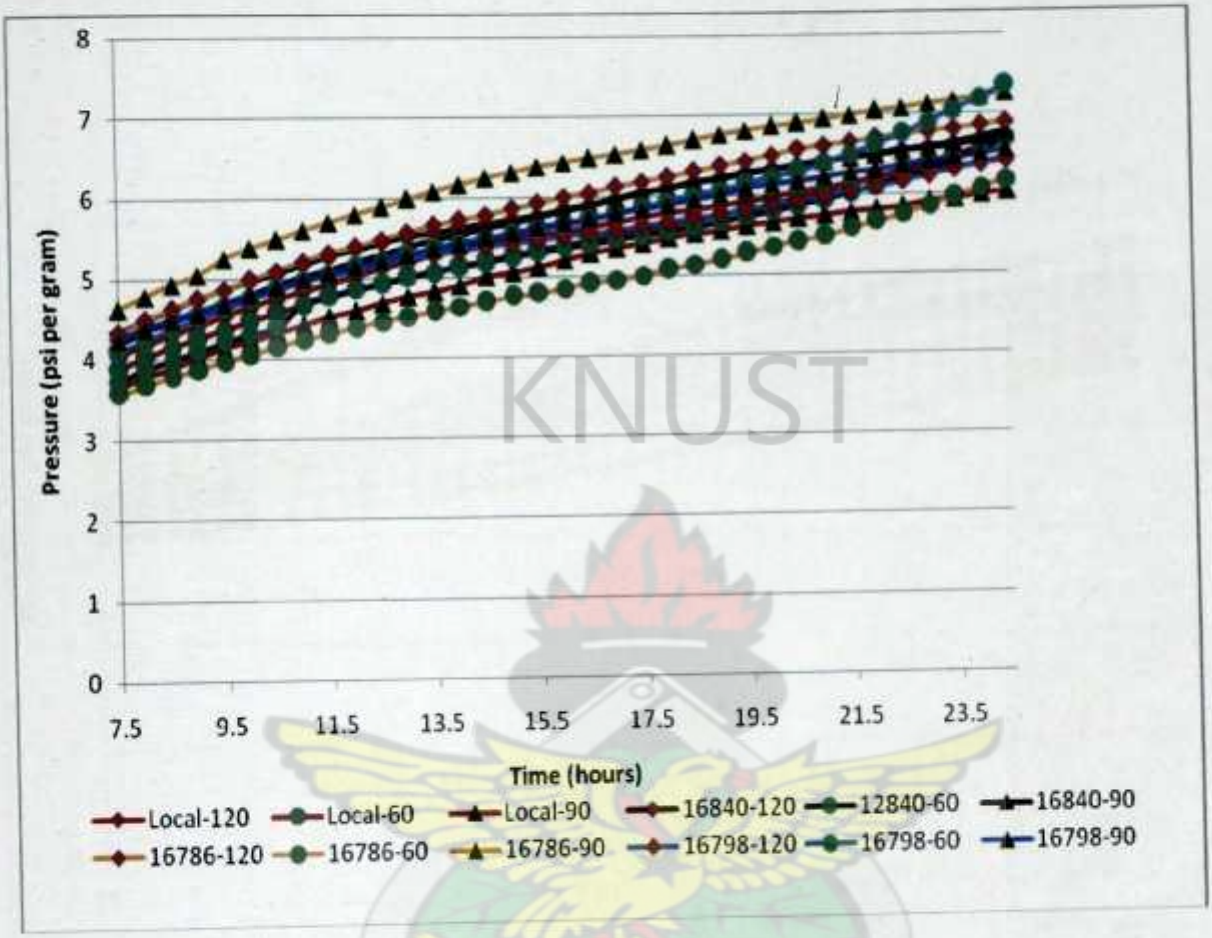


Figure 1c Accumulated *in vitro* gas production for the four Varieties and three Harvest Day in Phase III

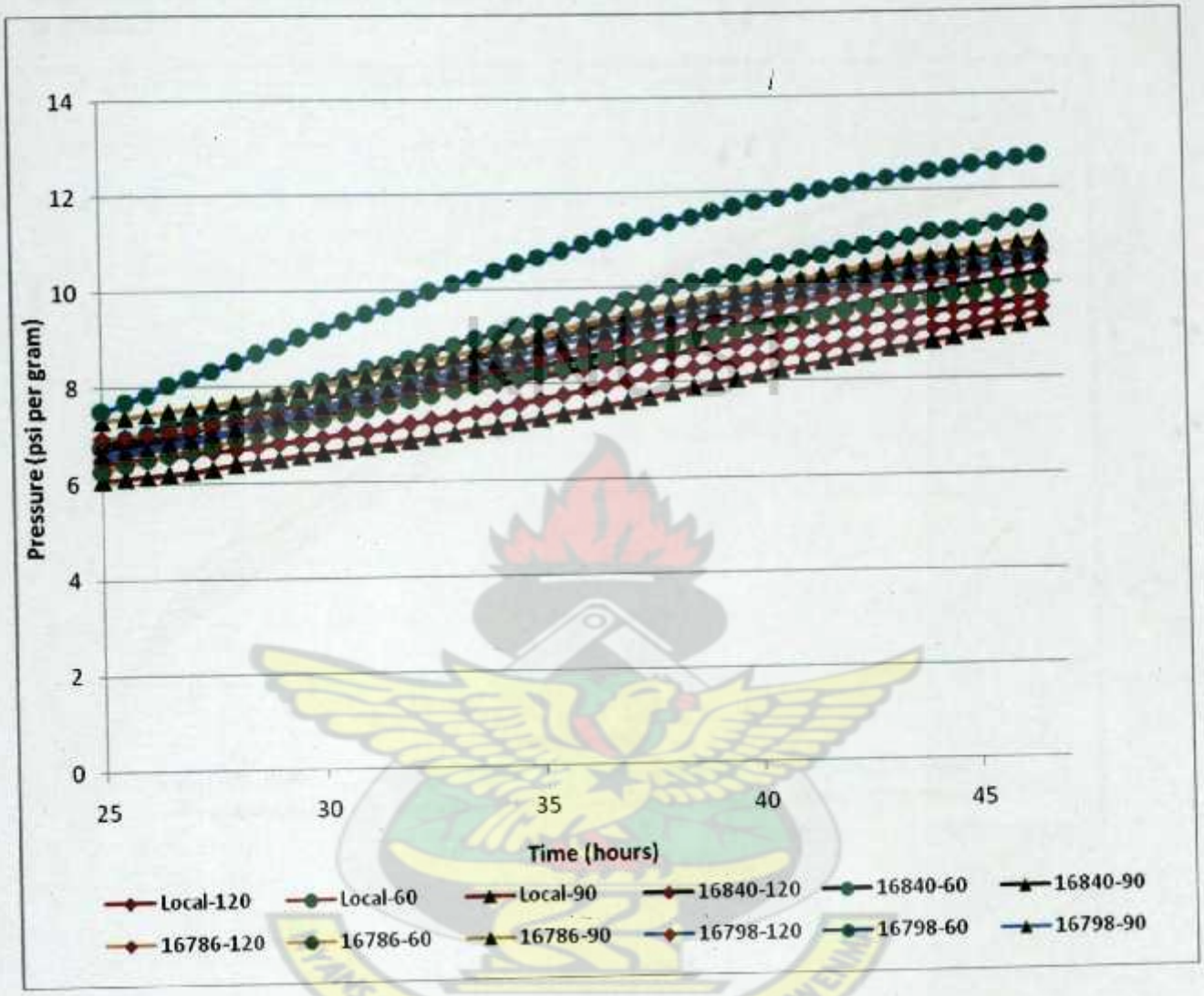


Figure 2a Accumulated *in vitro* gas production for two fractions and four varieties in Phase I

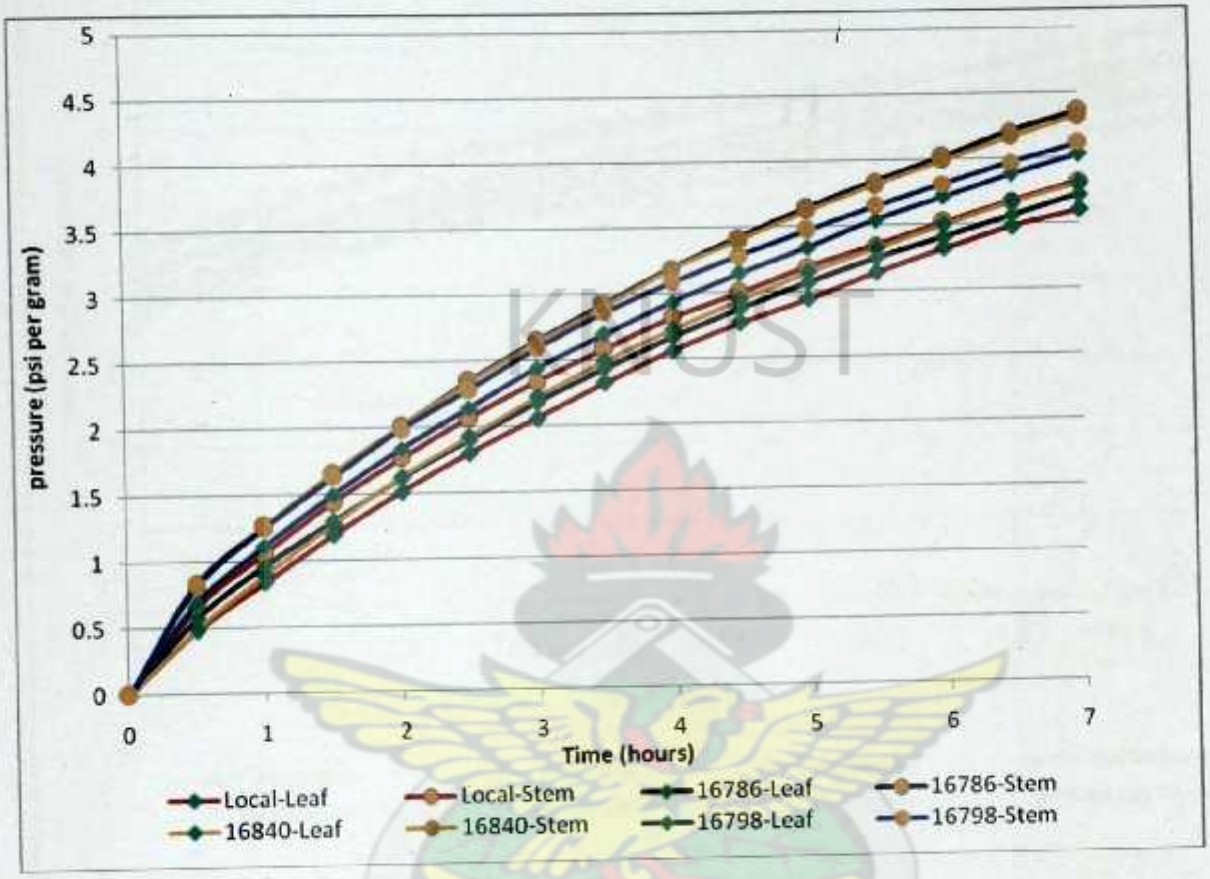


Figure 2b Accumulated *in vitro* gas production for two fractions and four varieties in Phase II

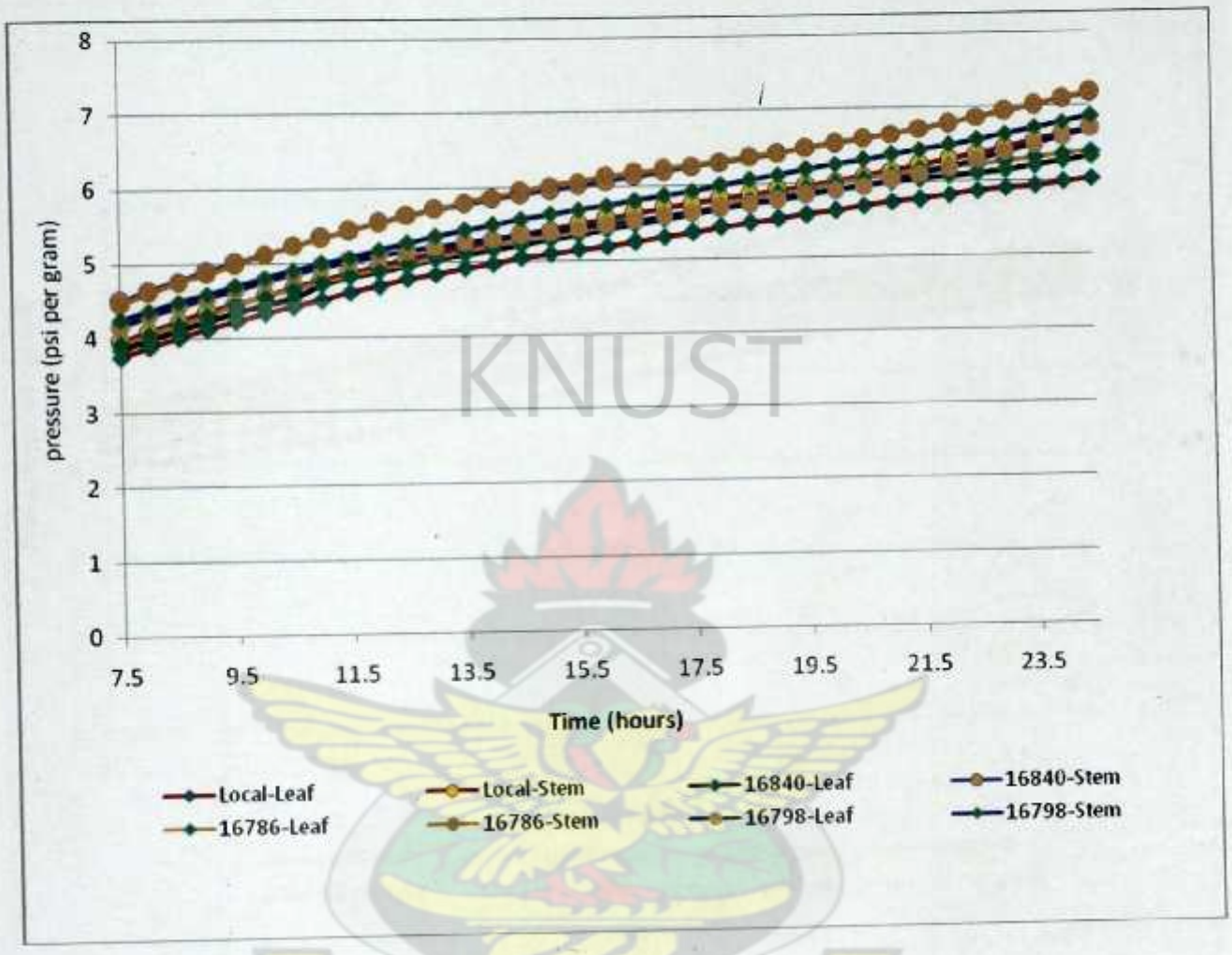


Figure 2c Accumulated *in vitro* gas production for two fractions and four varieties in Phase III

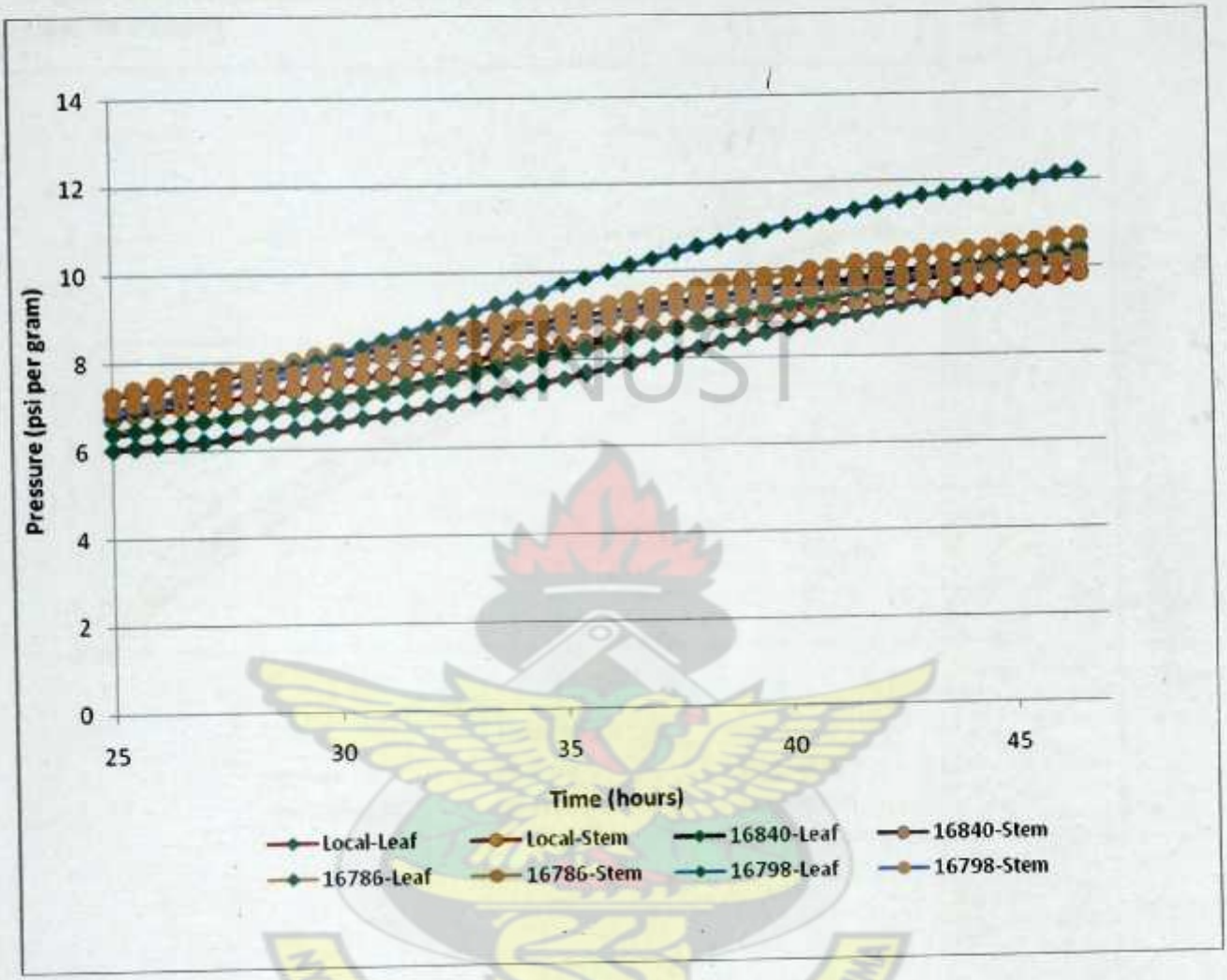


Figure 3a Accumulated *in vitro* gas production for two fractions and three harvest days in Phase I

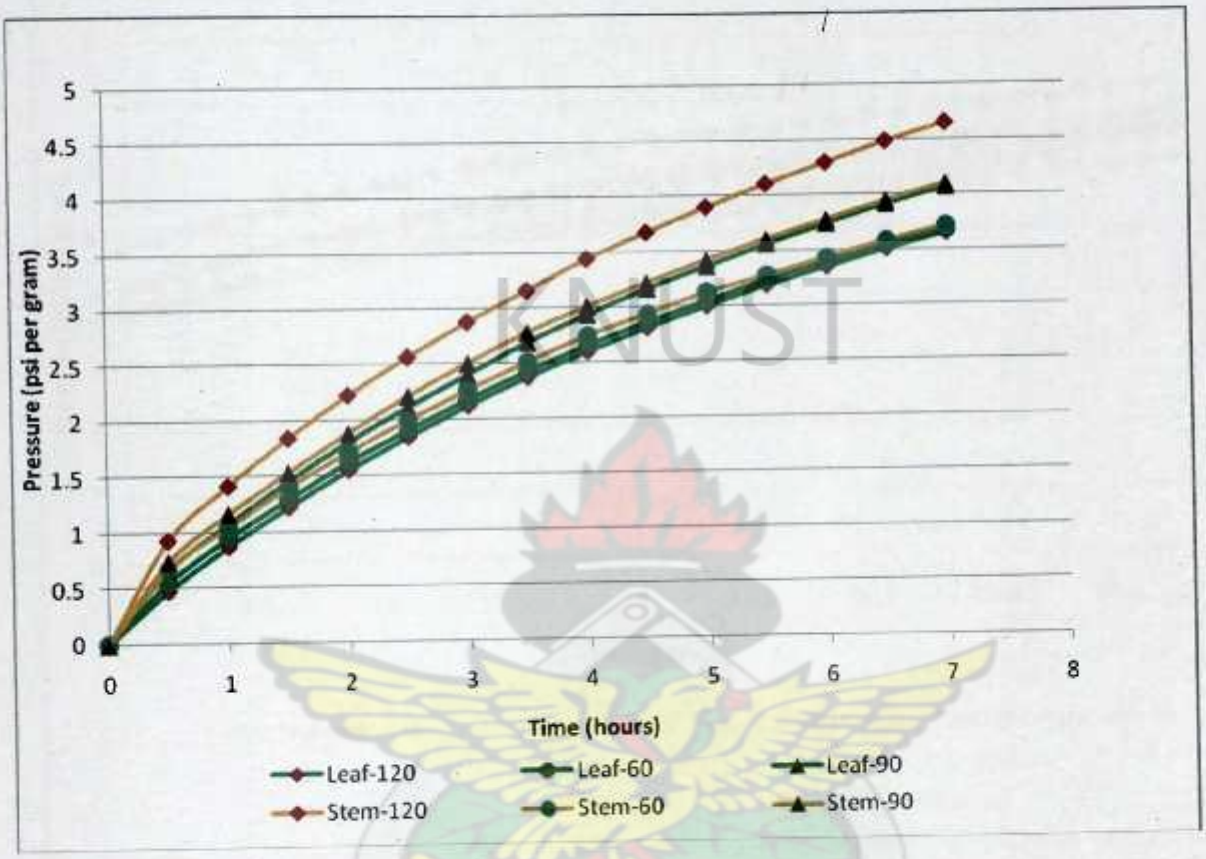


Figure 3b Accumulated *in vitro* gas production for two fractions and three harvest days in Phase II

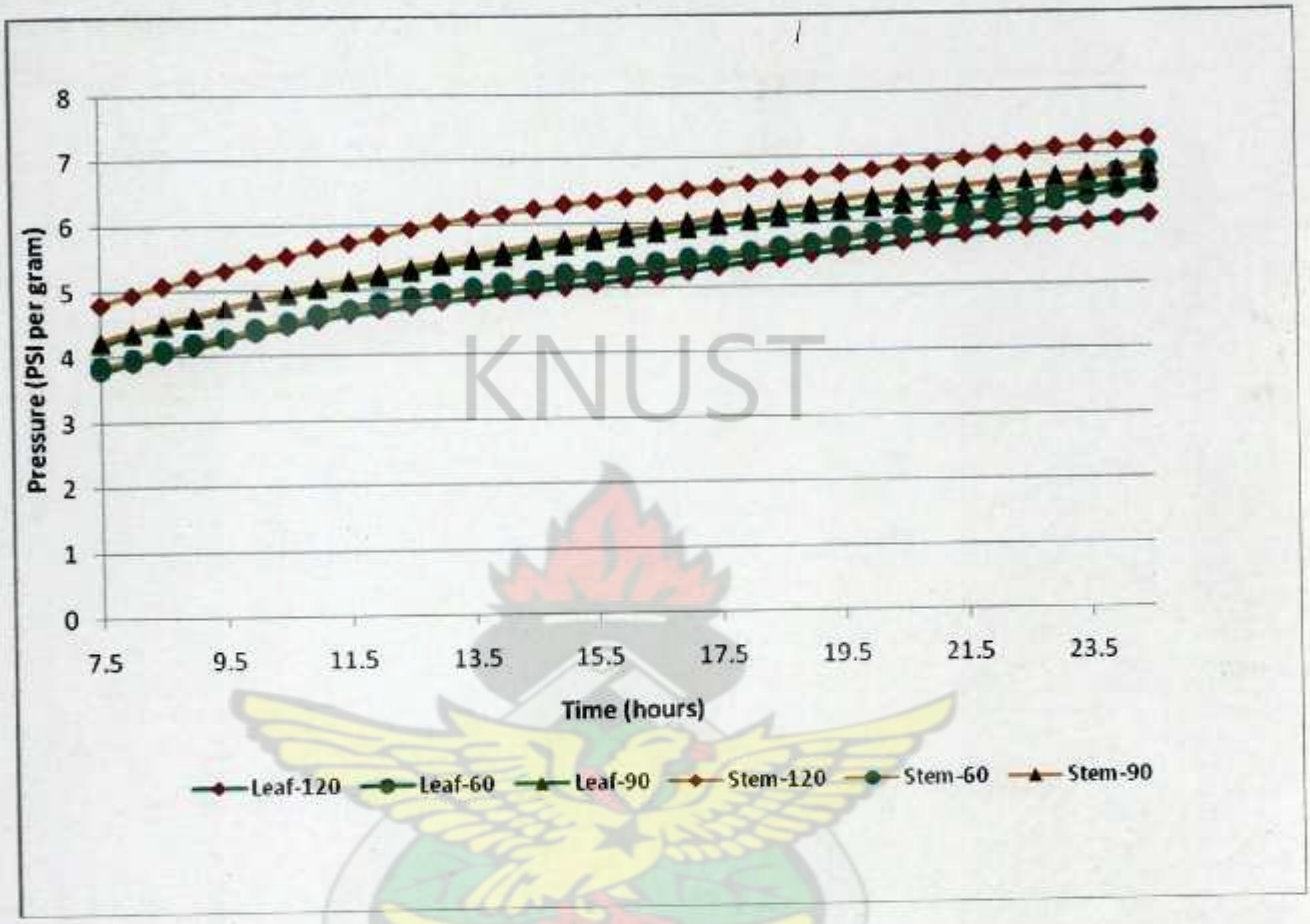


Figure 3c Accumulated *in vitro* gas production for two fractions and three harvest days in Phase III

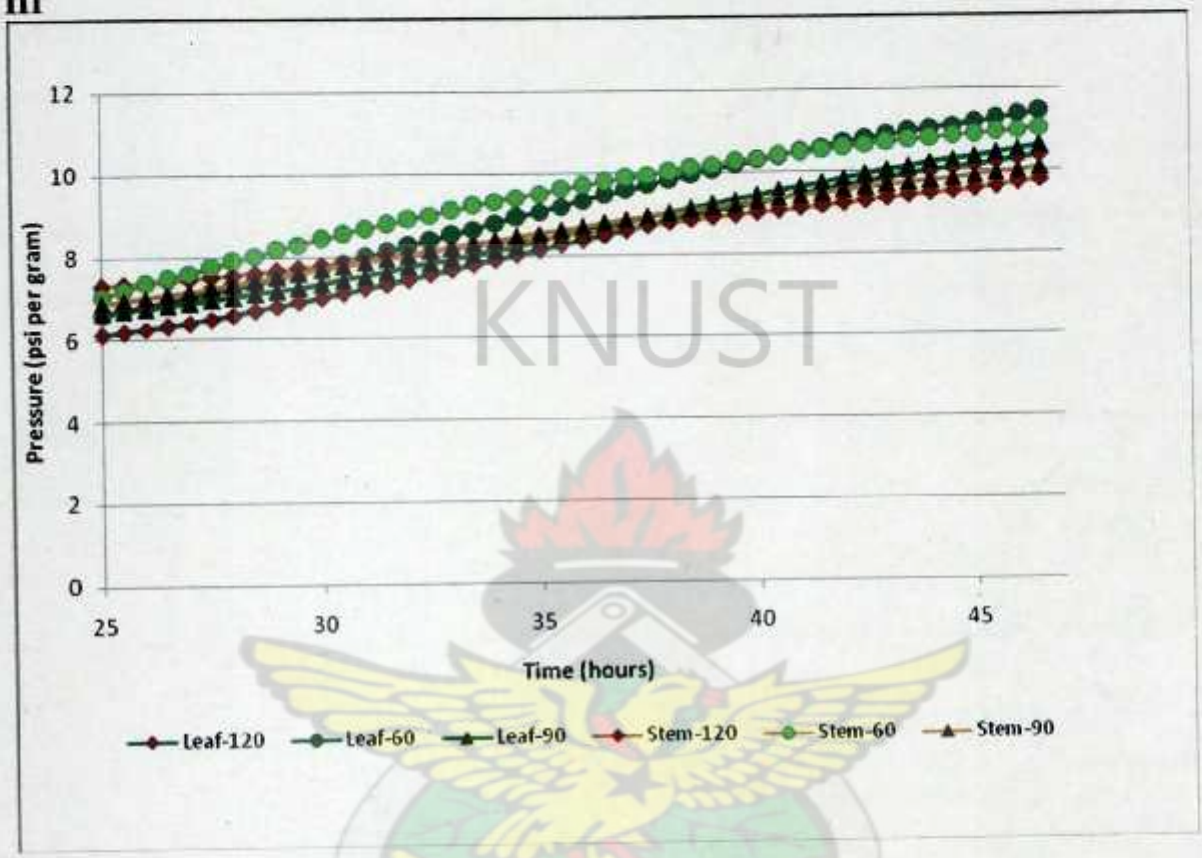
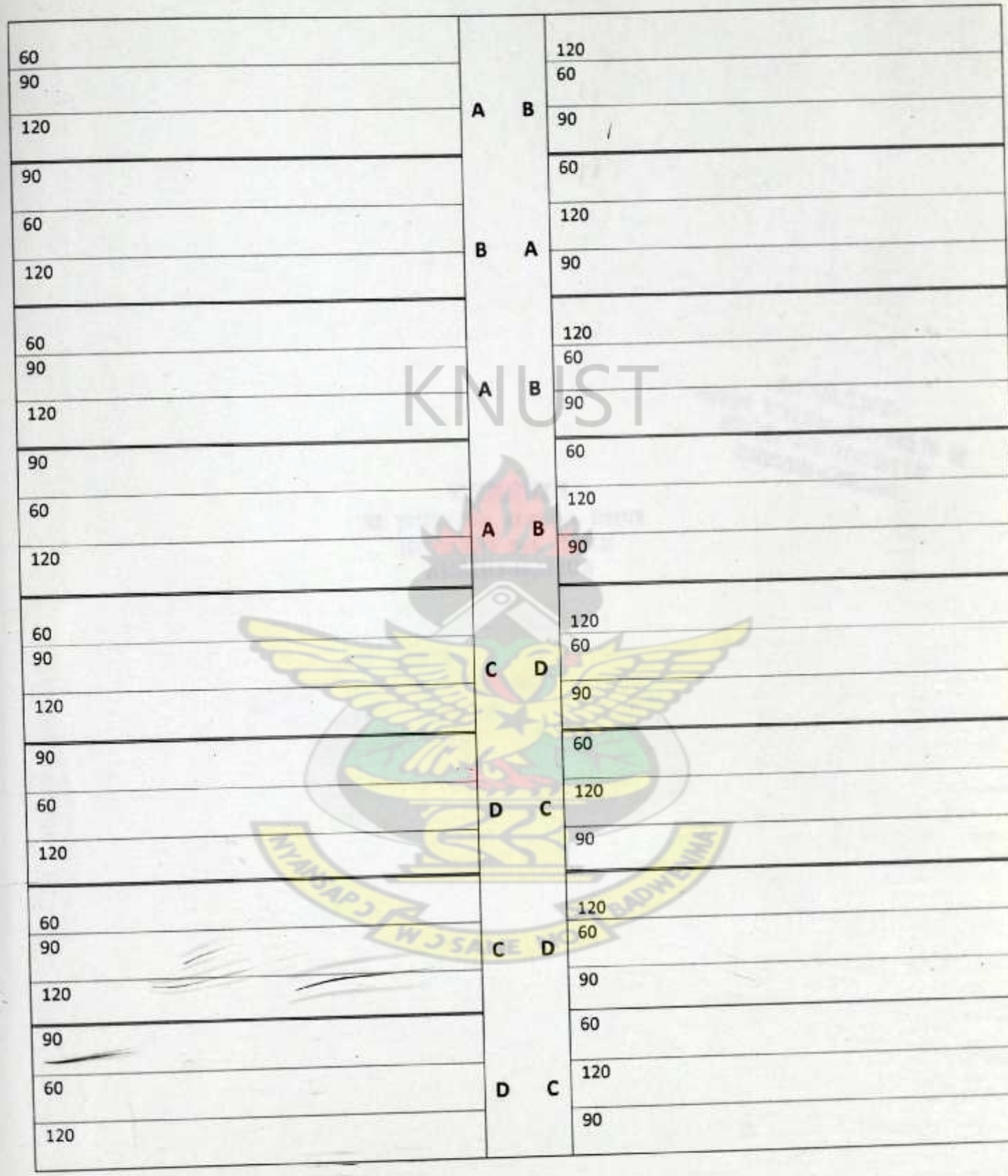


Figure 4 Field layout of Experiment I



VARIETY (A=LOCAL, B=16840, C=16786, D=16798)

HARVEST DAY (60=60 days harvest, 90=90 days harvest, 120=120 days harvest)