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GROWTH AND NUTRITIVE QUALITY OF PIGEON PEA (*Cajanus cajan*) FODDER AS INFLUENCED BY CUTTING REGIMES IN THE GUINEA SAVANNAH AGRO-ECOLOGICAL ZONE OF GHANA

BY

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DECLARATION

This is to affirm that this thesis is my genuine work and that all sources of materials used for this thesis have been duly acknowledged and has neither been submitted for a degree nor any aspect published by another person elsewhere.

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ABSTRACT

This study was conducted to evaluate the effect of cutting regimes on biomass yield and nutrient composition of pigeon pea (*Cajanus cajan*) fodder in the Guinea savannah agro-ecological zone of Ghana. The treatments were three cutting regimes (12, 16 and 20) weeks after planting (WAP) laid in a randomized complete block design. Agronomic data were taken on plant height, number of branches and stem diameter. At each harvest, the harvested samples were separated into leaf, stem and whole fractions for the determination of biomass yield, chemical composition and *in vitro* digestibility. Cutting regimes significantly ($P<0.05$) affected plant height, number of branches and stem diameter in both the initial establishment and regrowth. Biomass yield was significantly ($P=0.012$) affected by cutting regimes in the initial establishment but not the regrowth. The highest biomass yield was obtained in the harvest at 20WAP (6,515kgDM/ha) followed by 16 WAP and 12WAP (5,930kg/ha and 3,175kg/ha) respectively in the initial establishment. All chemical and digestibility parameters were significantly ($P<0.05$) affected by cutting regimes, fraction and their interactions except for dry matter which was only influenced by cutting regimes. The highest CP (235.8g/KgDM) was obtained in the leaf fraction in the harvest at 12WAP in the initial establishment whereas the leaf fraction at 20 WAP recorded the highest CP in the regrowth. In conclusion, harvesting at 20WAP produced the highest biomass yield but lower CP and organic matter digestibility in the initial establishment whereas harvesting at 20WAP produced higher biomass yield, organic matter digestibility and metabolizable energy in the regrowth.

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DEDICATION

This thesis is dedicated to my beloved sister Ms. Edith Afirifa Tenakwa for her prayers and support in my education.

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LIST OF ABBREVIATIONS

ADF	Acid Detergent fiber
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CP	Crude protein
DCP	Digestible Crude Protein
DM	Dry matter
DOM	Digestible Organic Matter
FAO	Food and Agriculture Organization
IVOMD	*In Vitro* Organic Matter Digestibility
ME	Metabolizable Energy
MOFA	Ministry of Food and Agriculture
N	Nitrogen
NDF	Neutral Detergent fiber
SCFA	Short Chain Fatty Acids
TDN	Total Digestible Nutrient

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CHAPTER ONE

1.0 INTRODUCTION

Ruminants derive a large proportion of their nutrients from growing natural pasture in most developing countries. The yield of these pastures are not adequate to meet ruminants' nutrient requirements especially in the dry season. This causes nutritional dissatisfaction and consequently decrease in animal productivity. Concentrate supplementation during this period of feed scarcity to ruminants is generally not a profitable practice due to high costs (Lukuyu *et al*., 2011).

One of the major constraints in the tropics to an efficient and increased ruminant production is the scarcity of feed. Farmers depend on crop residues and low-quality standing hay to feed their animals during the dry season (McDermott *et al*., 2010). Residues from crops are low in protein and high in lignocellulose, resulting in poor digestibility and insignificant voluntary intake. Subsequently, the energy and nitrogen intake of animals fed with such feedstuffs cannot support the requisite nutrient requirement for their performance (Mahesh and Mohini, 2013).

Trees and browse species have been used as livestock feed over the years. For example, *Albizia lebbeck*, *Leaucaena lecocephala*, *Entada Africana*, *Pterocarpus erinaceus*, *Securinega virosa* and others are being used as feed in Ghana (Ziblim *et al*., 2015). These forage species have been used for numerous purposes, such as food, shelter, wood or non-wood-based products, oil, biodiesel or medicines. Plant scientists are now concentrating on tree species that can offer good quality fodder

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particularly in dry periods where quality fodder and their availability becomes a major problem.

Asaolu *et al*. (2012) stated that multipurpose trees can also be used as low-cost protein supplements which can increase digestibility, voluntary intake and overall productivity of animals fed on low-quality feeds.

Pigeon pea (*Cajanus cajan*), is a perennial leguminous crop with several uses: grain, vegetable, animal feed, green manure, and firewood (Gowda *et al*., 2012). The shrub is used in the existing farming systems of northern Ghana as a border crop. Pigeon pea is a legume that has been documented to have 20-22% protein, 1.2% fat, 65% carbohydrate and 3.8% ash (Sharma *et al*., 2011). It particularly contains more sulphur-containing amino acids (cysteine and methionine) (Akande *et al.*, 2010). It is reported to be widely adaptable to different climates and soil, although it is primarily grown in African provinces that obtain between 500 and 1000 mm of rain in two periods (Choudhary *et al*., 2011). Pigeon pea continue to be one of the best drought-resistant legumes (Bidlack *et al*., 2006) and is often the only grain plant that provides some grain yield for the period of dry spells when other legumes such as field beans have wilted and possibly dried up (Sharma *et al*., 2011). Perhaps the high nutritional content of pigeon pea is the most appropriate reason why it should find a substantial place among poor smallholder farmers in Africa.

Fodder from *Cajanus cajan* has been documented to increase the intake of low-quality fodder which bring about high animal live weight (Shenkute *et al*., 2013).

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Cajanus cajan may be used in ruminant diets as a protein supplement even at higher levels of inclusion (Corriher *et al*., 2010). *Cajanus cajan* is very digestible and supply high quality protein (Corriher *et al*., 2007). Comparing to other grains, the protein and *in vitro* digestible dry matter of *Cajanus cajan* grains indicated that they can be substituted for maize or cottonseed meal in livestock diets. While its effectiveness is not as soybean, pigeon pea seed was able to accumulate valuable protein levels and digestible dry matter under varied growing conditions (Rao and Northup, 2009).

Additionally, some forages contain higher amounts of crude protein (CP) than other forages conventionally used in animal feed (Azim *et al*., 2011), which increases intake of roughage by ruminants. Shrubs can have better access to water that infiltrates through the top soil and infiltrates into the subsoil and are capable of producing large quantities and quality forage in dry sites (Whitford and Duval, 2019). Other shrub species are long-lasting, need low upkeep and boost the sustainability of the farming system.

However, the cutting of *Cajanus cajan* forage at different stages of their growth, the nutritional composition and how the stage at which they are harvested affect the growth performances of the plants and animals have not been thoroughly studied and documented in the savannah agro-ecological zone of Ghana, hence the need for this study.

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1.1 RESEARCH OBJECTIVES

1.1.1 General objective

The main objective of the study was to determine the effect of different cutting regimes on the growth, yield and chemical composition of pigeon pea (*Cajanus cajan)* fodder in the Guinea Savannah agro-ecological zone of Ghana.

1.1.2 Specific objectives

The specific objectives of the research were to;

- Determine the nutritional quality of *Cajanus cajan* at three (3) cutting regimes.
- Determine the plant height, number of branches and stem diameter of *Cajanus cajan* at three (3) cutting regimes.
- Ascertain the biomass yield of *Cajanus cajan* at three (3) cutting regimes.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 FEED SOURCES FOR LIVESTOCK PRODUCTION

Lack of affordable and acceptable feed represents a major constraint to smallholder livestock farmers' effectiveness and the overall profitability of livestock production systems (Ayantunde *et al*., 2005; Lukuyu *et al*., 2011) because of its direct impact on animal productivity.

Feed has been documented as the most essential component of livestock production systems, constituting up to 70% of the total cost of production (Makkar and Beever, 2013; Buza *et al.*, 2014). Additionally, animal feed availability and utilization have many-sided implications in terms of farm economics, environment, product quality, product safety, animal health and animal welfare (Makkar, 2016).

Feeds can be categorized according to some of their universal properties. Feedstuffs can be grouped as either concentrates or roughages (Birhan and Adugna, 2015).

Concentrates have low fiber content and high protein or energy content or both. On the other hand, roughage is a bulky feedstuff with a high fibre content and low nutrient density. Cereals, crop residues, pastures, agro-industrial by-products and others form these two categories of feedstuff.

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2.1.1 Natural pastures

Natural pasture supplies the majority of livestock feed which is composed of native forage species. Natural pastures are often subjected to overgrazing. These natural pastures are primarily found on rangelands.

Rangelands comprise of indigenous forage plants which are grazed severely. Grazing takes place on permanent foraging areas, fallow land and on land succeeding harvest. The palatable species are always selected first by desired animals and such forages are always high in nutrients. As a result of their nutritive value, overgrazing can wipe them from the rangeland (Van Soest, 2018).

Availability and nutritive constituents of the indigenous pastures varies. These variations occur according to elevation, rainfall, soil type and cropping intensity. Natural pastures have been reported to be occupied by low palatable species (Solomon and Alemu, 2009).

The total forage production in Ghana is projected at 10,600,000 tonnes of which some 70% originate from grassland herbage (Oppong-Anane, 2001). Oppong-Anane (2001) assessed that the permanent natural pastures of Ghana's total land area is about 15%. Inclusion of the uninhibited Savannah forest area of 71,000 km^2 (30% of the total land area) increases possibly accessible area of land for production of pasture to 45% of the total land cover (Oppong-Anane, 2001). The crop-free areas and land for supplementary use is also estimated to be 2.5% of the total land area (Quaye *et al*., 2010).

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The coastal areas have growing seasons which last for seven months with a dry season of five months. Meanwhile, the northern savannah's growing season constitute five months and seven months of dry period. The pattern of growth of forages takes the rainfall trend within the different agro-ecological zones. The total dry matter yields annually have not changed much over the years. Approximately 80% of the yields are attained within the cropping season in both zones (Oppong-Anane, 2001). Shrub forages and forage trees are high in protein minerals and vitamins as well. (Oppong-Anane, 2001).

The quality in terms of nutritional composition of natural pastures also varies within the year. Protein content of the herbage is high by the onset of the rains but drops in the dry season. Thus, the protein content ranges between 8-12% dry matter in the rainy season and drops to 2-4% dry matter in the dry season (Oppong-Anane, 2001). Levels of phosphorus also decline from 0.16% in the rainy to 0.06% in the dry season.

2.1.2 Crop Residue

Crop residues comprise of plant or crop resources which remain after they have been harvested and processed. As the human population keep increasing, greater proportion of the land is used to cultivate food crops to meet the demand of the population (Harris, 2002). This results in the production of large volumes of crop residues (Ansah *et al*., 2006).

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Bulk amounts of crop residues are used in many countries as animal feed, but much remains wasted for a variety of reasons or used for different purposes (Tesfaye and Chairatanayuth, 2007). Mussatto *et al*. (2011) stated that above 1000 million tonnes of cereal residues are generated each year in the unindustrialized countries. About the use of crop residues for feeding livestock, Devendra and Leng (2011) stated that in both technologically advanced and unindustrialized countries, residues from crops is about 24% of the total feed energy appropriate for ruminants. It was specified that if all crop residues were carefully utilized, the overall production would provide 3.4 tons and 6166 Mcal metabolizable energy (ME) per year in the whole world. Studies by Valbuena *et al*. (2012) reported that in various areas of semi-arid sub-Saharan Africa countries, remains from crop processing give as much as 45% of the feed consumed by ruminants each year and 80% during times of severe feed shortages.

A country like Ghana is capable of saving about 186 million kg of livestock weight that is usually lost during the 120-day dry period from its 2.3 million tonnes and beyond of cereal crop residues produced every year (Ansah, 2015).

Residues of crops cultivated in the savannah zone that serve as feed for ruminants include sorghum, millet, cowpea, groundnut and some tubers like yam and cassava (Sanon and Kanwe, 2010). Millet which is a principal grain crop cultivated in the dry semi-arid zone of most West African countries yields residues estimated to be 1000 to 2000 kg DM/ha (Corbeels *et al*.,2014). Groundnut haulms remain one of the most important crop residues utilized as feed for ruminants.

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However, the accessibility and exploitation of these crop residues is dependent on the percentage of crop cultivated and the yield of the plant parts as well as the type of crop. Straws of leguminous crops usually have higher nutritional value, a higher forage quality and are thus nutritionally superior to cereal straws. Stovers have greater nutritional content than straws as regards consumption and digestibility of organic matter (Walli, 2004). Duncan *et al*. (2016) reported that cereal residues are utilized *In situ* whereas residues from leguminous crops are used as stall feeds. Haulms of cowpea are stated to produce 400 to 1200 kg/ha in Mali and 200 to 500 kg/ ha in Burkina Faso when intercropped with sorghum (Valbuena *et al*., 2015). 400 to 700 kg/ha have been reported in Mali and 700 to 1500 kg/ha in Nigeria for groundnut. Devendra and Leng (2011) specified that if all the possible accessible crop residues could be exploited for feeding, each ruminant would obtain above 9kg DM and about 17 Mcal ME/day, thus essentially covering requirements. However, a much lower level of exploitation is possible because of difficulties of gathering, conveyance, storage and processing, alternative uses, seasonal availability, and more importantly, their poor feeding value.

Koura *et al*. (2016) reported that cereal crop residues have higher structural carbohydrates (NDF: 84.98 ± 2.34% DM) and very low CP (4.16 ± 0.69% DM) while legume residues are richer in CP (11.77 ± 4.49% DM) and lower structural carbohydrates (NDF: 56.12 ± 10.32% DM), but more lignified (ADL: 9.33 ± 2.97 and 12.36 ± 2.88% DM for cereal and legume, respectively). The energy content in cereal residues are lower (ME: 5.86 ± 0.73 MJ/kg DM) than in legume (ME: 10.89

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± 3.89 MJ/kg DM) crop residues (Koura *et al*., 2016). Devendra and Leng (2011) supported other reports that residues from farm and crop are low in metabolizable energy and crude protein.

Main limitation to make use of the straws by ruminants is the interconnecting of cell wall polysaccharides with the tough biopolymer lignin (up to 15%) which avoids their microbial biodegradation in the rumen (Mahesh and Mohini, 2013). This decreases the overall nutritive value for ruminants. A significant distinction in the superiority of straws in terms of digestibility as well as metabolizable energy occurs among different cultivars of rice, wheat, barley and stovers of sorghum, pearl millet along with groundnut haulms (Blümmel *et al*., 2010).

2.1.3 Agro-industrial by-products

Agro-industrial by-products originate from the treating of commercial crops in the food processing industry and the fibre industry. They have high nutrient content particularly protein (especially those obtained from animals) and sometimes in starch and are generally low in fibre (Sanon and Kanwe, 2010). The amounts of agro-industrial by-products produced is determined by the availability of the material being processed and the industrial unit capacity. Kabi *et al*. (2013) reported that agro-industrial by-products play an important role in the supply of metabolizable energy and CP to smallholder dairy farms in sub-Saharan Africa, which are essential components for optimum productivity in the feeding of dairy cattle.

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Fishmeal is a nutritious feed supplement derived from the body of fish. It comprises around 10% moisture, 55% protein, 6.9% fat, and 25% mineral salts, mostly 5.4% Ca and 3.4% P. It also contains vitamin A, vitamin D and few vitamin B-group leaders. The ultimate source of vitamin B12 is fishmeal (Hansen *et al*., 2007).

Molasses is a viscous by-product acquired through the processing of sugarcane or sugar beets into sugar. One major source of fermentable carbohydrate is molasses (Faber *et al*., 2011) and is also a freely accessible source of sugar and phosphorus to the animal body. In various stages of development, citrus by-products, such as fresh citrus pulp, citrus silage, dried citrus pulp, citrus meal and fines, citrus molasses, citrus peel and citrus-activated sludge were used as substitute feeds for ruminants (Bampidis and Robinson, 2006). By-products from mango such as mango-seed kernels contain 6% DCP and 70% TDN, and 5–7% tannin and can be recycled as an ingredient in livestock foods (Sanon and Kanwe, 2010).

The key organic compounds in several agro-wastes are lignocelluloses with a dense, partly crystal-like structure comprising of linear and crystalline polysaccharides, cellulose, non-cellulose and non-crystalline heteropolysaccharides, hemicelluloses, and non-crystalline lignin branching (Glasser *et al*., 2000).

However, the quantity of by-products used as animal feed is influenced by the abundance of resources and the technological equipment used to produce, maintain and develop them. These makes exploitation of agro-industrial by-products limited.

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2.2 LIMITATIONS ASSOCIATED WITH THE UTILIZATION OF NATURAL FORAGES

2.2.1 Quantity and quality of forages

Many natural pastures in Ghana are underutilized and the grasses are characterized by very fast growth with high feeding only in the initial part of the wet season, decreasing rapidly afterwards and being exceptionally small in the dry season. Even where dry matter supply is sufficient in the dry season, protein, vitamins, and minerals are highly deficient. In terms of quantity and consistency, the unavailability of feed negatively impedes the economic and reproductive efficiency of grazing animals (Oppong-Anane, 2001). Natural grazing is the most important source of animal feed and in the plains, livestock production rely on it for their feed. However, grazing lands cannot provide the nutrients requirements of animals mainly in the dry season because of their characteristic low output and quality.

2.2.2 Uncontrolled grazing

Grazing which involves extra fouling and trampling of forages have negative effects on both the forages and the soil. This is particularly observed in areas that are close to water source. Agricultural soils are continuously in threat of degradation with reduction in infiltration, reduced permeability and a decrease in water-holding ability (Rugadya, 2006). Though the undesirable impact of overgrazing by indigenous herds of cattle may appear negligible, the invasion of transhumant livestock influences a country to drastic overgrazing and damage to the soil that

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would be difficult to reverse (Rugadya, 2006). The cattle also create a possible means of introducing certain disease pathogens into the indigenous livestock (MOFA, 2015).

2.2.3 Bush and weeds interference

Fire is a common constituent of tropical habitats in which its non-existence has caused bush intrusion (Rugadya, 2006). In areas of pastoralism, misinterpretation of the indigenous information has led to restraint of controlling fire. Bush fires normally occur because of illegitimate and unrestricted bush burning after harvest to eradicate muribund foliage, or for purpose of hunting. Bush fires occurrence can be associated with human existence and ruminant density, since fire occur normally in areas with high density of ruminants (Archibald, 2016).

Natural lands which have been overgrazed are occupied by unpalatable or indigestible weeds and wooded species of plants. This is due to the reason that, the desirable and palatable species are continually grazed by the animals leaving the undesirable species.

2.3 ORIGIN, DISTRIBUTION AND DESCRIPTION OF *CAJANUS CAJAN*

Pigeon pea (*Cajanus cajan* (L.) Millsp) is a perennial multipurpose associate of the family Leguminosae. Studies conducted by Mallikarjuna *et al*. (2012) indicated that pigeon pea developed from its closest wild relative *C. cajanifolius*. However, the only domesticated species under Cajaninae is *Cajanus cajan*. The exact origin of

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pigeon pea is still debatable. Most likely, migrants of the 19th century who came to Africa to become railway workers and shopkeepers brought the crop from India to East Africa. It then went up the Nile River to West Africa and finally to the Americas (Hillocks *et al*., 2000). *Cajanus cajan* is present in both hemispheres from 30 ° N to 30 ° S and from sea level to 2000 m above sea level (Ecocrop, 2016).

The legume is grown exclusively in rain-fed environments with variable temperatures, elevations and latitudes (Silim *et al*., 2006). The crop is described to have a wide range of flexibility to diverse climates and soil (Odeny, 2007) although it is mostly cultivated in areas of Africa that obtain 500 –1000mm range of rain in two seasons (Choudhary *et al*., 2011). *Cajanus cajan* is adapted to a varied array of soil types. Well-drained soils give the best growth and will not do well in soaking situations and can be cultivated in a range between pH 4.5–8.4 (Cook *et al*., 2005). *Cajanus cajan* develops best in warm environments (65–86°F), and can do well in temperatures exceeding 95°F (Cook *et al*., 2005). 64°F of Soil temperature and above is essential during planting, as reduced temperatures will prolong the period of being established (Silim and Omanga, 2001). It is especially heat-tolerant, and grows best in areas without frost. While delicate to frostiness, pigeon pea continue to grow in temperatures around 0°C but large plants can withstand light cold conditions (Flower and Ludlow, 1986). The capacity of *Cajanus cajan* to survive severe drought better than other legumes is attributed to its deep seated roots (Battaglia and Covarrubias, 2013) and regulation of osmotic activities in the leaves

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(Subbarao *et al*., 2000). The legume also continues photosynthetic role in stress conditions.

Cajanus cajan continue to be one of the most drought-tolerant legumes (Bidlack *et al*., 2006) and is however the only crop which yields some grain in dry periods when other legumes have wilted and may have dried up (Odeny, 2007). *Cajanus cajan* is known globally as the sixth most valuable legume food crop (Odeny, 2007).

Cajanus cajan has deep tap roots which prolong upright to two meters and extend horizontally through adjacent roots. The Root is well established as high as 60cm deep in the soil profile (Sekiya and Araki, 2013). The root proliferation is related with the length of crop and growth pattern (Adjei-Nsiah, 2012). Tall varieties produce roots that elongate and grow deeper, while spreading forms produce more distributing and compact root systems (Singh, 2005). Also, the plant's deep root structure enables the moisture to be extracted from deep strata of the soil and enable the crop produce more yield (Odeny, 2006). The deep root system allows the crop to grow well under drought conditions with annual rainfall of 635 mm (Baryeh and Mangope, 2003).

A woody stem originates from the base of each petiole. *Cajanus cajan* exhibit great flexibility by means of regulating its branching actions depending on the availability of space between plants but branching pattern is determined by the genetic constitution (Frankel and Galun, 2012).

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The leaves are arranged spirally and are pinnately trifoliate in nature. The terminal petiole is greatly variable and reaches a length of 10-20 mm while the adjacent petiole is usually 2-3 mm long. Leaf size differs from 6-17 cm and there is the presence of simple or glandular hairs on them (Ranganathan *et al*., 2001). Leaves and stems are pubescent. The leaflets are oblong, lanceolate about 5-10cm in length and 2-4cm breadth.

The flowers are clustered in racemes of about 5 to 10 at the climaxes or axils of the twigs. Its flowers are papillonaceous and are largely yellow in colour (Bekele-Tesemma, 2007). Seeds are bivalve and found in pods of about 4 cm long, 1 cm wide, 3-4 mm thick with upper suture swollen. Usually the pods are 5-9cm in length and 12-13 mm wide, smooth and red, green or brown containing 2 - 9 seeds (Mula and Saxena, 2010). The husks of the seeds bear deep tilted furrows pointing out the septa among the seeds (Singh, 2005).

The plant has a lifespan of equal to 5 years and its reproduction with some tetraploid is 60% autogamous and generally diploid. Most *Cajanus cajan* plants have oblong, straight or sickle shaped pods. The majority of seeds are ovoid in shape with red, black and brown colours (Hluyako *et al*., 2017).

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2.4 IMPORTANCE OF *CAJANUS CAJAN*

2.4.1 Soil nutrient enhancement

Cajanus cajan has been considered a potential crop to sustain soil fertility due to its adaptability and tolerance to low soil fertility and capacity to recycle nutrients (Adjei-Nsiah, 2012). *Cajanus cajan* has been reported to fix high amounts of nitrogen into soil (Olujobi and Oyun, 2012). It also has the capacity to mobilize nutrients especially phosphorus from the deep soil horizons and to enhance soil organic matter concentration through litter fall and the decomposition of roots (Adu-Gyamfi *et al*., 2007).

Mtambanengwe *et al.* (2004) stated that, *Cajanus cajan* has the potential to fix nitrogen of 46KgN/ha for the short period varieties and 150KgN/ha for the long duration varieties. Abunyewa and Karbo (2005) reported an increase of 48.5% total soil nitrogen on *Cajanus cajan* fallow after a two-year period whiles Diekow *et al*. (2005) stated 28% increase in soil nitrogen. There was a significant improvement of soil nitrogen and phosphorus when *Cajanus cajan* was intercropped with millet as reported by Guggari and Kalaghatagi (2005). Sakala *et al*. (2000) and Snapp and Silim (2002) stated that *Cajanus cajan* has the capacity to increase soil organic matter of 1-4.5 t/ha from leaf biomass and senescent material. However, the contribution to soil organic matter is site specific and based on conditions as residue management and the extent of the crop in the planting field (Kätterer *et al*., 2011). It has been documented that, the higher the soil organic matter content, the larger

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the stability of soil aggregates especially in soils that contain minerals (Kimetu and Lehmann, 2010). Three years continuous intercropping of wheat and *Cajanus cajan* led to accumulation of carbon in the soil profile by 13.9% (Singh *et al*., 2005). This accumulation of carbon increased when nitrogen and phosphorus fertilizers were applied. Diekow *et al*. (2005) also reported similar findings in a long-term trial. In the trial, maize-*Cajanus cajan* cropping systems increased soil carbon by 26% after seventeen (17) years of cropping.

Cajanus cajan possesses a strong deep rooting system which penetrates through the soil therefore acting as biological plough and which loosens the soil and helps in infiltration and aeration enhancement. *Cajanus cajan* cropping has been reported to increase soil moisture storage (Chirwa *et al*., 2004). This is attributed to the plants ability to thrive well in drought environment and poor soil conditions. Tenakwa *et al*. (2019) also reported higher plant height of Napier grass when it was intercropped with *Cajanus cajan.* The Napier grass in the *Cajanus cajan* intercrop may have benefited from the available nitrogen fixed by the legumes.

2.4.2 Food and nutrition

Cajanus cajan is a significant foodstuff that can be cultivated in rain-fed environments with least inputs. *Cajanus cajan* contains protein, starch, calcium, magnesium, crude fiber, fat and minerals (Saxena *et al*., 2010). *Cajanus cajan* is described to have quality protein and is a good basis of amino acids excluding methionine (Sharma *et al*., 2011). When combined with cereals, *Cajanus cajan*

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make a balanced human food (Akporhonor *et al*., 2006). It is used in mixture with soya bean to produce one of the popular fermented, flavouring product soy sauce (Muangthai *et al*., 2009). The chemical constituent of *Cajanus cajan* has been estimated to contain protein of 21.5% whiles the fibre content is about 2.5% (Akande *et al*., 2010). It has appreciable quantity of the basic amino acid phenylalanine; which has been documented to have sickling properties, tryptophan which is associated with the treatment of hypertension (Ajaiyeoba *et al*., 2005), methionine and lysine, the last mentioned, among different properties help in retention of calcium (Ahmad *et al*., 2008). *Cajanus cajan* contains minerals like potassium, magnesium, calcium and is altogether low in sodium. The low sodium substance may be one reason it is utilized in enthno-drug for the treatment of hypertension. It additionally have vitamins, for example, vitamin A, niacin and limited quantity of thiamin, riboflavin, folate and pantothenic corrosive (Akande *et al*., 2010).

Cajanus cajan flour is an incredible segment in the snack industry and has been approved to increase the dietary benefit of pasta without influencing its sensory properties (Torres *et al*., 2007). In some cases, young pods are reaped (before the seeds create) and cooked like French beans in curries. Other food products derived from *Cajanus cajan* are crisp sprouts, tempe, ketchup, noodles, snacks and several others (Saxena *et al*., 2002).

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2.4.3 Medicinal properties

Chemical constituent investigations have indicated that *Cajanus cajan* have a lot of pharmacological properties. The chemical compounds of *Cajanus cajan* compose of isoguercitrin, quercetin, quercetin-3-methyl ether and 3-hydroxy-4-prenyl-5methoxystilbene-2-carboxylic acid (Green *et al*., 2003). The rich content of phenolic and flavonoids contained in leaf extracts are promising for their bioactivity such as antipasmodic, anti-imflammatory, antimicrobial and antioxidant activities (Zu *et al*., 2010).

Wu *et al*. (2009) stated that extracts from pea leaves can be beneficial natural antioxidants and potentially applicable as medicinal products by the health food industry. Pinostrobin, a synthetic flavanone derived from the leaves, has anti-inflammatory property and inhibits brain depolarization by sodium channels (Nicholson *et al*., 2010). Cajanol, an isoflovanone located in the roots is found to possess anticancer chemicals which prevents cancer (Luo *et al*., 2010). Cajanuslactone, an extract from *Cajanus cajan* leaves is found to possess good antibacterial property against *S. aureus* (Kong *et al*., 2010).

The hydro-alcoholic extracts of the above ground parts of *Cajanus cajan* were assessed to contain anthelmintic properties which prevent intestinal parasites and round worms (Pal *et al*., 2008). It was also reported that the crude ethanolic extracts of roots contain genistein, genistin, longstylin C, longistylin A and cajanol, which are antioxidant and anticancer chemical (Pal *et al*., 2011). *Cajanus cajan* is used in

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the controlling of discomforts as traditional Chinese medicine and as a relaxing agent (Ahsan and Isalam, 2009). Yuan-gang *et al*. (2010) indicated that *Cajanus cajan* is being used for handling diabetes, sores, skin, bedsores, measles, jaundice, dysentery and other illness, for expelling bladder stones and alleviating menstrual period.

2.5 ESTABLISHMENT OF *CAJANUS CAJAN*

Cajanus cajan may be grown to produce seed, forage or both. Some cultivars have been grown for double purposes (Rao *et al*., 2003). *Cajanus cajan* cultivation can be propagated solely or along with cereal crops or with other grain legumes such as cowpea (Bekele-Tesemma, 2007). *Cajanus cajan* are adapted to a broad kinds of soil situations and types. They prefer well drained soils and do not tolerate waterlogged ground. The crop develops well in all kinds of soils, ranging from sandy to heavy loams, the best being well drained medium heavy loams.

It needs a pH level ranging from 4.5 – 8.4. Increased acidity prevents nodulation; plants become chlorotic, with die-back disease (Hluyako *et al*., 2017).

Cajanus cajan is best established by seeding directly into a well-prepared seedbed. Seed inoculation is generally not required; however, a rhizobium cowpea group strain can be used. Planting can be done by placing the seeds about 4 to 5 cm deep; the deeper figure being in hand dibbling. Seeds can be spread at 45 to 67 kg / ha seed rate, preferably a maize planter can be used for seeding. The plants are planted at a spacing of 70 to 90cm for long and medium duration plants and germination

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will depend upon soil temperature. At soil temperatures above 60°F, *Cajanus cajan* will germinate within two (2) weeks if sown directly in the ground (Hluyako *et al*., 2017).

Supplementation of water by irrigation for the first two growing months under dry conditions of less than 400 mm of average rainfall per annum as a perennial plant. The irrigation must stop from flowering to harvesting to decrease the injury done by pests and diseases (Hluyako *et al*., 2017). *Cajanus cajan* has a very diverse growing forms. Depending on the cultivar, the place and period of sowing, flowering can take place between 65 - 100 days to about 430 days (Mesapogu *et al*., 2012).

Cajanus cajan can be established as an annual shrub or a perennial plant. It has been successfully used with cereals and legumes in alley cropping systems. The plant will live on up to five years under good management. When the plant grows older, the stem becomes woodier, and leaf regeneration declines (Silim and Omanga, 2001).

Cajanus cajan is resistant to drought, and because of its deep root system, can survive under very dry conditions. It has been found to develop over a dry season of six months (Cook *et al*., 2005), however, flowering will be hindered and seed yields will decline under long periods of drought (Silim and Omanga, 2001). The crop is less adapted to moist wet conditions.

Cajanus cajan normally shows slight reaction to nitrogen and phosphorus fertilizers and needs ample calcium, potassium and magnesium. It has the potential to thrive in a drought-prone climate and low-input farming systems, and to provide good

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economic returns. The peas' root nodules enrich the soil with some 40 kgN/ha added. Pigeon peas can be used as crop for green manure (Hluyako *et al*., 2017).

2.5.1 Weeds, diseases and pests' control

Due to its rate of growth in its initial stage, *Cajanus cajan* is sensitive to competition with weeds, particularly in the initial 45 to 60 days of growth. One of the most important contributing factors to high yields is active weed control at the early stages of growth of the plant, especially during the first 4 to 8 weeks (Hluyako *et al*., 2017). It can restrict weed growth in the field once the crop is well established. Some pigeon pea varieties are said to be unaffected by the root-knot nematode. Also, it has been stated that *Cajanus cajan* roots inoculated with useful mycorrhizal fungi not only enhance the plant's nutrient resource but promote the plant's nematodes and disease resistance. Such pests are also found naturally in the soil (Sharma *et al.*, 2011). When established as a green manure crop, pigeon pea will harbor few insect pests. Conversely, if allowed to develop pods, pigeon pea may draw pod borers and *agromyza* fruit flies. Pigeon pea can be a host of root-knot and reniform nematodes but this varies between cultivars (Saxena *et al*., 2010). Insect pests which include pod-sucking bugs, pod fly and pod borers and diseases like rust, downy mildew and *Cercospora* leaf spot which have an opposing impact on the output of pigeon peas; they also promote poor-quality seed production. Pests and diseases decrease the number of plants standing. However, they can be metigated by using pest-resistant cultivars, crop rotations, elimination of weeds and inoculation with a rhizobial cowpea group strain and cereal intercropping (Hluyako *et al*., 2017).

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2.5.2 Reproduction

Pigeon peas are prone to day-lengths demanding day-lengths below 12.5 h for flowering and seed development. Nevertheless, several variations have been produced to the day with different responses. Short varieties can begin to bloom in 60 days after establishment, while the taller cultivars flower considerably later, 180–250 days after planting.

Pigeon pea has cleistogamous flowers preferring self-pollination. In pigeon pea, however, 14-20 per cent of natural outcrossing occurred (Saxena *et al*., 2016). Pigeon pea is also pollinated entomophilically by cross. There is occurrence of self-pollination in the bud before the flowers open, while the insects are used to perform cross pollination. Saxena and Singh (2000) recorded low incidence of self-fertilization after they pollinated flower buds without emasculation with remote pollen. Pigeon pea is protogynous and the stigma is open 68 hours before anthesis and receptivity to stigma is kept even 20hours after anthesis (Choudhary *et al*., 2012).

A number of pollinators are attracted by large and bright colored flowers coupled with the company of nectar. Due to insects in pigeon pea, an average 20 per cent crossing out was observed (Saxena *et al*., 1990). Fertilization happens after pollination, within 48-54 hours. Many variables, such as genotype flowering habit, insect population presence, temperature, humidity, wind velocity and wind direction, influence natural outcrossing at a given time and space (Saxena *et al*.,

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1990). The outcrossing rate varies from place to place depending on the extent and climatic conditions of the pollinator bees. Outcrossing at different locations ranged from 20 to 70 per cent (Saxena *et al*., 2010). High rate of out crossing in pigeon pea creates difficulties in the conservation of varietal purity.

2.6 GENETIC DIVERSITY OF PIGEON PEA (*Cajanus cajan*)

To undertake molecular breeding in any seed, genomic tools such as molecular markers, genetic maps and transcriptomic or genome sequence data are the fundamentals. Despite the existence of substantial variability for different traits between pigeon pea landraces and varieties, effective molecular breeding programs are still in development. The determinations have focused only lately to improve some genomic resources in pigeon pea.

Wild pigeon pea relatives are known to have many significant agricultural genes that offer resistance to a variety of biotic and abiotic tensions such as sterility mosaic disease (SMD), phytophthora blight, root-knot nematode, pod borer, pod fly, salinity and drought (Rao *et al.,* 2003; Bohra *et al.,* 2010).

Wild pigeon pea species aid as a key basis of genes for resisting insects and disease that can be introgressed into the genotypes that are cultivated. Wild pigeon pea (*Cajanus volubilis*) was identified as the source of resistance to mosaic sterility disease (Mallikarjuna *et al*., 2011). Breeding by hybridization with wild relatives for improved plant types in pigeon pea has met with low success rate (Saxena *et al*., 2006). For effective crop improvement, research efforts in the future need to

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emphasise on developing a noble understanding of the crop at the molecular level. Existing standard cultivars have become heterogeneous due to regular out-crossing for many economic characters, such as disease tolerance and others.

Recently, more and more attempts are being made to develop pigeon pea by using wild relatives to transfer male sterile cytoplasm from its related species. Morphological characters have historically been used to identify pigeon pea cultivars and their wild relatives which require the plants to grow to full maturity.

Historically, farmers from the landraces have preferred desirable traits in pigeon pea to match their production systems and uses. The pigeon pea varieties now have four distinct durations, each of which is suitable for a specific agro-ecosystem. These include the extra short cultivars which mature in less than 100 days, the short cultivars which matures in 100 –120 days, medium cultivars which mature in 140 – 180 days and the long cultivars which mature in above 200 days.

Also, unique resistant African *Fusarium* lines were published (Gwata *et al*., 2006). In excess of 15 improved cultivars have been produced for Africa over the last 10 years but published in only a few countries. As noted, the majority of pigeon pea production in all additional African countries requires the use of traditional varieties.

The long-term genotypes usually grown in Africa use specific sources of phosphorus more efficiently (Yang *et al*., 2006) than their counterparts for the short-term. The essential phosphorus concentration requirement for dry biomass generation in pigeon pea is little relative to other major protein fodder or food crops such as

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soybean owing to its effective incorporation of external orthophosphates into deposited phosphate (Adu-Gyamfi *et al*., 1990).

There have been records of wild relatives showing large variability in their response to salinity (Subbarao *et al*., 1991) and thus signify genetic resources for improving this characteristic in cultivated pigeon pea. There was also known resistance to acid soils (Choudhary *et al*., 2011).

2.7 BIOMASS YIELD OF *CAJANUS CAJAN*

Forage yield and grain differ extensively depending on the ecological settings and the care given to the crop. Perennial varieties which are tall are amenable to pruning as fodder, but also as green manure (2.6 % nitrogen). The time needed to attain maturity can vary greatly due to seed variety, temperature, and photoperiod. *Cajanus cajan* is a short-day plant which requires 12.5 hours of daylight to initiate flowering and seed production (Cook *et al*., 2005). *Cajanus cajan* requires 65-80 days to flower and an additional 50-75 days to produce mature seeds (Silim and Omanga, 2001), although several varieties have been established to flower earlier. It is known to be 60% self-pollinated (Cook *et al*., 2005), but varieties cultivated within 2–3 miles may be cross-pollinated (Silim and Omanga, 2001).

Cajanus cajan is a significant seed production plant: seed yields worldwide range from 0.5 to 2 t/ha (Ecocrop, 2016). In extreme South Sahel environments, 650 kg/ha of beans could be produced as human food (Mula and Saxena, 2010). Yields of seeds were estimated to be as much as 5 t/ha in India and around 3-4 t / ha in Indonesia

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under optimal conditions (Mula and Saxena, 2010). In Asia, yields were 700 kg / ha in sole cultivation in marginal areas while maize intercropping systems yielded only 175 kg / ha (Mula and Saxena, 2010). Adebayo *et al*. (2017) reported seed yields of 1,024 kg/ha, 1,936 kg/ha and 1,912kg/ha when *Cajanus cajan* was planted with planting distances of 30cm x 30cm; 45cm x 45cm and 60cm x 60cm respectively.

The yield of forage varies from DM 20-40 t / ha. Rates of fodder and stalks as high as 24 t DM / ha were recorded in Sahel, and further analysis of the use of pigeon pea as a forage plant in this area has been suggested (Mula and Saxena, 2010). Under optimum conditions, up to 40 t DM / ha could be expected (Bode *et al*., 2018). Forage yield of 8.8–10.5 t/ha and 7.0 - 7.8 t/ha were also recorded by Alexander *et al*. (2007) in 2003 and 2004 respectively. Studies carried out in Australia, Colombia, China and India reported that *Cajanus cajan* produced 30-50 t/ha of fodder yield as reported by Sharma *et al*. (2011).

2.8 NUTRITIVE VALUES AND QUALITIES OF *CAJANUS CAJAN*

Cajanus cajan has the ability to grow in harsh environment with severe drought stress and poor soil fertility even under defoliation. Nutritionally *Cajanus cajan* holds high contents of proteins and vital amino acids like lysine, methionine and tryptophan. Dry *Cajanus cajan* seeds contain protein (20-22%), carbohydrate (57.3%), fat (1.5%) and ash (8.1%). Two globulins are found in the protein of *Cajanus cajan*, cajanin and concajanin constituting 58% and 8% respectively (Saxena *et al*., 2002). In combination with cereals, *Cajanus cajan* grains makes a

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well-balanced human food which makes the crop potential to reduce hunger and malnutrition while maintaining sustainable productivity of smallholder cropping systems (Saxena *et al*., 2010).

The nutritional contents of some foods are enhanced by the inclusion of pigeon pea in most parts of India where it supplies vitamin B, calcium and phosphorus (Mula and Saxena, 2010).

Cajanus cajan is a good source of dietary minerals such as calcium, phosphorus, magnesium, iron, sulphur and potassium, and water-soluble vitamins, particularly thiamine, riboflavin and niacin. *Cajanus cajan* also contains more nutrients, ten times fat, five times vitamin A and three times vitamin C relative to ordinary peas (Sharma *et al*., 2011). The seeds are being used as feed for animals, and good fodder value for post-harvest items such as haulms, leaf and young stems. Pigeon pea has a long cooking time and low nutritional value relative to cowpea for humans (Amaefule and Obioha, 2001). Amaefule and Onwudike (2000) reported that *Cajanus cajan* contain more amount of nitrogen (21-30% CP), thus makes it eligible as a appropriate protein source for ruminants. *Cajanus cajan* provides major amount of energy through carbohydrates (60-70%), lipids (1-7%), dietary fibres and minerals (2-5 %). Also the oil from the seeds contain adequate levels of thiamine, riboflavin and niacin (Torres *et al*., 2006).

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2.9 *CAJANUS CAJAN* AS LIVESTOCK FEED

Many legume crops are being extensively utilized for animal production in terms of feed. One of the preferred species of legume utilized is *Cajanus cajan*. As a multi-purpose plant species, *Cajanus cajan* serve several functions in animal feeding. Mean nitrogen content in *Cajanus cajan* forage is estimated to be between 3.4% to 3.6%, indicating that *Cajanus cajan* forage can serve as an active supplement to nitrogen deficient feedstuffs (Alexander *et al*., 2007). The foliage and husks are valued and appetizing protein-rich diet for animals. Orwa *et al*. (2009) reported that bees feed on *Cajanus cajan* leaves to yield a honey that have a characteristic colour in the honey comb. Omokanye *et al*. (2001) stated that fresh *Cajanus cajan* leaves were most preferred by sheep compared to other eight browse species. Green leaves and young branches of *Cajanus cajan* are used in rabbitries in western and eastern African countries including Uganda as protein rich forage (Djago *et al*., 2010).

Husks of pods of *Cajanus cajan* is reported to be exceptional replacements to rice bran as a diet for Shrimps in relation to existence, development, fertility, and Nauplius making (Yoganandhan *et al*., 2000). Upon processing, by-products like seed coats, broken bits and powder are useful feeds for pigs, poultry and cows (Saxena *et al*., 2002).

Seed and pod meal comprise 5-10% crude protein and 2-4% fat and ash. Studies conducted in the Philippines by Arif *et al*. (2017) revealed *Cajanus cajan* to be a low-cost source of poultry feed. Poultry birds fed 15% of *Cajanus cajan* seeds and

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85% of broiler mash produced a weighter and higher daily weight gain, increased feed conversion competence and good quality carcasses (Arif *et al*., 2017).

Cajanus cajan produces rapid forage and can be used as a perennial forage crop for short durations. The leaf and young pods can be harvested and stored, or freshly fed (Sharma, 2011).

2.10 DIGESTIBILITY AND DEGRADABILITY OF *CAJANUS CAJAN*

Cajanus cajan forage is rich in proteins, but its high fibre components (especially ADF and lignin) decrease digestibility and restrict its use. It can be regarded as a medium to low-energy forage in terms of quality.

Dry matter digestibility of fresh and dried (hay) *Cajanus cajan* leaves are within the range 50-60% (da Silva *et al*., 2009). *In vivo* organic matter digestibility of *Cajanus cajan* hay was found to be near to that of cowpea hay (55-56%) but lower to that of the other legume hays such as soybean (Foster *et al*., 2009). *Cajanus cajan* fed as haylage seems clearly to be less digestible when compared to other warm season legumes also fed as haylage (Foster *et al*., 2009). Pigeon pea forage is considered as low *in situ* ruminal dry matter, NDF and nitrogen disappearance kinetics when compared to other warm-season legumes or poor-quality forage hays, with much lower possibly degradable fraction and much greater undegradable fraction (Corrêa *et al*., 2012; Foster *et al*., 2011). This limits their possible use for high-production animals like dairy cows. Rao *et al*. (2002) reported in vivo dry matter digestibility of 44% when sheep was fed on pods of *Cajanus cajan* alone. Similar values were

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recorded when goats were fed on *Cajanus cajan* crop residues with *in vivo* dry matter digestibility being in the range of 47 – 54%.

2.11 PHYTOCHEMICAL PROPERTIES OF *CAJANUS CAJAN*

Phytochemicals are important chemicals occurring virtually at different concentrations and in plants and their various parts. In *Cajanus cajan*, phytochemical compounds contain many bioactive substances, such as tannins, sugar reduction, anthroquinone, triterpenoids, alkaloids, phenols, saponins and flavonoids (Titanji *et al*., 2008).

Alkaloids occur in *Cajanus cajan* seed extracts, fresh and dry leaf extracts. The existence of alkaloids is due to the anti-tumor, anti-inflammatory and anti-microbial effects. Alkaloids are also used as medicinal agents and their derivatives for their analgesic and bactericidal effects (Castilhos *et al*., 2007). The presence of these compounds is also directly related to the beneficial anti-sickling properties that *Cajanus cajan* possesses.

There are clear scientific proofs of flavonoid's intrinsic ability to modify the body's reaction to allergens, viruses and carcinogens. Flavonoid have been referred to as the biological response modifiers of nature. Flavonoids are very essential, as the free radical scavengers are named. They prevent damage to oxidizing cells and protect against all forms of cancer (Salah *et al*., 1995). These minimize heart attack levels and are distributed as phenolic compound groups. Flavonoids occur in fresh and dried leaf extracts, but are absent in *Cajanus cajan* seed extracts. The existence of

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flavonoids causes anti-parasitic, anti-bacterial and anti-fungal activities (Sen *et al*., 2005). Flavonoids are also diuretic and have anti-allergic, anti-cancer, anti-inflammatory effects (Evans, 2009). Saponosides decrease hypercholesterol, hyperglycemia and have an inflammatory effect according to Ngbede *et al*. (2008). Quinones have antispasmodic, anticoagulant, and antihypertensive properties (Tosun *et al*., 2009). Extraction of the *Cajanus cajan* flavonoid fraction strengthened cytotoxic and genotic results in animals caused by mutagenic agents (Titanji *et al*., 2008). Quercetin is one of the flavonoids extracted from *Cajanus cajan*, a very active compound against mutagen-induced cells and mutagenicity in the hepatic cells of rats, thus providing protection against DNA damage and germ chromosomal changes (Titanji *et al*., 2008). Some flavonoids have also been reported to act like certain coumarins in inhibiting the development of giant cells in cultures infected with HIV (Evans, 2009).

Tannins enhance the digestibility and palatability of the forage by interacting with proteins and, as a result, the tannin content is considered to be a significant feature of leguminous crops and was used as a selection criterion for pigeon pea improvement programmes (Godoy *et al*., 2005). It is known that tannin sacs are normal in Caesalpinoideae and exhibit antiviral, antibacterial, and anti-tumor activity. It has also been reported that some tannins can selectively inhibit HIV replication, and are also used as a diuretic. Plant tannins are also a commercial source of tannic acids and tanning agents (Evans, 2009). Tannins are indicated for

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their astringency and bitter taste, fastening wound healing and inflaming the mucus membrane (Okwu and Okwu, 2004).

Plant saponins have been in use for their detergent properties for many years. It is used as mild detergents and in the staining of intracellular histochemistry to allow access to intracellular proteins by antibodies. This is used in medicine for hypercholesterolaemia, hyperglycaemia, antioxidants, anticancer, anti-inflammatory and weight loss (Ngbede *et al*., 2008). It has also been reported that this compound has anti-hypercholesterol, anti-inflammatory, cardiac depressant properties and appears to kill or inhibit cancer cells without killing normal cells in the process (Okwu, 2001). Sodipo *et al*. (1991) reported that the properties of saponnins are anti-carcinogens, immune modulatory activity and cholesterol lowering. It is also stated that it has antifungal properties. Some glycosides of saponins are cardiotonic and others are contraceptives and precursors for other sex hormones (Evans, 2009).

Because of their hydroxyl groups, phenols are essential constituents of plants due to their scavenging ability on free radicals. Thus, plant phenol content can lead directly to its antioxidant activity (Michalak, 2006).

2.12 DETERMINATION OF FEED QUALITY

Under most feeding methods in most developing countries, insufficient protein supply post-ruminally is a significant restrictive factor that prevents animal growth and development. Different methods of determining remaining microbial protein

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production in the rumen are based on the use of microbial determinants, which include the use of post-ruminally fistulated animals to conclude digestive flow (Makkar, 2002). Under most study and realistic conditions in developing countries, the cannulation technique is rather cumbersome and has many limitations on its applicability (Chen *et al*., 1990).

Increasing interests in the effective utilization of feed high in fibre has led to maximization in the use of techniques in reviewing fermentation kinetics. This procedure has the advantage in providing vital information on digestion kinetics of both soluble and insoluble parts of feedstuffs (Singh *et al*., 2007).

A number of groups have utilized *in vitro* gas techniques as favorable method of determining feed quality (Lowman *et al*., 2002; Getachew *et al*., 1998a). However, the weaknesses of earlier methods are discussed by the *in vitro* gas methods in using light of syringes (Blümmel *et al*., 1997) which gives off an imprint of being preferred and suitable for use in many states.

In vitro techniques such as the methods of Tilley and Terry and the methods of nylon bags are based on gravimetric readings after the substrate has vanished, whereas the volume of gas emphasizes the presence of fermentation products. In the gas process, the kinetics of fermentation can be tested on a single sample, allowing a fairly small amount of sample to be used or better yet larger amounts of samples to be evaluated simultaneously (Makkar, 2002).

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The *in vitro* gas method was considered more effective in determining the effects of anti-nutritional factors as compared to the *in Sacco* approach (Makkar *et al*., 1995). *In vitro* gas is focused on the quantification of material degradation or production of microbial proteins using interior or exterior markers and the production of gas or short chain fatty acid (SCFA) in an *in vitro* rumen fermentation system focused on syringes (Trei *et al*., 1970; Makar, 2004).

Three research strategies which include enzymatic, crude nutrient and gas measuring system were studied as signs of net energy content of feed resource in view of *in vivo* digestibility and it was concluded that, the gas measuring technique was better than the other two systems analyzed (Aiple *et al*., 1996).

Given the process used in *in Sacco*, the factors are mixed in the rumen after being drained from the nylon vessel, which can significantly affect rumen fermentation, but the *in vitro* gas system will better examine nutrient-anti-nutrient and anti-nutrient-anti-nutrient relationships (Makker, 2002).

The approach for *in vitro* gas defined by Menke *et al*. (1979) focuses on fermentation in 100 ml capacity calibrated syringes containing feedstuffs and buffered rumen fluid. The gas generated on incubating 200 mg / DM feed within 24 h is exploited along with the stages of other material components to expect digestibility of organic matter, *in vivo* and metabolizable energy.

Blu and Ørskov (1993) changed the strategy for Menke *et al*. (1979) to a method where feed was incubated in a precise thermostatic water shower as a replacement

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for a rotor in an incubator. Makkar *et al*. (1995) and Blümmel *et al*. (1997) further refined the technique by increasing the measured feedstuff from 200mg to 500mg and increasing the buffer volume from 30ml to 40ml. This shift was better clarified by Getachew *et al*. (1998b) and Cone *et al*. (1996) where it was stated that in the 30 ml context the linearity between the quantity of substrate incubated and the quantity of gas produced is lost when the volume of gas exceeds 90 ml as a result of the medium buffer; and in the 40 ml outline, the linearity is lost when the volume of gas exceeds 130 ml. Occurrence that the measuring of gas creation surpasses 90 ml when using the 30 ml outline and 130 ml in using the 40 ml outline, measuring of feed which is incubated should be diminished. This is due to the reason that the fall of the buffer reduces pH of the incubating medium; afterwards, the fermentation is restrained (Wilkins 1974; Sanderson *et al*., 1997).

Gas provided is the direct gas created accordingly of fermentation and the anomalous gas supplied from the buffering of short chain fatty acids. Around 50% of the collective gas is delivered from buffering of the short chain fatty acids whiles the others are developed precisely from fermentation. Measurement of CO_2 created from buffering of short chain fatty acids is 60% of the cumulated gas produced at high molar propionate. Each mole of short chain fatty acid produced from aging discharges 0.8-1.0 mol of CO_2 from the sustained rumen liquid arrangement, depending upon the measure of phosphate buffer available (Mauricio *et al*., 1999; Makkar, 2002).

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Consequently, the molar ratio of propionate acetate was used to determine substrate-related variances. Speedily fermentable carbohydrates produce comparatively higher propionate than acetate, and the reverse occurs when carbohydrates which are fermentable are slowly incubated (Stevenson *et al*., 1997; Schofield, 2000).

The kinetics of gas production depend on the relative level of the feed particles dissolvable, insoluble and degradable, and undegradable (Pell and Schofield, 1993; Pell *et al*., 1998). Numerical definitions of gas production profiles allow the investigation of details, the valuation of differences in substrates and media and the fermentability of dissolvable and slow fermentable feed parts. Prototypes of different kinds were used to represent profiles of gas production. France *et al*. (2000) combined the formation of gas profiles with assessments of substrate loss and ruminal passage levels and derived estimates of ruminal degradation rates, thus linking gas production to proper rumen events.

2.13 FACTORS AFFECTING THE DIGESTIBILITY OF FORAGES

One of the essential components of forage quality is digestibility (Ball *et al*., 2001). Elements such as environment (Crasta and Cox, 1996), morphology of plants and growth can influence the digestibility and forage quality (Burns *et al*., 1997). Food intake, pH, type and quantity of rumen microbial communities are the other variables affecting the digestibility rate (Rasjid, 2012).

Subsequently, there is an alternative reaction arrangement, particularly since rumen degradation can be a useful forecast of livestock intake, digestibility and *in vitro*

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rumen digestibility (De-Boever *et al*., 2003). In perspective of digestion, digestion depends on physical characteristics and data about rumen degradation on each type of feed. To determine consumption and feed digestibility, the feed is closely connected with the plant cell wall basis (Toharmat *et al*., 2016). Pangola plant acid detergent fiber (ADF) and NDF material appeared to be usually affected by season as compared to geographical region or genotype, and their CP content was largely influenced by geographical region and period (Chen *et al*., 1997). Chen *et al*. (1999) stated that the ADF and CP quality of pangola grass was more associated with the cumulative temperature than with the harvest intervals. On the other hand, Elephant Grass content from ADF, NDF and CP was more influenced by harvest duration than environmental place or season (Wang *et al*., 2003). Pangola grass and Elephant Grass had substantial adverse correlation coefficients with their ADF and NDF content, both *in situ* digestibility of dry matter and real digestibility of *in vitro* dry matter (Chen *et al*., 2003). Van Soest (2018) also noted that the decline in grass digestibility is due to the rise in cell wall percentage and concentration of lignin. As with other C4 grasses, the digestibility of vegetative Rhodes grass is restricted by its high NDF content and by the chemical bonds among its polysaccharides, lignin and phenolic acid cell walls (Akin *et al.*, 1992).

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This research was conducted at the Horticultural experimental field, Nyankpala Campus of the University for Development Studies. Nyankpala campus falls within the Tolon district of the Northern Region of Ghana within the Guinea savanna Agro-ecological zone. Geographically, Nyankpala campus falls within latitude 9°24′N and longitude 0°59′W. Nyankpala is 16km (10 miles) away from Tamale, the capital of Northern region with an altitude of 167m above sea level.

The study area has mean annual rainfall of 1043mm and temperatures generally fluctuate between 15˚C (minimum) and 42˚C (maximum) with a mean annual temperature of 28.5˚C. The mean annual day time relative humidity is 54%.

The study area's vegetation is typical of grassland. The area's terrain is relatively level, with a sandy-loam soil type (SARI, 2007).

3.3 Source of Planting Materials

Local varieties of *Cajanus cajan* seeds commonly found growing in the northern zone of Ghana were procured from the market and used for the study. The seeds were selected based on visual appearance (seed coat, seed size, seed weight, colour and other physical purity).

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3.4 Experimental land preparation and planting

Poultry manure was first applied on the field at a rate of 0.4 kg/m^2, then was ploughed with a tractor. The *Cajanus cajan* seeds were planted at about 4cm deep in the soil.

3.2 Experimental design

The field was divided into eighteen (18) plots in a randomized complete block design with each plot measuring 15m^2 (5m×3m).

Three treatments were assigned which were;

- A cutting regime of twelve (12) weeks after planting at a height of 50cm.
- A cutting regime of sixteen (16) weeks after planting at a height of 50cm.
- A cutting regime of twenty (20) weeks after planting at a height of 50cm.

3.5 Agronomic Practice

Eradication of weeds was carried out manually using a hoe (hand weeding).

3.6 Data Collection

3.6.1 Days to germination, plant height, number of branches and stem girth diameter

Germination were observed four (4) days after planting. Five (5) plants were randomly selected on each plot excluding those on the borders where plant height,

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number of branches and stem girth diameter were taken starting from the sixth week after planting. This was to ensure that, the plants were firmly rooted for growth and also to avoid breakages and damages to the weak stems.

Plant height was measured from the base of the plant to the tip of the plant with a measuring tape whiles stem girth diameter was taken from the circumference of the base of the selected plant using veneer caliper. Number of branches was measured by simple arithmetic counting.

Plant height, number of branches and stem girth diameter measurements were repeated during the regrowth phases.

3.6.2 Harvesting and Herbage yield (Dry Matter)

The plants were harvested at 12, 16 and 20 WAP (Weeks After Planting) using a machete at a height of 50cm above ground and the total harvest per plot was weighed. Sub-samples (200g) were taken from each plot and chopped into short lengths (2-5cm) for dry matter determination using the AOAC (1990) procedure. This involved drying in an oven at 60°C for 48h.

Herbage yield of each plot 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 harvest from each plot.

$$\text{Herbage yield (KgDM/ha)} = \frac{\%\ \text{Dry matter}}{10000} \times \text{Fresh weight (Kg)}$$

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3.7 Sample Preparation for Chemical Analysis and *In Vitro* Digestibility

Sub samples of the harvest from each plot was taken, divided into whole plant, stem and leaf fractions. The samples were chopped into smaller length to facilitate drying and milling. The chopped samples were milled using a Hammer mill (Brabender, Germany) to pass through a 2 mm and then 1 mm sieve screens sequentially for laboratory analysis as proposed by Goering and Van Soest (1970) and AOAC (1990).

3.7.1 Crude protein and Ash

Crude protein and ash determination was done using the AOAC (2000) method.

3.7.2 Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF)

NDF and ADF were determined limited of residual ash through sodium sulfite and α- amylase using the procedure of Van Soest *et al*. (1991) and this was done using Ankom^{200} fibre analyser (Method 5 for ADF and method 6 for NDF).

3.8 *In vitro* Gas Production

3.8.1 Experimental Design

The design for the *in vitro* gas analysis was a factorial design in a randomized complete block. A 3 x 3 factorial design was used with each replicated two (2) times in each period. The factors included cutting regimes of 12, 16 and 20 WAP and three botanical fractions which were leaf, stem and whole.

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3.8.2 *In vitro* Gas Experiment Procedure

The *in vitro* gas method of Theodorou *et al*. (1994) was adopted with some modifications in this experiment as used by Alagma (2016).

The digestible organic matter (DOM) was calculated using the equation DOM (%) = 16.49 + 0.9042 GP + 0.0492 CP + 0.0387ash by Menke (1988).

Metabolizable energy was calculated using the equation

ME (MJ/ kg DM) +2.20 + 0.136 *GP+0.057 *CP according to Menke *et al*. (1979). Where, GP= gas production (ml/200mg DM at 24 h) and CP= Crude protein (g/kg DM).

3.9 DATA ANALYSIS

The analysis of variance (ANOVA) from Genstat 18th edition was used for analyzing the biomass yield, plant height, number of branches and stem collar girth in the field experiment. The *in vitro* gas digestibility trial and chemical composition parameters were analysed using two- way ANOVA. F-test means which were significant were separated at 5 % significance level using Tukey's test.

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CHAPTER FOUR

4.0 RESULTS

4.1 Growth and Biomass yield of *Cajanus cajan*

4.1.1 Plant height

The results on the plant height of *Cajanus cajan* at different cutting regimes are shown in figure 1. The plants harvested at 12WAP had the least height in the initial establishment with no significance difference between the heights of plants harvested at 16WAP and 20WAP. The regrowth followed a different trend in height with plants harvested at 12WAP recorded the heighest plant height.

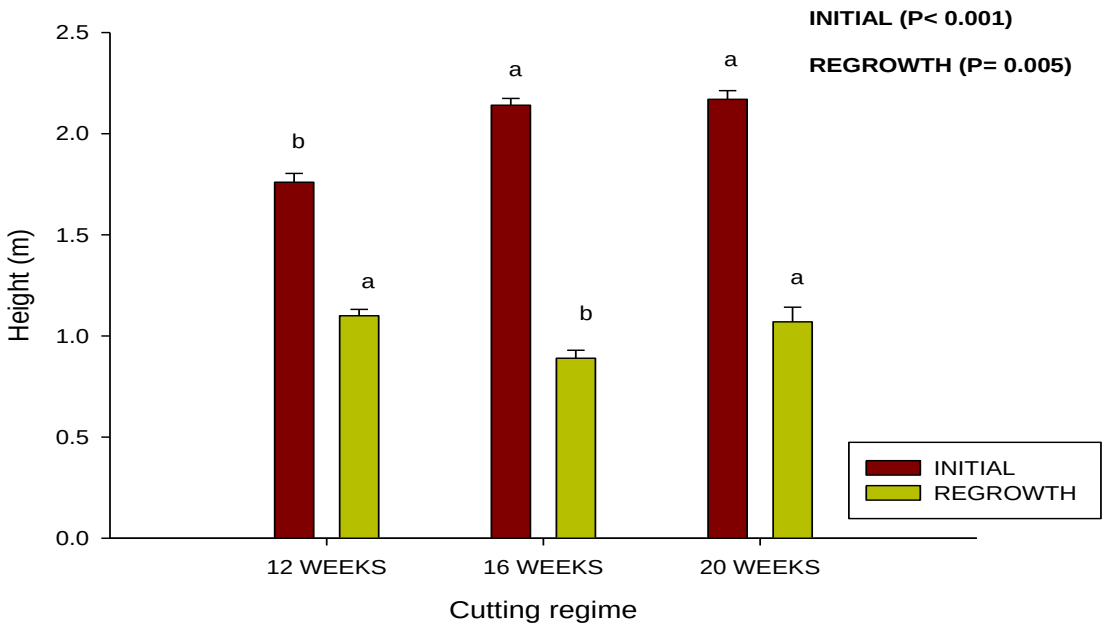

Figure 1. Plant height of *Cajanus cajan* at three cutting regime of initial establishment and regrowth

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4.1.2 Number of branches

Figure 2 below show the results on the number of branches of *Cajanus cajan* at different cutting regimes. Plants harvested at 16WAP had the highest number of branches in the initial establishment with no significant difference in plants harvested at 12WAP and 20WAP. Plants harvested at 12WAP however had the highest number of branches in the regrowth.

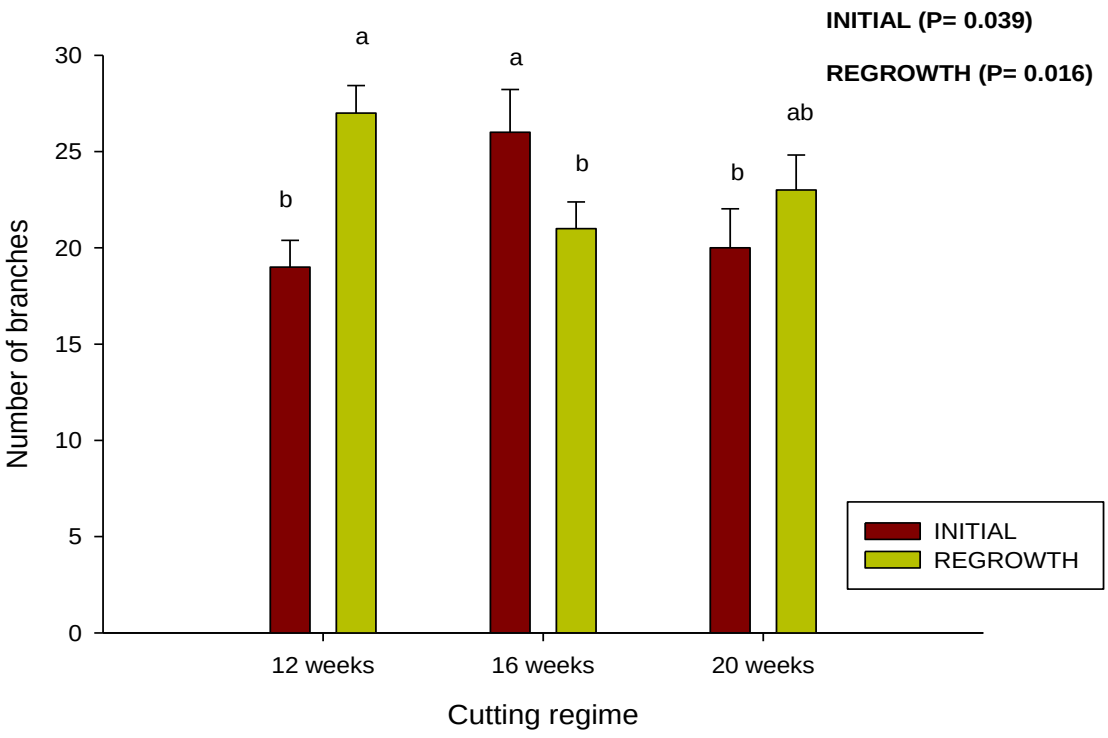

Figure 2. Number of branches of *Cajanus cajan* at three cutting regime of initial establishment and regrowth

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4.1.3 Stem diameter

The results on the stem diameter of *Cajanus cajan* at different cutting regimes are shown in figure 3. The highest stem diameter was recorded in the plants harvested at 16WAP followed by 20WAP with plants harvested at 12WAP recording the least stem diameter in the initial eastablishment. There was significant difference between plants harvested at 12WAP and 16WAP but there was no difference between that of plants harvested at 12WAP and 20WAP. The regrowth followed similar trend where there was significant difference between plants harvested at 12WAP and 16WAP.

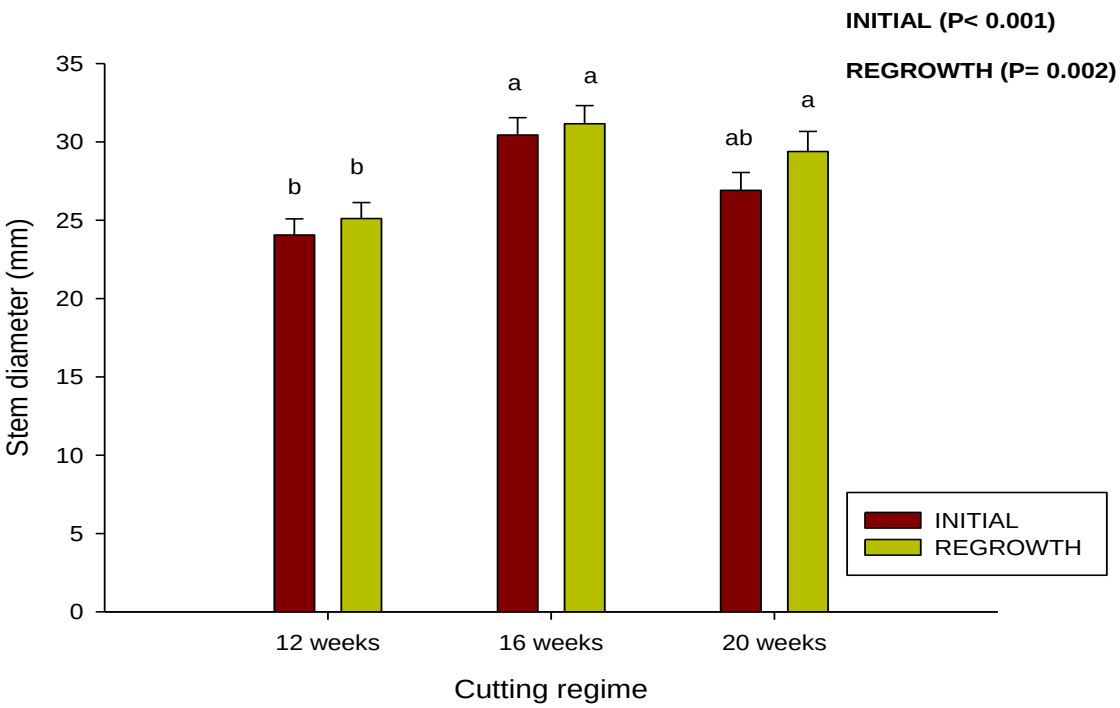

Figure 3. Stem diameter of *Cajanus cajan* at three cutting regime of initial establishment and regrowth

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4.1.4 Biomass yield

Figure 4 shows the biomass yield of *Cajanus cajan* at different cutting regimes. Harvesting at 20 WAP had the highest biomass yield (6515kg/ha) with 12 WAP harvesting recording the least (3175kg/ha) in the initial establishment. There were no significant differences between plants harvested at 16 WAP and 20 WAP. In the regrowth, harvesting at 20 WAP had the highest biomass yield though there was no significant difference (P = 0.461) between the cutting regimes.

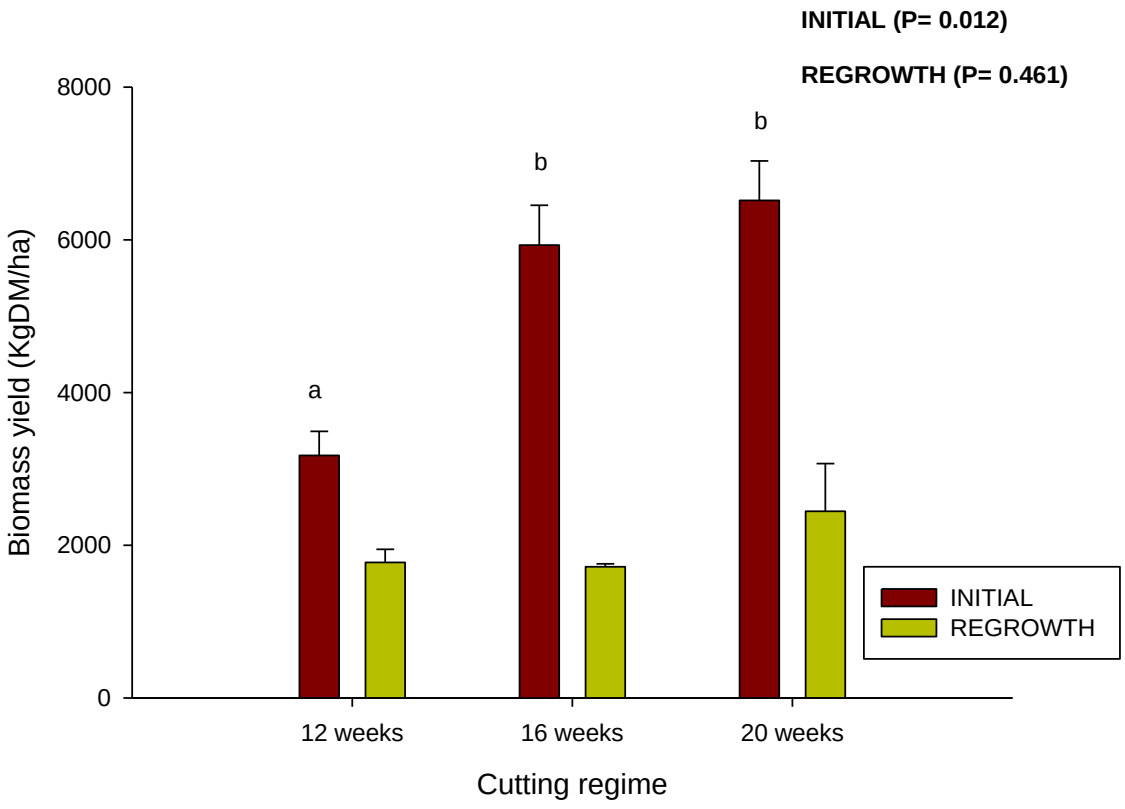

Figure 4 Biomass yield of *Cajanus cajan* at three cutting regime of initial establishment and regrowth

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4.1.5 Total crude protein

The total crude protein of *Cajanus cajan* at different cutting regimes are shown in figure 5. Harvesting at 16WAP had the highest total crude protein (1,246kg/ha) with 12WAP harvesting recording the least total protein in the initial establishment. There was no significant difference in total crude protein between plant harvested at 16WAP and 20WAP. In the regrowth however, harvesting at 20WAP had the highest total crude protein but there was no significant difference in total crude protein between different cutting regimes.

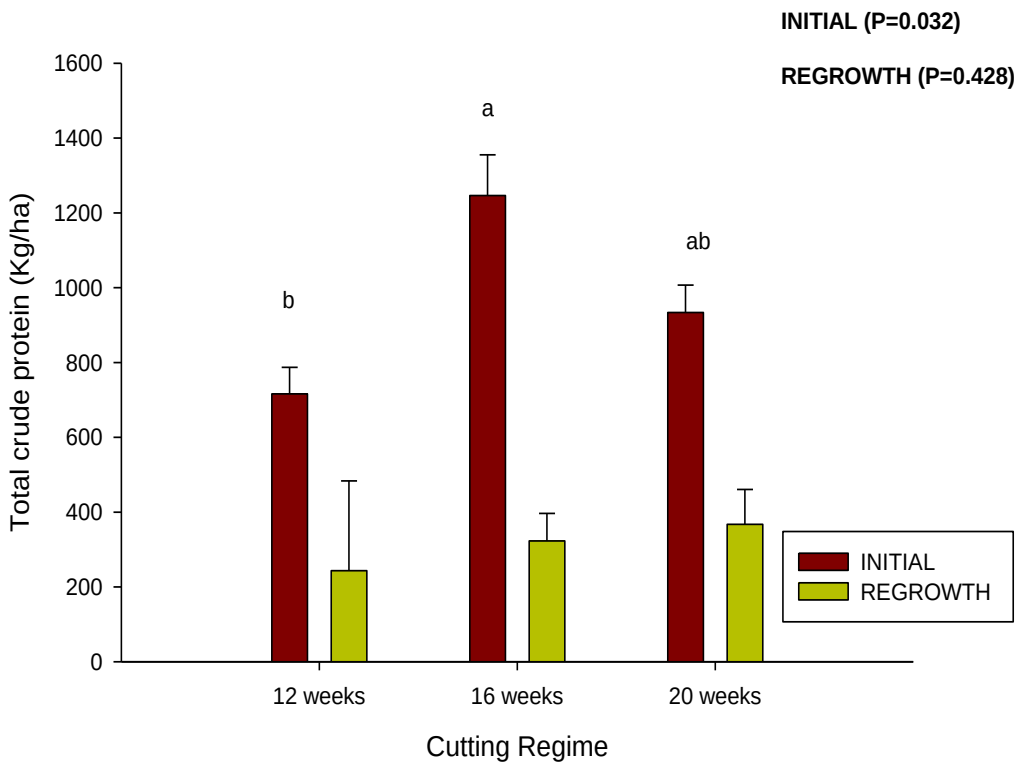

Figure 5. Total CP of *Cajanus cajan* at three cutting regimes of initial establishment and regrowth

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4.1.6 Metabolizable energy

The total metabolizable energy of *Cajanus cajan* at different cutting regimes is represented in figure 6. Harvesting at 20 WAP recorded the highest total metabolizable energy (30,281.73MJ/ha) followed by 16 WAP harvesting with 12 WAP harvest recording the lowest total metabolizable energy in the initial establishment. Significant difference was observed between harvesting at 12WAP and 16WAP and between that of 12WAP and 20WAP in the initial establishment. Harvesting at 20 WAP had the highest total metabolizable energy in the regrowth although there were no significant difference between cutting regimes.

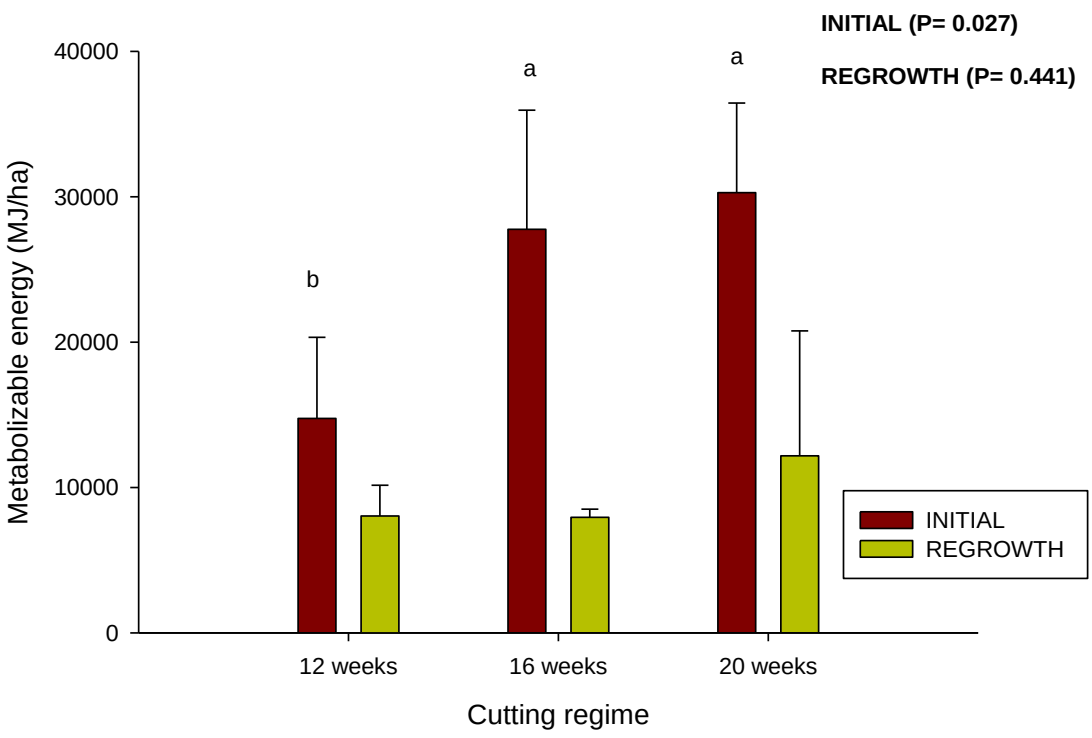

Figure 6. Total metabolizable energy of *Cajanus cajan* at three cutting regimes of initial establishment and regrowth.

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4.2 Chemical composition and *in vitro* gas digestibility of *Cajanus cajan*

4.2.1 Chemical composition

The results on the chemical composition of *Cajanus cajan* harvested at different cutting regimes after initial establishment is shown in Table 1.

There was a significant interaction effect on all parameters except dry matter. The highest CP (235.8g/KgDM) was obtained in the leaf fraction of plants that were harvested 12 WAP while the least (89.1g/KgDM) was recorded in the stem fraction of plants harvested at 20 WAP in the initial establishment. The NDF and ADF increased as the cutting regime increased with higher concentrations recorded in the stem fractions.

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Table 1. Chemical composition of *Cajanus cajan* as influenced by cutting regime on dry matter basis (g/kgDM) at initial establishment.

CUTTING REGIME	BOTANICAL FRACTION	PARAMETERS (g/kgDM)					
		DM	CP	NDF	ADF	HEM	ASH
	Leaf	273.3	235.8^{a}	382.9^{g}	234.4^{f}	149^{h}	70^{b}
C 12	Stem	279.3	133.9^{g}	604.2^{c}	354.4^{c}	250.5^{b}	45^{e}
	Whole	286.3	225.4^{c}	456.7^{e}	260.9^{e}	195.7^{d}	75^{a}
	Leaf	396.7	234.3^{b}	382.9^{g}	213.2^{i}	170.2^{g}	55^{d}
C 16	Stem	424.6	126.4^{h}	654.7^{b}	435.9^{b}	218.3^{c}	60^{c}
	Whole	397.5	210.2^{d}	391.6^{f}	217.4^{h}	174^{e}	60^{c}
	Leaf	465.9	184.4^{e}	333.4^{h}	222.2^{g}	111.3^{i}	60^{c}
C 20	Stem	491.9	89.1^{i}	723.6^{a}	468.5^{a}	255.4^{a}	45^{e}
	Whole	482.9	143.3^{f}	480.8^{d}	307.4^{d}	173.2^{f}	45^{e}
S. e. d		11.10	0.27	0.15	0.39	0.21	0.04
	Cutting regime	< .001	< .001	< .001	< .001	< .001	< .001
P value	Fraction	0.023	< .001	< .001	< .001	< .001	< .001
	Cutting regime x Fraction	0.284	< .001	< .001	< .001	< .001	< .001

Means with different superscript are significantly different at P<0.05; CP= Crude Protein, DM= Dry Matter, NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, HEM= Hemicellulose, P-value = 0.05

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Table 2 below shows the chemical composition of *Cajanus cajan* at different cutting regimes at regrowth. There were significant differences in the interaction effect in all parameters except for dry matter which was influenced by only cutting regime. The highest dry matter (459.9g/Kg) was recorded in the stem fraction of harvesting at 12 WAP and the least recorded in the whole fraction in plants harvested at 16 WAP in the regrowth. The CP in the leaf fraction of the regrowth increased with increasing age. The highest and least CP were recorded in the leaf and stem fractions in harvesting at 20 WAP. The stem fractions recorded the highest NDF and ADF concentration with the highest NDF recorded in the stem fraction of harvest at 20 WAP whiles the stem fraction of harvest at 16 WAP recorded the highest value of ADF in the regrowth.

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Table 2. Chemical composition of *Cajanus cajan* as influenced by cutting regime on dry matter basis (g/kgDM) at regrowth.

CUTTING REGIME	BOTANICAL FRACTION	PARAMETERS (g/kgDM)					
		DM	CP	NDF	ADF	HEM	ASH
	Leaf	453.5	163.5^{c}	320.2^{i}	220.5^{g}	100^{h}	70^{e}
RC 12	Stem	459.9	127.3^{g}	563.7^{b}	381.9^{b}	182.2^{b}	50^{h}
	Whole	450.4	137.3^{f}	387.8^{f}	224.6^{f}	163.5^{d}	65.1^{f}
	Leaf	384.2	154.2^{d}	425.9^{d}	277.9^{d}	148.1^{f}	90.1^{a}
RC 16	Stem	397.1	100.4^{h}	555.7^{c}	388.9^{a}	166.5^{c}	60.1^{g}
	Whole	375.1	187.9^{b}	404.3^{e}	255.1^{e}	148.6^{f}	85.1^{b}
	Leaf	413.9	194^{a}	352.9^{g}	196.1^{h}	156.5^{e}	80^{c}
RC 20	Stem	448	80.8^{i}	584.9^{a}	377.2^{c}	207.5^{a}	65.1^{f}
	Whole	425.1	150.5^{e}	339.9^{h}	220.5^{g}	120.5^{g}	74.9^{d}
S. e. d		15.69	0.31	0.16	0.18	0.32	0.05
	Cutting regime	< .001	< .001	< .001	< .001	< .001	< .001
P value	Fraction	0.104	< .001	< .001	< .001	< .001	< .001
	Cutting regime x Fraction	0.732	< .001	< .001	< .001	< .001	< .001

Mean with different superscript are significantly different at P<0.05; CP= Crude Protein, DM= Dry Matter, NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, HEM= Hemicellulose

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Figure 7 below shows the CP concentration between the initial establishment and the regrowth harvested at 12 WAP. The CP content of the leaf fraction was higher in the initial establishment compared to the regrowth. There was 30.6% decrease in CP between the initial establishment and regrowth. In the stem fraction, the percentage decrease was 5% between the initial establishment and the regrowth.

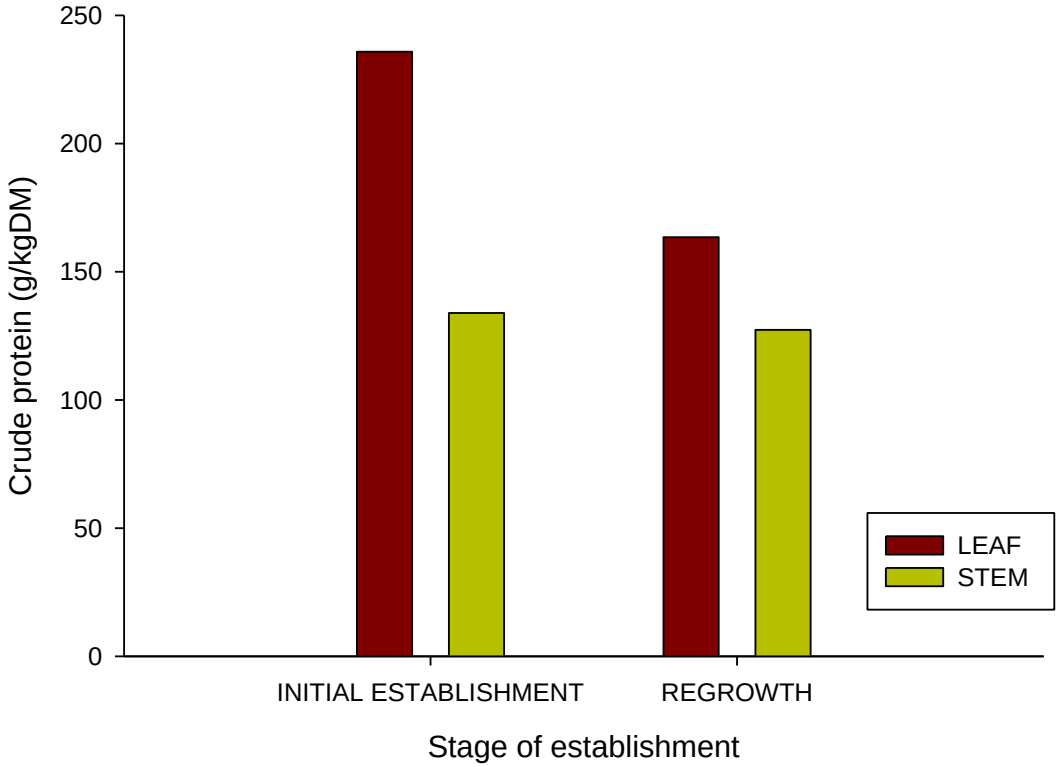

Figure 7. Crude protein content of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 12 WAP

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Figure 8 shows the CP concentration of *Cajanus cajan* between initial establishment and regrowth harvested at 16 WAP. The CP concentration of both the leaf fraction and the stem fraction in the initial establishment were higher than that of the regrowth. There was a decrease of 34.2% in the leaf fraction between the initial establishment and the regrowth whiles there was a decrease of 20.5% in the stem fraction between the initial establishment and the regrowth.

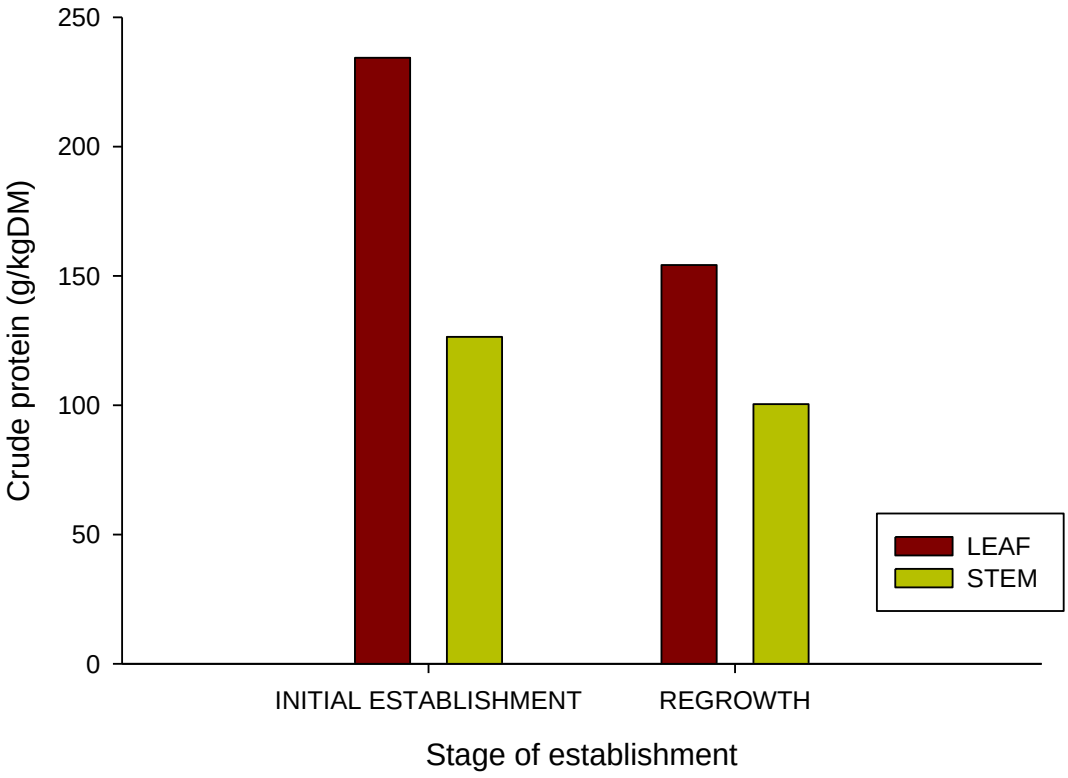

Figure 8. Crude protein content of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 16 WAP

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The CP concentration of *Cajanus cajan* harvested at 20 WAP are shown in figure 9 below. There was an increase of 5.2% between the initial establishment and the regrowth in the leaf fraction. In the stem fraction however, there was a decrease of 9.3% between harvesting in the initial establishment and the regrowth.

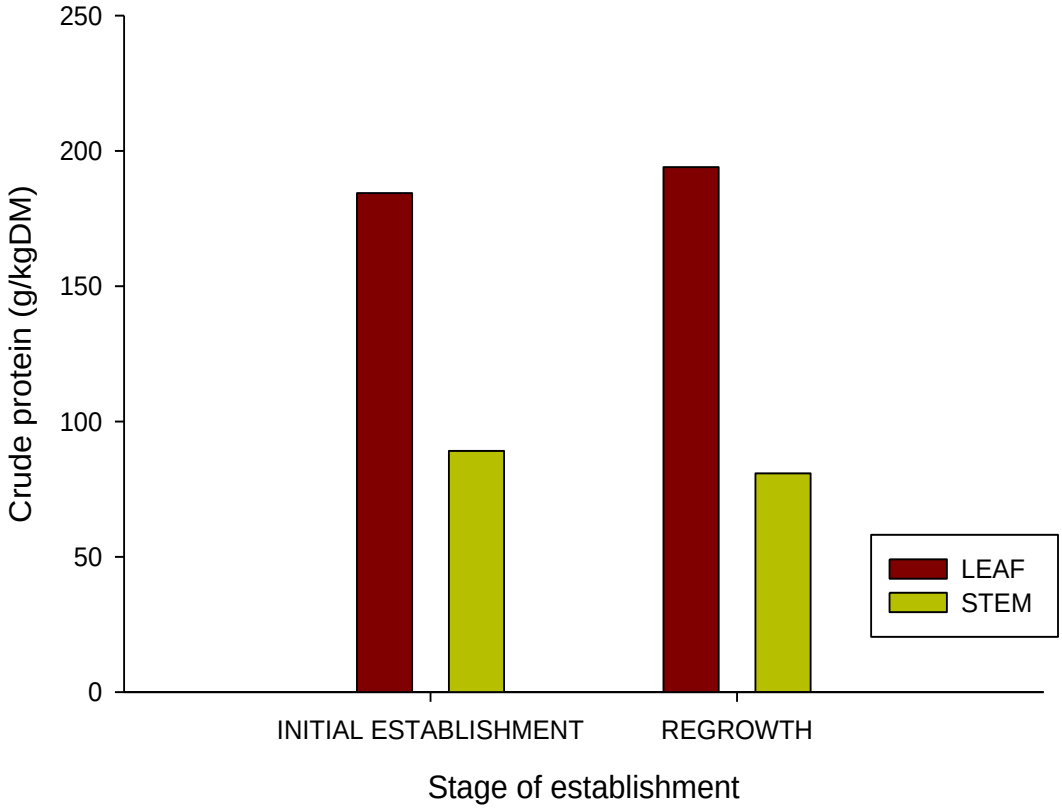

Figure 9. Crude protein content of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 20 WAP

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Figure 10 shows the NDF concentration between the initial establishment and regrowth of *Cajanus cajan* harvested at 12 WAP. The initial establishment had higher concentration of NDF in both the leaf fraction and the stem fraction than the regrowth. Whiles there was a decrease of 16.3% of NDF in the leaf fraction, the stem fraction reduced at 6.7% between the initial establishment and the regrowth.

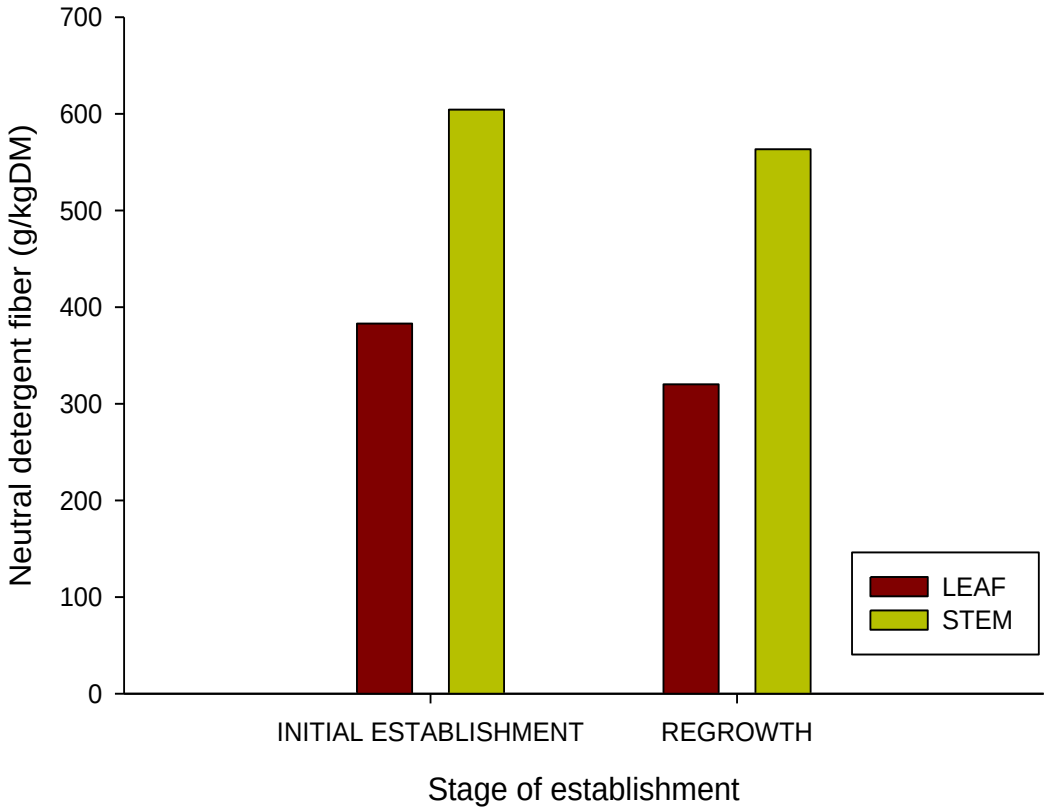

Figure 10. NDF content of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 12 WAP

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The NDF concentration of *Cajanus cajan* harvested at 16 WAP in the initial establishment and regrowth are shown in figure 11 below. In the leaf fraction, the regrowth had a higher NDF concentration than the initial establishment whiles there was a higher concentration of NDF in the stem fraction of the initial establishment than the regrowth. There was a percentage increase of 11% in NDF concentration between the initial establishment and the regrowth in the leaf fraction whereas the stem fraction decreased 15% between the initial establishment and the regrowth.

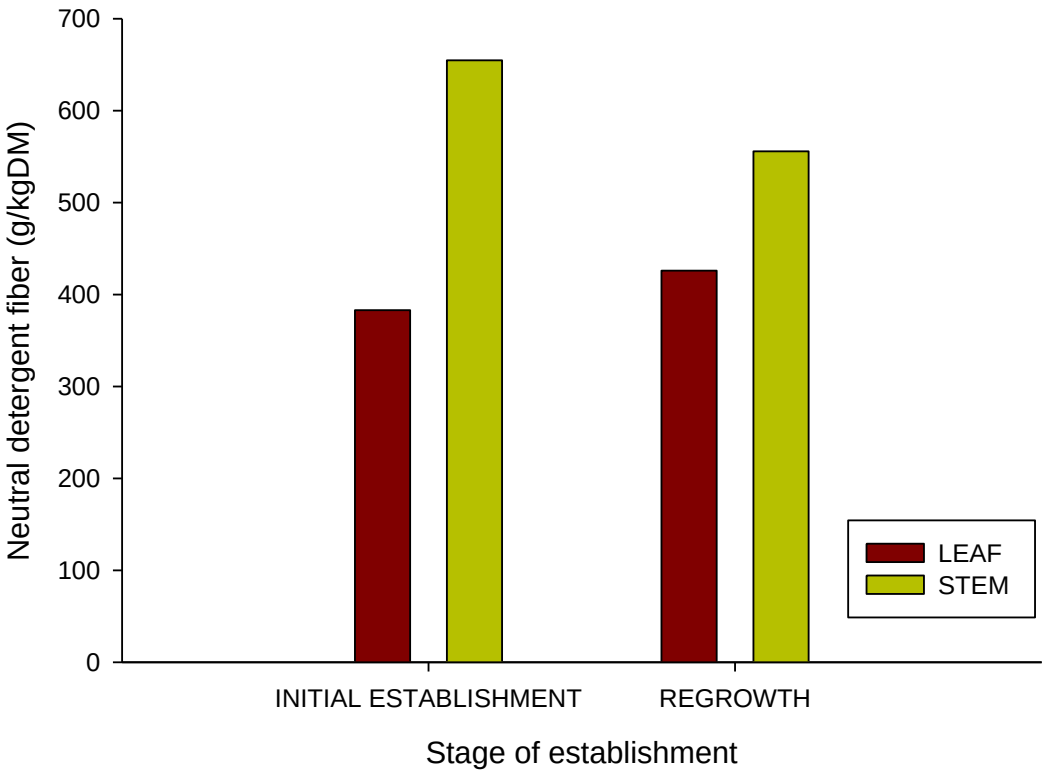

Figure 11. NDF content of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 16 WAP

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The results on the NDF concentration of *Cajanus cajan* harvested at 20 WAP for initial establishment and regrowth are shown in figure 12. The NDF concentration of the regrowth was higher than the initial establishment in the leaf fraction. There was a percentage increase of 5.8% from the initial establishment to the regrowth. In the stem fraction, the initial establishment had higher concentration of NDF than the regrowth where there was a percentage decrease of 19% from the initial establishment to the regrowth.

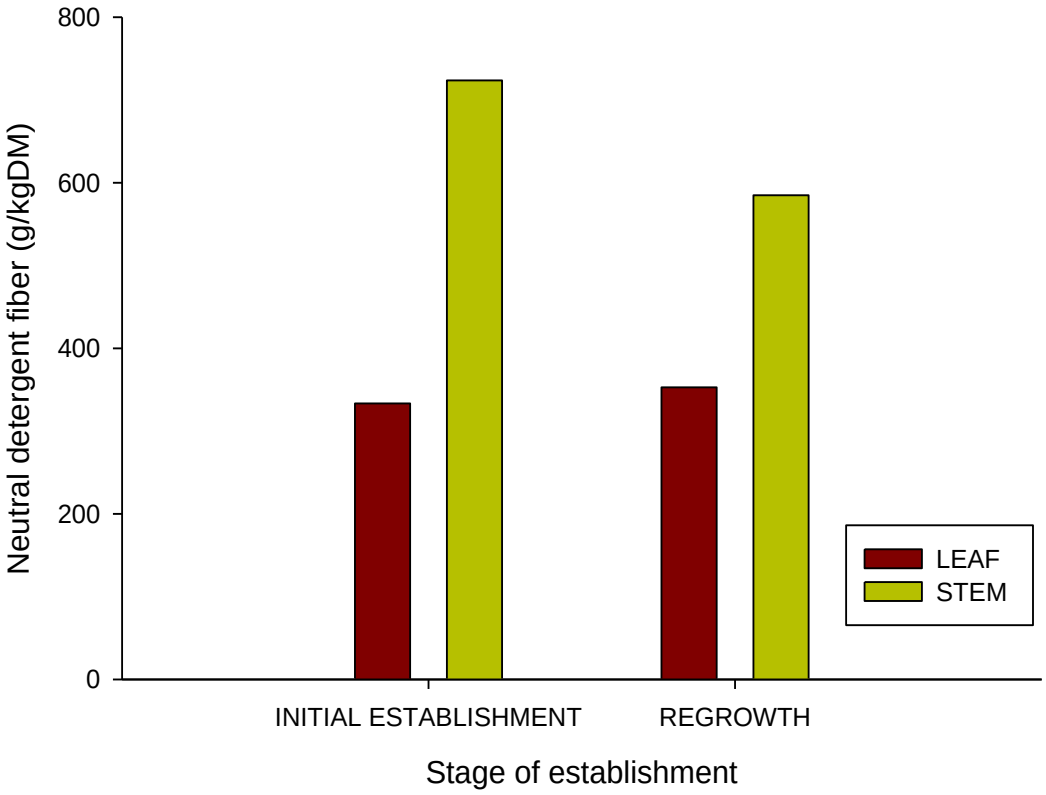

Figure 12. NDF content of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 20 WAP

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Figure 13 below shows the ADF concentration of *Cajanus cajan* at initial establishment and regrowth harvested at 12 WAP. In the leaf fraction, the initial establishment had higher concentration of ADF than the regrowth whereas the reverse occurred in the stem fraction. Whiles the leaf fraction had a percentage decrease of 5.9% in the concentration of ADF between the initial establishment and the regrowth, the stem fraction recorded a 7.7% increase between the initial establishment and the regrowth.

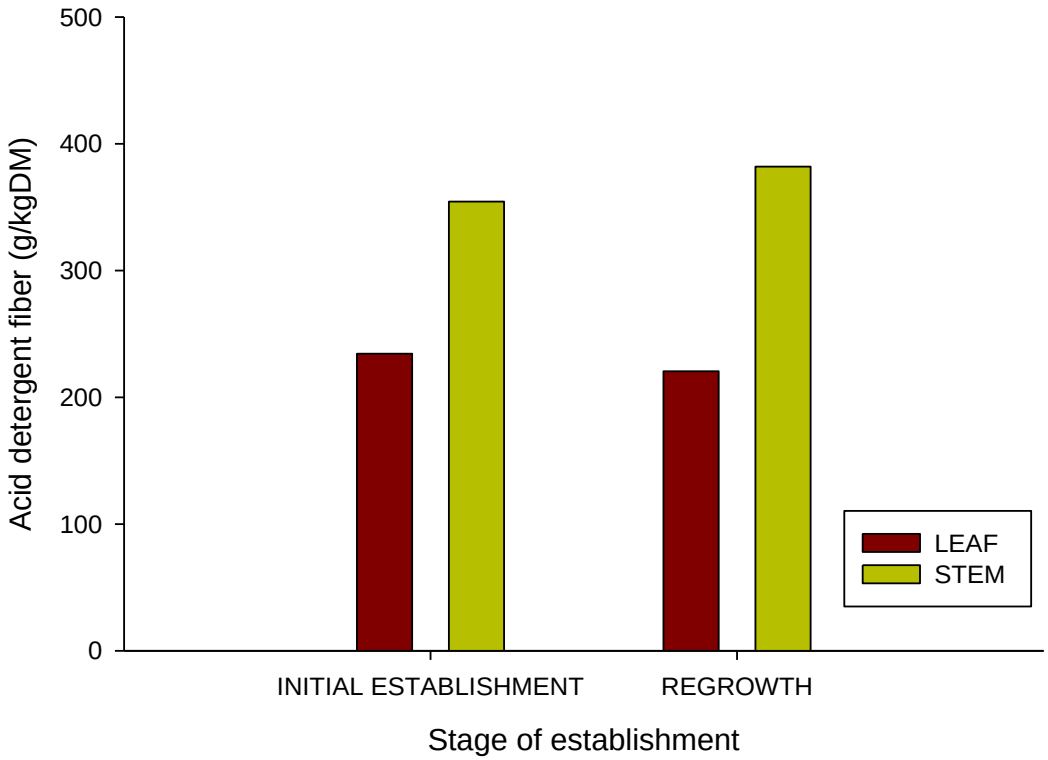

Figure 13. ADF content of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 12 WAP

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Figure 14 shows the ADF concentration of *Cajanus cajan* between initial establishment and regrowth harvested at 16 WAP.

The ADF concentration of the leaf fraction at regrowth was higher than the initial establishment but the concentration of ADF reduced in the regrowth for the stem fraction. There was a percentage increase of 30% in the leaf fraction between the initial establishment and the regrowth whiles there was a percentage decrease of 10.7% in the stem fraction between the initial establishment and the regrowth.

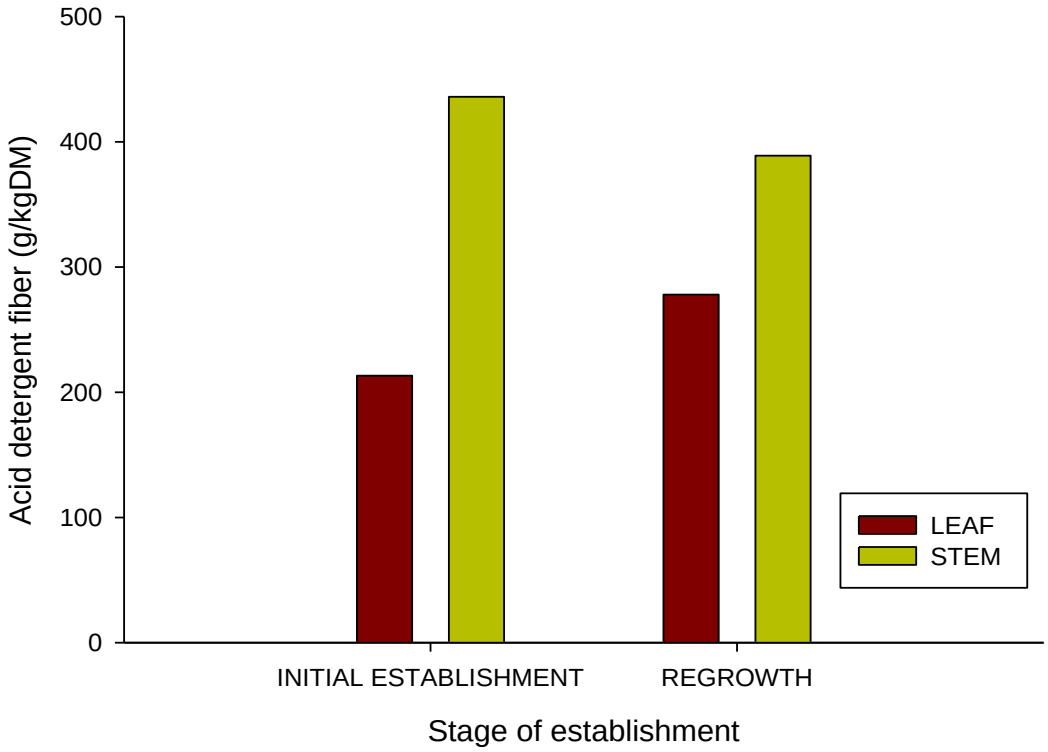

Figure 14. ADF content of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 16 WAP

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The ADF concentration of *Cajanus cajan* between initial establishment and regrowth harvested at 20 WAP is shown in figure 15. The ADF concentration of both the leaf fraction and the stem fraction in the initial establishment were higher than those of the regrowth. There was a percentage decrease of 11.7% in the leaf fraction between the initial establishment and the regrowth whereas there was a percentage decrease of 19.4% in the stem fraction between the initial establishment and the regrowth.

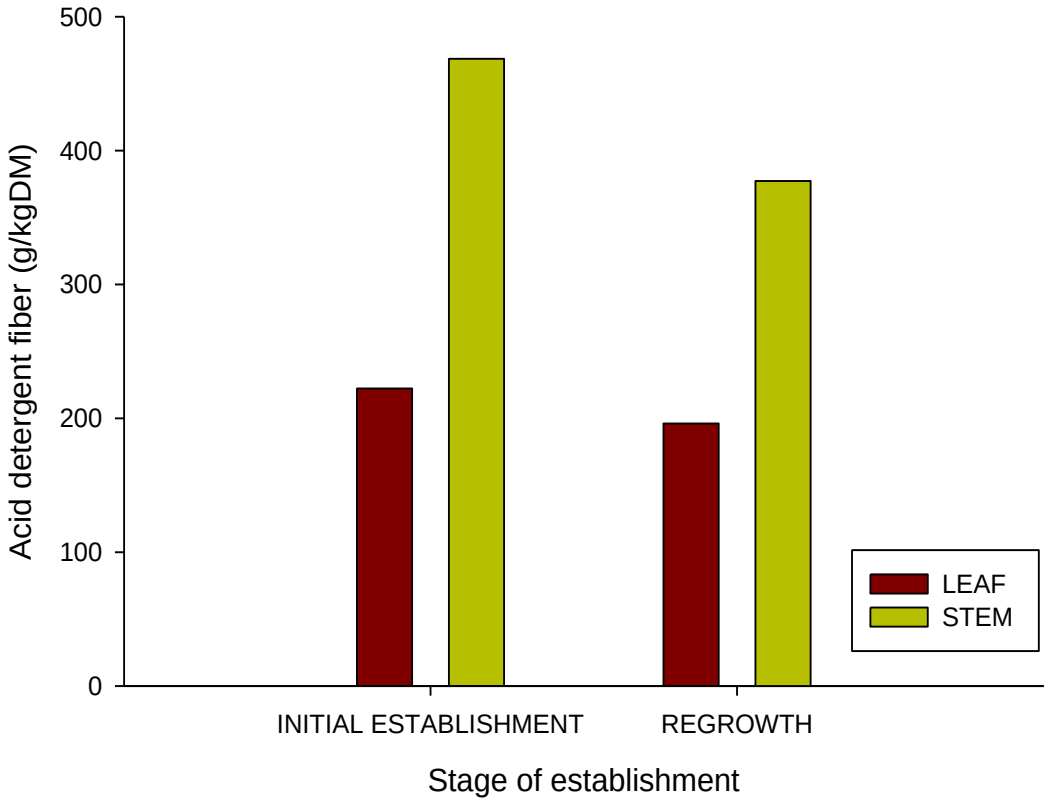

Figure 15. ADF content of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 20 WAP

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4.2.2 Gas production, *in vitro* digestibility, SCFA and Metabolizable energy

The results on the gas production, *in vitro* digestibility, short chain fatty acids and metabolizable energy of *Cajanus cajan* at different cutting regimes at initial establishment are presented in table 3.

There was a significant ($P < 0.05$) interaction effect on all parameters measured. The whole fractions generally produced higher gas at 24 hours followed by the stem fractions whereas the leaf fractions recorded the least. The highest gas production was recorded in the stem fraction (18.6g/ml) of harvesting at 16 WAP in the initial establishment. The short chain fatty acid followed the same trend as that of the gas produced at 24 hours.

In general, the leaf fractions had higher *in vitro* digestibility compared to the whole and stem fractions although the highest (46.6%) digestibility was recorded in the whole fraction of harvest at 12 WAP. However, *in vitro* digestibility decreased as the cutting regime increased.

Metabolizable energy of the leaf fractions were also higher compared to the stem and whole fractions. The highest metabolizable energy was recorded in harvesting at 12 WAP. There was also decrease in metabolizable energy as the cutting regime increased.

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Table 3. *In vitro* gas production, *In vitro* digestibility, short chain fatty acids and metabolizable energy of *Cajanus cajan* as influenced by cutting regime at initial establishment.

CUTTING REGIME	BOTANICAL FRACTION	PARAMETER			
		GAS @ 24(g/ml)	*IVOMD* (%)	SCFA (mmol/L)	ME (MJ/KgDM)
C 12	Leaf	16.8^{abc}	46^{a}	0.024^{abc}	17.9^{a}
	Stem	15.3^{c}	36.5^{f}	0.022^{c}	9.3^{h}
	Whole	17.8^{ab}	46.6^{a}	0.025^{ab}	17.4^{b}
C 16	Leaf	15.4^{c}	44.1^{bc}	0.022^{c}	17.6^{ab}
	Stem	18.6^{a}	42.2^{cd}	0.026^{a}	12.3^{f}
	Whole	18.2^{ab}	45.6^{ab}	0.026^{a}	16.6^{c}
C 20	Leaf	15.1^{c}	41.5^{d}	0.021^{c}	14.7^{d}
	Stem	16.4^{bc}	39.2^{e}	0.023^{bc}	11.6^{g}
	Whole	18^{ab}	41.5^{d}	0.025^{ab}	12.8^{e}
S. e. d		0.57	0.51	0.001	0.08
P value	Cutting regime	0.028	< .001	0.028	< .001
	Fraction	< .001	< .001	< .001	< .001
	Cutting regime x Fraction	< .001	< .001	< .001	< .001

Means with different superscript are significantly different at P<0.05, *INVOMD* = *In vitro* organic matter digestibility, SCFA = Short chain fatty acids, ME = Metabolizable energy

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Table 4 shows the results on the gas production, *in vitro* digestibility, short chain fatty acids and metabolizable energy of *Cajanus cajan* at different cutting regimes after regrowth.

There was significant differences in the interaction effect between cutting regime and fraction in all the parameters analysed. The stem fractions generally produced more gas at 24 hours with highest (21.8g/ml) gas production recorded in the stem fraction of harvest at 20 WAP in the regrowth. The gas productions however increased as the cutting regime increased. The *in vitro* digestibility also increased with increasing cutting regime where the highest *in vitro* digestibility was observed in harvest at 20 WAP regrowth. Metabolizable energy also followed the same trend but the leaf fractions had greater metabolizable energy. The highest (15.5MJ/KgDM) metabolizable energy was recorded in the leaf fraction of harvest at 20 WAP.

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Table 4. *In vitro* gas production, *In vitro* digestibility, short chain fatty acids and metabolizable energy of *Cajanus cajan* as influenced by cutting regime at regrowth.

CUTTING REGIME	BOTANICAL FRACTION	PARAMETER			
		GAS @ 24 (g/ml)	***IVOMD* (%)**	**SCFA (mmol/L)**	**ME (MJ/KgDM)**
RC 12	Leaf	17.2^{bc}	42.8^{abc}	0.024^{bc}	13.8^{b}
	Stem	17.7^{bc}	40.7^{c}	0.025^{bc}	11.8^{e}
	Whole	17.1^{bc}	41.2^{c}	0.024^{bc}	12.3^{d}
RC 16	Leaf	15.8^{c}	41.8^{bc}	0.022^{c}	13.1^{c}
	Stem	21.4^{a}	43.1^{abc}	0.031^{a}	10.8^{f}
	Whole	17.7^{bc}	45.1^{a}	0.025^{bc}	15.3^{a}
RC 20	Leaf	16.7^{c}	44.2^{ab}	0.024^{c}	15.5^{a}
	Stem	21.8^{a}	42.7^{abc}	0.031^{a}	9.7^{g}
	Whole	20.1^{ab}	44.9^{a}	0.028^{ab}	13.5^{bc}
S. e. d		0.85	0.77	0.001	0.11
	Cutting regime	0.002	< .001	0.002	< .001
P value	Fraction	< .001	0.01	< .001	< .001
	Cutting regime x Fraction	0.004	0.006	0.004	< .001

Means with different superscript are significantly different at P<0.05, *INVOMD* = *In vitro* organic matter digestibility, SCFA = Short chain fatty acids, ME = Metabolizable energy

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The *IVOMD* of *Cajanus cajan* harvested at 12 WAP between initial establishment and regrowth is shown in figure 16. There was 7% decrease in *IVOMD* between the initial establishment and the regrowth in the leaf fraction. In the stem fraction however, the regrowth recorded higher *IVOMD* than the initial establishment where was 11.5% increase between initial establishment and the regrowth.

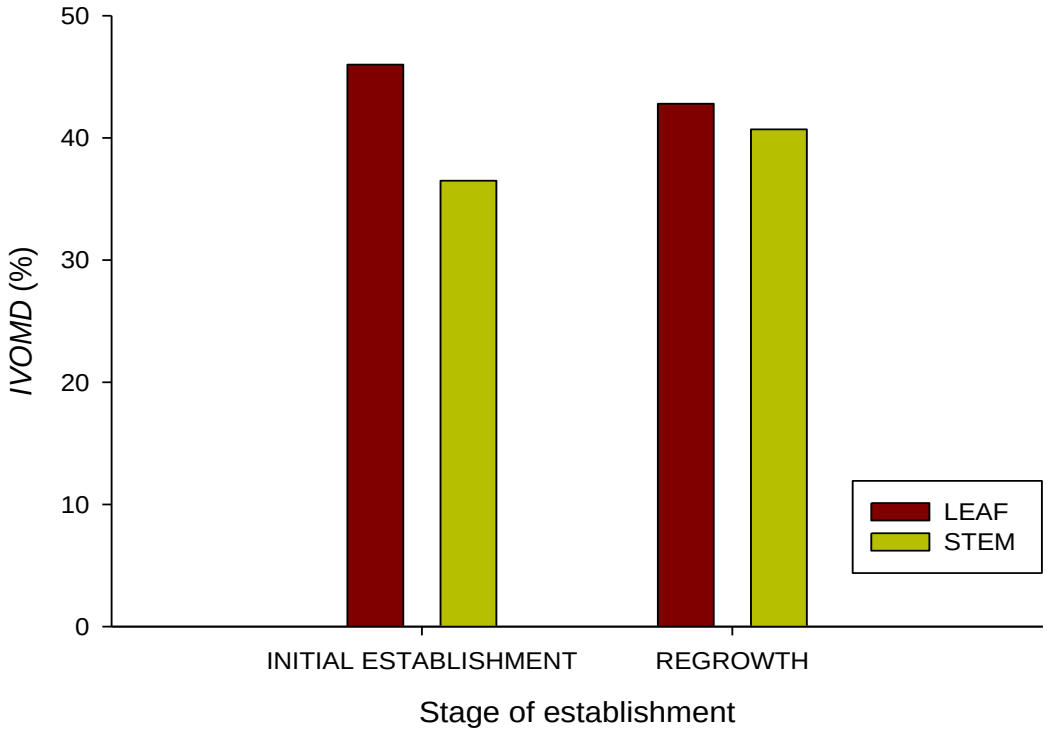

Figure 16. *IVOMD* of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 12 WAP

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Figure 17 shows the *IVOMD* of *Cajanus cajan* between initial establishment and regrowth harvested at 16 WAP. The *IVOMD* of the leaf fraction of the initial establishment was higher than the regrowth but in the stem fraction, the *IVOMD* of the regrowth was higher than the initial establishment. Whiles there was 5.2% decrease in the leaf fraction between the initial establishment and the regrowth, the stem fraction increased in 2% between the initial establishment and the regrowth.

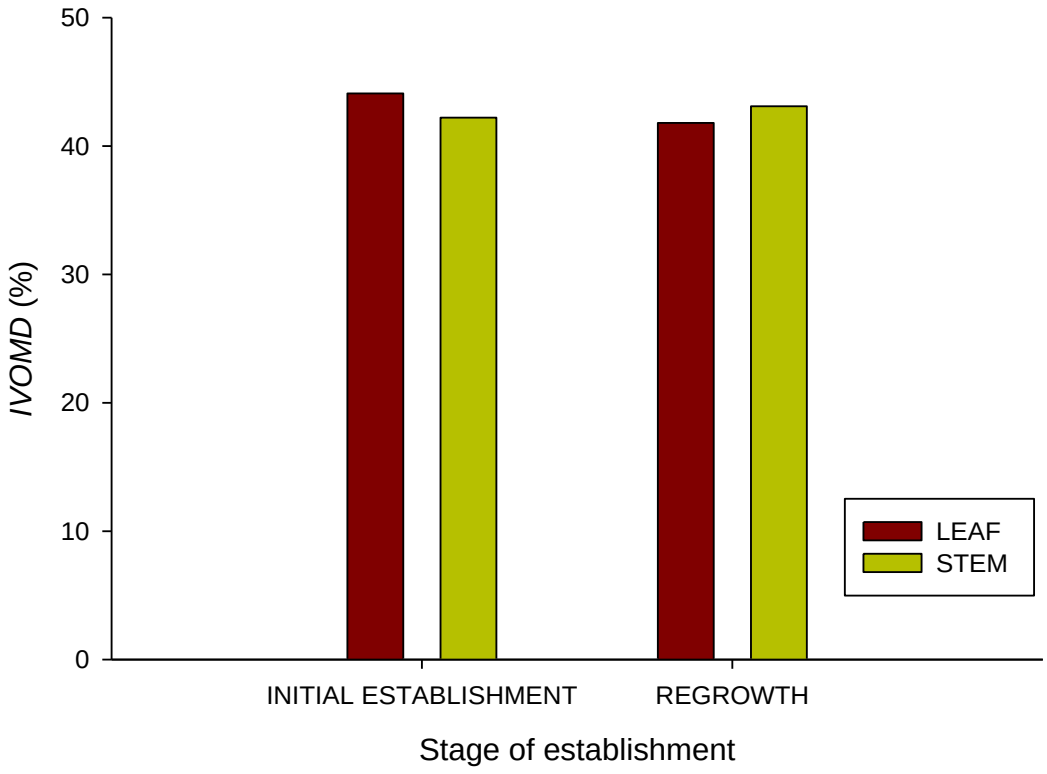

Figure 17. *IVOMD* of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 16 WAP

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The results on the *IVOMD* of *Cajanus cajan* harvested at 20WAP for initial establishment and regrowth are shown in figure 18. Whiles the leaf fraction increased by 6.5%, the stem fraction increased by 8.9% between the initial establishment and the regrowth.

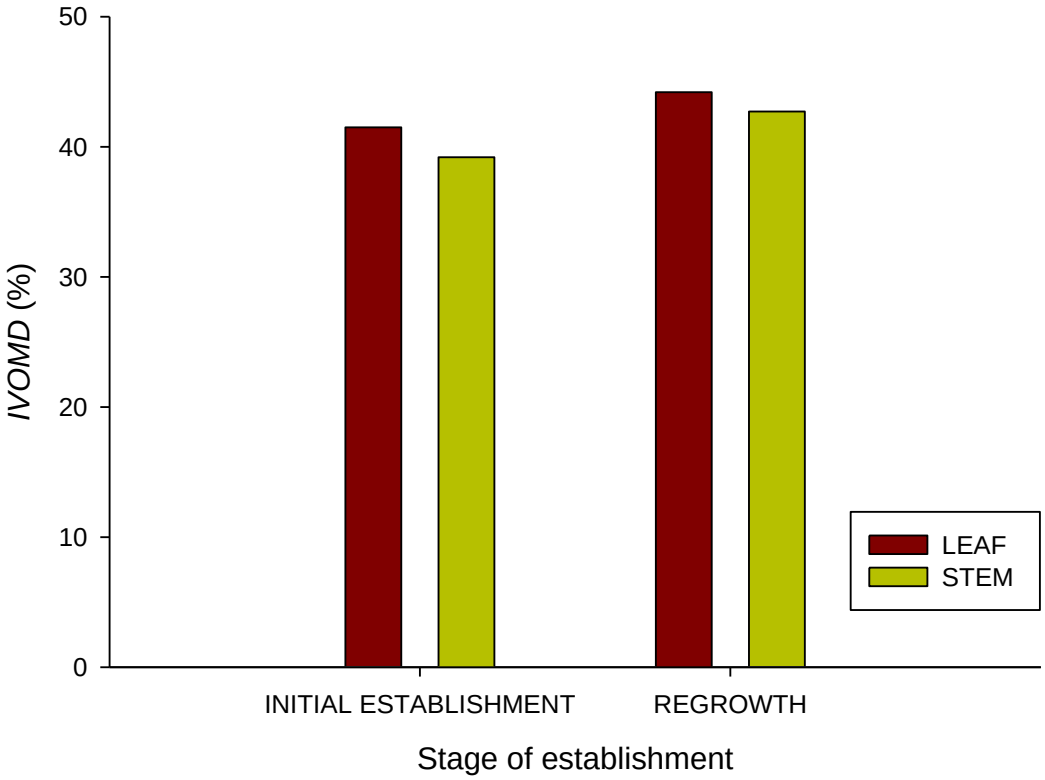

Figure 18. *IVOMD* of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 20 WAP

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Figure 19 below shows the metabolizable energy of *Cajanus cajan* at initial establishment and regrowth harvested at 12 WAP. In the leaf fraction, the initial establishment had higher concentration of metabolizable energy than the regrowth where the reverse occurred in the stem fraction. Whiles the leaf fraction had a decrease of 23% in metabolizable energy between the initial establishment and the regrowth, the stem fraction recorded 26.8% increase between the initial establishment and the regrowth.

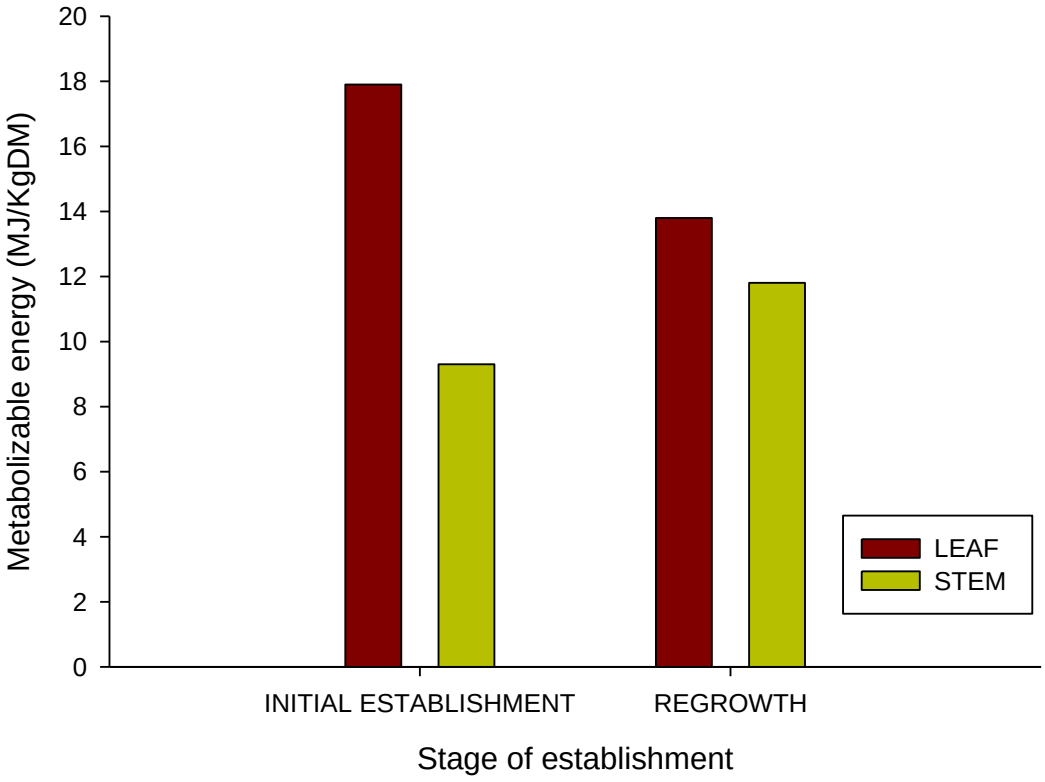

Figure 19. Metabolizable energy of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 12 WAP

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Figure 20 shows the metabolizable energy of *Cajanus cajan* between initial establishment and regrowth harvested at 16 WAP. The metabolizable energy of both the leaf fraction and the stem fraction in the initial establishment were higher than that of the regrowth. There was a decrease of 25.5% in the leaf fraction between the initial establishment and the regrowth whiles there was a decrease of 12% in the stem fraction between the initial establishment and the regrowth.

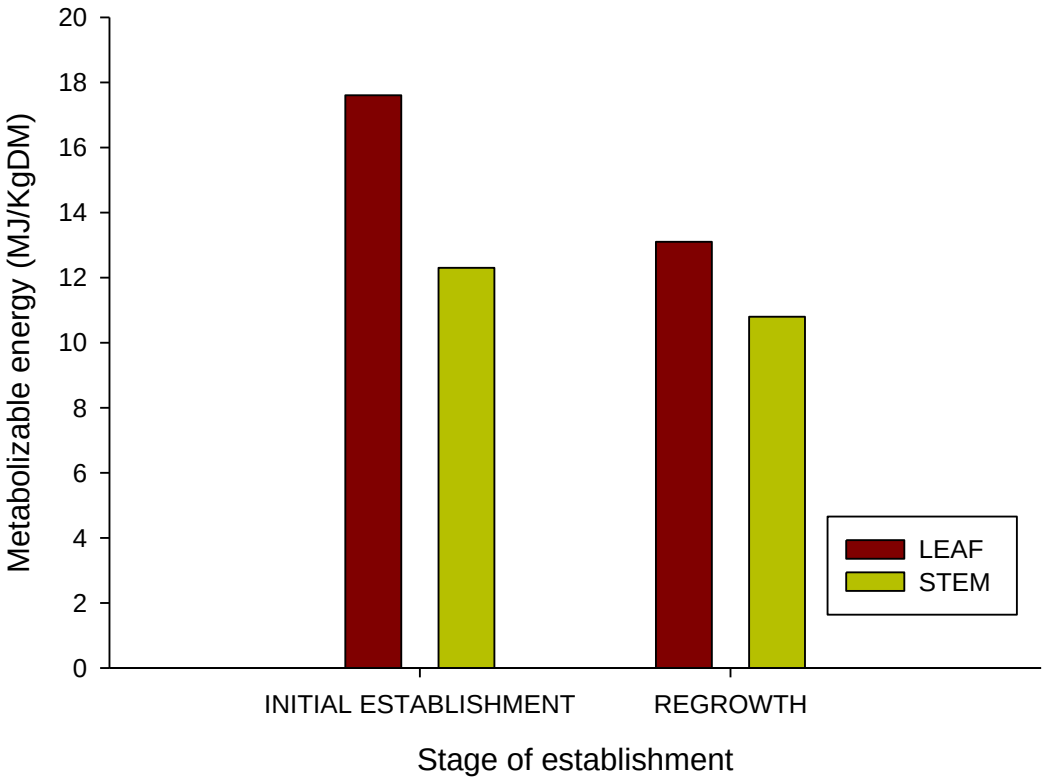

Figure 20. Metabolizable energy of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 16 WAP

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The metabolizable energy of *Cajanus cajan* harvested at 20WAP is shown in figure 21. The metabolizable energy in the leaf fraction in the regrowth was higher than the initial establishment whereas metabolizable energy in the stem fraction was higher in the initial establishment than the regrowth. There was an increase of 5.4% in the leaf fraction between the initial establishment and the regrowth whiles there was a decrease of 16.3% between the initial establishment and the regrowth in the stem fraction.

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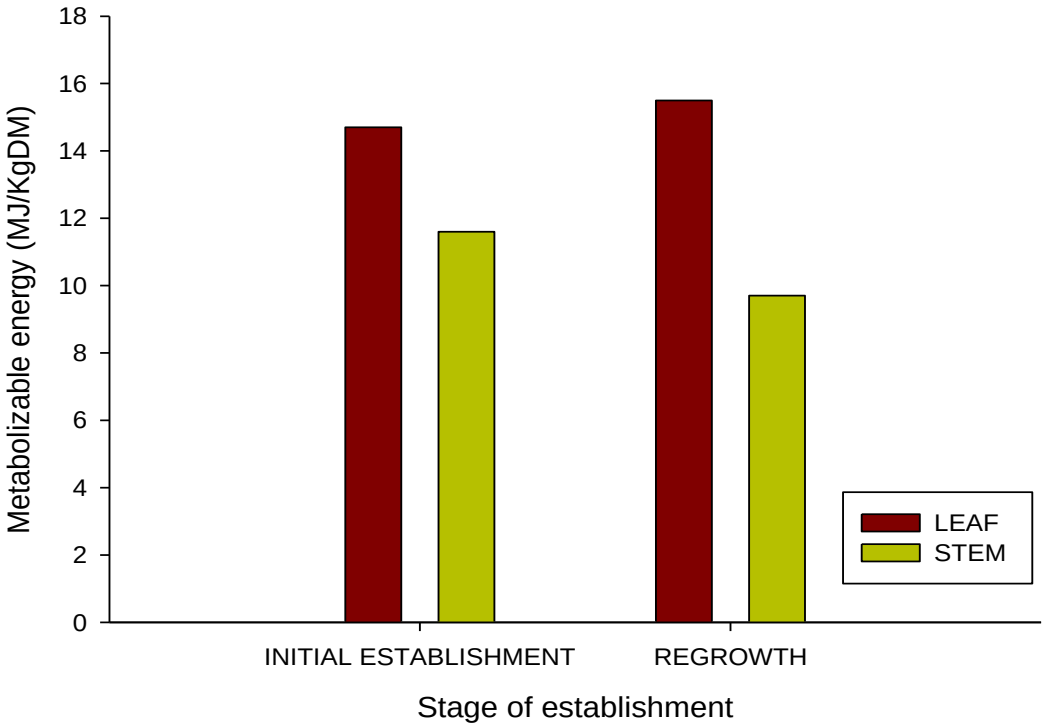

Figure 21. Metabolizable energy of *Cajanus cajan* in leaf and stem fraction of harvest from initial establishment and regrowth at 20 WAP

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CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Growth and yield of *Cajanus cajan*

The results obtained in this study generally indicates that *Cajanus cajan* can be cultivated and used as forage for ruminants in the Guinea savanna zone of Ghana.

The results obtained in plant height and number of branches is in agreement with the findings of Shoaib *et al*. (2013) who indicated that harvesting stage is the most important factor that affects growth morphology in forage plants. Increase in plant height and number of branches as the plant ages also conforms to the findings of Babu *et al*. (2015) who also reported increased plant height and number of branches of *Cajanus cajan* in the first year of establishment.

Variations in the initial establishment and regrowth in relation to plant height, number of branches and stem diameter might be attributed to factors such as season and climate after harvest which can positively or negatively affect this trait. Chiariello *et al.* (1991) suggested that perennial plants employ a stress-tolerance strategy that includes short stature and high partitioning to belowground structures in unfavorable conditions. This adaptation may generally explain the lower plant height obtained in the regrowth. Porter and Bidlack (2011) stated that, *Cajanus cajan* plants that receive water frequently attained higher plant height, number of branches and stem diameter than plants that received little water. This postulate

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could have been the case since the regrowth period coincided with the dry season. The trend in number of branches obtained in this study disagrees with the findings of Molla *et al*. (2018) who stated that as the plant ages, there is higher tendency of development of new tillers. This contradiction in findings may have occurred due to differences in climatic conditions such as rainfall and temperature in the environments where the study was carried out. Higher number of branches in the regrowth at 12WAP indicates stable productivity as stated by Assuero and Tognetti (2010) after periods of unfavourable conditions. Significant difference observed in the biomass yield in the initial establishment agrees with the findings of Bode *et al*. (2018) who also observed differences in biomass yield at different harvesting interval. Higher biomass yield in longer cutting intervals in both initial establishment and regrowth conforms to what was also obtained by Bode *et al.* (2018). The trend of the biomass yield may also be assigned to the trend of plant height and number of branches obtained at their various stages of harvest. Lower biomass yield at 12WAP and 16WAP in the regrowth indicates that the plants were at a critical point where water stress was initiated. Geiger and Servaites (1991) stated that plants postpone dehydration by decreasing productivity or inhibiting protein synthesis and it is possible that under the conditions of this experiment, postponement of dehydration in pigeon pea occurred for the continual survival of the plant. Behboudian *et al*. (2001) also reported decrease in plant height with corresponding decrease in biomass yield in chickpea when the plants were water stressed. However, lower biomass yield of *Cajanus cajan* in the regrowth agrees

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with the report from Cook *et al*. (2005) who stated that forage yield of *Cajanus cajan* declines through their life cycle.

5.2 Chemical composition of *Cajanus cajan*

Higher dry matter values recorded in longer cutting regime may be attributed to higher accumulation of dry matter at harvest. This conforms to McDonald *et al*. (2002) who stated that stage of maturity of forages at harvesting is the most important factor that influences the dry matter yield and nutritional quality. The high CP levels in the leaf fractions agrees with earlier reports from Tang *et al*. (2008) about the influence of botanical fraction and age on CP levels. As forages mature, the CP decreases and structural carbohydrates concentrations increase. The stems become tougher and more fibrous and protein and energy levels decrease. A similar finding was also reported by Kayani *et al*. (2007) who reported that the CP concentrations in the stems decreased as the plants mature. On the other hand, the lowest CP recorded at 20WAP in the initial establishment was due to reduction of leaf to stem ratio due to shedding of leaves as the plant ages. The CP contents reported in this study is within the range of CP values reported by Alexander *et al*. (2007) for 200 pigeon pea germplasms. The CP contents reported in the leaf fractions are in line with CP contents reported by Foster *et al*. (2009) and Jokthan (2006). With the exception of the stem fractions, the CP values reported in this study for the leaf and whole fractions are within the range required for maintenance and growth of ruminants (NRC, 2007) and can therefore be used as feed supplementation for ruminants. However, the CP contents in the stem fractions are within the

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minimum CP requirement (60-80 g/kg) for sustenance of microbial growth (Weiss *et al*., 1992). The CP in the leaf fraction of the regrowth interestingly increased with increase in cutting regime. There was a percentage increase of 5.2% in the leaf fraction of the regrowth in harvesting at 20 weeks, a trend which deviated from what was observed in the initial establishment. While CP concentration in the leaf decreased by 0.6% from harvesting at 12 WAP to harvest at 16 WAP, the decrease was higher (21%) between harvesting at 12 WAP and 20 WAP in the initial establishment. The percentage change of CP in the leaf fractions in the regrowth deviated from what was observed in the initial eastablishment. While there was a percentage decrease by 5.6% from harvesting at 12WAP to 16 WAP in the regrowth, there was a significant increase (15.7%) in CP between harvesting at 12 WAP and 20 WAP in the regrowth. There was also (20%) increase in CP in the leaf between harvesting at 16 WAP and 20 WAP in the regrowth.

This increase in CP may have have occurred through remobilization of nutrients from decomposed organic matter from shed leaves. Wedin and Russelle (2007) stated that, plants obtain most of their nutrients from the pool of dissolved nutrients in the soil. This solution is constantly being replenished from sources which include inputs like fertilizers and manure, decomposing organic matter and release of nutrients held weakly by the soil particles.

The trend in interaction effect of NDF, ADF and hemicellulose agrees with report from Lounglawan *et al*. (2014) that increasing the cutting regime increased the crude fiber, ADF, NDF and acid detergent lignin (ADL) percentage in the plants. Lower

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NDF and ADF recorded in the leaf fractions compared to the stem can be attributed to the accumulation of vascular tissues in the development of the stem as it ages. The high ADF in the stem can suppress intake as has been reported by Gusha *et al*. (2015). There was a percentage decrease of 12.9% of NDF in the leaf fraction as cutting regime increased from 12 WAP to 20 WAP in the initial establishment. In the stems of the initial establishment however, there was 8.3% increase of in NDF concentration as the cutting regime increased from 12 WAP to 16 WAP and a 19.7% increase in NDF concentration as the cutting regime increased from 12 WAP to 20 WAP. In the regrowth, there was an increase of 33% NDF concentration in the leaf fraction as the cutting interval increased from 12 WAP to 16 WAP. The stem fraction also recorded a 5.2% increase in NDF concent as cutting regime increased from 16 WAP to 20 WAP. The ADF content in the leaf fraction decreased by 9% between harvesting at 12 WAP and 16 WAP but the stem increased 32% when cutting regime increased from 12 WAP to 20 WAP in the initial establishment. In the regrowth however, the ADFconcentration in the leaf increased by 26% when cutting regime increased from 12 WAP to 16 WAP.

The ash content obtained in this study is in agreement with Baars and Geleti (2000) who reported that mineral contents of plants declined during the maturing process due to natural dilution and translocation of nutrients from vegetative part to the root system. However, the concentrations of ash reported in this study disagrees with the findings of Roothaert (2000) who found in Kenya that ash concentration increased with age in many indigenous fodder trees and shrubs. High ash in the leaf fractions

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is also an indication that the forages contains adequate minerals for the animals to utilize.

5.3 *In vitro* Gas production, *IVOMD*, SCFA and ME of *Cajanus cajan*

In vitro gas production is an indirect way of measuring microbial fermentation of feed and is related to the extent of digestibility.

In vitro gas production was consistently higher in the leaf fraction of the early harvest than the late harvest. This difference could be attributed to the expansion in the middle lamella with increase in age. The middle lamella is composed of vascular tissues usually lignified (Wilson and Hatfield, 1997). This chemical structure of the middle lamella has been reported to slow down microbial fermentation leading to lower production of gas (Guo *et al*., 2001). This reflects in the trend of *IVOMD* of leaf as it matures. However, the gas production values reported in this study are comparable with those reported for other multipurpose tree forages (Larbi *et al*., 1998).

Generally, *IVOMD* was higher in the whole fraction followed by the leaf fraction and the stem fraction recording the least values for all the cutting regimes in both the initial establishment and the regrowth. *IVOMD* indirectly measures the extent of organic matter digested in the rumen by the anaerobic microbes. CP degradation is required to supply the amount of nitrogen needed by the fermenting microbes. The high *IVOMD* in the whole fraction is an indication that the CP that supplies the needed nitrogen was not limiting even as they decreased while increasing the cutting

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regime. The values of *IVOMD* recorded in this study are comparable with values recorded by Boga *et al*. (2014) for *Lotus corniculatus*, *Trifolium alexandrinum* and *Medicago sativa* which are also forage legumes but lower than values reported for alfalfa (Denek and Deniz, 2004) . The trend in SCFA may also be attributed to the extent of degradability of the plant as the cutting regime increases as reflected in the gas produced at 24 hours.

In general, ME was higher in the leaf fractions in both the initial establishment and regrowth and their values decreased as the cutting regime increased. The trend is attributed to the increase in the accumulation of cell wall constituents as the plant ages. As plant ages and become more fibrous, digestibility reduces. Higher ME constituents in the leaf fraction is an indication of better fermentation to release maximum energy (Ball *et al*., 2001). Nevertheless, the ME recorded in this study in both the initial establishment and the regrowth are higher than values reported for *Lotus corniculatus*, *Trifolium alexandrinum* and *Medicago sativa* (6.6 – 7.8MJ/kgDM) by Boga *et al*. (2014) and Abdulrazak *et al*. (2000) for six acacia tree species (11.1 – 13.1MJ/kgDM). The ME recorded in this study are higher than 7MJ/kgDM and therefore suitable for maintenance level of beef cattle and acceptable for dairy cattle and rapidly growing calves.

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results obtained in the study indicates that *Cajanus cajan* forages harvested at different cutting regimes affect the plants differently.

In the initial establishment, the highest plant height and biomass yield were recorded in harvesting at 20 WAP whiles harvesting at 16 WAP had the highest number of branches and stem diameter. Dry matter increased as the cutting regime increased with the stem fractions recording higher dry matter contents. The leaf fraction of harvest at 12 WAP yielded the highest CP whiles the NDF and ADF concentrations increased as the cutting regime increased. The leaf fraction of harvest at 12 WAP yielded the highest *IVOMD* and ME. Harvest at 16 WAP also yielded the highest total ME and total CP.

In the regrowth however, harvest at 20 WAP had the highest biomass yield, total CP and total ME. Increasing cutting regime also increased the concentrations of CP, NDF and ADF. The leaf fractions of harvest at 20 WAP also had higher *IVOMD* and ME in the regrowth. Meanwhile, biomass yield and chemical composition was lower in the regrowth compared to the initial establishment.

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It can therefore be concluded that, cutting regime has great influence on the nutritional composition of *Cajanus cajan* in the Guinea Savannah agro-ecological zone of Ghana.

6.2 Recommendations

Based on the results obtained in the study, it is recommended that;

- For higher biomass yield, harvesting of *Cajanus cajan* fodder should be carried out sixteen (16) WAP in its initial establishment whiles harvesting at twenty (20) WAP in its regrowth will produce higher biomass yield and higher CP for livestock productivity in the savanna agro-ecological zone of Ghana.
- To boost growth and nutrient composition of *Cajanus cajan* fodder, fertilizing the regrowth should be considered.

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APPENDICES

Appendix 1. ANOVA tables for *Cajanus cajan* agronomic data

Variate: Biomass yield (Initial Establishment)					
Source of variation	Df	s.s	m.s	v.r	F pr.
Rep stratum	2	1492909.	746454.	1.27	
Treatment	2	19089932.	9544966.	16.25	0.012
Residual	4	2349520.	587380.		
Total	8	22932361.			

Variate: Plant height (Initial Establishment)					
Source of variation	Df	s.s	m.s	v.r	F pr.
Rep stratum	2	0.08467	0.04234	1.68	
Treatment	2	1.57932	0.78966	31.36	<.001
Residual	40	1.00724	0.02518		
Total	44	2.67123			

Variate: Number of branches (Initial Establishment)					
Source of variation	Df	s.s	m.s	v.r	F pr.
Rep stratum	2	60.04	30.02	0.50	
Treatment	2	422.98	211.49	3.51	0.039

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Residual	40	2407.56	60.19		
Total	44	2890.58			

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Variate: Stem Diameter (Initial Establishment)					
Source of variation	Df	s.s	m.s	v.r	F pr.
Rep stratum	2	60.88	30.44	1.65	
Treatment	2	306.54	153.27	8.29	<.001
Residual	40	739.56	18.49		
Total	44	1106.98			

Variate: Biomass yield (Regrowth)					
Source of variation	Df	s.s	m.s	v.r	F pr.
Rep stratum	2	471193.	235596.	0.46	
Treatment	2	977527.	488763.	0.95	0.461
Residual	4	2064499.	516125.		
Total	8	3513219.			

Variate: Plant height (Regrowth)					
Source of variation	Df	s.s	m.s	v.r	F pr.
Rep stratum	2	0.43416	0.21708	6.70	
Treatment	2	0.38742	0.19371	5.98	0.005
Residual	40	1.29572	0.03239		
Total	44	2.11730			

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Variate: Number of branches (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	323.78	161.89	5.06	
Treatment	2	298.00	149.00	4.66	0.016
Residual	38	1216.32	32.01		
Total	42	1828.79			

Variate: Stem diameter (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	70.11	35.06	1.69	
Treatment	2	290.70	145.35	7.01	0.002
Residual	40	829.70	20.74		
Total	44	1190.51			

Appendix 2. ANOVA tables for *Cajanus cajan* chemical Analysis

Variate: Dry matter (Initial Establishment)					
Source of variation	Df	s.s	m.s	v.r	F pr.
Rep stratum	2	654.7	327.3	1.77	
Treatment	2	185269.4	92634.7	501.31	<.001
Fraction	2	1791.6	895.8	4.85	0.023
Treatment x Fraction	4	1022.2	255.5	1.38	0.284
Residual	16	2956.6	184.8		

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Total	26	191694.4			

Variate: CP (Initial Establishment)					
Source of variation	Df	s.s	m.s	v.r	F pr.
Rep stratum	2	2.8985	1.4493	12.73	
Treatment	2	8496.6674	4248.3337	37317.61	<.001
Fraction	2	50461.7452	25230.8726	2.216E+05	<.001
Treatment x Fraction	4	11544.2459	2886.0615	25351.33	<.001
Residual	16	1.8215	0.1138		
Total	26	70507.3785			

Variate: NDF (Initial Establishment)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	2.696E-01	1.348E-01	3.57	
Treatment	2	1.302E+04	6.508E+03	1.725E+05	<.001
Fraction	2	4.200E+05	2.100E+05	5.566E+06	<.001
Treatment x Fraction	4	2.623E+04	6.558E+03	1.738E+05	<.001
Residual	16	6.037E-01	3.773E-02		
Total	26	4.593E+05			

Variate: ADF (Initial Establishment)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	2.763E-01	1.381E-01	0.59	
Treatment	2	5.384E+03	2.692E+03	11445.03	<.001
Fraction	2	1.948E+05	9.739E+04	4.140E+05	<.001
Treatment x Fraction	4	2.819E+04	7.048E+03	29962.23	<.001
Residual	16	3.764E+00	2.352E-01		

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Total	26	2.284E+05			

Variate: Hemicellulose (Initial Establishment)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	1.534E+00	7.670E-01	11.03	
Treatment	2	2.134E+03	1.067E+03	15342.36	<.001
Fraction	2	4.392E+04	2.196E+04	3.158E+05	<.001
Treatment x Fraction	4	6.609E+03	1.652E+03	23759.58	<.001
Residual	16	1.113E+00	6.954E-02		
Total	26	5.266E+04			

Variate: Ash (Initial Establishment)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	5.816E-02	2.908E-02	8.11	
Treatment	2	8.135E+02	4.067E+02	1.134E+05	<.001
Fraction	2	7.124E+02	3.562E+02	99338.87	<.001
Treatment x Fraction	4	1.332E+03	3.329E+02	92849.47	<.001
Residual	16	5.737E-02	3.586E-03		
Total	26	2.858E+03			

Variate: Dry matter (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	4176.9	2088.4	5.66	
Treatment	2	21999.3	10999.6	29.79	<.001
Fraction	2	1935.6	967.8	2.62	0.104

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Treatment x Fraction	4	747.1	186.8	0.51	0.732
Residual	16	5907.4	369.2		
Total	26	34766.2			

Variate: CP (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	0.9807	0.4904	3.32	
Treatment	2	168.6896	84.3448	570.40	<.001
Fraction	2	23524.6607	11762.3304	79544.88	<.001
Treatment x Fraction	4	9840.6215	2460.1554	16637.24	<.001
Residual	16	2.3659	0.1479		
Total	26	33537.3185			

Variate: NDF (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	1.089E-01	5.444E-02	1.26	
Treatment	2	8.262E+03	4.131E+03	95632.98	<.001
Fraction	2	2.317E+05	1.158E+05	2.682E+06	<.001
Treatment x Fraction	4	1.738E+04	4.345E+03	1.006E+05	<.001
Residual	16	6.911E-01	4.319E-02		
Total	26	2.573E+05			

Variate: ADF (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	7.119E-01	3.559E-01	6.77	

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Treatment	2	8.842E+03	4.421E+03	84061.57	<.001
Fraction	2	1.354E+05	6.768E+04	1.287E+06	<.001
Treatment x Fraction	4	4.092E+03	1.023E+03	19453.74	<.001
Residual	16	8.415E-01	5.259E-02		
Total	26	1.483E+05			

Variate: Hemicellulose (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	1.4052	0.7026	4.35	
Treatment	2	750.0474	375.0237	2324.39	<.001
Fraction	2	13006.5274	6503.2637	40307.17	<.001
Treatment x Fraction	4	10255.4815	2563.8704	15890.85	<.001
Residual	16	2.5815	0.1613		
Total	26	24016.0430			

Variate: Ash (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	4.110E-02	2.055E-02	3.82	
Treatment	2	1.320E+03	6.599E+02	1.225E+05	<.001
Fraction	2	2.309E+03	1.155E+03	2.144E+05	<.001
Treatment x Fraction	4	2.331E+02	5.828E+01	10821.72	<.001
Residual	16	8.617E-02	5.386E-03		
Total	26	3.862E+03			

Appendix 3. ANOVA tables for *Cajanus cajan in vitro* gas production

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Variate: Gas at 24 hours (Initial Establishment)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	13.1388	6.5694	13.50	
Treatment	2	4.3847	2.1924	4.51	0.028
Fraction	2	23.6144	11.8072	24.27	<.001
Treatment x Fraction	4	17.5680	4.3920	9.03	<.001
Residual	16	7.7841	0.4865		
Total	26	66.4900			

Variate: *IVOMD* (Initial Establishment)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	11.1017	5.5508	13.86	
Treatment	2	48.8399	24.4199	60.95	<.001
Fraction	2	146.2157	73.1078	182.48	<.001
Treatment x Fraction	4	74.1514	18.5379	46.27	<.001
Residual	16	6.4101	0.4006		
Total	26	286.7188			

Variate: SCFA (Initial Establishment)					
Source of variation	Df	s.s	m.s	v.r	F pr.
Rep stratum	2	2.711E-05	1.355E-05	13.50	
Treatment	2	9.047E-06	4.523E-06	4.51	0.028
Fraction	2	4.872E-05	2.436E-05	24.27	<.001
Treatment x Fraction	4	3.625E-05	9.062E-06	9.03	<.001
Residual	16	1.606E-05	1.004E-06		
Total	26	1.372E-04			

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Variate: ME (Initial Establishment)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	0.323832	0.161916	16.35	
Treatment	2	30.091223	15.045612	1519.43	<.001
Fraction	2	161.201193	80.600596	8139.72	<.001
Treatment x Fraction	4	40.178949	10.044737	1014.40	<.001
Residual	16	0.158434	0.009902		
Total	26	231.953632			

Variate: Gas at 24 hours (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	0.055	0.027	0.02	
Treatment	2	21.291	10.645	9.65	0.002
Fraction	2	63.554	31.777	28.79	<.001
Treatment x Fraction	4	26.738	6.685	6.06	0.004
Residual	16	17.658	1.104		
Total	26	129.296			

Variate: *IVOMD* (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	0.0544	0.0272	0.03	
Treatment	2	27.2392	13.6196	15.30	<.001
Fraction	2	10.9524	5.4762	6.15	0.010
Treatment x Fraction	4	19.5513	4.8878	5.49	0.006
Residual	16	14.2460	0.8904		
Total	26	72.0434			

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Variate: SCFA (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	1.125E-07	5.625E-08	0.02	
Treatment	2	4.393E-05	2.196E-05	9.65	0.002
Fraction	2	1.311E-04	6.556E-05	28.79	<.001
Treatment x Fraction	4	5.517E-05	1.379E-05	6.06	0.004
Residual	16	3.643E-05	2.277E-06		
Total	26	2.668E-04			

Variate: ME (Regrowth)					
Source of variation	df	s.s	m.s	v.r	F pr.
Rep stratum	2	0.00614	0.00307	0.17	
Treatment	2	0.74515	0.37258	20.04	<.001
Fraction	2	59.57068	29.78534	1601.79	<.001
Treatment x Fraction	4	28.30571	7.07643	380.55	<.001
Residual	16	0.29752	0.01860		
Total	26	88.92519			

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Plate 1. *Cajanus cajan* harvested to a staple height

Plate 2. Determination of NDF and ADF with Ankom200 fibre analyser

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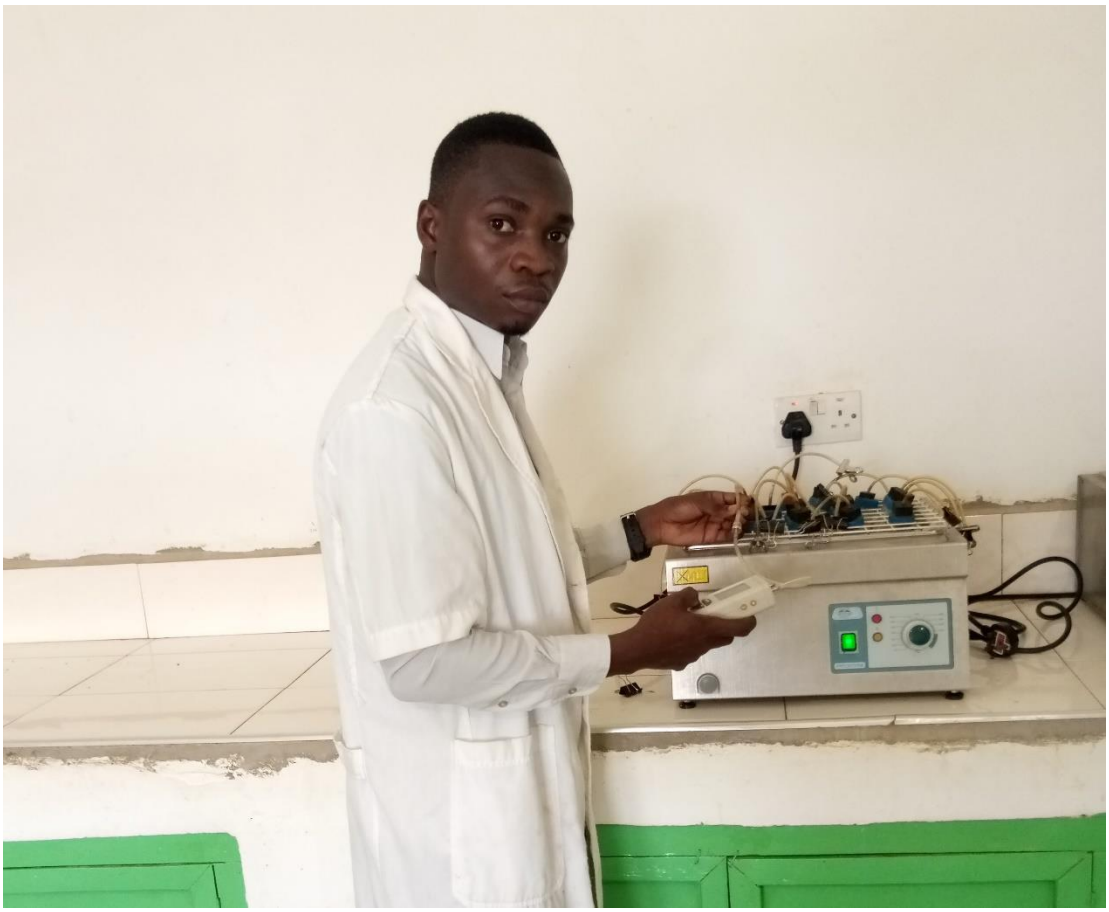

Plate 3. Gas reading of experimental samples in water-bathe