SOME FACTORS INFLUENCING THE BIOMASS YIELD AND NUTRITIONAL VALUE OF IMPROVED COWPEA HAULM AS RUMINANT FEED

by

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DECLARATION

This is to affirm that this thesis has been authored by me and has neither been submitted for a degree nor any aspect published by another person elsewhere. All cited literature in the text has been well referenced and any assistance received in writing the thesis is duly acknowledged.

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ABSTRACT

Small scale livestock farmers in Ghana commonly use native grass and crop residues as feed for their ruminant livestock. These feeds however, have low nutritive value and subsequently affect productivity of ruminants. Cheap and affordable source of supplementation (i.e. leguminous forages) is therefore needed. Climatic conditions affect the quantity and quality of the forage. To address some of the problems encountered by small scale livestock farmers in the country, the research examined the effects of cultivar, season and year of cultivation on grain and haulm yield of cowpea. This aimed at providing data on yield expectations of the selected cowpea cultivars as well as aid in risk assessments. To further enhance food security (i.e. feed availability especially during the lean season), the study compared the common storage practices by farmers to an ideal method that maintains the haulm quality and quantity during storage. Owing to the numerous insect pests of cowpea, pesticides are applied by farmers to bring insects population below economic injury. There have been growing concerns regarding pesticide ingestion by ruminants and its effect on rumen fermentation. This was tested by varying pesticide concentration based on the residue levels in the cowpea haulm on *in vitro* gas production by the haulms. To evaluate the haulms' use as supplement, they were offered to sheep given a basal diet of maize stover and the nutritional assessments done.

Five separate experiments were conducted in this research to address these concerns. The first trial (Agronomy Experiment 1) tested the seasonal and yearly effects on grain and haulm yield of four cultivars of cowpea (SORONKO, IT93K-2309, IT86D-716, and IT93K-2045-93) established on arable land at the Department of Animal Science, KNUST for three years (2005, 2006 and 2007). The study revealed that, yield estimates of the cultivars were significantly ($P < 0.001$) influenced by season and year of cowpea establishment. The interaction of season and year however, was significant ($P < 0.001$) for grain and haulm yield. The research revealed that, the grain yield of cultivar IT86D-716 was higher than SORONKO, IT93K-2309 and IT93K-2045-93, and would therefore be a cultivar of choice for wet season cowpea cultivation. However, since cowpea is sensitive to drought during the pod filling stage, the cultivar which tolerated the drought conditions (i.e. IT93K-2045-93) and compared well with the other 3 cultivars in terms of grain and haulm yields would be the ideal cultivar for both wet and dry season cowpea establishment.

The second experiment (Agronomy Experiment 2) tested the effects of 3 methods of storage on DM loss in cowpea haulms, and retention of nitrogen, neutral detergent fibre, acid detergent fibre and nitrogen bound to fibre levels as a function of storage time. Losses of haulm DM, NDF and ADF remaining in the four cowpea cultivars were similar ($P > 0.05$) but differences in CP and NDIN existed among the cultivars ($P < 0.05$). The haulms lost more DM with roof storage ($P = 0.002$) than with the shed storage with increasing storage time ($P < 0.001$). Concentration of ADIN in the cultivars with roof storage was 1.3 times greater than for those kept in the shed ($P < 0.001$). Storage method interacted with week for all the parameters measured, because the impact of storage became greater with time ($P < 0.001$). The study showed that the shed system of storage improves the availability and quality of fodder in respect of dry matter and nutrient composition. Therefore, the shed storage system is suggested to farmers as a way of ensuring nutrient retention in cowpea haulm and haulms availability all year round.

Experiment 3 (Animal Experiment 1) investigated the chemical composition and the nutritive value using both *in situ* and *in vitro* gas production techniques. Cultivar effect was significant ($P < 0.0001$) with regard to CP, ash, NDF, NDIN, ADF, ADL, ash free CF ($P = 0.01$) and EE ($P = 0.0067$). Gas production data revealed that, cultivar effect on readily fermentable portion represented as "*a*" and rate of gas production "*c*" tended to approach significance ($P = 0.0784$ (*a*); $P = 0.0856$ (*c*)). However, the greatest gas production (27.97 ml gas/200 mg DM) at a rate of 7.6 % h^{-1} was observed in the cultivar IT93K-2045-93. All the cultivars had similar ($P = 0.989$) pools of digestible fibre and disappeared at the same rates ($P = 0.778$). Assessment of CP degradation showed that cultivar IT93K-2045-93 had a greater percent soluble material than IT93K-2309 ($P < 0.001$). The amount of ruminal digestible protein (RDP) was greater ($P = 0.024$) in IT93K-2309 than in IT93K-2045-93, however, the latter disappeared at greater rates ($P < 0.001$) than the former. The potential utility index (PUI) estimated from the data obtained in Agronomy Experiment 1 and Animal Experiment 2 was significantly higher ($P < 0.0001$) in cultivar IT93K-2045-93 than for the other cultivars in the dry season. This was so for the same cultivar in the wet season except that IT93K-2045-93 and IT86D-716 were similar ($P = 0.058$). It was concluded that, cultivars IT93K- 2045-93 and IT93K-2309 have a set of desirable attributes with greater grain and haulm yields, potential utility

index, and nutritive value compared to IT86D-716 and SORONKO and may serve the diverse needs of farmers.

Experiment 4 (Animal Experiment 2) assessed the effects of pesticide namely Lambda cyhalothrin, Dimethoate and Cypermethrin residues on *in vitro* gas production and some predicted fermentation parameters (i.e. short chain volatile fatty acid and microbial protein supply). Application of Cypermethrin resulted in no significant differences ($P > 0.05$) in gas production while application of Dimethoate and lambda Cyhalothrin increased gas accumulation beyond control levels with increasing concentration. Pesticide residue application on gas production showed that the three pesticides considered did not inhibit gas production and fermentation parameters at the 40 µl pesticide application. It is therefore important to note that, high levels of pesticides, presumably beyond that encountered in the field or lethal dose (LD_{50}), will be required for inhibition of rumen microbial activities to occur.

In the final experiment (Animal Experiment 3), four rumen fistulated rams were used in a Latin square design to assess the degradation, intake and digestibility of maize stover (MS) when sheep were fed a supplement of cowpea haulm. Significant differences ($P < 0.05$) in the intake of MS were observed as the level of supplement increased. Dry matter intake of the basal diet and the supplement varied between 13.5 and 18.3; 5.01 and 7.02 g DM kg^{-1} LW respectively whereas digestibility ranged between 31.6 and 75.6%. Substitution of basal diet by supplement occurred when supplement was fed beyond 14.64 g DM kg^{-1} LW. Total DM intake was similar for the supplemental levels of 7.32 and 21.96 g DM kg^{-1} LW, regardless of the differences in supplement DM intake. The DM degradability parameters of maize stover were significantly influenced ($P < 0.001$) by cowpea haulm supplement. However, no difference in the potentially digestible fraction was observed between cowpea haulm at highest level (14.64 vs. 21.96 g DM kg^{-1} LW) of supplementation. The results suggest that, where limited quantities of cowpea haulm are available, it is possible to offer small amounts i.e. 150g/ d cowpea haulm to improve intake when animals are consuming poor quality fodders.

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LIST OF ABBREVIATIONS

%	Per cent
^0C	Celsius
ADF	Acid Detergent Fibre
ADIN	Acid Detergent Insoluble Nitrogen
ADL	Acid Detergent Lignin
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
APRI	Agricultural Production and Research Institute
DAS	Department of Animal Science
$(M)^{0.75}$	Metabolic weight
c	Rate of Degradation
CF	Crude Fibre
CV	Coefficient of variation
CP	Crude Protein
CRI	Crop Research Institute
CSIR	Council for Scientific and Industrial Research
DDD	Dichlorodiphenyldichloroethane
Df	Degree of Freedom
DDT	Dichlorodiphenyltrichloroethane
DM	Dry Matter
DMD	Dry Matter Digestibility
DMI	Dry matter Intake

EE	Ether Extract
FAO	Food and Agricultural Organization
GC-ECD	Gas Chromatography – Electrochemical Detection
h	Hour
H_2SO_4	Sulphuric Acid
ha	Hectare
HCl	Hydrochloric Acid
HDML	Haulm Dry Matter Loss
HR	Haulm Remaining
HYW	Haulm Yield Weight
IITA	International Institute for Tropical Agriculture
IVDMD	*In Vitro* Dry Matter Digestibility
IVOMD	*In Vitro* Organic Matter Digestibility
kg	Kilogram
KNUST	Kwame Nkrumah University of Science and Technology
LD_{50}	Lethal Dose
LW	Live weight
mcg	Millicentigram
mg	Milligram
ml	Millilitre
MP	Microbial Protein
MS	Maize Stover
MSD	Mean Significant Difference
N	Nitrogen

NaOH	Sodium Hydroxide
NDF	Neutral Detergent Fibre
NDIN	Neutral Detergent Insoluble Nitrogen
NFE	Nitrogen Free Extract
NH_3	Ammonia
NH_3-N	Ammonia Nitrogen
NS	Not Significant
OMD	Organic Matter Digestibility
ppm	Parts Per Million
r	Coefficient of Correlation
r^2	Coefficient of Determination
RDP	Rumen Degradable Protein
S	Sulphur
SCFA	Short Chain Fatty Acid
SE	Standard Error
SM	Storage Method
t	Tonne
VFA	Volatile Fatty Acid
WHO	World Health Organisation

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CHAPTER 1

1.0 INTRODUCTION AND SCOPE OF WORK

Livestock plays an important role in most small-scale farming systems throughout the world. They provide traction to cultivate fields, manure to maintain crop productivity, nutritious food products for human consumption and additional income for farmers (CGIAR, 1997). Notwithstanding the importance of livestock within the agricultural system, their production is constrained by scarcity and fluctuating supply of large quantity and high quality of feeds all year round. These problems are accentuated in regions of the world with distinct dry seasons. In Ghana, during the dry season available grasses become senescent, less palatable, and contain large proportions of indigestible fibre. According to Peacock (1996) these characteristics of pastures result in reduced intake or make it difficult for ruminants to consume enough material to support adequate performance, or in some cases, to utilize the material at all. The available feeds are often limiting in fermentable nitrogen, glucogenic precursors, by-pass protein, and dietary long chain fatty acids (Preston and Leng, 1987) and are characterised by crude protein levels of less than 70 g/kg. Rate of passage is slow and feed resides in the rumen for a long time before moving out, thereby reducing feed intake (Peacock, 1996). Consequently, growth performance and milk production are affected, and calving, kidding and lambing intervals are extended (Zinash and Seyoum, 1989).

Osuji *et al.* (1993) reported that dietary supplements were required in order to attain adequate levels of glucose and glycogenic compounds to support high ruminant productivity from low quality tropical forage. However, conventional animal feeds (i.e. cereal and protein based diets – maize and wheat bran, fish meal and soy bean meal) that ensure high productivity may be in short supply owing to the high demand

for human consumption. With increasing consumption and demand for livestock products, as a result of growing economies, rising incomes and changes in lifestyle, urbanization and the associated shrinking land area, future hopes of feeding billions of people and ensuring food security will depend on the better utilization of non-conventional feed resources.

Leguminous crops represent such feed resources which could help ensure food security. Legumes thrive in marginalized and degraded soils resulting from intensification of crop production to feed the ever increasing population and play a vital role in improving soil fertility and checking of erosion, and also some are quite drought tolerant. Nutritionally, the haulm contains greater levels of protein and minerals than crop residues and enhances fibre degradation in the rumen, hence improved animal performance. Cowpea (*Vigna unguiculata* L.Walp) is a grain legume and a valuable component of the traditional cropping systems in the semi-arid tropics (Singh *et al.*, 1997). It is one of the most drought resistant food legumes serving as an insurance crop to sustain production during drought periods. Cowpea is consumed in many forms. Nielsen *et al.* (1997) reported the utilization of its young leaves, green pods and green seeds as vegetable while the dry seeds are used in the preparation of several foods. The haulms, according to Singh *et al.* (2003), are used as nitrogenous feed supplement for livestock. Work in Nigeria by Tarawali *et al.* (1996) showed that cowpea haulm contains 20-30% crude protein (CP) which 60-70% is digestible. Devendra and McLeroy (1982) reported cowpea haulm having ME content of 10-12 ME MJ/ kg DM; and supporting a growth rate of 50-80g per day, in a study with sheep. Therefore, cowpea's dual role as grain for human consumption and haulm for feeding livestock could reduce the human-animal competition for food grains as well as cost of livestock production.

With regards to cowpea's importance as grain and haulm, methodical breeding programmes which combined breeding for high yield potential for grain and haulm were initiated by the International Institute for Tropical Agriculture (IITA) in the 1990's. Similar breeding programmes i.e. breeding for improved dual-purpose cowpea with high grain and haulm yield, adaptation to the various ecological zones (Coastal, Forest and Transitional), as well as adaption to drought are being conducted on the experimental fields of the Council for Scientific and Industrial Research - Crop Research Institute (CSIR-CRI), at Fumesua in the Ashanti Region of Ghana. Cowpea cultivars with high agronomic characteristics were developed. To ensure enhanced nutritional characteristics of the identified cultivars of cowpea haulms, collaborative efforts with the Department of Animal Science, KNUST were instituted. Ten dual-purpose cowpea cultivars with high agronomic and nutritional features were identified in a previous studies (Antwi *et al.*, 2004); out of which four cultivars which ranked highest in grain yield and chemical composition i.e. SORONKO, IT93K 2309, IT86D-716 and IT93K 2045-29 were selected for this study.

Cowpea is grown in both the wet and dry seasons in Ghana and in some areas, the major cropping season. Although cowpea is adapted to drought conditions (Dadson *et al.*, 2005), some precipitation is required if premature leaf abscission prior to harvesting is to be avoided. While the dry season is characterized by low haulm yield, large quantities of haulms are available in the wet season of cowpea growth. Efforts are made by farmers in Northern Ghana and Nigeria to store surplus haulms for dry season feeding in a bid to ensure a stable supply of haulm for feeding throughout the year. Most farmers leave the haulms on the field as standing hay, or stack them on traditional structures or on a tree exposing them to losses due to effects of the

weather. Such losses, as described by Tripathi *et al.* (1995), vary from shattering of leaves, leaching of soluble nutrients by rain, and potentially large losses due to mold damage and bleaching by exposure to the weather. The losses aforementioned influence the quality of the haulm i.e. increased acid and neutral insoluble bound nitrogen and fibre concentrations (Rotz *et al.* (1991), and may reduce feed intake.

Cowpea is attacked by numerous insect pests which damage the crop from seedling emergence to storage, and are responsible for 20-100% of cowpea grain or foliage loss (Singh and Jackai, 1985). Cowpea thus cannot be grown successfully without at least one or two pesticide applications. Farmers having realized the dramatic benefits of insecticide application on cowpea productivity have resorted to continued and increased use of the pesticides without any concerted effort to abide by the recommendations of the manufacturers. The inception of pests triggers the application of insecticides by farmers regardless of the days to maturity. Inherent problems of accumulation and degradation of compounds in edible tissues associated with pesticide use is well recognized (Laben, 1968). Studies on pesticides use on the fields of cowpea have concentrated on degradation rates in plants and excretion products in the urine and feces of ruminants (Gutenmann *et al.*, 1968). However, how the ingestion of pesticide-contaminated haulms affects the microbiota functions in the gut is yet to be ascertained. The outcome of any such studies will be of significance to researchers and farmers.

1.1 Overview of thesis

Feed availability and quality of feed resources year round affect animal production within smallholder systems in Ghana. The available feed resources i.e. grasses are only productive during the rainy season and besides multi-purpose trees or dual-

purpose forages known of their high nutritional quality are not traditionally utilized within the smallholder sectors. This may be as a result of the greater interest in grain yield by plant breeders and little collaborative efforts between plant and animal researchers; hence, the limited research on haulm quality of dual-purpose legumes. In resolving the challenges of feed availability year round and utilization of the haulms of dual-purpose cowpea cultivars, the study considered the following experimentations:

(1) To identify cultivars with yield (grain and haulm) stability in any growing season or year, and availability of feed throughout in the year, agronomy experiments were set out. The first agronomy experiment sought to provide data on yield expectations of the selected cowpea cultivars as well as aid in risk assessment. This was achieved by cropping both in the wet and dry seasons of 2005, 2006 and 2007. To further aid in the selection of cultivars with high agronomic and nutritive characteristics, an index called potential utility index (Fleischer *et al.*, 1989) which integrates grain and haulm yield, and *in situ* degradability data (Ørskov *et al.*, 1980) were adopted.

(2) With the issue of feed availability, which is an immense problem in smallholder farming systems in Ghana, a storage experiment was undertaken to recommend the best storage practices that would make available feed of high quality year round to ensure improved animal performance. It is a common practice to see most farmers leaving crop residues after grain harvest on the field as standing hay or cut, rolled and stored on trees or roof. This practice according to Al-Mamum *et al.* (2002) may reduce the quality and quantity of haulm available for lean season feeding. A storage experiment was therefore designed to identify the best storage practice that would ensure feed

availability and hence food security. To arrive at this, the haulms of the cowpea cultivars were kept under three different conditions to simulate how farmers stored their haulms (i.e. roof, shed and field). The changes in quality during storage were monitored for 12 weeks.

(3) The concern over pesticide residues' influence on rumen fermentation parameters was raised in 2004 during a paper presentation at the 14th Biennial Conference of the Ghana Animal Science Association, at the Radach Memorial Centre, Tamale, Ghana. The study attempted to address this issue by varying the concentration of the pesticides used in cowpea insect control in the laboratory to ascertain pesticide effect on *in vitro* gas production and other predicted parameters. To obtain data that will be useful to researchers and farmers, the pesticides commonly applied by farmers in Ghana to control cowpea insects were used this study (i.e Lambda-cyhalothrin, Cypermethrin and Dimethoate).

(4) With regard to the nutritional quality of the haulms of the cowpea cultivars under study, *in vitro* gas production and *in situ* degradability techniques were used as an evaluation tool for nutritive value. In assessing the utilization of the cowpea haulms as feed supplement to a crop residue (i.e. maize stover), cultivar IT93K 2045-29, which ranked highest in agronomic characteristics and nutritive value were fed to rams in a 4 x 4 Latin square designed experiment.

The outcome of the thesis hopefully will be useful especially to smallholder farmers who cannot afford conventional supplements such as co-products of grain processing.

1.2 Research Objectives

The research therefore sought to address a major constraint in livestock production by developing feeding strategies that ensures availability and high quality feed year round. To realize this goal, the following objectives were set in this study:

- ascertain the influence of season and year of planting on the yield of grain and haulm among four cowpea cultivars namely, SORONKO, IT93K-2309, IT86D-716, and IT93K-2045-93
- assess the effects of cultivar on degradation characteristics and potential utility indexes of the haulms.
- determine the effect of varied concentrations of three pesticides namely Lambda-cyhalothrin, Cypermethrin and Dimethoate,on *in vitro* gas production levels of cowpea haulms.
- investigate the effects of three different storage practices on changes in nutrient composition as a function of time in storage and
- evaluate the effect of the use of cowpea haulm as feed supplement on feed intake, degradability and digestibility of a maize stover basal diet.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Crop residues

Millions of tonnes of cereal straws are produced annually on agricultural fields in Ghana. Amaning-Kwarteng (1992) reported of about 2.3 million tonnes of cereal crop residues produced annually in Ghana and added that, if their use as feed resource is optimized, the estimated livestock weight loss of 186 million tonnes during the dry season would be saved. Crop residues therefore assume great importance in ameliorating the feed deficit in animal nutrition in Ghana. However, some crop residues have limited feeding value. Osuji *et al.* (1995) reported that crop residues are invariably bulky, high in fibre, poorly degraded in the rumen, low in nitrogen and minerals resulting in very low intakes. In addition, crop residues generate low levels of ammonia (NH_3) in the rumen upon fermentation (Ørskov, 1995). In a study where maize stover was fed alone to sheep (Tolera and Sundstol, 2000), intake, digestibility, and microbial protein synthesis were reported to have declined. In a similar trial, Koralagama *et al.* (2008) observed weight loss in rams fed a sole diet of maize stover and this was ascribed to high levels of NDF (738 g/kg DM) and lignin (94 g/kg DM), as well as a low N concentration of 6.08 g/kg DM.

Ibrahim (1986) documented that better utilization or improvement of the feeding value of crop residues can be achieved by physical and chemical treatment or supplementation with conventional feedstuffs such as oilseed cakes. The author reported that grinding, chopping or steam treatment of straw were beneficial physical means of increasing voluntary intake. In addition, the author stated that digestibility and nitrogen content can be increased by chemical (e.g. using sodium hydroxide, potassium hydroxide, calcium hydroxide and sodium carbonate or with nitrogenous

compounds such as urea and ammonium hydroxide) treatment. However, the cost of treatment is high because it requires a lot of labour and the chemicals used are expensive. Besides, the availability of chemicals and conventional nitrogen supplements may pose problems for small-holder farmers. Thus the cost of treatment and supplement availability may prohibit the wide scale use of this technology by smallholder farmers.

Leguminous forage is a cheaper source of protein supplement for such low quality diets. Forage legumes are relatively good sources of degradable nitrogen and fermentable energy (Topps, 1995). So their inclusion in the diet is expected to improve the rumen environment and animal performance. The potential benefits of leguminous fodders as supplementary feed are explained below.

2.2 Supplementation with leguminous fodders

Supplementation aims at increasing essential nutrients or providing critical nutrients (i.e. energy and protein) lacking in the basal diet, and creates an optimum condition in the rumen resulting in better fermentation and microbial protein supply (Osuji and Odenyo, 1997). Leguminous foliage does survive, produce and store large quantities of green biomass that can serve as protein source (Jones, 1979). In a trial by Abule *et al.* (1995) using leguminous fodder as supplement to low quality crop residues, increases in concentration of NH_3-N, microbial-N and VFA were observed and these stimulated degradation due to an increased supply of nitrogen, fermentable carbohydrates, sulphur and other essential nutrients. Said and Tolera (1993) stated that leguminous crop residues improve protein supply to the host animal by increasing the supply of both degradable and undegradable protein depending on the level of tannins present in them, since tannins complex with the proteins thereby enhancing

supply of by-pass protein to the ruminant (Ash, 1990). Elliott and Topps (1963) reported that leguminous fodders with high content of rumen degradable nitrogen induce greater responses in food intake. This was confirmed in a study by Smith *et al.* (1990) where cowpea having the highest nitrogen content (comparable with pigeon pea and lablab hay) promoted the greatest intake of maize stover. The unique characteristics of cowpea are detailed below:

2.3 Features of cowpea

Cowpea is an important food legume and a valuable component of the traditional cropping systems in the semi-arid tropics covering Asia, Africa, Central and South America (Singh *et al.*, 1997). Cowpea contributes valuably towards human food and livestock fodder; in areas where land is scarce, its dual-purpose nature makes it an attractive crop. Singh *et al.* (2003) described cowpea as a drought tolerant and warm weather crop, well adapted to semi-arid regions of the tropics where other food legumes do not perform well. Sanginga *et al.* (2000) reports of the unique ability of cowpea to fix atmospheric nitrogen through its nodules, growing well in poor soils with more than 85% sand, less than 0.2% OM and low levels of phosphorous. Cowpea is noted for its quick growth and rapid ground cover which checks soil erosion; furthermore, when its root decay *in situ*, nitrogen-rich residues that improve soil fertility and structure are produced (Singh *et al.*, 2003).

2.3.1 Importance of cowpea as food and fodder

In developing countries, the contribution of cowpea to the nutrition and livelihoods of people cannot be overemphasized. Cowpea is a major source of proteins, minerals and vitamins (Bressani, 1985). Cowpea contains 200–300 g crude protein and about 600 g carbohydrate kg^{-1} seed. Arora and Das (1976) reported a range of values (g kg^{-1}

seed) for protein content (179 to 275), total soluble sugars (138 to 198), starch (507 to 670) and mineral matters (31 to 46) and seeds are also richer in leucine, lysine, phenylalanine and sulfur containing amino acid than Arachis hypogea seed (Orr and Wall, 1957). Cowpea is consumed in the form of its young leaves, green pods and green seeds as vegetables; whereas the dry seeds are used in a variety of food preparations (Nielsen *et al.*, 1997). Cowpea does impact positively on the nutrition and health of poor people whose diet is mainly starchy foods such as cassava and yam. The inclusion of cowpea in human diets is reported to improve the nutritional balance of the diet and enhance protein quality (Singh *et al.*, 2003).

Cowpea is equally important as nutritious fodder for livestock. At maturity, cowpea pods are harvested and haulms cut while green, rolled into small bundles and stored on roof tops or tree forks for use or sale as feed supplement in the dry season, particularly in Northern Nigeria (Singh and Tarawali, 1997). These authors further reported a price range of cowpea haulm on dry weight basis of between 50-80% of the price of grain, constituting an important source of income. The nutritional importance of cowpea haulms has been reported by several authors and is detailed in the Section 2.3.2 below:

2.3.2 Nutritional quality of cowpea haulm

The nutrient composition and nutritional characteristics of cowpea haulm from several authors is shown in Table 2.1. Savadogo *et al.* (2000) reported the crude protein content of cowpea roots and stems to be 7.7 and 7.8 % of DM respectively which was much lower than that of the leaves (14.6% of DM). This compares well with the CP values of 8.1, 5.9, 10.7, and 8.2 % DM respectively for the root, stem, leaves and the whole haulm of groundnut reported in the same experiment. In their experiment, the

whole haulm contained 91.8% OM and 12.4% CP, and had an IVOMD of 67.5 %. Digestibility varied from 63.9% (stems) to 72.0% (leaves). Camara (1996) and Kaasschieter *et al.* (1998) also reported CP values of cowpea haulms ranging from 7.8 to 21.7%. The data reported by these authors varied widely from those presented in Table 2.1.

The observed variations in the nutrient composition of the cowpea haulm reported above may be ascribed to inherent genetic characteristics (Badve *et al.*, 1994; Singh and Schiere, 1995; and Subba Rao *et al.*, 1994), environmental factors such as soil characteristics and rainfall and crop management (level of fertilizer application, plant density, stage of maturity at harvest, methods of harvesting, and storage) (Harika and Sharma, 1994; Walli *et al.*, 1994).

2.3.3 Effect of cowpea haulm supplementation on intake, degradability, digestibility and animal performance

The effect of cowpea haulm supplement on intake, digestibility and animal performance has been investigated (Savadogo *et al.*, 2000; Koralagama *et al.*, 2008). In the study by Koralagama *et al.* (2008), increasing levels of cowpea haulm supplementation induced greater intake of a basal diet of maize stover with no apparent effect of substitution when the low (150g) and high (300g) inclusion rates of cowpea haulms were compared. Their findings supported the outcome of the study by Bonsi *et al.* (1994) but contrasted the findings of Savadogo *et al.* (2000), who reported that sorghum stover intakes declined linearly with level of supplementation at the rate of 0.424 g g^{-1} of cowpea haulm offered. In related studies, increases in intake of a basal diet of teff (*Eragrostis tef*) straw (Abule *et al.*, 1995) as well as supply of microbial N (Osuji and Odenyo, 1997) were observed with cowpea haulm supplementation.

Table 2.1 Chemical composition and nutritional characteristics of cowpea haulm (g/kg)

Composition	Authors								
	I	II	III	IV	V	VI	VII	VIII	Mean
DM	944	897	909	920	-	-	-		917.5
OM	819	904	910.9	860	-	-	-	918	882.4
Ash	181		89	140.0	-	-	385		198.6
CP	168	137	141.5	193.7	185	268.5	268	124	185.7
EE	-	-	-	-	-		16		16
NDF	419	516	507.2	463		503			481.6
ADF	367	464	386.7	384	-	-	-		400.4
Lignin	101	116	-	78.8	-	-	-		98.6
Calcium	-		10.9	12.2	-	-	-		11.6
Phosphorus	-	-	-	4.10	-	-	-		4.1
IVOMD	-	-	-	-	-	-	-	675	675
IVDMD		-	-		669	615.5	503		595.8
Degradation constants									
a	-	-	-	190	-	-		-	190
b	-	-	-	559	-	-		-	559
c	-	-	-	0.064	-	-		-	0.064

Where I = Koralagama *et al.* (2008); II = Coppock and Reed (1992); III = Chakeredza *et al.* (2002); IV = Abule *et al.* (1995); V = Sanghi and Raj (1983); VI= Ram *et al.* (1990) VII = Buamah (1971) and VIII = Savadogo *et al.* (2000).

The increased intakes of basal diets of teff straw and maize stover in the various studies may be due to the increased supply of readily degradable carbohydrate and nitrogen. The ready supply of carbohydrate is reported by Silva and Ørskov (1988) to have stimulated ruminal fibre degradation and enhance production of rumen ammonia.

In a study by Chakeredza *et al.* (2002), where haulms of cowpea, groundnut, cotton seed meal and maize meal were used as supplement to maize stover diet, the results showed no significant dietary differences ($P > 0.05$) for either dry matter or organic

matter degradabilities. They concluded that degradation kinetics of maize stover is not influenced by supplementation of the diets used, although factors like ammonia concentration that is thought to enhance microbial activity were significantly increased over time. In contrast to the study by Chakeredza *et al.* (2002), Abule *et al.* (1995) reported an increased rate of degradation of teff straw in calves supplemented with cowpea haulm and reduced mean retention time of the teff straw than in calves fed teff straw alone. Their study agreed with the results obtained by Bonsi *et al.* (1994) who reported similar trends due to supplementation with Sesbania or leucaena.

In the trial by Koralagama *et al.* (2008), apparent digestibility of maize stover improved from 450g/kg to 550g/kg and 510g/kg when cowpea haulm was offered at 150 and 300g/ day respectively. Similar digestibility values and liveweight gains were obtained from higher (300g) and lower (150g) levels of cowpea haulm supplementation by the same authors.

In another trial by Singh *et al.* (2003), where rams were fed graded levels (0, 200, 400 or 600g) of cowpea haulm as supplement to a basal diet of sorghum stover, an average daily gain of 31.3g was observed with the 200g cowpea supplement. Increases in the supplement level to 400 and 600g, however, resulted in diminishing returns on daily weight gain. The findings of their studies showed no additional value to animal performance but a waste of feed resources when excess cowpea haulm is offered as supplement above the 200g level.

2.4 Yield and factors influencing the yield of improved dual purpose cowpea

Cowpea grain is as important as the haulm (Singh *et al.*, 1997); hence, the importance of breeding for dual-purpose cowpea cultivars to suit the diverse needs of humans as

food and livestock as feed supplement. The yields of improved cowpea varieties have been investigated. Singh *et al.* (2003) reported a yield range of 1.79 - 2.58 and 1.20 - 5.99 t/ha, respectively. In an on-farm trial in Nigeria, several cowpea varieties were evaluated and the results showed a grain yield potential of over 1 t/ha and haulm yield of 4 - 10 t/ha (Singh *et al.*, 1997). In a related study, a most promising cowpea variety, IT89KD-288, which is an improved local variety in Nigeria that combines resistance to aphids, bruchids, thrips, nematodes, and some viruses, were released to small scale farmers. Results from the farmers' fields indicated a grain and haulm yield of 1.3 t/ha and 2.5 t/ha, respectively and these were higher than those of the local varieties (Singh and Blade, 1997).

The yields of cowpea grain and haulms are, however, affected by factors such as, season and year of production, water stress, and more importantly, insect pests. These factors influencing the yields of cowpea are discussed below.

2.4.1 Seasonal and year effects on yield and cowpea

Plant growth in semi-arid regions is limited by variations in the amount and duration of precipitation (Pandey *et al.*, 1984). Cowpea established in the major (wet) season is provided with sufficient moisture to meet its water requirement, especially during the reproductive phase. Seasonal effect becomes important in the growth of cowpea if the pod filling stage coincides with drought conditions especially during the dry season. Soil moisture availability, (i.e. deficit or surplus, a common occurrence in the dry and wet season, respectively) during the vegetative stage has little effect on seed yield if conditions improve and sufficient moisture is present during flowering and pod-fling (Muleba *et al.*, 1991). Thus, if rain or water for irrigation is limited in any particular

year, it is possible to withhold irrigation during the vegetative stage with negligible effects on seed yield (Ziska and Hall, 1983).

Kirksey *et al.* (2003) reported that, year of growth characterised by low precipitation especially prior to or during the growth period of cowpea, influenced the cowpea stand establishment and vegetative growth. That notwithstanding, reduction in the yields of cowpea was reported by Muleba *et al.* (1991) during periods with severe and protracted drought and was attributed to little or no precipitation during either flowering or pod-filling.

2.4.2 Effects of water on yields of cowpea

Cowpea is reported to be drought tolerant crop (Akyeampong, 1985). However, when cowpea was subjected to water stress at the vegetative stage in a trial by Warrrag and Hall (1984), it resulted in a reduced rate of leaf expansion and cessation of new leaf production. The stressed cowpea plant remained in a stunted state until they were re-watered. Besides, drought stress at the flowering stage caused senescence and abscission of mature basal leaves. Turk *et al.* (1980) also observed that induced drought during the vegetative stage followed by weekly irrigation just prior to flowering and continuing until 50% senescence, maximized cowpea grain yields. It is therefore inferred that, some amount of water is needed during both vegetative and reproductive stages if severe yield loss was to be avoided.

2.4.3 Pesticides use in cowpea production

Generally, cowpea production in Ghana is fraught with misuse and overuse of pesticides. Insect poses big problems in cowpea production and these have led many farmers to use chemical pesticides even if they have received no training in application techniques. The results on pesticides residue analysis have indicated water pollution, food contamination and accumulation of toxic compounds in human fluids of farmers.

Cowpea production is considered too risky an investment by many growers owing to the pest problems associated with it. Jackai and Adalla (1997) documented that insect pests damage cowpea from seedling emergence to storage. The insect pests (aphids, thrips, *Maruca* pod borer, pod bugs and bruchid) are responsible for 20-100% cowpea grains or foliage yield reduction (Singh and Jackai, 1985). According to them, the most damaging of all pests are the flower thrips, legume pod borer, and pod and seed suckers that occur during the flowering and podding stage. The pest problem on cowpea production in Africa is more severe than elsewhere, mainly because the pests are considered indigenous to the continent (Ng and Maréchal, 1985).

The most widely known form of pest control for cowpea is the use of insecticide applications. The insecticides have the benefit of improving cowpea yields by at least tenfold with 2 - 4 applications through reduction or controlling insects to levels below economic injury (Afun *et al.*, 1991). However, its effect on human health and the environment over a long period is immense as pesticides can cause health problems such as birth defects, nerve damage and cancer.

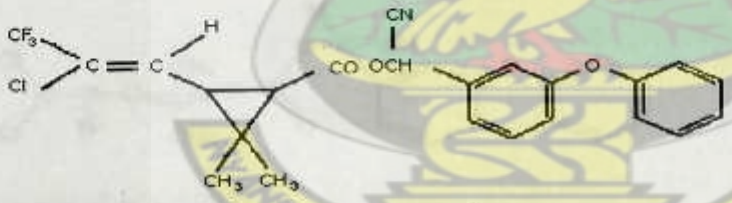
The most commonly used insecticides include endosulfan, lambda cyhalothrin, Cypermethrin, permethrin and Dimethoate. The description of endosulfan and

permethrin has not been incorporated in the thesis as the emphasis of the research is on the commonly used chemicals i.e. lambda cyhalothrin, Cypermethrin, and Dimethoate on the fields of cowpea in Ghana.

2.4.3.1 Lambda-cyhalothrin

The World Health Organization (WHO) (1990) describes Lambda-cyhalothrin as an organochlorine and pyrethroid insecticide applied on the agricultural fields to control a wide range of species of Lepidoptera, Hemiptera, Diptera, and Coleoptera. Its structure is shown in Figure 2.1. It is used on cowpea fields at an application rate of 0.8 l/ha to control aphids. The compound is a stomach and contact insecticide and shows adulticidal, ovicidal and, particularly, larvicidal activity. Residues in food arising from the use of Lambda-cyhalothrin on crops and in animal health are low, usually less than 0.2 mg/kg. This is as a result of low application rates and rapid degradation in the environment.

Figure 2.1 The structural formula of Lambda cyaholothrin



Source: WHO (1990).

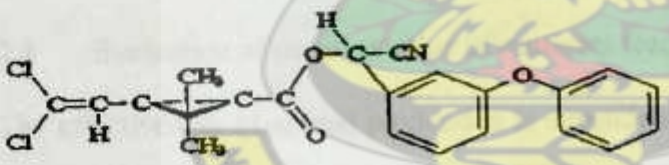
Lambda-cyhalothrin on soil surfaces and in aqueous solutions at pH of 5 degrades in sunlight with a half-life of approximately 30 days. The main degradation products are 3-(2-chloro-3,3,3-trifluoroprop1-enyl)-2-dimethylcyclopropane carboxylic acid, the amide derivative of cyhalothrin, and phenoxybenzoic acid. According to Dewey *et al.* (1962), the breakdown of organo-chlorine results in the release of inorganic chlorine

thereby reducing the toxicological attributes of the compound. The initial half-lives are in the range of 22 - 82 days. In plants, Lambda-cyhalothrin degrades at a moderate rate (half-life up to 40 days).

2.4.3.2 ·Cypermethrin

Cypermethrin is a synthetic pyrethroid insecticide (Figure 2.2) used to control many pests, including moth pests of cotton, fruit, and vegetable crops (Meister, 1992). The main pests of cowpea susceptible to Cypermethrin are thrips. When Cypermethrin was applied to strawberry plants with light rain on day 3, 40% of the pesticide remained after one day, 12% remained after three days, and 0.5% remained after seven days (Belanger, 1990). When Cypermethrin was applied to wheat, residues on the wheat were 4 ppm immediately after spraying and declined to 0.2 ppm 27 days later. No Cypermethrin was detected in the grain. Similar residue loss patterns have been observed on treated lettuce and celery crops (Westcott and Reichle, 1987).

Figure 2.2 structural formula of Cypermethrin



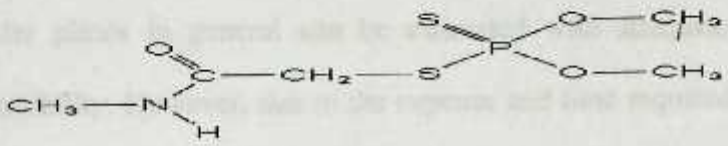
Source: FAO (1992).

2.4.3.3. Dimethoate

Dimethoate is an organophosphate ester (Figure 2.3) and a cholinesterase inhibitor. It is applied on a cowpea field at a rate of 1.95 l/ha to control pod sucking insects. It decomposes to give products which are more toxic than the original substance (Casida *et al.*, 1976) and is active as a contact and systemic insecticide (FAO, 1982).

Dimethoate has a broad spectrum effect due to its contact and systemic action when applied to both animals and plants. As a result it is being adopted for use against a wide variety of pest species on virtually every crop except a small number that show phytotoxic reaction to some Dimethoate formulations.

Figure 2.3 Structural formula of Dimethoate



Source FAO (1982)

According to Cook (1957), hydrolysis of organophosphate (e.g. Dimethoate) by rumen microbial flora resulted in less toxic compounds in their studies. The products from the hydrolysis of the parent compound (Figure 2.3) by rumen microbes to inorganic sulphur and phosphorus may have influenced the fermentation activities in this research (Chapter 7).

2.5 Evaluation of nutritive value of ruminant feedstuffs

The effectiveness of animal production is dependent upon the optimum utilization of feed for metabolic processes of maintenance and production. Therefore, in determining the biological efficiency, it is pertinent to have an indication of the feed's suitability in meeting the animal's requirement. Several feedstuffs (conventional and non-conventional) are available for animal production; in order to establish their suitability for meeting animal's requirement, it is important to have an indication of their nutritive value. Owing to this, techniques of feed assessments such as chemical, biological and analytical have been evolved. This has made it possible for feed assessment and comparisons so that planning of feeding systems as well as

formulation of feedstuffs to meet animal requirements can be made with accuracy. Some of these techniques for assessing the nutritive value of feeds are discussed in the following sections:

2.5.1 *In vivo* digestibility

Digestibility is a measure of the nutritive value of feed. The nutritional value of the fodder plants in general can be estimated with adequate precision from *in vivo* digestibility. However, due to the expense and time required to conduct animal trials, alternative biological procedures (i.e. *in vitro* and *in situ* techniques (sections 2.5.2 & 2.5.3), respectively) to predict organic matter digestibility have been developed in the past 40 years (Gosselink *et al.*, 2004). Givens *et al.* (1989) obtained a good correlation when *in vivo* digestibility was predicted from enzymatic (pepsin-cellulase) methods ($r^2 = 0.68$) and *in vitro* (inoculum) technique ($r^2 = 0.74$) using 124 dried samples of clamp silages from different farms. Fonseca *et al.* (1997) predicted digestibility and voluntary intake of ewes using 12 feedstuff from *in sacco* degradability. Their study showed that, DM degradation after 96 or 72 h incubation were the best predictors of roughages OMD (r^2 = 073 and 0.70, respectively) and obtained a high correlation when DM intake ($r^2 = 0.68$) and live weight changes ($r^2 = 0.82$) of ewes were predicted. High relationship therefore exists between *in vivo* digestibility and the biological procedures and can thus predict accurately digestibility, intake and animal performance without the conventional *in vivo* digestibility trials which requires high economic cost and long period of time for feed evaluation.

2.5.2 *In vitro* methods

The adoption of *in vitro* techniques in the assessment of nutritive value of feed is rapidly soaring in most laboratories. This is because of the laborious nature and high

cost associated with the use of digestibility trials especially where large numbers of samples are to be evaluated. Besides, the methods have the advantages of being less costly, less time consuming, good reproducibility and also correlates well with values measured in *in vivo* trials. The use of the *in vitro* techniques such as the two stage *in vitro* digestibility, rumen simulation technique, ANKOM Daisy technique, *in vitro* gas production, in the assessment of feed is more meaningful than chemical methods (Van Soest, 1994) since the microorganisms and enzymes used as innocula are more sensitive to factors influencing the rate and extent of digestion.

The two - stage *in vitro* method pioneered by Tilley and Terry (1963) simulates the activities in the rumen and lower digestive tract. The forage or substrate is anaerobically fermented in a buffered rumen fluid for 48 h in the first stage. This is followed by 48 h of acid-pepsin digestion to digest undegraded plant cell and microbial protein. The Tilley and Terry (1963) technique has the advantages of examining many samples at one time with the use of simple apparatus and gives highly reproducible results. The analysis provides an estimate of *in vitro* digestibility from which *in vivo* digestibility can be predicted. However, with this method, data regarding degradation kinetics is not provided.

There have been a growing concern regarding animal welfare issues and this has raised the ethical debate as to whether we should continue to surgically modify animals for routine feed evaluation. This has led to the development of high capacity *in vitro* or enzyme-based systems that have the potential to greatly reduce the number of such animals required. The use of cellulolytic enzymes as alternatives to rumen fluid is clearly an attractive prospect as their use gives results comparable to those of inoculum methods. The use of enzymes obviates the need for fistulated animals and anaerobic procedures, thereby simplifying analytical methodology.

The enzymatic methods, according to Theodorou *et al.* (1994), have the advantages over the Tilley and Terry *in vitro* method in that, the former does not require animals as inoculum donors and also the need to maintain anaerobic conditions is eliminated. But as with the latter, the enzymatic method does not offer data on the digestion kinetics of substrates. This introduces some difficulties in differentiating substrates with similar degradabilities but differing degradation kinetics. Owing to the shorter retention times of fast degrading feeds, improvement of the method will be of importance, as feeds with a higher rate of degradation will generally be consumed in higher quantities. Also with the Tilley and Terry (1963) method, degradation of substrates is underestimated in situations of pepsin-insoluble residues or microbial contamination. As a result, the method was modified by Goering and Van Soest (1970) to include a third step in which the residues after 48 h incubation are washed with neutral detergent solution to remove any contamination or to estimate true dry matter digestibility.

Akhter *et al.* (1996) developed a method that replaces the rumen fluid in the Tilley and Terry (1963) procedure with fecal inoculum. This technique might have arisen because of the difficulties in obtaining licenses elsewhere to surgically prepare fistulated animals and also concerns of animal welfare. Fistulated animals are also expensive to keep or maintain. With this technique, comparatively good predictions of *in vivo* digestibility are obtained.

Another *in vitro* technique recently introduced to simplify the estimation of *in vitro* digestibility is the ANKOM Daisy technology (1998). The ANKOM technique offers a rapid and more convenient way to determine the *in vitro* digestibility of feeds. The technique involves digesting forage samples in bags placed within glass jars and

rotated in an insulated chamber. The system allows for investigating the effects of changes in the rumen environment on the digestibility of feeds, such as the addition of a substance.

The ANKOM technique has the advantage of reduced labour input associated with the estimation of *in vitro* digestibility in that, the need for filtration is avoided. The ANKOM technique according to Julier *et al.* (1999) and Wilman and Adesogan (2000) gives relatively accurate predictions of *in vitro* apparent and true digestibilities.

The Rusitec developed by Czerkawski and Breckenridge (1977) is an invaluable technique for the study of the rumen metabolism and assessment of ruminant feedstuff. According to them, the Rusitec is a unit with four vessels secured to the base of Perspex water tank with a 'bayonet' fitting. The feed material is put into nylon bags, sealed by plastic binders and placed into a polyethylene container. The container is moved up and down by means of stainless steel rod and passage of the liquid is made possible through the holes punched in the container. The Rusitec, as described by Cheng and McAllister (1997), comprises a rumen without walls. Mandsfield *et al.* (1995) have attributed the inconsistencies that exist between measurements of fermentation characteristics made in Rusitec and those made *in vivo* to the absence of absorption across a semi-permeable membrane (rumen wall).

2.5.2.1 Gas Production technique

Feed fermentation in the rumen is associated with the evolution of gas, mainly carbon dioxide and methane. Menke *et al.* (1979), on the assumption that the quantity of gas produced from *in vitro* incubation of feedstuffs with rumen fluid is closely related to digestibility, and hence the energy value of feed to ruminants, proposed the gas

production system to evaluate feedstuffs. With this system, the substrate is incubated (the incubation media are rumen fluid and a buffer) in a calibrated gas-tight glass syringe fitted with a plunger to allow gases evolved (CH_4 and CO_2) to be retained and recorded manually over a selected time depending on the type of substrate being incubated. Based on the volume of gas accumulated over time, different empirical equations were developed by Menke and Steingaß (1988) to predict *in vivo* digestibility from chemical composition and *in vitro* gas production.

Wilkins (1974), in a different approach adopted the pressure transducer method to measure accumulated head-space gases resulting from microbial fermentation. Theodorou *et al.* (1994) further exploited the pressure transducer technique and recognized the potential of this technology to offer the advantage of studying the fermentation kinetics of soluble and insoluble fractions of feed. Thus, the methodology provides an estimate of rate and extent of feed degradation. Feed fermentation in this system results in the production of short chain fatty acid (SCFA) microbial biomass and gases (CO_2 and CH_4). The gas produced arises directly from substrate degradation by rumen microbes and indirectly from the reaction of volatile fatty acid end products with the bicarbonate fraction of the buffer used in media preparation (Beuvink and Spoelstra, 1992). The gas produced gives an indication of feeds that have been degraded.

2.5.2.2 Applicability of gas technique

The *in vitro* gas test is widely used in feed evaluation to predict *in vivo* digestibilities and energy content of feed (Menke and Steingaß, 1988). The amount of fermentative gas, although nutritionally unimportant, represents the degradable portions of feed incubated. This was affirmed by Menke and Steingaß (1988) in the same studies

where gas produced from *in vitro* substrate incubation highly correlated with substrate degradation and was proportional to volatile fatty acid production. Dijkstra *et al.* (2005) have demonstrated its use in estimating the rate and extent of ruminal organic matter degradability. The gas production technique's potential in predicting *in situ* DM disappearance and some degradation parameters has also been documented by Kamalak *et al.* (2005).

The use of *in vitro* gas production profile in estimating organic matter digestibility, ruminal microbial protein synthesis, dry matter intake and energy content of feeds has been documented by Getachew *et al.* (2002) in the ensuing pages.

2.5.2.3 Estimation of organic matter digestibility

The appropriateness of gas production as a measure of OMD has been shown by Khazaal *et al.* (1993), Cone *et al.* (1998) and Rymer and Givens (2002). The gas produced on incubation of 200 mg feed dry matter after 24 h together with the levels of other chemical constituents are used to predict digestibility of organic matter determined *in vivo* using the equation of Menke and Steingaß (1988) expressed as

$OMD = [14.88 + (0.889 \times GV_{24}) + (0.45 \times CP) + (0.0651 \times Ash)]$.

Where OMD = Organic matter digestibility; GV_{24} = Gas volume in ml from 200 mg dry sample after 24 hour; CP = Crude protein

The OMD measured *in vivo* is closely correlated with that predicted from gas production, crude protein and ash contents.

2.5.2.4 Energy content of feed

Gas measurement is a better index of energy content as it is a direct measure of microbial activity. In estimating the energy content of feeds, the gas method combined with chemical constituents of the feed, gives a better estimate than using chemical constituents alone. Accurate measurements of ME and NE content of feeds

however, require *in vivo* digestibility measurements from ruminants fed at a maintenance intake level. High costs make this approach to estimating ME content inappropriate for routine feed evaluation. Therefore, a number of equations have been developed to predict ME and NE from chemical composition or *in vitro* digestibility (López *et al.*, 2000). Some of these are:

$ME = 2.2 + (0.136 \times GV_{24}) + CP \times 0.057$

$NE = (2.2 + (0.0272 \times GV_{24} \times 5) + (0.057 \times CP) + (0.149 \times EE)) / 14.64) \times 9.14.$

Where ME is the Metabolizable energy; NE = Net energy; EE = Ether extract

2.5.1.5 Estimation of dry matter intake

The use of *in vitro* gas production technique in estimating DMI has been reported in the literature (Blümmel and Ørskov, 1993; Blümmel *et al.*, 2005). Blümmel and Ørskov (1993) adopted the exponential model $P = a + b\ (1-e^{-ct})$ to predict feed intake in cattle. The outcome of the studies showed that total gas production (a+b) value strongly correlated with intake ($r = 0.88$) and digestible dry matter intake ($r = 0.93$).

2.5.2.6 Estimation of ruminal microbial protein synthesis

Protein of microbial origin usually accounts for a major part of the protein available for absorption from the GIT in ruminants. Current protein evaluation systems share a common framework and predict microbial synthesis from estimates of rumen degradable OM or energy and rumen degradable N. Several techniques have been described in estimating microbial protein supply (IAEA, 1997). They include purine derivatives (namely allantoin, uric acid, xanthine and hypoxanthine) excretion techniques, use of spot urine measurement and the use of plasma or milk purine derivatives (PD). The PD method has the advantage of being non-invasive and simple to use. However, the use of this technique is not wide spread in developing countries because of its expensive nature and the difficulties associated with the laboratory

techniques in estimating microbial protein synthesis (Menke *et al.*, 1979). The use of gas production techniques in predicting microbial protein supply (MPS) has been suggested by Blümmel (2000). Thus, microbial biomass protein (MBP) may be obtained by multiplying organic matter degradation (estimated using the gas production technique) by a microbial yield factor, as suggested by Dhanoa *et al.* (2000) and Grings *et al.* (2005) in the equation below.

$$MBP = TSD - (\text{gas volume} \times SF) \quad I$$

where TSD is the true substrate degradability defined by Goering and Van Soest (1970) and SF is the stoichiometrical factor given as 2.20 (Blümmel, 2000).

2.5.2.7 Limitations of *in vitro* fermentation methods

The *in vitro* rumen fermentation methods according to Weiss (1994) are subject to multiple sources of variation, such as type of fermentation vessels, the composition of the buffer-nutrient solution, the conditions of incubation (anaerobiosis, pH, temperature, stirring), the sample size or the sample preparation (drying, grinding, particle size). Marten and Barnes (1980) reported inoculum as the greatest source of variation in these techniques. To overcome errors arising from the use of inoculum from fistulated donor animals, the use of faecal samples as an alternative source of fibrolytic microorganisms has been considered (Omed *et al.*, 2000).

With the Rusitec, both the infusion of the buffer solution into the vessel and the removal of the liquid effluent are continuous. There are, however, no provisions for continuous feed supply and solid particles outflow from the vessel and so is considered as a semi-continuous flow system.

Though the ANKOM technique represents a faster and a more convenient way to determine the *in vitro* digestibility of feeds, data generated from sample digestibility can be influenced by sample size and processing method, the proximity of the

incubation jars to the heat source and the extent to which individual bags are submerged throughout the incubation (Adesogan, 2002).

The Menke's system only measures the gas produced during substrate incubation. The gas produced reflects loss in energy in the form of CO_2 and CH_4 which has the potential of harming the environment. That notwithstanding, gas measurement is only an indication of SCFA production and fails to account for the rate and extent of substrate degradation (Makkar, 2002). It is known that the partitioning between SCFA and microbial biomass is unsteady owing to the variation of biomass per unit production of ATP. Therefore, the adoption of *in vitro gas* production test in estimating nutritive value might select against maximum microbial biomass yield by favouring substrates with proportionally high SCFA yield (Blümmel *et al.*, 1997).

These inherent drawbacks of the *in vitro* gas production techniques may be overcome by combining gas measurements with quantification of the undegraded residues.

2.5.3 *In situ* degradability technique

The *in situ* technique (Ørskov *et al.*, 1980) has been extensively used over the last three decades for measuring ruminal degradation of feedstuffs. The widespread attention given to this technique since its inception is because it can be readily used in developing countries as it is not reliant on a steady electricity supply, and more importantly, because it is one of the few techniques that describe the kinetics of feed degradation in the rumen. This method is based on the incubation of nylon bags with feed sample in the rumen of an animal fitted with a rumen fistula. The nylon bags are then withdrawn after various intervals of time, washed and dried. Degradability of dry matter, nitrogen, energy etc., can thus be measured against time. Ørskov and McDonald (1979) described the rumen degradability parameters according to the equation: $P = a + b\,(1 - e^{-ct})$

where P is the dry matter loss at time t; and a, b and c are constants.

Blümmel and Ørskov (1993) stated that, if substrate degradation proceeds without delay the intercept 'a' is considered as the soluble fraction of the substrate (washing loss), 'b' represents the insoluble but potentially degradable fraction and 'c' is the rate constant of 'b' or the rate of feed degradation. Thus the 'c' gives an indication of the transit time of feed in the gastro-intestinal tract and level of feed intake (i.e. feeds with higher 'c' will generally be consumed in higher quantities).

The *in situ* technique assesses how the rumen environment impacts on feed degradation and has provided relatively good predictions of forage intake and digestibility (Ørskov, 2000). The technique has also improved the understanding of nitrogen (N) supply to ruminants and their microbes and does provide information on DM disappearance of feed or rumen degradation properties of feeds needed for proper inclusion in the diet through formulation programs.

Many factors have been described as influencing the degradabilities of feed *in situ*. They are classified into animal characteristics (Ganev *et al.*, 1979), substrate characteristics (Figroid *et al.*, 1972) and bag characteristics (Varga and Hoover, 1983). These factors may account for the differences in degradability and kinetic measurements reported in the literature and are discussed in detail.

2.5.3.1 Effects of species on degradability

Substantial variation exists among animals, between replicates or across days within the same animals used in this method of feed assessment. Therefore, adjusting the number of animals would reduce variation due to animal as well as provide greater repeatability of digestion estimates. There is contrasting literature on the effect of species type on degradability. Playne *et al.* (1978) reported greater microbial activity

in cattle and therefore DM degrades faster in the rumen of cattle relative to sheep. On the contrary, little differences were observed in DM degradability between the animals in question (Ørskov *et al.*, 1983). Prigge *et al.* (1984) and Huntington and Givens (1997) showed that mature ruminant species when fed at maintenance, degrade DM of hay, soybean meal and fish meal similarly.

2.5.3.2 Influence of substrate characteristics on degradability

Characteristics of samples may hamper the accuracy of digestion measured *in situ*. Samples that assume gelatinous form may in part reduce the available surface area or completely block pores of nylon bags, thereby reducing digestibility (Stern *et al.*, 1983). Figroid *et al.* (1972) experimented on substrate characteristics on degradability by sieving ground sorghum grain into different particle sizes. They observed that, particle sizes in the range of 0.6 to 0.8 mm degraded faster *in situ* than particles of 0.4 to 0.6 mm. This was explained by the adhesive tendencies and clumping of the sorghum particles used by these authors.

2.5.3.3 Pore size of nylon bag on degradability

Rumen microorganisms and fluids enter the nylon bag through the pores of the bag. The microbes colonize and degrade feed particles in the nylon bag. The reduction in particle size of feed in the bag is aided by microbial fermentation and rubbing forces driven by the movements of the rumen wall and its contents. The degraded materials pass through the pores out of the bag. It is to be noted that, loss of particles from the bag can be attributed mainly to the interaction between bag pore size and sample particle size. Therefore, an appropriate pore size must allow the influx of rumen microorganisms, efflux of end products of digestion; and minimize the entry of residues of ruminal digesta and the exit of small (sample size) particles. High

estimates of degradability have been reported to be linked to increasing pore size (Nocek, 1985). When large pore sizes are used for instance, there is a high potential loss of particulate material from the bag. This leads to the assumption that the degradation of the escaped materials from the nylon bag is complete. Though, pore size positively influences rate of *in situ* degradation, minimal effect on extent of degradability has been reported (Udén and Van Soest, 1984). Therefore, attention must be given to end-product accumulation and decreased ability of nutrients, buffers, and other compounds to move through the nylon bag than mechanical loss of particles in the choice of bag's pore size. Literature has recommended a pore size ranging from 20-60μm (Ørskov, 1992; Lindberg, 1985; Nocek, 1988).

2.5.3.4 Nylon bag and sample size effects on degradability.

Literature has proven the influence of surface area (SA) and sample size (SS) on *in situ* degradability characteristics of feed sample. It was shown by Van Hellen and Ellis (1977) and Figroid *et al.* (1972) that high SS:SA ratio resulted in decreased *in situ* disappearance and vice versa. Mehrez and Ørskov (1977) reduced the SS:SA ratio from 54 to 16 mg/cm^2 and recorded an immense increase in DM degradability of barley at 24 h. Large SS:SA ratio may result in inadequate mixing and removal of digestion end products from *in situ* bags. This results in reduced DM digestibility and may underestimate the feed's potential as ruminant feed. However, Playne *et al.* (1978) observed a minimal effect on digestibility when SS: SA ratio was held constant as sample and bag sizes were varied. An SS: SA ratio of 10-20mg/cm^2 has therefore been recommended (Ørskov, 1992; Lindberg 1985 and Nocek, 1988). Expression of SS: SA ratio is given as:

SS: SA = sample size (mg)/ Bag width (cm) x Bag length (cm) x 2 (Vanzant *et al.*, 1998).

2.5.3.5 Limitation of *in situ* degradability studies

In terms of assessing how the rumen environment impacts on degradation, the *in situ* technique has no substitute to date. However, this method requires the use of at least 3 surgically modified animals to reduce variation. The technique is also flawed by excessive initial particle losses. Dhanoa (1988) reported that, in the assessment of fibrous diets, colonization of microbes at early stages underestimates the degradability of the sample. Equally, and like the Tilley and Terry (1963) methodology, it assumes that the substrate lost equates to that potentially available to the host animal which may not be so due to possible bonding of substrate with anti nutritional factors like tannins.

2.6 Pesticide and microbial interaction in the rumen

In discussing the interaction between microorganisms and pesticides, certain effects may become important. There may be potential germistatic or probable germicidal action of pesticides and its metabolites effects on rumen microbes. Any study that seeks to assess the effects of pesticide residues on ruminal ecosystem is crucial since the performance of the ruminant animal depends on the microbial activities in the rumen. Cook (1957) studied rumen microbial interactions with parathion, an organophosphate, in cattle. The author observed that rumen liquor plays an active role in the hydrolysis of parathion and metabolism of parathion by rumen microbes accounted for its apparent toxicity to cattle. Similar experiment on the use of dichlorodiphenyltrichloroethane (DDT) by Fries (1968) showed a high conversion efficiency of ^{14}C-DDT to less toxic dichlorodiphenyldichloro ethane (DDD) by rumen microorganisms. Barber and Nagy (1971) in a study where mixed and pure cultures of rumen micro organisms were used with Dieldrin, DDT, Sevin and Bordeaux mixture, reported that the pesticides lowered the growth rate of microorganisms at 10

or at 1 *ppm* and that, pure cultures, especially cellulolytic organisms, were more sensitive to pesticides than organisms growing in mixed cultures.

2.7 Effects of pesticides on rumen functions

2.7.1 Gas Production

Studies conducted by Williams *et al.* (1963) using rumen holotrich protozoa as an inoculum source showed that gas production was stimulated by the application of chlorinated hydrocarbon-, organophosphate-, and carbamate-containing insecticides. When rumen bacteria served as inoculum source, these compounds had no considerable effect on gas production. Trials later carried out by Williams *et al.* (1968) indicated that, atrazine, or simazine did not inhibit or stimulate gas production, nor was CO_2 production detected from protozoal metabolism.

2.7.2 Effect of pesticide on dry matter digestibility

An *in vitro* study on the toxicological effects of some pesticides (Table 2.2) on rumen functions by Kutches *et al.* (1970) showed that some of the pesticides were ineffectual ($P > 0.05$) in causing a reduction in IVDMD when they were dispensed at 100, 250, and 500 mcg/ml into fermentation vessels containing 1g ground fescue. It was realized that as the concentration increased to 750 and 1000 mcg/ml levels, significant differences ($P < 0.05$) were found in the degree of IVDMD inhibition. As suggested by Cook (1957), the hydrolysis of organophosphates to less toxic compounds by the rumen microbial flora may explain the insignificant differences obtained. This was further proven by Dewey *et al.* (1962). They observed the degradation of organo-chlorine in biologically active soils with the release of inorganic chlorides thereby decreasing the toxicological properties of the compound. In contrast, insecticides like 2,4-Dichlorophenoxyacetate, simazine and diuron

significantly depressed IVDMD at levels of 500 mcg/ml and progressively inhibited microbial activity as the pesticide concentration increased. Implications are that, high levels of pesticide application on the field would be required for reduction in IVDMD and aside that LD_{50} is exceeded if concentrations of inhibitory compounds reach 1000 ppm in the rumen.

Table 2.2 Influence of pesticides on percent *in vitro* Dry Matter Disappearance.

	Pesticide levels (mcg/ml)					
Treatment	0	100	250	500	750	1000
Insecticide						
DDT	30.80	30.92^{a}	29.69^{ab}	28.05^{ab}	26.02^{b}	25.74^{b}
Malathion	28.92	29.17	29.32	28.67	27.60	26.51
Sevin	28.97	29.91^{a}	28.40^{ab}	25.92^{abc}	25.07^{bc}	22.38^{c}
Toxaphene	27.95	28.51^{a}	27.85^{ab}	25.71^{abc}	22.37^{cd}	19.17^{d}
Herbicide						
Diuron	29.03	27.79^{a}	24.55^{a}	18.24^{b}	16.61^{b}	15.09^{b}
Simazine	29.49	28.98^{a}	25.76^{ab}	23.30^{bc}	20.83^{cd}	18.62^{d}

^{a,b,c,d} *Means having common superscripts or with no superscripts among the five levels and within each treatment are not significantly different ($P > 0.05$) according to Duncan's New Multiple Range test;*

Source: Kutches *et al.* (1970).

2.7.3 Pesticide effect on volatile fatty acids production

The production of VFA's from rumen fermentation is a major source of metabolic fuels for ruminants. The performance of the animal is affected if some factors alter or lower the quantities of VFA produced.

In a study conducted by Kutches *et al.* (1970) to assess the effect of pesticides on VFA production in the rumen, it was observed that, the levels of VFA production did not differ significantly when tested with 100, 250, and 500 mcg/ml of herbicides (Dicamba, Tordon, Simazine, Diuron except 2,4,5-Trichlorophenoxyacetate) and

insecticides (Sevin, Malathion, Toxaphene). However, increments of 2, 4, 5-Trichlorophenoxyacetate beyond 500 to1000 mcg/ml resulted in a significant decrease of VFA production.

2.8 Changes in quality and quantity in straw during storage

Large quantities of cowpea haulms are available after grain harvest in Ghana and Nigeria. The haulms are cut while green, rolled into small bundles and stored on roof tops or tree forks for use and sold as feed supplement in the dry season especially in Northern Nigeria (Singh and Tarawali, 1997), while other farmers leave the haulms on the field as standing hay. Quantity and quality losses are reported during hay storage which might be as a result of microbial respiration in the hay, culminating in dry matter loss and quality reduction (Buckmaster *et al.*, 1989). Shattering (losses) of leaves and bleaching by exposure to sunshine (Tripathi *et al.*, 1995) and leaching of soluble, nonstructural carbohydrates from plant tissues by rain (Collins *et al.*, 1987 and Al-Mamum *et al.*, 2002) have been documented. The loss of non-structural carbohydrate during storage could result in an increase in the concentration of fibre components in the hay material decreasing its potential intake and digestibility after storage (Buckmaster *et al.*, 1989).

The beneficial effect of improved storage methods on hay quality over traditional methods of storage was affirmed by Al-Mamum *et al.* (2002). They reported that poor storage could facilitate spoilage due to mould growth. They also observed a higher NFE ($P < 0.01$) content of rice straw when kept under improved (shed) storage than when stored on trees or roofs. Though their findings indicated no significant ($P > 0.05$) effect of storage methods on OM, CP, CF and EE contents of rice straw, improved storage method significantly ($P < 0.01$) recorded increased NFE, IVDMD

and IVOMD of rice straw compared with those of traditional storage method. Their study indicated that an improved storage system could store well, excess fodder from the major season harvest intended for lean season feeding.

2.9 Inference from literature

Crop residue abounds on the agricultural fields in Ghana after harvesting the economic grain parts. However, they are bulky, high in fibre, poorly degraded in the rumen, resulting in very low intakes. Crop residues generate low levels of ammonia (NH_3) in the rumen upon fermentation which is necessary to ensure an efficient digestion process. The effects of the supplementation of nitrogen on intake and digestibility of poor quality roughages has been well documented, however, the influence of supplements on degradability and utilization of crop residues has not been extensively investigated. Since most farmers rearing livestock are small-holders, high cost of conventional nitrogen supplements will discourage them from benefitting from this knowledge. Therefore supplement used by such farmers should be obtained with little or no cost. Cowpea haulm has been cited in the literature review as a co-product to grain production, hence its use as supplement will be economical to farmers.

The nutritional value of feedstuffs is estimated with adequate precision from *in vivo* digestibility. However, due to the expense and time required to conduct animal trials, alternative procedures as *in vitro* and *in situ* have been adopted in the assessment of nutritive value of feed. Studies have indicated high relationship between *in vivo* digestibility and *in vitro*/ *in situ* techniques and can thus predict accurately the digestibility of feedstuff without the conventional *in vivo* digestibility trials which requires high economic cost and long period of time for feed evaluation.

The production of cowpea is fraught with seasonality, year and insect pests. Seasons and years characterised by extreme climatic variables of high temperatures, solar radiation, saturated vapour pressure deficits, little rainfall amounts during vegetative stage has little effect on grain yield, however, yield is reduced when harsh climatic variables meet the flowering and pod filling stages. Therefore, if water for irrigation is limited in any particular year, it is possible to withhold irrigation during the vegetative stage with negligible effects on seed yield.

Insects account for yield losses in cowpea production and they are responsible for 20 - 100 % yield losses. Control of the insects below their economic threshold has been effected through pesticide applications. However, pesticide use over a long period comes with its attendant human related health hazards as cancer etc. Research on degradation rates on of pesticide in plants and excretion products in the urine of ruminants have been extensively done, however, how the ingestion of pesticide-contaminated haulms affects the microbial functions in the gut is yet to be fully addressed.

The literature review has shown the seasonality of leguminous fodders. The fodder becomes available in large quantities in a period of the year. However, some farmers leave the haulms after grain harvest as standing hay or cut and store them on roof exposing them to the inclement weather. This practice reduces the haulm quality and quantity available for dry season feeding. Storage of the fodder for lean season feeding has been reviewed but limited information is available on the amount of nutrient retention during and after storage is limited.

CHAPTER 3

3.0 MATERIALS AND METHODS – GENERAL

3.1 Overview

This chapter outlines the various procedures and protocols used in this study. Five experiments were set out and grouped as Agronomy and Animal Experiments. The Agronomy experiment comprised determining the effects of cultivar, year and season on yield of grain and haulm of 4 improved cultivars of cowpea, and changes in the nutrient composition of cowpea haulms of these cultivars in storage. The Animal experiments assessed the chemical composition, gas production and degradation characteristics of haulms of the improved cowpea cultivars, the effect of pesticide use during cultivation on *in vitro* gas production from haulms of cowpea, and effects of feeding graded levels of cowpea haulm as a supplement to maize stover offered to sheep on intake, degradability and digestibility of maize stover.

Materials from Agronomy Experiment 1 were obtained as described in Section 3.3.1 and the materials for assessments of the Agronomy experiment 2 and the Animal experiments were also obtained after grain harvest (Section 3.3.3.3). References were made to the relevant sections of this Chapter in the various experiments, where necessary.

3.2 Experimental sites

The Agronomy Experiments 1, 2 and Animal Experiment 3 were carried out at the Department of Animal Science, Kwame Nkrumah University of Science and Technology, (KNUST) Kumasi (at location $1^{0}36$'N, 6043'W). The Animal experiments 1 and 2 were conducted at the Animal Production Research Institute

(APRI) – Agriculture Research Centre, Noubaria, Egypt and Alexandria University, Alexandria, Egypt.

3.3 Agronomy Experiments

Two agronomy experiments were undertaken. The following sections describe how the materials were sourced and the cultural practices used in the establishment and harvesting of the cowpea.

3.3.1 Source of cowpea cultivars and establishment

Seeds of four improved cowpea (*Vigna unguiculata* (*L.*) *Walp.*) cultivars (SORONKO, IT93K-2309, IT86D-716, IT93K-2045-93) were obtained from the CSIR - CRI, Fumesua, Kumasi, Ghana. The four cowpea cultivars were selected based on their biomass yield (Antwi *et al.*, 2004) and were sown on the arable fields at the Department of Animal Science, KNUST, in May for wet season production and in September for dry season production, for 3 consecutive years i.e. 2005, 2006 and 2007. The mean monthly weather pattern during the years of cowpea production is presented in Appendix I.

3.3.2 Land preparation and field layout

A 1-acre field was mechanically slashed of weeds, ploughed, harrowed, demarcated and pegged. A randomized block design was used with four replicates in blocking the field into 16 plots. Each plot measuring 17 m x 1.8 m was divided into 6 rows, with a 1m border on all sides. Within rows, seeds were sown at 20 cm intervals, with three seeds per hill and spaces between rows measured 60 cm in length resulting in 0.12 m^2 area per plant. This procedure was repeated for all the 3 years of study. The plot

layout and pictorial view of the cowpea field are shown in Figure 1 and Plates 1 -3, respectively in Appendix II.

3.3.3 Cultural practices

3.3.3.1 Weed control and thinning

Weeds were controlled by hand hoeing during the third and sixth week after planting while thinning was done at the 14th day of sowing.

3.3.3.2. Pesticide management

Three pesticides namely Lambda cyhalothrin, Cypermethrin and Dimethoate were employed to simulate the practices adopted by farmers in controlling insect pests. Pesticides were applied at the inception of pest invasion. Cowpea was sprayed as recommended by manufacturers, at an application rates (ml ha^{-1}) of 800 (Lambda cyhalothrin[1] against pre-flowering insects); 1,500 (Cypermethrin[2]) and 1,950 (Dimethoate[3]) against post-flowering insects.

3.3.3.3 Harvesting of cowpea

Cowpea seeds were harvested 79 days post-establishment from the central rows (i.e. two middle rows excluding the border rows) of each plot of area 17m x 1.8m. The seeds were conveyed to a newly constructed barn for air drying. Threshing was done two weeks after harvesting. Haulm yield was determined from the materials in the same central row. This was done by cutting with machete, 2 cm above ground level and weighed for each plot. The haulms were then kept for Agronomy and Animal experiments 2 and 3, respectively.

[1] Karate (PAWA), 2.5EC. Distributed by Chemico Limited, Tema. Batch # 20060723
[2] Cypercal 50 EC. Distributed by Calli Ghana Company Limited, Accra North. Batch # 3GH00003
[3] Cerox, ai 400. Distributed by Agrana Ghana Limited, Tema. Batch# BG07-001C

3.4 Sample preparation.

Representative materials of the cowpea haulms from the central rows were air dried and milled through a 2-mm screen. Samples were stored in a plastic containers and placed in a freezer (-20°C) pending analysis in the Nutrition Laboratories of APRI and Alexandria University, Egypt.

3.5 Analytical procedures

Cowpea haulm were analyzed for DM, CP, EE and fibre components. Detailed description is found in Appendix IV.

3.6 Animal Experiments

Three animal experiments were conducted as stated in Section 3.1. The following practices and procedures were used in conducting the animal experiments.

3.6.1 Animals, housing and feeding

Three rumen-fistulated rams (45±2 kg average live weight) were used for the gas production and degradability studies. The rams were kept in individual pens and fed a standard hay of berseem (*Trifolium alexandrinum*) *ad libitum* in two identical meals daily, at 8.30 and 16.30 h.

3.6.2 Sample collection

3.6.2.1 Rumen fluid

The rumen liquor was collected from fistulated sheep into CO_2-pregassed and warmed vacuum flask. The fluid was strained through 4 layers of cheese cloth into a measuring flask, sealed with aluminum foil and incubated at 39°C in a water bath.

3.6.3 Nylon bag studies

3.6.3.1 Determination of DM and CP disappearance

The DM disappearance *in situ* was assessed using the nylon (42µm pore) bag technique described by Ørskov *et al.* (1980). Duplicate samples of ground cowpea haulms weighing 5g each were incubated in the three fistulated Barki rams for 3, 6, 12, 24, 48, 72 and 96 hours. The sheep were fed berseem hay *ad libitum*. The nylon bags were rinsed with tap water on removal at the end of each incubation period and stored in a freezer at –20°C pending analysis. The frozen samples were thawed and washed to eliminate microorganisms associated with the residues. The bags were then dried at 55°C for 48 hours. A set of bags containing each of the feed samples that were not incubated (0 hour) was washed and dried under similar conditions to estimate the readily soluble fraction.

3. 6.3.2 Degradability as determined with the nylon bag

The DM and CP disappearance from bag of each cultivar at each incubation time was estimated as nutrient concentration in the original samples less nutrient concentration of residues after incubation and was used to calculate the kinetics of ruminal fermentation according to the formula of Ørskov and McDonald (1979). i.e.

$$P = a + b\,(1-e^{-ct})$$

Where p = cumulative amount of DM, and CP degraded by time, t
a = readily soluble fraction
b = potential degradable fraction
c = rate constant for the degradation of b

3.6.4 Gas production

3.6.4.1 Sample preparation and experimentation

Representative samples of dried cowpea haulms were milled through a 1 mm screen and kept in plastic containers until needed for the experiment. Approximately 210 mg triplicate samples of the dry matter (DM) of each sample were placed in 100 ml graduated glass syringe filled with 10mL of rumen fluid, and 20mL of buffer (McDougall, 1948). Pistons were lubricated with Vaseline and inserted into the syringes. The rumen fluids were sampled, before feeding berseem hay, from the rumens of sheep with permanent rumen fistula. Rumen digesta was squeezed through four layers of cheesecloth, homogenized and kept at 39^{0}C in a water bath under continuous flushing with CO_2 before use. This was diluted with a culture medium (Makkar *et al.*, 1995; FAO/ IAEA, 2000), containing bicarbonate buffer, macro-mineral, micro-mineral, resazurine and a reducing solution. The buffered rumen fluid (30ml) was pipetted into each syringe and syringes were immediately placed in a water bath at 39^{0}C (Blümmel and Ørskov, 1993). Syringes were shaken at hourly intervals and gas volumes were recorded at 3, 6, 12, 24, 48, 72 and 96 h of incubation and corrected for blank syringes incubated in each run.

The model below was used for the calculation of gas production (GP):

$$GP = b\,(1\text{-}Exp\,(^{-ct}))$$

Where b = potential gas production
c = rate of gas production
GP = gas produced at time t (Siaw *et al.*, 1993)

3.7 Statistical Analysis

The generalized linear model (GLM) and the PROC MIXED procedure of SAS (1999) were used in all the experiments conducted except gas production and

degradation constants were determined by a curve fitting procedure of PROC NLIN, available in Version 8 of SAS (SAS, 1999).

CHAPTER 4

4.0 AGRONOMY EXPERIMENT 1

4.1 EFFECTS OF CULTIVAR, YEAR AND SEASON ON YIELD OF GRAIN AND HAULM OF COWPEA

4.1.1 INTRODUCTION

Cowpea is grain legume crop and also a valuable component of the traditional cropping systems in the semi-arid tropics (Singh *et al.*, 1997). Cowpea is an important source of human food and livestock fodder. It is also one of the most drought resistant food legumes serving as an insurance crop to sustain production during droughts (Singh *et al.*, 2003).

In Ghana, there are two major cropping seasons (wet and dry) during which farmers grow cowpea to enhance their chances of achieving a significant grain and fodder food / feed security. Unlike the wet season, the dry season is characterized by high temperature, solar radiation, saturated vapour pressure deficit, little or no rainfall, and increasing soil moisture deficit, which all limit plant growth and performance (Agele *et al.*, 2006). In addition to environmental influences on yield, performance of cowpea is also affected by insect pests. Insect pests have been cited for damage to the crop from seedling emergence to storage (Jackai and Adalla, 1997); thus complete protection by pesticide applications is recommended (Afun *et al.*, 1991). However, how the ingestion of pesticide contaminated forages or haulms influence gas production and predicted fermentation parameters is thoroughly discussed in Animal Experiment 2

Preliminary experiments in collaboration with CSIR-CRI involving ten (10) dual-purpose cowpea cultivars (Antwi *et al.*, 2004), showed a significant effect of site

(Fumesua, Pokuase and Wenchi) on yield of grain and nutrient composition of haulms. Out of the ten cultivars, four cultivars were selected for this study based on their agronomic features and chemical composition; variations due to season and year on yield of grains and haulms in the year 2004's study were not ascertained. This, if known, may serve as a representation of possible yield in all years and aid in risk assessment. It may also help to offer more specific advice to farmers to take advantage of both high grain and haulm yield as food for humans and feed for livestock, as well as make recommendations that may hold for all seasons in any year of cowpea establishment. In order not to select against grain or haulm yield an index called potential utility index has been suggested by Fleischer *et al.* (1989). This has implication for both plant breeders and animal nutritionists on the release and adoption of cultivars for use by farmers.

The objective of agronomy experiment 1 was to ascertain the influence of cultivar, and season and year of growth on agronomic characteristics of four dual purpose cowpea cultivars.

4.1.2 MATERIALS AND METHODS

The location of the trial, cultivars used, the cultural practices adopted in cowpea establishment and measurements taken have been outlined in Sections 3.2. - 3.3.3.3 of Chapter 3.

4.1.3 Experimental design

A randomized complete block design was used. The four cultivars of cowpea i.e. SORONKO, IT93K 2309-11, IT86D-716 and IT93K 2045-29 cultivars were planted in four different blocks located in various areas of the field during the wet and dry

seasons for three years (2005, 2006 and 2007); each block contained four replicated plots of the four cultivars examined.

4.1.4 Harvesting and yield estimation

The harvesting of cowpea grain and haulm has been described earlier in Section 3.3.3.3. The expressions for estimation of grain and haulm yield, as well as potential utility index are presented in Appendix III.

4.1.5 Statistical analysis

Analysis of variance (ANOVA) was performed by using GLM and PROC MIXED procedures of SAS (SAS, 1999) to calculate the cultivar effects on grain and haulm yield. The statistical model included the effect of season, year and the season x year interaction as follows.

$$Y = \mu + \tau_i + A_j + B_k + R(A*B) + (AB)_{jk} + \varepsilon_{ijk}$$

where Y is the grain and haulm yields, μ is the overall mean, τ = fixed effect of treatment, A is multiple years (random), B is season (fixed effect), R is the replications (random effect), and ε is random residual error term.

4.1.7 RESULTS

4.1.7.1 Overview

Experimental conditions differed between the seasons in all 3 years of experimentation. For instance, the growth period in the dry season of year 2005 was marked by drought while excessive rains characterised the 2007 growing seasons (Appendix I, Tables 1-3).

4.1.7.2 Seasonal and year effects on grain and haulm yields of cowpea

The results of season and year effects on grain and haulm yields are presented in Table 4.1. The results indicated that, yield estimates were significantly ($P < 0.0001$) influenced by year of cowpea establishment. Seasonal effect also resulted in different ($P < 0.0001$) grain and haulm yields. There was a significant ($P < 0.0001$) season x year interaction for grain and haulm yield. The interaction was due to a significant ($P < 0.001$) lower yield differences that characterised the seasonal yields more in year 2005 than 2006 and 2007. Grain yield of cultivar IT93K-2045-93 was similar ($P = 0.1084$) to IT86D-716 and the differences only tended to approach significance ($P = 0.0764$) with respect to IT93K-2309. Cultivar IT93K-2045-93, on the other hand, differed significantly ($P = 0.0017$) from SORONKO. With regard to haulm yield, the cultivars ranked as follows; IT93K-2309 > IT93K-2045-93 > SORONKO > IT86D-716.

Generally, yield estimates in the wet season were significantly ($P < 0.0001$) higher than those obtained from the dry season. Grain and haulm yields were 2.06 and 2.51 times higher in the wet season than in the dry season respectively ($P < 0.0001$). Grain and haulm yield values recorded for years 2006 and 2007, though similar (grain; $P <$

0.7708: haulm; P < 0.3585), were significantly different from the yield estimate of 2005 (P < 0.0001).

Table 4.1 Least square means (± s.e) of season and year effect on cowpea grain and haulm (t ha^{-1}) yields.

Cultivar	Season	Year	GY	HY
SORONKO			1.62^{b}	6.78^{c}
IT93K-2309			1.71^{ab}	10.98^{a}
IT86D-716			1.72^{ab}	5.91^{d}
IT93K-2045-93			1.82^{a}	9.74^{b}
	Wet		2.31^{a}	11.94^{a}
	Dry		1.12^{b}	4.76^{b}
		2005	1.22^{b}	5.61^{b}
		2006	1.96^{a}	9.61^{a}
		2007	1.98^{a}	9.84^{a}
Statistical Significance				
Year			****	****
Season			****	****
Y x S			****	****

Means with the common letters (a,b,c) within columns are not significantly different at P < 0.0001; **** (*P* < 0.0001)

Where GY = Grain yield; HY = Haulm yield; s.e = standard error

4.1.7.3 Grain yield

The least square means of grain yield in all years and seasons of cowpea establishment are presented in Table 4.2. The effect of cultivar was significant for all years (2005, P = 0.0435; 2006, P = 0.0235; 2007, P = 0.0012) in the wet season, and dry season (2006, P = 0.0247; 2007, P = 0.0021) except for year 2005 where cultivar effect was not significant (P = 0.1165). The mean wet season grain yields recorded in the three years' growing periods were 2.13, 2.26 and 2.55 t ha^{-1} for years 2005, 2006

and 2007, respectively, while those of the dry season grain yields were 0.32, 1.40 and 1.67 t ha^{-1} for years 2005, 2006 and 2007, respectively.

Table 4.2 Least square means (±s.e) showing grain yields of improved dual purpose cowpea cultivars grown in the wet and dry seasons

Cultivar	Grain yield (t ha^{-1})					
	Year					
	2005		2006		2007	
	Seasons					
	Wet	Dry	Wet	Dry	Wet	Dry
SORONKO	2.03^{b}	0.28^{a}	2.49^{a}	1.37^{b}	2.40^{c}	1.18^{b}
IT93K-2309	1.98^{b}	0.37^{a}	1.96^{b}	1.95^{a}	2.45^{bc}	1.57^{a}
IT86D-716	2.43^{a}	0.27^{a}	2.45^{a}	1.31^{b}	2.80^{a}	1.09^{b}
IT93K- 2045-93	2.08^{b}	0.34^{a}	2.13^{ab}	2.04^{a}	2.57^{b}	1.78^{a}
Mean	2.13	0.32	2.26	1.67	2.55	1.40
MSD	0.35	0.10	0.31	0.57	0.15	0.31
CV	9.51	18.48	9.96	22.28	3.82	13.90

Within column means with common letters (a,b,c) are not significantly different according to the Waller-Duncan k-ratio t-test with t=100.
Where MSD = mean significant difference; CV = coefficient of variation; s.e = standard error

The grain yield of cultivar IT86D-716 in the wet seasons of crop establishment was higher than ($P < 0.05$) for all the other cultivars, except in 2006 when it was similar to IT93K-2045-93 and SORONKO. The three other cultivars, namely IT93K- 2045-93, IT93K-2309 and SORONKO had similar ($P > 0.05$) grain yield in the wet season of the year 2005. Conversely, the grain yields of IT93K-2045-93 and IT93K-2309 significantly ($P < 0.05$) differed from SORONKO in 2007 and were similar in the

wet seasons of 2006 and 2007. SORONKO on the other hand, out yielded ($P < 0.05$) IT93K-2309 in the wet season of 2006.

In the dry season, yields of IT93K-2045-93 and IT93K-2309 outstripped ($P < 0.05$) those of IT86D-716 and SORONKO in 2006 and 2007. Yield similarities, however, were observed between cultivars IT93K-2045-93 and IT93K-2309; and IT86D-716 and SORONKO.

4.1.7.4 Cowpea haulm yield

The least square means of haulm yield in all years and seasons of cowpea establishment are shown in Table 4.3. Cultivar effect in the wet season was significant for all years (2005, $P < 0.0001$; 2006, $P = 0.0007$; 2007, $P < 0.0001$). The same observation was made for dry season yields in 2006 ($P = 0.0002$) and 2007 ($P < 0.0001$) except year 2005 where cultivar effect was not significant ($P = 0.3573$). The mean haulm yields of cultivars in the wet season of growth were within the range of 9.45 and 13.81 t ha^{-1} while that of the dry season ranged from 1.77 to 6.65 t ha^{-1}.

The haulm yield of IT93K-2309 exceeded ($P < 0.05$) that of IT93K-2045-93 in the wet seasons of all the years under study, but yields of both cultivars significantly ($P < 0.05$) differed from SORONKO and IT86D-716.

Table 4.3 Least square means (± s.e) showing haulm yields of improved dual purpose cowpea cultivars grown in wet and dry seasons

Cultivar	Haulm yield (t ha^{-1})					
	Year					
	2005		2006		2007	
	Seasons					
	Wet	Dry	Wet	Dry	Wet	Dry
SORONKO	6.14^{c}	1.75^{a}	11.81^{b}	4.91^{b}	12.05^{c}	4.03^{b}
IT93K-2309	14.40^{a}	1.97^{a}	15.92^{a}	8.43^{a}	17.25^{a}	7.90^{a}
IT86D-716	5.27^{c}	1.33^{a}	9.00^{c}	5.07^{b}	11.46^{c}	3.33^{b}
IT93K-2045-93	11.98^{b}	2.01^{a}	13.59^{b}	8.18^{a}	14.47^{a}	7.90^{a}
Mean	9.45	1.77	12.5	6.65	13.81	5.87
MSD	2.13	1.07	2.32	1.25	0.90	1.07
CV	15.08	31.89	11.89	12.36	4.38	12.20

Means within column bearing common letters (a,b,c) are not significantly different according to the Waller-Duncan k-ratio t-test with t=100.

Where MSD = mean significant difference; CV = coefficient of variation; SE = standard error

4.1.8 DISCUSSION

4.1.8.1 Grain and haulm yield

The mean grain yield in the wet season observed in the 3 years of cowpea establishment agrees with the yield range (1.79-2.58 tha^{-1}) reported by Singh *et al.* (2003) who evaluated the grain yields of cowpea cultivars at IITA –Kano Research Farm, Minjibir, Nigeria. Yield variation among cultivars may be due to inherent genotypic characteristics. Furthermore, cultivars' yield differences in the years of growth may be ascribed to variations in weather patterns. The annual yield variation

emphasizes the need for replicating the years of cowpea establishment. This may serve as a representation of yield expectation in any year of cowpea growth as well as aid in risk assessment. The higher grain yield and low haulm yield performance recorded by IT86D-716 is a reflection of carbohydrate accumulation for grain formation rather than biomass production. The relatively lower grain yield of the other three varieties was probably because of excessive vegetative growth at the expense of grain formation and this reflected in their higher haulm yields. The growth periods of the cultivars in the minor season coincided with drought conditions in all the years of crop establishment. Cultivars IT93K- 2045-93 and IT93K-2309 however, appeared to be more tolerant of the harsh conditions and outperformed SORONKO and IT86D-716 in terms of grain and haulm yields. The immense yield differences between the two seasons were as earlier mentioned, due to the terminal drought in the dry season of growth which led to the abscission of leaves during pod filling. Terminal droughts that coincide with pod set and pod filling stages of cowpea is reported by Dadson *et al.* (2005) to reduce yield performance owing to senescence of leaves, hence the differences in yield observed in the two seasons.

4.1.8.2 Seasonal and year effect on grain and haulm yield

The marked yield difference between seasons is explained by extreme climatic variables that characterized the dry seasons. This partly explains the significant seasonal and year interaction. The pod filling stages of the four cowpea cultivars coincided with high temperatures, and solar radiation, and saturated vapour pressure deficits in all the dry seasons in the 3 years of crop establishment. This observation accounted for the high CV recorded for the 2005 dry season yield. Harsh climatic conditions, according to Agele *et al.* (2006) limit plant growth and yield performance, hence the greater yield differences between the two seasons. Lower rainfall receipt

during the vegetative stage of the cultivars in the dry season in part, resulted in the cessation of leaf production and expansion (Akyeampong, 1985), senescence and abscission (Karamanos, 1980). This accounted for lower grain and haulm yield relative to the yields in the wet season. The reduction in grain yield supports the assertion by Rawson and Turner (1982) that grain production correlates positively with leaf area and that yield declines owing to reduction in leaf area induced by drought stress. Differences in cultivars' performance in different years of establishment are indicative of variable weather conditions that characterised cowpea production in both seasons. Where rainfall receipt was optimum (2007) especially during the pod filling stages, yield output was comparatively good.

4.1.9 CONCLUSION

The grain yield of cultivar IT86D-716 was higher than SORONKO, IT93K-2309 and IT93K-2045-93, and would therefore be a cultivar of choice for wet season cowpea cultivation. However, since cowpea is sensitive to drought during the pod filling stage, the cultivar which tolerated the drought conditions (i.e. IT93K-2045-93) and compared well with the other 3 cultivars in terms of grain and haulm yields would be the ideal cultivar for both wet and dry season cowpea establishment.

CHAPTER 5

5.0. AGRONOMY EXPERIMENT 2

5.1 CHANGES IN THE NUTRITIVE VALUE OF COWPEA HAULMS IN STORAGE

5.1.1 INTRODUCTION

The harvest, storage, sale, as well as feeding of leguminous fodders as supplement are important components of livestock farming particularly in the Northern part of Ghana (I Mustafa 2007, pers. Comm., 18 October). In the wet season when these leguminous fodders are available in large quantities, efforts are made by the farmers to store any excess haulm for dry season feeding in a bid to ensure stable supply of supplements throughout the year, thus enhancing food security. The farmers either leave the haulms as standing hay or harvest and cart to homesteads and stack them on trees or roofs. This storage practice may cause considerable loss and damage to the haulms, resulting in insufficiency of haulms for dry season feeding. Losses of nutrients as a result of this storage practice have been described by Tripathi *et al.* (1995); these vary from the shattering (loss) of leaves, leaching of soluble nutrients by rain, and bleaching by over exposure to sunshine.

The harvest of the major season cowpea crop coincides with extreme environmental conditions such as high rainfall, wide temperature variations, as well as, high relative humidity. These events may cause considerable damage to the cowpea haulm if the appropriate storage method is not adopted. To maintain the quality of the cowpea haulm during storage for dry season feeding, improved storage methods must be adopted since these will enable farmers to store haulms for longer periods as well as ensuring availability of haulms all year round for livestock feeding. An improved

storage method prevents the exposure of haulms to rainfall and sunshine; and thus loss of dry matter, total nitrogen and fibre components is reduced.

Agronomy experiment 1 revealed that significant quantities of cowpea haulm (i.e. 11.94 t/ha) are available from the major cropping seasons but this could be as low as 4.76 t/ha in the minor season. An appropriate practice that stores excess haulm as well as maintain the quality of the haulm for dry season feeding would ensure good feed availability all year round and hence improved food security. Agronomy experiment 2 therefore was aimed at investigating the effects of field, roof and shed storage, as well as duration of storage on weight loss in haulms, and retention of crude protein, neutral detergent fibre, acid detergent fibre and acid detergent insoluble nitrogen.

5.1.2. MATERIALS AND METHODS

5.1.2.1 Overview

The source of experimental material has been outlined in Section 3.3.1 of Chapter 3. The experiment was conducted in the wet season of the year 2007 and the haulms after grain harvest were used for this study.

5.1.2.1 Harvesting and sampling

Two outer rows (i.e. the 1st and 6th rows of each plot) of the established cowpea stand per plot, measuring 17m x 1.2m, were harvested and one half stored under shed while the other half was kept on the roof of the shed. The remaining two rows (i.e. 2nd and 5th rows) were left on the field as standing hay for monthly sampling. At day 0, and

weeks 4, 8, and 12, haulms were sampled from the three storage practices for chemical analysis.

5.1.2.2 Construction of improved storage facilities

Three traditional sheds with floors which were 0.6 m high from the ground level were constructed with the following dimensions: length - 3 m, height - 2.4 m, and width - 1.2 m (Appendix II, Plate 4). The location of these sheds was close to the place earmarked for the field storage data collection.

5.1.2.3 Experimental design

A strip-split plot design with three replications for the main plots was used in this study. The main plot was the two storage practices used (roof and shed) and the four cowpea cultivars with four replications in each storage type were the subplots with the sampling period i.e. 0, 4, 8 and 12 weeks as a repeated measure on the experimental unit being the strip plot.

5.1.2.4 Chemical analysis

Dry cowpea haulms were milled through a 2-mm screen and subsequently analysed for dry matter (DM), nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent insoluble nitrogen (ADIN). The nitrogen content was determined by the Kjeldahl procedure and the crude protein was quantified by multiplying the nitrogen content in the haulm by a factor of 6.25; while the ADF, NDF, concentration of nitrogen in ADF, and lignin analyses were conducted using the protocols outlined by Galyean (1997).

5.1.2.5 Statistical analyses

Changes in haulm weight, in both roof and shed systems, were analysed by PROC MIXED of SAS (SAS, 1999) as a split plot with three replications, and repeated measures (weeks) with replications being random and all other effects fixed. Mean concentrations of N, NDF, ADF, ADIN obtained from the haulms of the cultivars in the 3 storage methods were regressed against time in storage.

5.1.3 RESULTS

5.1.3.1 Overview

The harvest of the cowpea haulm coincided with heavy rains in July and August 2007 (Appendix I, Table 1.) which soaked the pile of the haulm on the field intended for the storage experimentation. Subsequent drying of the haulm prior to storage and carting to the storage area led to some losses of the leaves. However, 10 kg each of the 4 cultivars were weighed and nutrient composition determined (i.e. at time 0 weeks). Nutrient retention was expressed as the percentage of haulm remaining in the roof and the shed storage type. The roof and shed storage was therefore used for the assessment of the nutrient retention and dry matter loss during storage. To ascertain the changes in chemical composition during storage, the haulms of the four cultivars were bulked and scatter graphs were used to explain the observations made. It is to be noted that, this assessment combined the field storage and that of the shed and roof storage methods.

5.1.3.2 Dry matter loss and cowpea haulm remaining in storage.

Table 5.1 shows haulm DM losses and quantity of haulm remaining in the four cowpea cultivars at the 12th week. The cultivars showed similarities ($P > 0.05$) in haulm DM losses during storage however, the haulms significantly lost more DM

during roof storage (P = 0.0018) than that of shed storage with increasing storage period (P < 0.0001).

The amount of haulm remaining at the end of the trial (12 weeks) was similar (P = 0.4773) among the cultivars but marked differences (P = 0.0051) were observed in the two storage methods. The shed type of storage retained more of the haulms (P = 0.0051) than the roof type. Significant interaction existed between storage method x weeks in storage (P < 0.0001), however, the interactions of cultivar x week (P = 0.8631) and cultivar x storage methods x weeks (P = 0.9879) were not significant for haulm remaining after the experiment.

5.1.3.3 Crude protein remaining in cowpea haulm

The amount of CP remaining (kg) in the haulm of cowpea is shown in Table 5.1. Cultivars IT86D-716 and IT93K-2309 had similar CP retention (P = 0.9289) but were different from cultivars IT93K-2045-93 (P = 0.0030) and SORONKO (P = 0.0302) which had similar (P = 0.2625) CP retention. The amount of CP retained in the haulms of cultivars kept in the roof was about 1.04 times greater than the value for those kept on the shed (P = 0.3003). There existed no significant storage x week (P = 0.6610); cultivar x storage x week (P = 0.1358) interaction. However, a trend towards an interaction was observed in cultivar x week (P = 0.0720) which was as a result of the slight increase in CP concentration in the cultivars with time in storage.

Crude protein concentration of haulm in roof storage increased slightly with time (Figure 5.1), while haulm in shed storage remained fairly constant throughout the period in storage.

Table 5.1 Least square means (±s.e) showing the effect of storage methods (SM) on haulm dry matter loss (HDML), cowpea haulm remaining (HR) and nutrient retention in cowpea haulms (kg).

Cultivar	SM	HDML	HR	CP	ADF	NDF	ADIN
IT86D-716		0.5106	8.7539	0.832 [a]	4.699	5.467	0.054 [bcd]
IT93K-2045-93		0.6294	8.4483	0.689 [b]	4.564	5.165	0.050 [c]
IT93K-2309		0.7383	8.1517	0.836 [a]	4.474	5.176	0.058 [b]
SORONKO		0.5561	8.6489	0.735 [b]	4.664	5.366	0.066 [a]
SE		0.1642	0.4007	0.040	0.219	0.252	0.003
	Roof	0.8344 [a]	8.0328 [b]	0.788 [b]	4.375 [b]	5.036 [b]	0.065 [a]
	Shed	0.3828 [b]	8.9686 [a]	0.757 [a]	4.825 [a]	5.551 [a]	0.049 [b]
	SE	0.1161	0.2834	0.028	0.155	0.178	0.002
Statistical Interaction							
Cultivar x week		NS	NS	NS	NS	NS	***
Storage x week		NS	***	NS	***	***	***
Cultivar x storage x week		NS	NS	NS	NS	NS	***

Means with the common letter within cultivars and storage methods are not significantly different based on comparison of least squares means within PROC MIXED of SAS. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Where s.e = standard error; CP = crude protein; ADF = acid detergent fibre retention; NDF = neutral detergent fibre retention; ADIN = Acid detergent insoluble nitrogen

The crude protein content of the cultivars left on field as standing hay however, declined with increasing weeks in storage. There was a strong relationship when crude protein in the cultivars was related to weeks in shed ($r^2 = 0.797$), and roof ($r^2 = 0.991$) storage and when left as standing hay on the field ($r^2 = 0.993$).

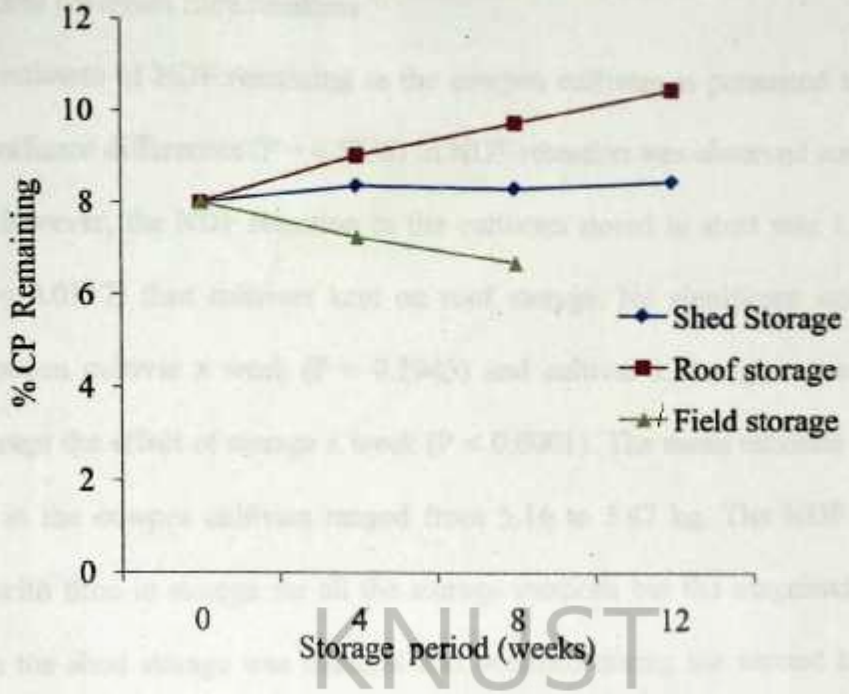


Figure 5.1 Changes in crude protein concentrations of cowpea haulms in 3 different storage methods

5.1.3.4 Acid detergent insoluble nitrogen

Concentration of ADIN significantly varied among cowpea cultivars ($P = 0.0016$) except for IT86D-716 which was similar to IT93K-2045-93 ($P = 0.2950$) and IT93K-2309 ($P = 0.2019$). Concentration of ADIN of cultivars in roof storage was 1.3 times greater than the value for those kept in the shed ($P < 0.0001$). Significant interactions ($P < 0.0001$) existed between cultivar x week; storage x week; and cultivar x storage x week. The ADIN increased sharply with time in roof storage while that of field storage increased steadily with time (Figure 5.2).

5.1.3.5 Neutral detergent fibre retention

The mean estimate of NDF remaining in the cowpea cultivars is presented in Table 5.1. No significant differences (P = 0.5738) in NDF retention was observed among the cultivars. However, the NDF retention in the cultivars stored in shed was 1.1 times higher (P = 0.0117) than cultivars kept on roof storage. No significant interaction existed between cultivar x week (P = 0.2945) and cultivar x storage x week (P = 0.9280) except the effect of storage x week (P < 0.0001). The mean estimate of NDF remaining in the cowpea cultivars ranged from 5.16 to 5.47 kg. The NDF content increased with time in storage for all the storage methods but the magnitude of the increase in the shed storage was minimal and declined during the second month of storage (Figure 5.3). Highest accuracy for predicting the NDF concentration in cultivars kept on the field (r^2 = 0.999) and roof (r^2 = 0.944) was observed except shed storage (r^2 = 0.201) where the NDF content was poorly predicted with time in storage.

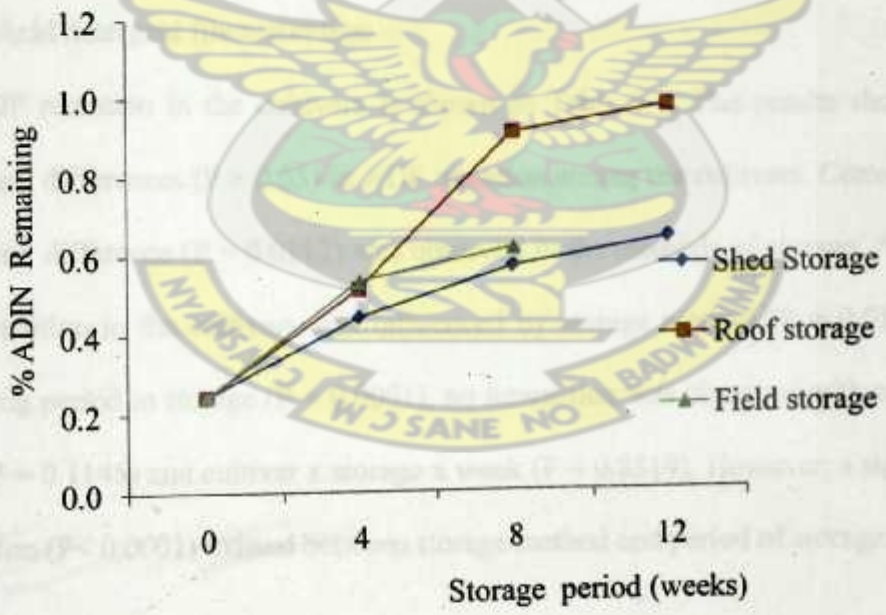


Figure 5.2 Changes in acid detergent insoluble nitrogen concentrations of cowpea haulms in 3 different storage methods.

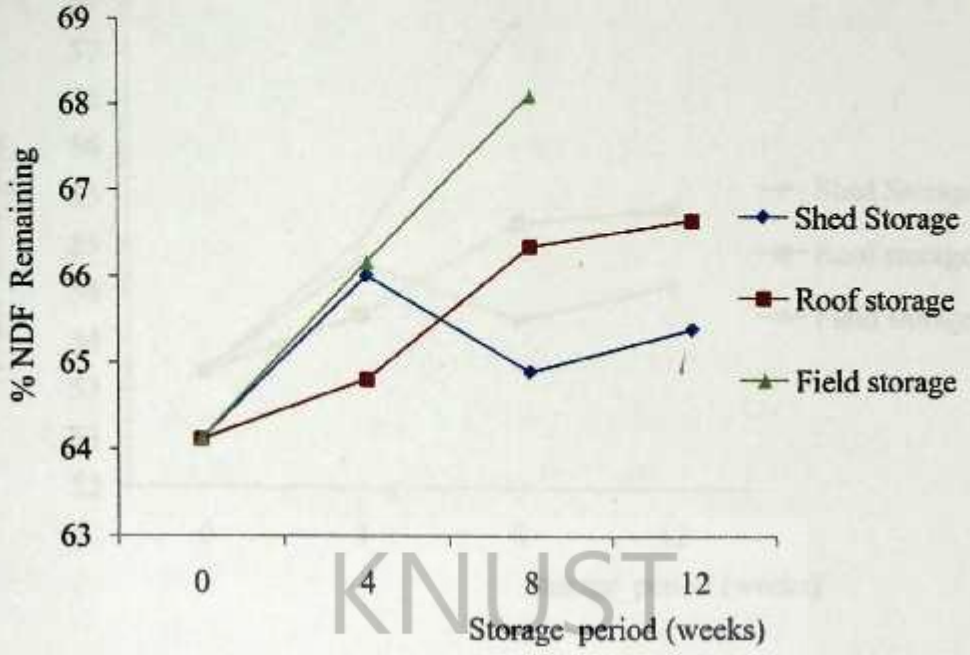


Figure 5.3 Changes in neutral detergent fibre concentrations of cowpea haulms in 3 different storage methods

5.1.3.6 Acid detergent fibre retention

The ADF retention in the cultivars is shown in Table 5.1. The results showed no significant differences ($P > 0.05$) in ADF retention among the cultivars. Conversely, a significant difference ($P = 0.0112$) was observed in the methods of storage. Although ADF retention in the cultivars was influenced by storage method ($P = 0.0112$) and advancing period in storage ($P < 0.0001$), no interaction was observed with cultivar x week ($P = 0.1145$) and cultivar x storage x week ($P = 0.8519$). However, a significant interaction ($P < 0.0001$) existed between storage method and period of storage.

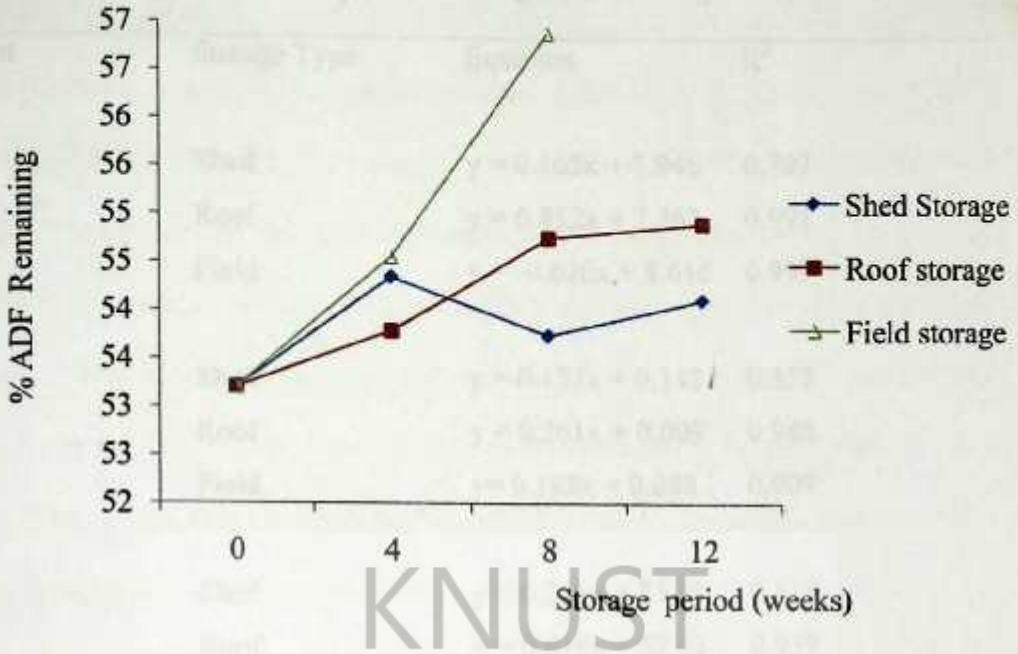


Figure 5.4 Changes in acid detergent fibre concentrations of cowpea haulms in 3 different storage methods

The estimated ADF retention in haulms of the cultivars ranged from 4.47 to 4.70 kg .The ADF content of the cultivars left as standing hay and those kept on a roof increased with time in storage (Figure 5.4), while cultivars kept in shed initially increased and gradually declined with time. The estimated ADF was positively related to weeks in storage of cultivars left as standing hay ($r^2 = 0.976$) and kept on roof ($r^2 = 0.939$). However, ADF was poorly predicted by advancing weeks in shed storage ($r^2 = 0.313$).

Table 5.2 Prediction equations for estimating nutrient concentrations (y) of cowpea haulms as a function of time (x) in storage

Nutrient	Storage Type	Equation	R^2
CP			
	Shed	y = 0.165x + 7.946	0.797
	Roof	y = 0.852x + 7.262	0.991
	Field	y = -0.626x + 8.616	0.993
ADIN			
	Shed	y = 0.133x + 0.142/	0.955
	Roof	y = 0.261x + 0.009	0.942
	Field	y= 0.188x + 0.088	0.909
ADF			
	Shed	y = 0.219x + 53.31	0.313
	Roof	y = 0.618x + 52.63	0.939
	Field	y = 1.866x + 51.16	0.976
NDF			
	Shed	y = 0.282x + 64.42	0.201
	Roof	y = 0.935x + 63.17	0.944
	Field	y = 0.282x + 64.42	0.999

Where R^2 = coefficient of determination

5.1.4 DISCUSSION

5.1.4.1 Dry matter loss during storage

The higher haulm DM loss observed in roof storage than shed agrees with the observations of Buckmaster *et al.* (1989). They asserted that dry matter loss was as a result of microbial respiration in the hay. The exposure of haulms in roof storage to rains and high temperatures in the storage period July – September 2007 (Appendix I, Tables 1-3) may explain the differences in DM loss between the storage methods. These climatic factors as outlined by Tripathi *et al.* (1995), Collins (1987) and Al-Mamum *et al.* (2002) result in bleaching and leaching of soluble and non - structural carbohydrate from haulms when exposed to the vagaries of the weather.

Weight of haulm remaining in the shed storage at the end of the trial was greater than for roof storage. This is in consonance with the study by Al-Mamum *et al.* (2002) who indicated that an improved storage system could store well excess fodder from the major season harvest intended for lean season feeding. The haulms kept in the shed had little exposure to rains; therefore wetting and subsequent drying which might have led to loss of soluble nutrients and hence dry matter loss was not seen in this storage system. This explains the greater haulm remaining in shed than in roof storage. The storage x week interaction for haulm remaining, however, was caused by shattering of leaves on the roof, with advancing weeks in storage

5.1.4.2 Crude protein concentration

The nutrients remaining in haulms (expressed as a percentage of haulm weight) gives an indication of the proportion of the nutrient in the haulm at any particular time in storage. It was observed that the haulms kept on the roof had increased concentration of crude protein than those in the shed storage. This increase is consistent with the studies of Rotz *et al.* (1991), who suggested that, in the event of rain, soluble N leaches at a slower rate than other constituents such as sugars, thereby causing N concentration to increase.

In an assessment of percentage nutrient that combined all the three (roof, shed and field) storage methods showed a decline in crude protein concentration in the haulms left as standing hay (Figure 5.1). This was attributed to increased cell wall content as the plant ages. The CP of the haulms in the shed remained constant while that of the roof increased with time in storage. According to Rotz and Muck (1994), concentration of nitrogen in heated hays (i.e. haulms in roof storage) increases because nonstructural carbohydrates are preferentially oxidized by plant enzymatic

processes and microorganisms associated with storage. The heat generated in the haulms kept on the roof after any incidence of rain explains the differences in CP concentrations in roof and shed storage systems.

5.1.4.3 Fibre components

Fibre components according to Rotz and Muck (1994) are not lost during storage of hay; however, fibre concentrations are thought to increase owing to preferential oxidation non –structural carbohydrates. Krishnamoorthy *et al.* (1982) and Licitra *et al.* (1996) have explained nitrogen bound to fibre as nitrogen associated with feed DM after extraction in neutral or acid detergent solution, and insoluble in water (Van Soest, 1987). The ADIN is considered to be ruminally undegradable (Sniffen *et al.*, 1992) and to have very low bioavailability (Licitra *et al.*, 1996). The reported 1.3 times higher ADIN in haulms kept on roof than shed is may be an indication of the extent of rain and heat damage of the haulms. The increase observed herein was ascribed to leaching of soluble nitrogen as well as decreased solubility of the nitrogen remaining. Maillard reaction could explain the increase in the recovery of N from ADF as a result of direct exposure to light (Rotz *et al.*, 1991). The significant interactions between the storage methods and week in storage was caused by increased concentration of ADIN in the cultivars in roof storage with time. The higher ADF and NDF recorded in shed storage is explained by the fact that, the percentage difference of the fibre components between haulms in shed and roof storage were not so wide and again haulm remaining under shed storage was significantly greater than that of roof. The increased fibre fractions in field storage depict higher lignification and cell wall contents with maturing plant.

5.1.5 CONCLUSION

Dry matter and nutrient composition were affected by storage method. The level of fibre increased and a higher proportion of the nitrogen became associated with the cell wall of cultivars left as standing hay and in roof storage. It was realised that, the shed system of storage improves the availability and quality of fodder in respect of dry matter and nutrient composition. Therefore, the shed storage system is suggested to farmers as a way of ensuring nutrient retention in cowpea haulm and haulms availability all year round.

CHAPTER 6

6.0 ANIMAL EXPERIMENT 1

6.1 CHEMICAL COMPOSITION, GAS PRODUCTION AND DEGRADATION CHARACTERISTICS OF HAULMS OF IMPROVED DUAL PURPOSE COWPEA CULTIVARS

6.1.1 INTRODUCTION

The major constraint to small-holder ruminant livestock production in Ghana is availability and quality of feed all year round. Ruminants survive on crop residues and unimproved swards deficient in nitrogen, energy, and minerals. This affects intake, digestibility, utilization and animal productivity. The deficiencies in these roughages are overcome partly by nitrogen supplementation. Leguminous fodders are promising and cheap source of nitrogen for use by smallholder farmers; among these is cowpea haulm. Cowpea haulm has been shown to increase microbial nitrogen supply in calves when used as supplement to teff straw (Abule *et al.*, 1995); promote intake of maize stover and improve ammonia concentration and degradation of maize stover (Chakeredza *et al.*, 2002).

In evaluating feedstuffs, it is useful to know the chemical composition of such feeds but this fails to provide information on feed degradation characteristics which determine its utilization and in turn the performance of animals (Blümmel *et al.*, 1997). A series of studies have shown that, degradation characteristics of feed in the rumen offers a crucial means of evaluating the nutritive value of several feedstuffs. *In situ* nylon bag (Ørskov *et al.*, 1980) and *in vitro* gas production (Menke and Steingaß, 1988) techniques have been used extensively for measuring ruminal degradation, screening of feedstuffs (Siaw *et al.*, 1993; Nsahlai *et al.*, 1994) and predicting digestible organic matter intakes (Kibon and Ørskov, 1993) because of a high degree

of correlation with *in vivo* digestibility values (Marten and Barnes 1980; AOAC, 1990).

Agronomy experiment 1 has shown that yields of 4 improved cowpea cultivars could be as high as 10.98 t/ha, but these levels could also vary with season and year of cultivation. It would be useful to assess the chemical composition as well as the nutritional value of the improved cowpea haulms. The objective of Animal experiment 1 was to assess the chemical composition, gas production profiles and *in situ* degradation characteristics of haulms of four improved dual - purpose cowpea cultivars.

6.1.2 MATERIALS AND METHODS

6.1.2.1 Location of Experiment

The chemical composition (with the exception of ether extract (EE) and lignin (ADL)) was assayed in the Nutrition laboratory of the Department of Animal Science (DAS), KNUST. The EE, and ADL contents; *in situ* dry matter and crude protein digestibility; and *in vitro* gas production studies were carried out in the Nutrition laboratories of the Animal Production and Research Institute, Noubaria, Egypt and Alexandra University, Egypt, respectively. The laboratory assays lasted for two months.

6.1.2.2 Source of cowpea haulm

The source of the cowpea haulm is as stated in Section 3.3.1 of Chapter 3

6.1.2.3 Morphological fractionation of cowpea haulm

Leaves were manually detached from the cowpea plants grown on the arable fields of DAS after harvesting, and the stems were chopped and pooled to constitute the leaf and stem fractions, respectively.

6.1.2.4 Animals and feeding

Three rumen-cannulated Barki rams (45±2kg average live weight) were used. Each ram had *ad libitum* access to water and a feed of berseem hay (*Trifolium alexandrinum*) provided at 08: 30 h every day.

6.1.2.5 Experimental design

The design used for the gas production was the completely randomized design with three replications per treatment (i.e. 4 haulms of the cowpea cultivar). The DM and CP degradability were examined using a split-plot design to compare the effects of cultivar and plant fraction as well as their interactions. The whole haulm formed the main plot structure; while the leaf and stem fractions represented the sub plot factor, and the cultivars were the blocks.

6.1.2.6 Chemical analysis

Samples of cowpea haulms were analyzed for DM, OM, EE, NDF, ADF and CF. The detailed procedures involved have been described in Appendix IV.

6.1.2.7 *In vitro* gas production

The procedure adopted for this technique has been outlined in Section 3.6.4 of Chapter 3.

6.1.2.8 Nylon bag studies

The morphological fractions of the cowpea haulms as described in 6.1.2.3 above were milled through 2mm sieve and 5.0g samples were put in the nylon bags (5x10 cm, 40µm pore size). Duplicate samples of each fraction were incubated for 3, 6, 9, 12, 24, 48, 72 and 96 hours. Section 3.6.2.1 of Chapter 3 outlines the procedures adopted for washing of bags, calculation of sample disappearance and degradation constants.

6.1.2.9 Statistical analysis

The data obtained from the gas production technique were subjected to GLM PROC of SAS (1999) while the *in situ* degradability data were analyzed with a split plot analysis using the GLM and PROC MIXED procedures of SAS (1999). The degradation constants were determined by a curve fitting procedure of PROC NLIN, available in Version 8 of SAS (SAS, 1999). When differences (i.e. $P < 0.05$) occurred, treatment means were compared by least square means.

6.1.3 RESULTS

6.1.3.1 Overview

The analysis of cultivars for their chemical composition was done in Ghana and representative samples of the cowpea haulm were assessed for their gas production profiles and degradability data in Egypt at the Alexandria University, and Animal Production and Research Institute. Two cultivars that recorded high CP, gas production profile, DM degradabilities and low fibre fractions were selected and their morphological fractions (stem and leaf) were further evaluated for DM and CP degradabilities. The chemical composition of the Berseem hay used as standard hay is given as 86.75(OM), 1.67 (EE), 12.18(CP), 26.45(CF), 59.12(NDF), 36.43(ADF), and 7.26 (ADL).

6.1.3.2 Chemical composition

The chemical composition of the morphological fractions of the haulms of the cowpea cultivars are shown in Table 6.1. Cultivar effect was significant ($P < 0.0001$) with regard to CP, ash, NDF, NDIN, ADF, ADL, ash free CF ($P = 0.01$) and EE ($P = 0.0067$). The CP content in the whole haulm of the cultivars was highly variable ($P = 0.0010$), ranging from 148.2 g/kg DM (SORONKO) to 229.6 g/kg DM (IT93K-2309); compared with 240.9 g/kg DM (SORONKO) to 342.1 g/kg DM (IT93K-2045-93) for leaves; and 57.6 g/kg DM (IT86D-716) to 98.4 g/kg DM (IT93K-2045-93) for stem. Some significant ($P = 0.0003$) amount of nitrogen was found to be associated with NDF in the haulm of the cultivars. Concentration of NDIN was comparatively higher in leaves than in whole haulm or stems. Lower levels of NDIN were recorded in the whole haulms of IT93K-2045-93 and SORONKO than IT93K-2309 and IT86D-716. The NDIN level in the leaves of IT86D-716 was 2.2, 1.9 and 1.7 times higher than ($P < 0.0001$) SORONKO, IT93K-2309 and IT93K-2045-93, respectively. The difference in the concentration of NDIN in IT93K-2309 and IT93K-2045-93, however, tended to approach significance ($P = 0.0864$). On the contrary, NDIN levels obtained in the stem of all the cultivars were similar ($P = 0.2635$).

6.1.3.3 Gas production

The results of the gas production data for the four cultivars are shown in Table 6.2. Cultivar effect on readily fermentable fraction represented as "a" and rate of gas production "c" tended to approach significance ($P = 0.0784$ for "a"; $P = 0.0856$ for "c"). However, there were significant differences ($P = 0.0001$) in the potential gas production "b" among the four cultivars used in this study.

Table 6.1 Least square means (±se) of chemical composition (g/kg DM) of cowpea haulm.

Fraction Cultivars	CP	EE	CF	Ash	NDF	NDIN	ADF	ADL
Leaves								
SORONKO	240.9^{c}	38.2^{c}	125.3^{c}	49.1^{b}	193.7^{c}	8.5^{c}	154.6^{d}	19.5^{c}
IT93K-2309	304.8^{b}	41.9^{a}	139.0^{b}	47.4^{b}	239.9^{b}	10.1^{b}	178.5^{c}	21.7^{b}
IT86D-716	298.8^{b}	31.4^{d}	153.9^{a}	65.9^{a}	241.2^{b}	18.8^{a}	192.2^{b}	26.4^{a}
IT93K-2045-93	342.1^{a}	45.3^{a}	120.1^{c}	32.0^{c}	257.8^{a}	10.8^{b}	200.5^{a}	22.2^{b}
CV	3.52	3.83	4.00	6.33	2.68	4.10	1.99	1.66
MSD	20.23	2.91	10.57	5.93	12.05	0.94	6.94	0.71
Stem								
SORONKO	72.1^{e}	16.0^{b}	322.1^{b}	94.1^{a}	700.6^{a}	5.3^{a}	571.2^{a}	48.7^{b}
IT93K-2309	92.6^{b}	16.6^{ab}	323.2^{b}	96.9^{a}	691.0^{a}	5.6^{a}	547.6^{ab}	47.8^{b}
IT86D-716	57.6^{d}	12.5^{c}	345.5^{a}	106.1^{a}	674.0^{a}	5.4^{a}	558.4^{a}	51.5^{a}
IT93K-2045-93	98.4^{a}	17.7^{a}	313.2^{b}	79.0^{b}	696.5^{a}	5.9^{a}	530.4^{b}	46.0^{c}
CV	2.16	5.40	2.00	7.45	2.31	6.26	2.25	0.94
MSD	3.30	1.67	13.06	14.40	35.60	0.78	25.97	0.88
Whole								
SORONKO	149.2^{c}	22.4^{b}	293.7^{b}	69.1^{a}	460.6^{b}	6.4^{b}	372.8^{a}	35.4^{b}
IT93K-2309	229.6^{a}	26.0^{a}	293.6^{b}	72.0^{a}	500.0^{a}	7.4^{a}	373.7^{a}	34.6^{b}
IT86D-716	190.1^{b}	20.6^{c}	324.6^{a}	74.5^{a}	463.3^{b}	7.7^{a}	346.4^{b}	36.8^{a}
IT93K-2045-93	229.1^{a}	21.9^{b}	275.3^{c}	52.5^{b}	466.5^{b}	6.2^{b}	368.6^{ab}	30.9^{c}
CV	7.10	2.42	1.85	8.33	2.55	2.65	2.93	2.01
MSD	27.88	1.06	10.67	11.36	24.96	0.35	22.76	1.34

Means with the common superscripts (a,b,c) within columns are not significantly different according to the Waller-Duncan k-ratio t-test with t=100.

Where CV = Coefficient of variation; MSD = Mean significant difference.

Table 6.2 Least square means (± s.e) of the effect of four cultivars of cowpea haulms on cumulative *in vitro* gas production (ml gas/200mg DM).

	Cultivars				SE
	1T93K-2309	IT93K-2045-93	IT86D-716	SORONKO	
GP Parameters					
a	2.29^{a}	1.43^{b}	0.89^{b}	1.89^{a}	0.309
b	21.76^{b}	26.54^{a}	16.54^{d}	18.84^{c}	0.407
c (h^{-1})	0.07^{b}	0.08^{a}	0.08^{a}	0.07^{b}	0.003

Means with the common superscripts (a,b,c) within rows are not significantly different according to the Waller-Duncan k-ratio t-test with t=100.

Where a = readily fermentable fraction; c = rate of gas production (GP) from the slowly fermentable fraction, b; SE = standard error

The cumulative gas production as a function of incubation time is presented in Figure 6.1. The highest gas production was observed in cultivar IT93K-2045-93 followed by 1T93K-2309, SORONKO and IT86D-716. The rate of gas production among the cultivars (Table 6.2) however, varied from 6.7% h^{-1} (1T93K-2309) to 8.1% h^{-1} (IT86D-716).

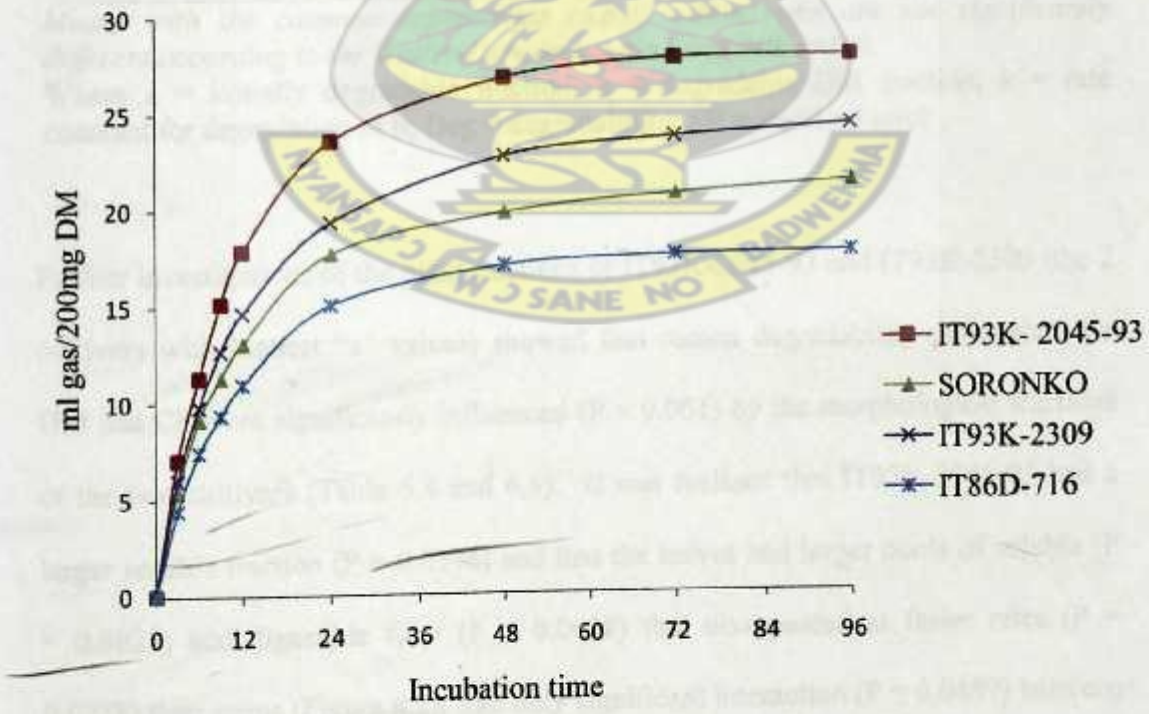


Figure 6.1 Gas production profiles of four cultivars of cowpea haulm

6.1.3.4 Degradation Characteristics

The results of the degradability studies involving the four cowpea cultivars is shown in Table 6.3. The cultivars differed (P = 0.0009) in the quantity of readily soluble material but no significant differences were found in the potentially digestible fraction (P = 0.9887) and the rate of degradation (P = 0.7783). The cultivar IT93K-2045-93 had the highest percent soluble material (24.3%) while cultivars IT93K2309, IT86D-716 and SORONKO recorded 20.7, 18.6 and 18.3% readily soluble materials respectively. Similar pools (P = 0.9887) of digestible fibre (represented as "b") that disappeared at the same rates (P = 0.7783) were observed among the cultivars.

Table 6.3 LS means of DM degradabilities (%) of four cultivars of cowpea haulms.

	Cultivars				SE
	IT93K-2309	IT93K-2045-93	IT86D-716	SORONKO	
Deg. Parameters					
a (%)	20.7^{b}	24.3^{a}	18.6^{c}	18.3^{c}	0.81
b (%)	44.1	44.3	44.1	44.1	0.83
k (h^{-1})	0.06	0.06	0.07	0.06	0.01

Means with the common superscripts (a,b,c) within rows are not significantly different according to the Waller-Duncan k-ratio t-test with t=100.
Where a = initially degradable fraction; b = degradable DM fraction; k = rate constant for degradation of b; Deg = degradability; SE = standard error

Further investigations of the plant fractions of IT93K-2045-93 and IT93K-2309 (the 2 cultivars with highest "a" values) showed that rumen degradability parameters for DM and CP were significantly influenced (P < 0.001) by the morphological fractions of the two cultivars (Table 6.4 and 6.5). It was realized that IT93K-2045-93 had a larger soluble fraction (P = 0.0396) and that the leaves had larger pools of soluble (P = 0.0023) and digestible fiber (P = 0.0638) that disappeared at faster rates (P = 0.0238) than stems (Figure 6.2). The only significant interaction (P = 0.0489) between

cultivar and fractions of cowpea occurred for digestion rate. The rates of digestion for leaves and stems in IT93K-2309 were not as divergent as the rates of digestion for leaves and stems in IT93K-2045-93 (P = 0.0489). It is to be noted however, that otherwise the rates, did not differ significantly between the two cultivars (P = 0.5121)

Table 6.4 Dry matter degradation parameters for haulms of two cowpea cultivars with highest "a" values

Parameter Estimates			
Parameter			*SE*
	Whole Sample Analysis		
Cultivar	*IT93K-2309*	*IT93K-2045-93*	
a	20.7305	24.2575	0.6749
b	44.0648	44.3264	0.8079
k	0.05967	0.06563	0.00534
	Separated Plant Fraction Analysis		
Fraction	*Leaf*	*Stem*	
a	25.2447	12.5600	0.7646
b	50.5596	48.3461	0.4154
k	0.10730	0.06017	0.00544
Cultivar	*IT93K-2309*	*IT93K-2045-93*	
a	17.4716	20.3330	0.7602
b	50.2157	48.6900	0.4154
k	0.07910	0.08833	0.00441
Interaction	*IT93K-2309*	*IT93K-2045-93*	
Leaf-a	23.4179	27.0714	0.8534
Stem-a	11.5253	13.5946	
Leaf-b	52.0377	49.0816	0.4154
Stem-b	48.3937	48.2984	
Leaf-k	0.09767	0.11690	0.00587
Stem-k	0.06053	0.05980	

Where a = initially degradable fraction; b = degradable DM fraction; k = rate constant for degradation of b; SE = standard error

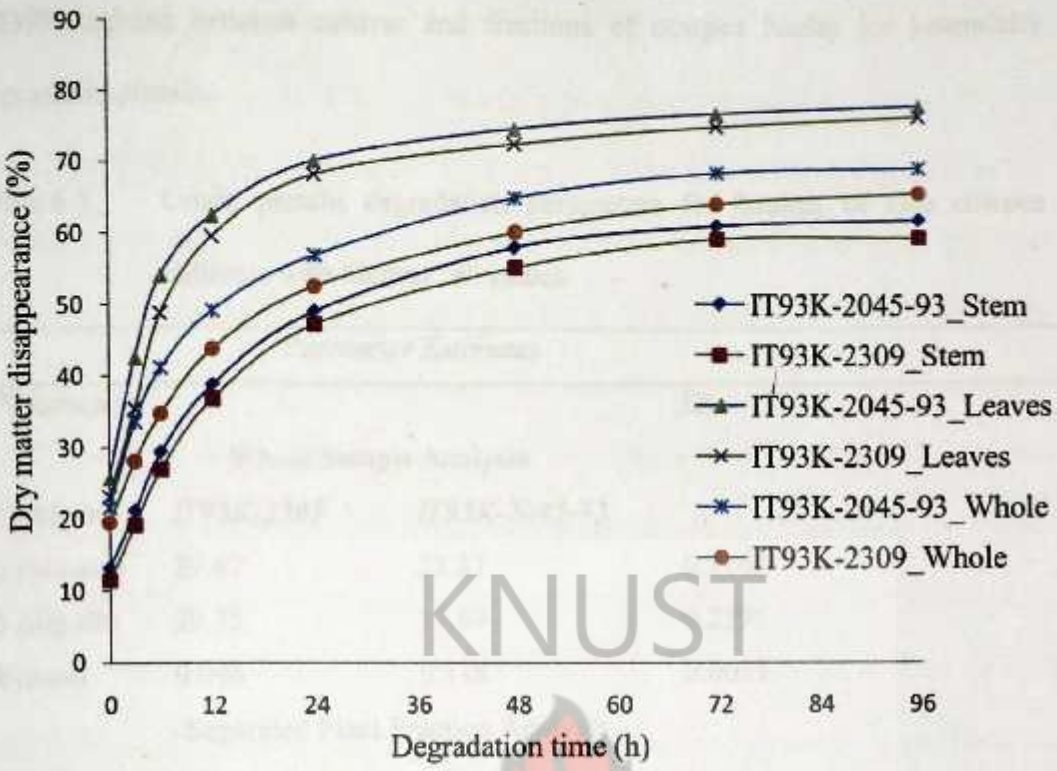


Figure 6.2 Disappearance of dry matter with time

Analysis of the CP degradation (Table 6.5) in the whole plant showed that significant differences ($P < 0.05$) existed in all the parameters estimated. Cultivar IT93K-2045-93 had a greater percent readily digestible protein than IT93K-2309 ($P = 0.0003$). The amount of ruminal degradable protein (RDP) was higher ($P = 0.0241$) in IT93K-2309 than IT93K-2045-93. However, the latter disappeared at greater rates ($P = 0.0002$) than the former.

An assessment that combined separated leaves and stems (Table 6.5) from the two cultivars led to the observation that the amount of CP readily degradable in the rumen was particularly higher for IT93K-2045-93 ($P = 0.0087$) and that leaves had larger pools of soluble ($P = 0.0012$) and potentially degradable protein ($P = 0.0774$) that also

disappeared at faster rates (P = 0.0030) than stems. Significant interaction (P = 0.0399) existed between cultivar and fractions of cowpea haulm for potentially degradable protein.

Table 6.5 Crude protein degradation parameters for haulms of two cowpea cultivars with highest "a" values

Parameter Estimates			
Parameter			*SE*
	Whole Sample Analysis		
Cultivar	*IT93K-2309*	*IT93K-2045-93*	
a (init sol)	27.67	33.37	0.1149
b (dig fib)	39.75	37.69	0.2295
k (rate)	0.096	0.118	0.0015
	Separated Plant Fraction Analysis		
Fraction	*Leaf*	*Stem*	
a	38.12	25.62	0.6262
b	36.02	32.34	0.8147
k	0.199	0.003	0.0084
Cultivar	*IT93K-2309*	*IT93K-2045-93*	
a	28.64	35.10	0.6623
b	34.83	33.53	0.7056
k	0.114	0.118	0.0086
Interaction	*IT93K-2309*	*IT93K-2045-93*	
Leaf-a	34.52	41.72	0.7278
Stem-a	22.75	28.49	
Leaf-b	37.86	34.19	0.9244
Stem-b	31.80	32.88	
Leaf-k	0.197	0.200	0.011
Stem-k	0.032	0.035	

Where a = readily degradable protein; b = potentially degradable protein; k = rate constant for degradation of b; SE = standard error

6.1.4 DISCUSSION

6.1.4.1 Chemical composition

Chemical evaluations of cowpea haulms have been established in studies by Savadogo *et al.* (2000) and Kaasschieter *et al.* (1998). This current study however, sought to undertake a more comprehensive chemical analysis of selected cultivars from a series of breeding programmes in Ghana. The results obtained in this study showed that the chemical composition of the cultivars differed from other published data (Abule *et al.*, 1995; Chakeredza *et al.*, 2002; Coppock and Reed, 1992; Koralagama *et al.*, 2008). The CP content is an important indication of nutritional quality since the cultivars are intended to be used as supplements for poor quality crop residues. The CP content among the cultivars was variable, with cultivar IT93K-2045-93 recording the highest. The reported CP values are higher compared to those reported by Savadogo *et al.* (2000). The differences in CP values from the reported data, and even among the cultivars, may be as a result of genetic improvement of the cultivars and inherent genetic characteristics (Badve *et al.*, 1994; Singh and Schiere, 1995; Subba Rao *et al.*, 1994); environmental factors such as soil characteristics and crop management (level of fertilizer application, plant density, stage of maturity at harvest, methods of harvesting, and storage) (Harika and Sharma, 1994; Walli *et al.*, 1994). The relatively higher CP in the leaves and the stem of the same cultivar (IT93K- 2045-93) may explain the extensive *in situ* degradation and high gas production of that cultivar. Cultivars IT93K- 2045-93 and IT93K-2309 had similar CP contents but differed in the quantity of soluble CP. This could be related to the rate of faster release of nitrogen from IT93K- 2045-93 given its low levels of NDIN.

The whole and the fractionated parts of IT93K- 2045-93 recorded the least crude fibre and lignin contents. This may also help to explain the high DM and CP degradabilities, as well as higher gas production levels of this cultivar.

6.1.4.2 *In sacco* DM and CP disappearance

The degradability parameters measured in this study are of paramount importance as they influence rumen fill and hence feed intake (Ørskov *et al.*, 1988). Differences therefore in the parameters estimated are suggestive that the effect of offering different cultivars of cowpea haulm as nitrogenous supplement on intake and animal performance may vary substantially.

The variations in the initially digestible dry matter among the cultivars may be related to differences in the chemical composition (Åman and Nordkvist, 1983) or variations in physical structure, such as the distribution of lignified cells within the tissues (Ramanzin *et al.*, 1991). Thus the high DM digestibility of IT93K- 2045-93 among the cultivars is a reflection of its low contents of lignin and crude fibre.

The larger pools of digestible fibre in the leaves that disappeared at faster rates (10.7% h^{-1}) than stems (6.0% h^{-1}) of the cultivars are indicative of the higher lignin content in the stem. This is in consonance with the assertion by Reed and Van Soest (1984) that, stems of dicotyledonous crop residues are characterised by high fibre, lignin and low nitrogen content, hence low digestibility.

The rapid ruminal protein degradation of cultivar IT93K- 2045-93 may result in the production of more peptides and amino acids (Broderick *et al.*, 1991). These end - products are incorporated into microbial protein during practical ruminant feeding.

Among the microbial consortium that are partially dependent on the supply of amino acids and peptides are the cellulolytic bacteria. Therefore, degradable fractions of protein sources that provide suitable substrates would induce a stronger bacterial response (McAllan and Smith, 1983) in ruminal feed fermentation. The results for the protein degradability of leaf and stem fractions reported in this work revealed that, ruminal protein degradability in the leaf was comparatively higher. This could be due to greater proteolytic activities of the ruminal microflora thereby evoking a higher bacterial degradation.

The higher nitrogen-bound protein to fibre in the leaves fraction of the cultivars relative to the stems may be an effective nutritional strategy in manipulating excess RDP in an attempt at reducing nitrogen losses in the form of ammonia nitrogen. Thus NDIN helps protect protein from complete degradation by proteolytic bacteria and provides greater protein supply to the small intestines. In this vein, cultivar IT86D-716 would have been the cultivar of choice, however, because of its high lignin and fibre, as well as low CP levels, its use as supplement would not be viable.

6.1.4.3 *In vitro* gas production.

The highest fermentative gas production was observed in IT93K-2045-93 and was followed by, IT93K-2309, SORONKO and IT86D-716 in decreasing order (Table 6.2). The least gas accumulation, which was from cultivar IT86D-716, may be as a result of high cell wall content (crude fibre and lignin). Lignin content is reported to be negatively correlated with gas production (Jung and Deetz, 1993). According to the authors, lignification of cell wall limits the functions of rumen microbial flora such as fermentation or enzymatic breakdown of forage polysaccharides. Since gas

production is associated positively with feed fermentation, cultivar IT86D-716 could be described as having low feeding value owing to its low gas production.

6.1.5 CONCLUSION

Nutritional value is a complex interplay of many factors, however, the assessment tools used (i.e. chemical composition, *in vitro* gas production and *in situ* degradability) in this experiment indicated higher CP, readily soluble DM and CP, lower NDF, ADF and NDIN of cultivars IT93K- 2045-93 and IT93K-2309. These cultivars would therefore serve as a source of nitrogenous supplement and improve the productivity of ruminants fed poor quality diet.

CHAPTER 7

7.0 ANIMAL EXPERIMENT 2

7.1 THE EFFECT OF PESTICIDE USE IN COWPEAS *IN VITRO* GAS PRODUCTION

7.1.1 INTRODUCTION

Cowpea is a nutritionally important legume crop grown for its leaves and grains for human consumption (Nielsen *et al.*, 1997), while the haulms are fed to livestock (Singh *et al.*, 2003) or incorporated into the soil. Cowpea haulm plays a major role as supplement for ruminants fed poor quality basal diets. Savadogo *et al.* (2000) reported improved performance of sheep when cereal straws were supplemented with the haulms of cowpeas. Cowpea production, however, is considered too risky an investment by many farmers because of numerous insect pests damaging the crop, from seedling emergence right to storage (Jackai and Adalla, 1991). Therefore, for a complete protection of the cowpea crop, applications of these pesticides; Lambda cyhalothrin, Dimethoate, and Cypermethrin recommended (Afun *et al.*, 1991) and commonly practiced in Ghana.

Inherent problems of pesticide residue accumulation and degradation compounds in edible tissues (Laben, 1968) associated with pesticide use are well recognized. Studies of the aforementioned pesticides have concentrated on their degradation rates in plants and excretion products in the urine and faeces of ruminants (Gutenmann *et al.*, 1968). However, how the ingestion of pesticide contaminated forages or cowpea haulms affects ruminal feed fermentation by microorganisms is of significance to researchers. Animal experiment 1 explored the effects of different cultivars on gas production of cowpea haulm. It did not however, address the possible effects of pesticide application on gas production by microbes.

Microbial protein accounts for as much as 90% of the amino acids reaching the small intestine, and energy from short-chain organic acids drives ruminant metabolism (Nocek and Russell, 1988). Rumen microbial fermentation supplies approximately 50-70 percent of the energy requirements of ruminants (Annison and Lewis, 1959). The role therefore of rumen microbes in ruminant nutrition, cannot be overemphasized. Consequently, any factor that alters the rumen microbial ecology adversely might influence digestion in the rumen, resulting in a reduced animal performance (Schwartz and Nagy, 1974).

The study for animal experiment 2 describes the effects of 3 pesticides at varied concentrations on *in vitro* gas production levels and predicted rumen fermentation parameters.

7.1.2 MATERIAL AND METHODS

7.1.2.1 Location of experiment

The experiment was conducted in the Nutrition and Chemistry Laboratories of Alexandra University, Alexandria, Egypt for a period of two months.

7.1.2.2 Analytical procedures

7.1.2.3 Extraction of pesticides from cowpea haulm

The extraction of pesticide residue was done according to the procedures of Fillion *et al.* (1995). The cowpea haulm was chopped and 50g was homogenized with 100 ml acetonitrile for 5 minutes, using a blender to achieve representative samples. Ten grams of sodium chloride was added in an 8 ml graduated cylinder and homogenized for 5 minutes. Approximately 13 ml of acetonitrile was transferred into a 15 ml graduated centrifuge tube. Into the tube, 3g of sodium sulphate was added, capped and shaken before centrifuging at high speed for 5 minutes. An aliquot of 10 ml (5g of

sample) was transferred to a clean 15 ml tube and evaporated to 0.5 ml, under nitrogen water bath at 35^{0}C. This was transferred into ENVI-Carb SPE[4] tube and eluted with 20ml acetonitrile/ toluene (3:1). The elute was transferred into a clean 15 ml tube and 50 µl internal standard added and brought to a volume of 2.5 ml with acetone. Half a ml of this was used for the gas chromatography analysis of the pesticide residues.

7.1.2.4 Gas Chromatography analysis

GC–ECD conditions: splitless injection of 1µl was carried out at 270 ^{0}C and splitless time 1.5 minutes. Column temperature was initially set at 80 ^{0}C and held for 2 min. It was then increased at a rate of 35 ^{0}C /min to 170 ^{0}C and held for 13.5 min. This was followed by 10 ^{0}C /min to 230 ^{0}C, held 7 min and was finally increased at rate of 10 ^{0}C /min to 300 ^{0}C for 3 min. The carrier gas flow rate was 2 ml/min.

Pesticide residue was estimated as:

$$\text{Pesticide residue (mg/kg)} = \frac{\text{peak height ratio in sample}}{\text{peak height ratio in standard}} + \frac{\text{[pesticide] in standard}}{\text{[sample] in final solution}}$$

Where [pesticide / sample] = concentration of pesticides and sample, respectively.

7.1.2.5 Stock solution

Stock solutions of the various pesticides ranging from 2.66 ml (Lambda cyhalothrin), 5.14 ml (Cypermethrin) to 6.68 ml (Dimethoate) were dissolved in 1l distilled water.

[4] ENVI-Carb - 120/400 mesh, surface area 100m2/g, was obtained from Supelco (Bellefonte,PA, USA).

Known volumes of 40, 80 and 120 µl were dispensed into gas syringes containing 210 mg pesticide-free cowpea haulm.

7.1.2.6 Source of inoculum

Rumen fluid for the *in vitro* fermentation studies were obtained from two rumen-fistulated Barki rams (45±2 kg average live weight) which had *ad libitum* access to berseem hay (*Trifolium alexandrinum*) at 08: 30 h every day. The rumen fluid was collected from the fistulated animals and strained through four layers of cheesecloth into bottles pre-gassed with CO_2.

7.1.2.7 *In vitro* incubation in glass syringe and gas measurements

The *in vitro* fermentation studies were carried out in 100 ml glass syringes fitted with a plunger. Each glass syringe contained 210 mg cowpea haulm as substrate to which 10 ml of rumen fluid, 20 ml of buffer (McDougall, 1948) and varied amounts of pesticides (40, 80 and 120 µl) were added. Gas volumes were recorded at 3, 6, 12, 24, 48, 72 and 96 h of incubation and corrected for blank syringes incubated in each run.

7.1.2.8 Calculations of IVOMD, ME, NE, SCFA and MP

In vitro organic matter digestibility (IVOMD, g/kg DM), metabolisable and net energy (ME, NE, MJ/kg DM) were predicted according to the following stoichiometric equations of Menke and Steingaß (1988) and Close and Menke (1986):

$$\text{IVOMD (g/kg DM)} = [14.88 + 0.889\ \text{IVGP} + 0.45\text{CP (\%DM)} + 0.0651\ \text{Ash}]$$

$$\text{ME (MJ/kg DM)} = 2.20 + (0.136\ \text{IVGP24}) + 0.057 \times \text{CP}$$

$$\text{NE (MJ/kgDM)} = (2.20 + (0.0272 \times \text{IVGP 24}) + (0.057 \times \text{CP}) + (0.149 \times \text{EE}))/14.64$$

$$SCFA = [-0.00425 + 0.0222 \times \text{IVGP24 (ml/0.5g DM)}] \text{ X } 100$$

$$MP = 1.93 \times \text{IVOMD} / 10$$

Where: IVGP = Gas volume measured at 24 h; and OMD = Organic matter digestibility

7.1.2.9 Experimental design and statistical analysis

A complete randomized design with 4 replications was employed in the trial. A non-linear curve fitting and PROC MIXED procedures were used in SAS (1999) for the analysis of *in vitro* gas production. Waller k-ratio test was used to separate treatment means.

7.1.3 RESULTS

7.1.3.1 Overview

The cowpea haulm samples obtained from 2007 wet season harvest were analyzed for pesticide residue. The haulms contained detectable concentrations of some of the pesticides. Cypermethrin was detected at the highest concentration with a peak value of 1.8 µl and Dimethoate was detected at a maximum concentration of 1.38 µl while Lambda cyhalothrin was not detected in the samples. The volumes of pesticide residues in this experiment were varied above the levels detected in the haulms (i.e. 40, 80 and 120 µl) to ascertain levels that may influence gas production by rumen microbes.

Gas production in general was significantly influenced ($P < 0.05$) by pesticide application. Introduction of incremental levels of Lambda cyhalothrin reduced gas evolution while increasing levels of Dimethoate resulted in a significant ($P < 0.05$) accumulation of gas. Cypermethrin application however, yielded no noticeable change in gas production. Lambda cyhalothrin residues resulted in a reduction of

some of the predicted parameters while the other two pesticides were ineffectual in causing a depression of any of the parameters estimated.

7.1.3.2 Effect of Lambda cyhalothrin on *in vitro* gas production

Significantly higher gas production level was measured for cowpea haulm spiked with 40 µl Lambda cyhalothrin compared to untreated samples. This significantly declined ($P < 0.05$) as pesticide concentration increased to 80 µl (Table 7.1). An increase in gas production was however observed again as the concentration of the pesticides further increased to 120 µl. The rates of gas production varied among the treatments. A decreasing trend followed by an increasing trend was observed with increasing concentration.

Table 7.1 Effect of lambda cyhalothrin on gas production kinetics of cowpea haulm incubated with buffered rumen fluid *in vitro* for 96 h

Treatment	Gas production profiles			Predicted Parameters				
	GP	*c*	*l*	SCFA	ME	NE	OMD	MP
BH	35.06^{a}	0.085^{a}	-0.016^{d}	68.07^{a}	7.47^{a}	4.76^{a}	51.07^{a}	98.57^{a}
CP	24.46^{d}	0.057^{b}	0.017^{d}	43.80^{e}	6.90^{b}	4.70^{a}	48.20^{e}	93.07^{c}
$LC_{40\mu l}$	28.87^{b}	0.042^{c}	0.169^{c}	46.47^{b}	6.27^{c}	4.27^{b}	44.37^{d}	85.77^{d}
$LC_{80\mu l}$	26.24^{c}	0.053^{b}	0.409^{b}	43.33^{c}	7.00^{b}	4.83^{a}	49.10^{b}	94.67^{b}
$LC_{120\mu l}$	27.92^{b}	0.058^{b}	0.692^{a}	44.80^{c}	6.90^{b}	4.70^{a}	48.20^{c}	93.10^{c}
CV	2.29	9.01	0.55	0.50	3.04	2.18	0.34	0.17
MSD	1.15	0.01	0.024	0.43	0.39	0.19	0.29	0.28

Means within column bearing common letters (a,b,c,d) are not significantly different according to the Waller-Duncan k-ratio t-test with t=100.

Where MSD = mean significant difference; CV = coefficient of variation; GP= gas production; *c* = rate of gas production; *l* = lag phase; SCFA = short chain fatty acid; ME = metabolizable energy; NE = net energy; OMD = organic matter digestibility; MP = microbial protein. BH = berseem hay; CP = cowpea haulm with no pesticide; LC = lambda cyhalothrin

7.1.3.3 Dimethoate effect on gas production

Residues of Dimethoate tested for gas production showed an increased gas accumulation beyond control levels with increasing concentration (Table 7.2). Gas measured from the treated straw was 1.6 times higher than the gas produced from untreated cowpea haulm. Rates of gas production, however, remained similar among the various concentrations of Dimethoate.

Table 7.2 Effect of Dimethoate on gas production kinetics of cowpea haulm incubated with buffered rumen fluid *in vitro* for 96 h

Treatment	Gas production profile			Predicted Parameters				
	GP	*c*	*l*	SCFA	ME	NE	OMD	MP
BH	35.06^{a}	0.085^{a}	-0.013^{d}	68.07^{a}	7.47^{a}	4.77	51.07^{a}	98.57^{a}
CP	24.47^{d}	0.058^{b}	0.017^{c}	43.80^{c}	6.90^{b}	4.70	48.20^{c}	93.07^{b}
$D_{40\mu l}$	28.77^{c}	0.053^{b}	0.676^{b}	45.97^{b}	7.03^{b}	4.80	49.07^{b}	94.67^{b}
$D_{80\mu l}$	29.05^{bc}	0.051^{b}	-0.173^{e}	46.10^{b}	7.00^{b}	4.77	49.07^{b}	94.77^{b}
$D_{120\mu l}$	29.93^{b}	0.051^{b}	0.797^{a}	46.17^{b}	7.03^{b}	4.77	49.10^{b}	94.80^{b}
CV	2.23	18.92	2.35	0.34	2.71	1.99	0.23	0.17
MSD	1.56	0.02	0.02	0.30	0.39	0.23	0.19	0.27

Means within column bearing common letters (a,b,c,d,e) are not significantly different according to the Waller-Duncan k-ratio t-test with t=100.

Where MSD = mean significant difference; CV = coefficient of variation; GP= gas production; *c* = rate of gas production; *l* = lag phase; SCFA = short chain fatty acid; ME = metabolizable energy; NE = net energy; OMD = organic matter digestibility; MP = microbial protein; BH = berseem hay; CP = cowpea haulm with no pesticide; D = Dimethoate

7.1.3.4 Effect of Cypermethrin on gas production

The gas produced by the control was significantly lower ($P < 0.0001$) than obtained from those spiked with Cypermethrin which recorded similar rates in gas production

(Table 7.3). No significant differences (P > 0.05) were, however, observed for the gas produced from the various levels of Cypermethrin incubated with cowpea haulm.

Table 7.3 Effect of Cypermethrin on gas production kinetics of cowpea haulm incubated with buffered rumen fluid *in vitro* for 96 h

Treatment	Gas production profile			Predicted Parameters				
	GP	*c*	*l*	SCFA	ME	NE	OMD	MP
BH	35.06^{a}	0.085^{a}	-0.013^{d}	68.07^{a}	7.47^{a}	4.77	51.07^{a}	98.57^{a}
CP	24.47^{c}	0.058^{a}	0.017^{c}	43.80^{c}	6.90^{b}	4.70	48.20^{c}	93.07^{c}
$C_{40\mu l}$	27.62^{b}	0.052^{b}	0.287^{a}	46.13^{b}	6.97^{b}	4.77	49.07^{b}	94.77^{b}
$C_{80\mu l}$	27.37^{b}	0.047^{b}	0.237^{b}	46.17^{b}	6.97^{b}	4.77	49.17^{b}	94.90^{b}
$C_{120\mu l}$	26.81^{b}	0.053^{b}	-0.067^{e}	43.77^{c}	6.87^{b}	4.70	48.17^{c}	92.97^{c}
CV	10.27	18.89	10.27	0.24	2.99	1.95	0.12	0.27
MSD	1.14	0.02	0.02	0.21	0.43	0.22	0.11	0.45

Means within column bearing common letters (a,b,c,d,e) are not significantly different according to the Waller-Duncan k-ratio t-test with t = 100.

Where MSD = mean significant difference; CV = coefficient of variation; GP= gas production; *c* = rate of gas production; *l* = lag phase; SCFA = short chain fatty acid; ME = metabolizable energy; NE = net energy; OMD = organic matter digestibility; MP = microbial protein. BH = berseem hay; CP = cowpea haulm with no pesticide; C = Cypermethrin

7.1.3.5 Estimated gas production parameters

The predicted short chain fatty acids (SCFA, mM), metabolisable energy (ME, MJ/kg DM), (NE, MJ/kg DM), organic matter digestibility (OMD, %) and microbial protein (MP, g/kg DOM) are presented in Tables 7.1 – 7.3. The mean SCFA predicted from gas production at 24 h was 49.59 mM. The highest SCFA was observed with the standard hay and was 1.5 times greater than the other treatments.

Cypermethrin concentrations up to 80 µl significantly (P < 0.05) improved SCFA production and declined to a level similar to the untreated haulm when the

concentration increased to 120 µl. Similar results were obtained for organic matter digestibility and microbial protein synthesis.

The ME-content of the standard hay significantly ($P < 0.05$) differed from all the Cypermethrin treated and untreated haulms ($P < 0.05$). The NE contents of all the treatments nonetheless, remained the same. Though the SCFA, ME, NE, OMD and MP were not influenced ($P > 0.05$) by Dimethoate at all concentrations, they were 1.04, 1.02, 1.02, 1.02, 1.02 times greater than the SCFA, ME, NE, OMD and MP estimates for the control, respectively.

7.1.4 DISCUSSION

7.1.4.1 Effects of pesticide residues on *in vitro* gas production

A remarkable trend was observed in the effects of the three pesticide concentrations on gas production. The increase in gas production associated with increasing concentrations of Dimethoate residue is in consonance with the report by Williams *et al.* (1963), who observed an increase in gas production when an organophosphate was applied. This could be due to the hydrolysis of the organophosphate to less toxic compounds (Cook, 1957), as well as the potential release of inorganic sulphur, nitrogen and phosphorous in the compound by rumen micro flora which use these as substrates for microbial proliferation, hence the increase in gas production. On the contrary, Lambda cyhalothrin significantly depressed gas production when the concentration increased from 40 to 80 µl. This could be ascribed to the nature of the compound (organochlorine) and the possible role of rumen microbes in its breakdown and metabolism to more toxic products which might have influenced the growth of more sensitive microorganisms and hence a reduction in gas production.

Gas production was stimulated by the application of Cypermethrin; however, gas volumes recorded remained similar at all levels of pesticide concentrations. Hydrolysis of the Cypermethrin residues to less toxic compounds (Cook, 1957) may explain the insignificant differences in the volumes of gas produced.

7.1.4.2 Effect of pesticides on predicted gas production parameters

Short chain fatty acid (SCFA) arises from carbohydrate fermentation and is directly related to gas production (Getachew *et al.*, 2002). It is also an indicator of energy availability to the animal. The predicted SCFA values from all the three pesticides concentrations used revealed a high SCFA at 40 µl. The high gas production level at this concentration than the control may have contributed to the high SCFA; and this affirms the postulate that the volume of gas produced reflects SCFA levels.

Pesticide application was ineffectual in influencing the predicted metabolisable and net energy values from gas production and chemical composition. However, the difference observed between the standard hay and the control may be as a result of the differences in chemical composition and gas production.

It has been reported in the literature that, the amount of microbial protein can generally be predicted based on organic matter digested in the rumen and is a factor of microbial efficiency (Dijkstra *et al.*, 1998). It was realised in this study that, a direct causal relationship existed between organic matter digestibility and microbial biomass production in all the treatments. An increase in organic matter digestibility resulted in a concomitant increase in microbial biomass with the reason being that, more of the degraded organic material might have been incorporated into the microbial mass. Although, no effect was noted with Dimethoate on organic matter digestibility and

microbial protein supply, it is significant to note that Lambda cyhalothrin and Cypermethrin reduced organic matter digestibility and microbial protein supply at pesticide concentration of 120µl. This may be interpreted to indicate that cellulose or fibre digesting bacteria are more sensitive to this concentration of the pesticides used.

7.1.5 CONCLUSION

Pesticide residue application on gas production showed that the three pesticides considered did not inhibit gas production and fermentation parameters at the 40 µl pesticide application. It is therefore important to note that, high levels of pesticides, presumably beyond that encountered in the field or lethal dose (LD_{50}), will be required for inhibition of rumen microbial activities to occur.

CHAPTER 8

8.0 ANIMAL EXPERIMENT 3

8.1 EFFECTS OF FEEDING GRADED LEVELS OF COWPEA HAULM AS SUPPLEMENT TO MAIZE STOVER DIET: DEGRADABILITY, INTAKE AND DIGESTIBILITY PARAMETERS OF MAIZE STOVER

8.1.1 INTRODUCTION

Deficiencies of protein, energy and minerals are the main nutritional factors limiting productivity of sheep in tropical regions. Moreover, insufficient nitrogen supply for ruminal microbes result in low microbial protein synthesis and intestinal amino acid absorption which can limit forage intake and impair animal performance (i.e. growth, capacity for maintaining live weight and reproduction). Owing to inherent nutrient deficiencies, native grasses and cereal crop residues (the main feed resources in Ghana), these cannot sustain effective animal production or even maintenance, when fed alone. Thus, provision of appropriate supplementary feedstuffs would be an important step to enhance the productivity of sheep under smallholder and pastoral production systems in Ghana.

Cowpea is a grain legume utilized as human food and its haulms as livestock feed (Singh and Tarawali, 1997). The haulms of cowpea contain more nitrogen than cereal straws and have been shown to improve intake of low quality forages (Smith *et al.*, 1990 and Abule *et al.*, 1995), average daily gain and carcass dressing percentage of sheep (Koralagama *et al.*, 2008), as well as the supply of microbial nitrogen (Osuji and Odenyo, 1997). Silva and Ørskov (1988) attributed improved intake and degradability of a basal diet to increased supply of readily degradable carbohydrate and nitrogen from the supplement, thus stimulating ruminal fibre degradation and enhancing passage rates.

Animal experiment 1 has shown that the readily soluble DM and CP of cultivar IT93K-2045-93 was higher than all the cultivars assessed. It would be important to know how the haulms of this cultivar would influence the intake and degradability of maize stover when used as a supplement. The animal experiment 3 therefore aimed at assessing the effects of feeding graded levels of the haulms of cultivar IT93K-2045-93 as a supplement on the degradability, intake and digestibility of maize stover offered as basal diet to sheep.

8.1.2 MATERIALS AND METHODS

8.1.2.1 Location of experiment

The experiment was conducted at the Department of Animal Science (DAS), KNUST, Kumasi, Ghana. The cowpea and the maize were grown on the arable fields of the DAS (Section 3.2.2) and harvested during the 11th and 22nd week, respectively. The experiment lasted for 90 days.

8.1.2.2 Animals and experimental design

Four Djallonké rams with a mean initial weight of 22.28 ±2.71 kg were used. The rams were fitted with rumen cannulae[5] and randomly assigned to a basal diet of maize stover and three different levels of cowpea haulm (150, 300 and 450 g/d) supplementation over a four period in a (4x4) Latin square design. Each period lasted for 9 days. Animals were rested for a week after each period and allowed to accustom to new treatments in the subsequent period for another week.

[5] Nepean Rubber Mouldings Pty Ltd - Macam Division, Baulkham Hills, Australia

8.1.2.3 Diet and treatments

The sheep were kept individually in pens with wooden slatted floors with dimensions 1.2 m wide and 2.4 m long. The haulms of the cultivar IT93K-2045-93 and the maize stover were chopped into 4-5 cm length and fed to the rams. A two-week trial prior to the main experiment was carried out where sheep was offered maize stover *ad libitum* and 150 g wheat bran daily as supplement. The rams were then adapted to the experimental diets for a further two weeks where sheep were offered maize stover *ad libitum* and supplemented daily with 150 g (7.32 g DM kg^{-1} LW), 300 g (14.64 g DM kg^{-1} LW) or 450 g (21.96 g DM kg^{-1} LW) cowpea haulm. The supplement was offered at 08:00 h while half of the basal diet was offered at 10:00 h and again at 16:00h. In instances where the supplement was not completely consumed, it was kept in a separate plastic container to allow animals more time for supplement consumption. However, it is to be noted that, supplements were withdrawn before feeding of maize stover. Water and mineral lick were available *ad libitum*.

8.1.2.4 Degradability studies

Degradation of maize stover in ewes offered different levels of cowpea haulm as supplement was assessed during days 1– 4 according to the technique described by Ørskov *et al.* (1980). Bags were withdrawn from the rumen after they had been incubated for 3, 6, 12, 24, 48, 72 and 96 h. Bags were washed, dried at 55^{0}C for 48 h and weighed.

8.1.2.5 Intake, digestion trials and measurements

Rams were accustomed to the digestibility crates for three days prior to intake and digestion trials, during each period following a week adaptation period. The quantities of feed offered and refused were recorded daily; the difference was calculated as feed

intake. The total feed offered, refused and feces voided were bulked for each ram for estimation of digestibility.

8.1.2.6 Chemical analysis

Samples were dried at 55^{0}C for 48 h and finely ground using a laboratory mill (Wiley Mill, UK) to pass through a 1 mm screen. The DM in nylon bag residues was determined by subjecting samples to a temperature of 55^{0}C for 48 h in an oven.

8.1.2.7 Statistical analysis

Data were analyzed as a replicated 4 x 4 Latin square using PROC MIXED of SAS (1999) according to the following model.

$$Y_{ij(k)} = \mu + P_i + \tau_j + A_{(k)} + \varepsilon_{ij(k)}$$

Where Y_{ijkl} = measured dependent variable;
μ = overall mean;
Pi = fixed effect period i (i = 1,..,4);
$\tau_{(j)}$ = fixed effect of diet j (j = 1,.,4);
A_k = random effect of animal
e_{ijkl} = residual variation

The mean separation was done using Waller-Duncan k-ratio t-test. The effect of the amount of cowpea supplemented was partitioned into orthogonal contrast using SAS (1999).

8.1.3 RESULTS

8.1.3.1 Overview

Humid atmospheric conditions coincided with the drying of the cowpea haulm and the maize stover. This resulted in a prolonged drying process of the feeds. In some cases spots of mould growth were observed on the cowpea haulm and the maize stover.

The dry matter intake was expressed on the basis of metabolic body weight $(M)^{0.75}$ and liveweight (LW) of the rams. When treatments, expressed on both $(M)^{0.75}$ and LW were contrasted, similar statistical significance was obtained except for supplemental levels of 150 versus 300 g where statistical significance differed for maize stover intake. Therefore, the expression on $(BW)^{0.75}$ and LW is interchanged in the explanation of the results except where probabilities are dissimilar between treatments. The intake of the basal diet of maize stover was significantly ($P < 0.001$) influenced by the supplement. The graded levels of supplement promoted higher intake of supplement DM ($P < 0.001$). Substitution of basal diet by supplement occurred when supplement was fed beyond 300 g.

8.1.3.2 Feed intake and digestibility

The intake and digestibility of maize stover (MS) and supplement is shown in Table 8.1. The results indicated significant differences ($P < 0.05$) in the intake of MS as the level of supplement increased. Dry matter intake of the basal diet, and the supplement varied between 13.5 and 18.27; and 5.01 and 7.69 gDM kg^{-1} LW, respectively. Supplementation with cowpea haulm resulted in higher ($P < 0.05$) intakes of MS, however, the difference between supplement 150 and 300 g only tended to approach significance ($P < 0.0860$).

Table 8.1 Effect of supplementation level (g) on feed intake and digestibility

	DMI									DMD (%)
	g/d			gDMkg^{-1}LW/d			gM$^{0.75}$/d			
SL	MS	Sup	Total	MS	Sup	Total	MS	Sup	Total	
0	298.2^{c}	0^{c}	298.2^{c}	13.5^{b}	0^{c}	13.5^{c}	38.4^{c}	0^{c}	38.4^{a}	31.6^{c}
150	362.4^{b}	109.6^{b}	472.0^{b}	16.5^{a}	5.0^{b}	21.5^{b}	46.9^{b}	14.3^{b}	61.1^{b}	71.0^{b}
300	401.8^{a}	152.4^{a}	554.2^{a}	18.3^{a}	7.0^{ab}	25.3^{a}	57.0^{a}	19.9^{ab}	71.9^{a}	74.1^{ab}
450	374.7^{ab}	168.2^{a}	542.8^{a}	17.0^{a}	7.7^{a}	24.6^{ab}	48.2^{b}	21.8^{a}	70.0^{a}	75.6^{ab}
Statistical significance										
Treatment										
0 v150	**	**	**	**	***	**	*	**	**	***
0 v 300	**	***	***	**	***	***	***	***	***	***
0 v 450	**	***	***	**	***	***	**	***	***	***
150v 300	*	*	*	NS	NS	*	**	NS	*	NS
150v450	NS	**	*	NS	*	NS	NS	*	NS	*
300v 450	NS	NS	NS	NS	NS	NS	*	NS	NS	NS

Within column means with the common letter (a,b,c) are not significantly different (P > 0.05;) comparison of least squares means within PROC MIXED of SAS. * *P < 0.05;* ** *P < 0.01;* *** *P < 0.001.* Where SL: Supplement level; DMI: Dry matter intake; Sup: supplement; MS: Maize stover; DMD: Dry matter digestibility.

Dry matter intake of supplement varied between 14.25 and 21.83 g M $^{0.75}$/d. Total DM intake was similar (P = 0.0939) among the supplement's level of 7.32 and 21.96 g DM kg^{-1} LW, regardless of the difference in supplement DM intake. This is attributable to decreased intake of MS with increasing levels of cowpea haulm intake, thus the highest level of supplement intake was significantly (P < 0.0266) different from the lowest level of intake. This observation implies substitution of the basal diet DM by cowpea haulm DM at the highest level of supplement offered.

Apparent dry matter digestibility significantly ($P < 0.05$) increased with cowpea supplementation at all levels relative to the control group with apparent dry matter digestibility ranging between 31.55 and 75.62%.

8.1.3.3 Dry Matter degradation parameters of maize stover

The DM degradability parameters for maize stover were significantly influenced ($P < 0.001$) by cowpea haulm supplementation (Table 8.2). The results showed that cowpea haulm supplemented diets differed significantly ($P < 0.05$) from the control diet in both the readily soluble and potential degradable fractions of the maize stover. However, no significant difference ($P < 0.05$) was observed between cowpea haulm at the 14.64 and 21.96 g DM kg^{-1} LW levels of supplementation. The rate of disappearance "*c*" significantly increased (3.4-7.3%) with increasing level of cowpea haulm supplementation ($P < 0.0001$).

The amount of DM readily soluble in the rumen represented as "*a*" ranged from 6.17 to 8.47% while the potentially digestible fraction of the maize stover DM averaged 51.26%.

Table 8.2 Rumen DM degradability of maize stover (MS) incubated in nylon bags in rumen of ewes fed MS alone or MS supplemented with graded levels of cowpea haulm.

Parameter	MS (0g)	Cowpea haulm supplement (gDMkg^{-1} LW) 7.32 (150g)	14.64 (300g)	21.96 (450g)	P > F
a	6.17^{ab}	8.33^{a}	7.52^{a}	8.47^{a}	0.0414
b	48.00^{c}	50.07^{b}	53.56^{a}	53.40^{a}	0.0003
$c(h^{-1})$	0.034^{d}	0.0517^{c}	0.0659^{b}	0.073^{a}	0.0001

Within column means with common letter (a,b,c,d) are not significantly different (P > 0.05;) comparison of least squares means within PROC MIXED of SAS.

Where a = initially degradable fraction; b = degradable DM fraction; c = rate constant for degradation of b;

found in a study by Koralagama *et al.* (2008) who reported that, all levels of cowpea haulm supplementation resulted in a greater intake of a basal diet of maize stover with no apparent effect of substitution when the low (150g) and high (300g) inclusion rates of cowpea haulm were compared; but was in agreement with the results of Savadogo *et al.* (2000), who reported that sorghum stover intakes declined linearly ($P < 0.01$) with levels (0.424 g g^{-1}) of cowpea supplementation.

8.1.4.2 Digestibility

The apparent DM digestibility value of 31.55% for the MS control diet in this work was lower than that of 45.2% reported by Koralagama *et al.* (2008). On the contrary, when MS was supplemented with graded levels of cowpea haulm, the intake results recorded were comparable to those found by the same authors. Siaw *et al.* (1993) and Balogun *et al.* (1998) have reported that drying of forages causes losses in water-soluble carbohydrates due to respiration, Maillard reactions and possibly decomposition. Therefore, the low apparent digestibility of the maize stover could have been influenced by drying of the feed prior to being offered to the rams.

8.1.4.3 Ruminal degradation of maize stover

The percentage of readily soluble material for MS of 6.17 for the control diet and the mean value of 8.11 obtained for the cowpea supplements (Table 8.2) compares favourably (7.01 and 8.14, respectively) with the data obtained by Chakeredza *et al.* (2002). Likewise, the digestible fibre disappeared at rates similar to those obtained by them.

The relatively higher extent and faster rate of degradation of maize stover in the supplemented diets compared to the control could be due to proliferation of rumen

microbes and improved activities of cellulolytic bacteria, owing to the probable higher supply of peptides and amino acids. It is documented in the literature that, the activities of certain cellulolytic bacteria are stimulated by the end-products of proteolytic actions of ruminal microbes (Ndlovu and Buchanan-Smith, 1985). This might have increased the rate of degradation of the MS, as reported by McMeniman *et al.* (1988). The faster rate of degradation at the higher levels of supplementation may offer some explanation to the substitution effect of the basal diet by the cowpea haulm supplement.

8.1.5 CONCLUSION

Supplementation of cowpea haulms improved intake and digestibility, however substitution effect was observed when cowpea was offered at higher level. It is therefore concluded that, where limited quantities of cowpea haulms are available, it is still possible to offer only small amounts of cowpea haulms (150 g/ d) to improve intake when animals are consuming poor quality fodders.

CHAPTER 9

9.0 GENERAL DISCUSSION

9.1 INTRODUCTION

The assessment of agronomic (grain and haulm yield) characteristics of 4 improved cultivars of cowpea and the utilization of their haulms as supplement to poor quality basal diet of maize stover; retention of nutrients during storage under 3 methods and pesticide residues' effect on *in vitro* gas production were investigated in this thesis. This research also consisted of a series of trials which sought to ascertain the influence of season and year on grain and haulm yields of four cowpea cultivars, the changes in nutrient composition during storage, nutrient composition *in situ* and *in vitro* gas production profiles of 4 cultivars of the cowpea haulm, the effects of pesticide residues on rumen fermentation parameters as well as effects of haulm supplementation on intake of a of a maize stover diet.

The first trial (agronomy experiment 1) involving the determination of the yields of grain and haulm of 4 cowpea cultivars assessed the influence of season and year of planting on yield characteristics. This was aimed at making recommendations that may hold for all seasons and any year of cowpea establishment in Ghana. Agronomy experiment 2 focused on the effect of 3 storage practices on the quality of the haulms. The outcome of the study provided information on changes nutrient retention in the cowpea haulms over a period of storage. This provided an idea on how much nutrient would remain in the cowpea haulm when kept over a period of 12 weeks for dry season feeding.

Animal experiment 1 assessed the chemical composition and degradation characteristics of morphological parts (leaf, stem and whole plant) using the *in situ*

method as well as evaluating the cultivars for gas production profiles using the Menke' *in vitro* gas production technique. Equations were modeled to predict *in situ* values from *in vitro* gas production profiles. In areas where veterinary officers or personnel for rumen fistulation are scarce or there are difficulties in obtaining rumen cannula, the gas production models obtained in this study can be used to predict *in situ* data. Faecal matter could instead be used as the source of inoculum for the gas production technique rather than the rumen liquor.

Animal experiment 2 adopted the gas production technique to ascertain the effects of varying concentrations of residues of pesticides used in the establishment of cowpea on rumen fermentation parameters. This was used to predict organic matter digestibility and microbial protein supply.

The final study (Animal experiment 3) monitored feed intake, and digestion in sheep and the degradation of maize stover incubated in the rumen of rams fed graded levels of the cowpea cultivar IT93K-2045-93.

All the aforementioned experiments have been comprehensively and separately discussed and conclusions made. Below is a summary of the observations and assertions made in the study.

9.2 Effect of season and year of cowpea establishment on grain and haulm yield

The grain and haulm yields of the four cowpea cultivars were determined in the wet and dry seasons of the three year (2005-2007) period under study. Grain and haulm yield ranges of 2.13 – 2.56 and 8.58 – 13.35 t/ha, respectively were recorded among the cultivars in the wet season of the study, while for the dry season yield, minimum

and maximum yields of 0.89 and 3.2; 1.39 and 6.14 t/ha, respectively were observed for cowpea grain and haulm. The establishment of dual-purpose cowpea varieties results in high grain and haulm yields and is therefore, a viable option for increased human food supply while simultaneously decreasing the cost of raising ruminant livestock.

The agronomic features of IT93K-2045-93 and IT93K-2309 were better than for the other cultivars in any year of establishment except for the grain yield in the wet season where the other two cultivars (i.e. SORONKO and IT8160716) recorded higher yield. The relatively lower yield that characterised the dry season production is explained by terminal droughts resulting in leaf abscission during pod filling which led to lower grain and biomass accumulation (Dadson *et al.*, 2005). However, the higher haulm yields recorded by IT93K-2045-93 and IT93K-2309 might be attributed to a relatively higher resistance offered by the cultivars to drought conditions. Low haulm yields in the dry season underscores the need to establish an appropriate technique for storing haulms from relatively high wet season yields to make up for deficits in dry season thereby ensuring availability of haulm for supplement all year round.

The potential utility index (PUI) was estimated for the cowpea cultivars based on a pooled data from agronomy experiment 1 and animal experiment 2. This index combined the grain yield of individual cultivars and their respective haulm yields and also took into consideration, the *in situ* dry matter digestibility of the cowpea haulm at 48 h.

Table 9.1 shows the least square means of the grain and haulm yields and potential utility index of the four cultivars, in the wet and dry seasons. The potential utility

index was significantly higher in cultivar IT93K-2045-93 ($P < 0.0001$) than for all other cultivars during the dry season. This was also the case for the same cultivar in the wet season, except that IT93K-2045-93 and IT86D-716 were similar ($P = 0.058$).

Table 9.1 Least square means (± s.e) of cowpea grain and haulm yield (t ha^{-1}) and potential utility index.

Cultivar	Grain yield		Haulm yield		*In situ* DMD	PUI	
	Season						
	Wet	Dry	Wet	Dry	48 h	Wet	Dry
SORONKO	2.30^{b}	0.94^{b}	10.00^{c}	3.56^{b}	56.88^{c}	52.23^{b}	57.37^{b}
IT93K-2309	2.13^{c}	1.30^{a}	15.85^{a}	6.10^{a}	59.29^{b}	51.02^{c}	58.34^{b}
IT86D-716	2.56^{a}	0.89^{b}	8.58^{d}	3.24^{b}	57.21^{c}	54.43^{a}	57.88^{b}
IT93K-2045-93	2.26^{bc}	1.39^{a}	13.35^{b}	6.14^{a}	64.46^{a}	55.49^{a}	62.34^{a}
SE	0.13	0.42	1.35	1.54	1.93	0.5643	0.8579

Within column means having common letters (a,b,c,d) are not significantly different at $P > 0.0001$.

The PUI aids in the selection of high quality crop residue without sacrificing grain yield. Thus the PUI integrates grain yield and DM digestibility of the crop residue (haulm) in question. The cultivars with higher PUI reflect the relatively higher soluble pools of digestible material at 48h incubation and higher grain yield. The higher PUI values recorded by cultivars IT93K-2045-93 and IT86D-716 in the wet season of growth were as a result of higher grain yield performance than in the other two cultivars. The highest PUI recorded in the dry season by cultivar IT93K-2045-93 was as a result of higher grain yield and relatively higher soluble digestible material.

9.3 Effect of storage practices and length of cowpea haulm storage on nutrient composition or retention

This experiment (agronomy experiment 2) sought to identify how different storage techniques (shed, roof and field storage) influenced nutrient retention in cowpea haulm at any particular period in storage. The dry matter loss in haulms of the cultivars during the 12 weeks experiment ranged from 0.51 – 0.74 kg but the loss in the cowpea haulm kept on roof was about 2.2 times greater than for those stored in a shed. The two-fold increase in dry matter loss of haulms in roof storage was a possible result of leaching of soluble nutrients by rain and bleaching by over exposure to sunlight (Tripathi *et al.*, 1995). The low dry matter loss observed in cultivars during shed storage is an indication of little environmental influence on such materials in shed storage. A major advantage of this storage practice is its ability to store well excess fodder from a major season harvest intended for lean season feeding.

The experiment revealed that, aside lignification of cell wall content of cultivars left as standing hay, cultivars could only be utilized as feed for at most two months. This is because, the cowpea cultivars left on the field as standing hay wilted approximately 2 months after grain harvesting (Figure 5.1 – 5.4). According to the linear regression model developed, should some cultivar extend its wilting period, higher levels of bound protein [ADIN = 0.188 (weeks) + 0.088 (r^2 = 0.909)] and fibre components [ADF= 1.866x + 51.16 (r^2 = 0.976); NDF = 0.282x + 64.42 (r^2 = 0.999] will be obtained rendering, their usefulness as supplement less efficient.

9.4 Effect of pesticide residues on rumen fermentation parameters and gas production

The idea of residual effect of pesticide on rumen activities owe its genesis to discussions during a theatre presentation of an aspect of my thesis during the Ghana Animal Science Association Symposium held at Tamale in 2004. It was inquired by a participant whether the ingestion of pesticide residue in cowpea haulms could degrade to more toxic levels or compounds in the rumen. This hypothesis was tested by applying incremental levels (40, 80 and 120 µl) of cowpea pesticides namely, Lambda cyhalothrin, Cypermethrin and Dimethoate, on residue - free cowpea haulm and incubated in glass syringes. The study indicated that, the effect of the pesticides on rumen microbial function may only be effectual if the LD_{50} is exceeded. All the levels of pesticide applied in the study, though higher than what was determined in the cowpea haulms (≤ 1.8 µl), did not inhibit the gas production parameters measured. Rumen fermentation characteristics following the ingestion of pesticide contaminated forage would only be impaired if the application of pesticides exceeds the lethal dosage.

Predictions of fermentation parameters such as SCFA, ME, NE and MPS from gas production and chemical composition of cowpea haulm were made to assess pesticide residue effect on these parameters. Accurate measurement of these fermentation parameters requires *in vivo* measurements. However, the high cost involved make this approach impracticable for routine evaluation of feeds. The adoption of the gas production technique in prediction of the fermentative parameters therefore became more feasible, especially in less advanced countries where laboratories are seldom equipped with modern equipment to measure these variables. These parameters are important in that SCFA for example serves as a major source of metabolic fuels for

ruminants and MPS accounts for a major portion of the protein absorbed from the gastro intestinal tract. Hence, any factor that alters their production would affect the performance of the animal. The pesticide application did not inhibit the parameters estimated; for animal performance to be affected, excess dosage beyond recommended dose would have to be applied.

9.5 Gas production and *in situ* degradability

The volume of gas production was related to degradability of the cultivars, using correlation analysis. This was done by pooling the gas production and the degradability data of the four cultivars. High correlation ($r = 0.93$) was recorded (Figure 9.1). The relationship between the *in situ* and *in vitro* gas production of the four cultivars used in the study is expressed below

In situ = 19.58 + 1.87 gas ($r^2 = 0.90$; $P < 0.001$)

Regression analysis presented in Table 9.2 also showed a good coefficient of determination at all time points (r^2 ranged between 0.44 and 0.81; $P < 0.01$) with the exception of estimate at the 6th hour (r^2=0.26; $P = 0.09$).

Table 9.2 Linear regression to predict *in situ* DM degradability (DMD, %) from *in vitro* gas production

Time (hr)	Model	SE	r^2	Adj r^2	Significance
3	*In situ* DMD = 14.68+2.42 gas	0.78	0.49	0.44	0.0112
6	*In situ* DMD = 28.10 + 0.88 gas	0.47	0.26	0.19	0.0905
12	*In situ* DMD = 37.05 + 0.58 gas	0.17	0.53	0.48	0.0072
24	*In situ* DMD = 39.94 + 0.65 gas	0.16	0.64	0.60	0.0019
48	*In situ* DMD = 41.90 + 0.83 gas	0.19	0.66	0.63	0.0012
72	*In situ* DMD = 49.39 +0.73 gas	0.11	0.83	0.81	<.0001
96	*In situ* DMD = 54.51 +0.52 gas	0.10	0.71	0.69	0.0005

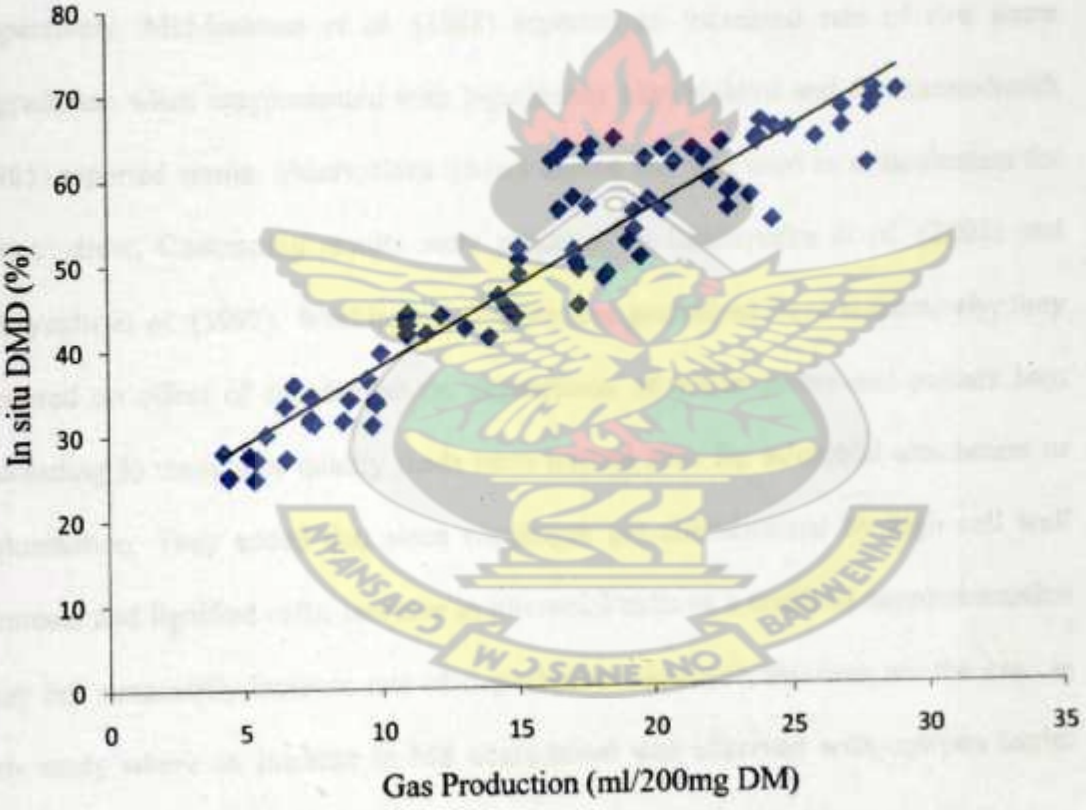


Figure 9.1 The relationship between in situ DMD and gas production

9.6 Effect of supplement on intake, degradability and digestibility

Supplementation has been the strategy used in manipulating the rumen environment when poor quality fodders are fed. Supplementation aims at increasing supply of certain nutrients, thereby creating optimum conditions in the rumen that ensure better fermentation, increased passage rate and supply of microbial protein. The effect of improved rumen ecosystem was evident on maize stover degradation when supplemented with cowpea haulm. Maize stover DM degradability in sheep fed unsupplemented or supplemented maize stover diet were respectively 329 and 478 g/kg after 24 h incubation, and 452 and 552 g/kg after 48 h. Likewise, dry matter digestibility for the control MS diet and the supplemented diet, were 315 and 735 g/kg respectively. McMeniman *et al.* (1988) reported an increased rate of rice straw degradation when supplemented with leguminous hay. Ndlovu and Buchanan-Smith (1985) reported similar observations when Lucerne hay was used as a supplement for barley straw. Contrasting results were reported by Chakeredza *et al.* (2002) and Manyuchi *et al.* (1997). Working on cowpea and groundnut hays respectively, they reported no effect of supplement on degradation of maize stover and pasture hay. According to them, low quality feeds have limited sites for microbial attachment or colonization. They added that since roughages are characterized by high cell wall contents and lignified cells, increase in microbial cells as a result of supplementation may not necessarily increase rate of degradation. However, this was not the case in this study where an increase in MS degradation was observed with cowpea haulm supplementation.

10. 0 GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

10.1 GENERAL CONCLUSIONS

Animals consuming poor quality roughages often fail to obtain the needed nutrients to even meet their maintenance requirements. In developing countries like Ghana, roughages, which form the bulk of ruminant livestock feed may lack vital nutrients and are poorly consumed.

Supplementation with nitrogen has been demonstrated to increase intake and digestibility of poor quality roughages. However, cost of conventional nitrogen supplements discourages small-holder farmers in adopting this technology. Cowpea haulm is generated by small holder farmers as a co-product to grain production, making its use as supplement to low quality diet economical. The research has revealed that a relatively small amount of cowpea haulm (5.01 g kg^{-1}LW) is needed to improve intake, digestibility and DM degradability of sheep consuming such roughages.

The study also showed significant cultivar differences in agronomic yield and nutritive value of cowpea haulm. Cultivars such as IT93K-2045-93 recorded high potential utility index, high readily soluble DM and CP, as well as good storage characteristics. This suggests the possibility of selecting for cowpea cultivars that combine high agronomic and nutritive characteristics of the grains and haulms to serve the diverse needs of humans as food and for ruminants as feed supplement.

The study revealed that, shed storage maintained the quality of the cowpea haulm and therefore a better option for cowpea haulm storage because of little environmental influence compared to field and roof storage. This storage method would ensure good feed availability all year round and hence improved food security.

Further experimentation on the effects of pesticide residues on gas production showed that the residual pesticide levels commonly present in haulm was ineffective in depressing the fermentation activities of the rumen microbes. Since cowpea cultivars used in this study had low concentration of residue, it can thus be said that pesticide residues under normal cultivation of cowpea would have a negligible effect on rumen microbial function.

10.2 RECOMMENDATIONS FOR FUTURE INVESTIGATION

1. The dry season, which is characterised by shortage of ruminant feed, can last for about 16 weeks and beyond in Ghana. The study adopted 12 weeks in assessing the changes in haulm quality during storage. Owing to long and pronounced lean season during certain times, any trial that seeks to assess the changes in nutritive value of feed as a function of time in storage should extend the duration of the storage study further.

2. Improved intake and digestibility of maize stover associated with cowpea supplement was attributed to its higher DM and CP degradability. Studies that take into account supplementation effects on rumen microbial counts and supply of ammonia - nitrogen, branched-chain fatty acids and total VFA's

could provide more explanation to other factors influencing intake and degradability of maize stover.

3. The study emphasized the importance of supplementation on degradability, intake and digestibility of roughage. The assessment of the supplement on growth and carcass characteristics will further add to the importance of cowpea haulm as ruminant feed supplement.

4. The pesticides used in this study were observed to have influenced gas production and other parameters such as the SCFA and MPS predicted. Further investigations to ascertain microbial counts and strains of bacteria that detoxify or are susceptible to inhibitory compounds in the residues seem desirable.

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APPENDICES

Appendix I: Weather pattern during experimental period

Table 1. Rainfall receipt pattern (mm)

Years	Months												
	Jan	Feb.	Mar	Apr.	May	Jun.	Jul.	Aug	Sept	Oct	Nov	Dec	Total
2005	.8	46	85	127	172	93	23	36	169	225	55	0	1037
2006	110	114	91	93	144	113	69	76	97	178	60	5	1150
2007	9	65	77	190	84	244	374	127	540	238	49	3	1999

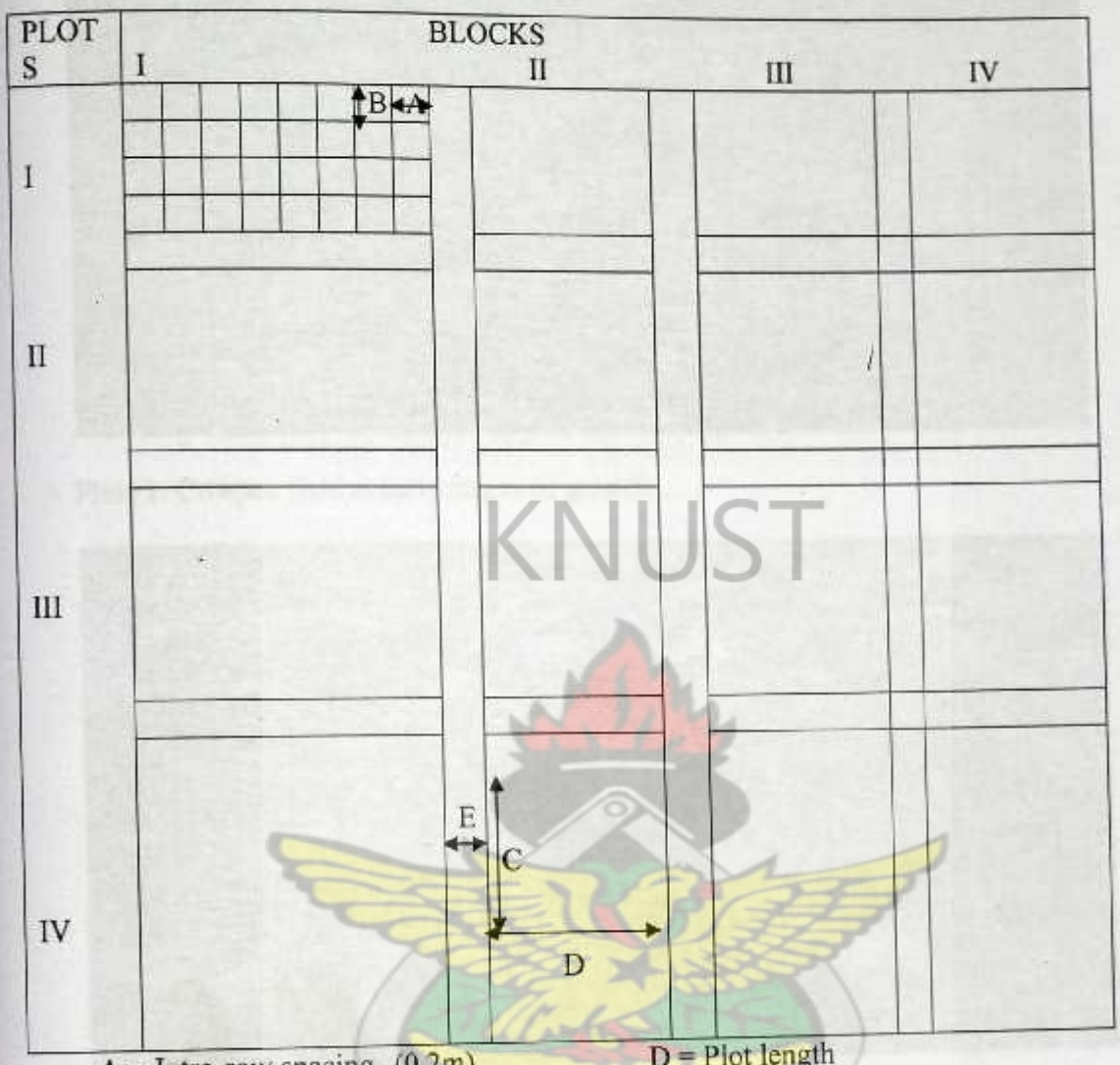
Table 2. Maximum temperature receipt (^{0}C)

Years	Months												
	Jan	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Total
2005	32.4	35.1	34.1	34.2	32.5	30.6	29.3	28.4	30.7	31.8	32.0	32.1	383.2
2006	32.6	35.0	32.9	34.3	32.2	31.4	30.3	29.2	30.1	31.5	32.3	32.7	384.5
2007	34.0	34.5	35.2	34.0	32.9	31.6	29.6	29.9	30.2	30.9	31.4	32.1	386.3

Table 3. Minimum temperature receipt (^{0}C)

Years	Months												
	Jan	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Total
2005	16.3	22.6	22.0	22.9	22.5	21.7	20.7	20.3	21.1	21.6	22.0	21.5	255.2
2006	21.2	22.5	21.8	22.5	22.0	20.6	20.8	20.5	22.1	21.7	21.8	21.8	259.3
2007	16.5	22.4	22.6	22.0	22.6	22.9	22.1	22.1	22.1	21.9	22.1	19.9	259.2

Appendix II



A = Intra-row spacing (0.2m)
(17m)
B =Inter-row spacing (0.6m)
C = Plot width (1.8m)
D = Plot length
E = Inter plots / Inter Blocks space (1m)

Figure 1. Layout of Cowpea field.

Plate 1. Cowpea field at early stages of growth

Plate 2. Cowpea at pod filling stage

Plate 3. Ripped cowpea at harvesting stage

Plate 4. Storage structure with shed and roof systems

Appendix III: Estimations of grain and haulm yield and potential utility index of cowpea

1.0 Grain/ haulm yield

$$\text{Grain Yield (t/ha)} = \left(\frac{10{,}000\text{m}^2}{\text{Plant area (m}^2\text{)}}\right)\text{yield}/1000$$

2.0 Potential utility index.

The potential utility indices of the different cultivars were calculated according to the formula described by Fleischer *et al.* (1989) i.e.

$$\text{Potential utility index} = \frac{(\text{Grain yield} + \text{Digestible haulm DM yield})}{\text{Total above ground biomass DM yield}} \text{ X } 100$$

The 48 h *in sacco* DM degradability was used for the calculation of the digestible cowpea haulm DM yield.

Appendix IV: Analytical procedures

1.0 Dry matter determination

Dry matter was determined by oven drying a known weight (2g) of cowpea haulm at 55^{0}C for 48h (AOAC, 1984). The samples in the oven were regularly removed and re-weighed till constant weight was reached. This was cooled in a dessicator and final weight determined. Dry matter was calculated as

$$\text{DM} = \frac{\text{Oven dried weight}}{\text{air-dried sample weight}} \text{ x } 100$$

1.1 Nitrogen in haulm and nylon bag residues

In the determination of nitrogen, 0.2g sample was weighed into digestion tubes and 1gm catalyst (10: 1 potassium sulphate and anhydrous copper sulphate) added. Concentrated H_2SO_4 (5 ml) was added and digested (moderately heated and gradually increased to 350^{0}C) till the solution became light green. Boiling continued for an hour and was allowed to cool. Distilled water (25 ml) was added to the digesta and distilled into 25 ml 4% boric acid. The distillate was titrated with 0.1N HCl to determine

nitrogen. This same procedure was also followed in the determination of nitrogen bound to fibre (ADF and NDF).

1.2 Ether extract

The weighed sample (2g) was hydrolysed in a 3 N HCl for 1 h, cooled at room temperature, filtered through a filter paper, rinsed with distilled water to remove all HCl, and oven dried at 55 ^{0}C overnight. The sample was placed in a Soxhlet extractor with an anhydrous diethyl ether and the heater coil turned high enough to evaporate 2-3 drops of ether per second in the condenser. Extraction was allowed for 24 h and samples oven dried at 55^{0}C for 48 h.

1.3 Determination of fibre components (CF, NDF, ADF and ADL)

The fibre fractions and lignin were determined following the protocols of Goering and Van Soest (1970). The CF level (s) was determined by weighing 1 g of the residues from the ether extract procedure into a Berzelius flask and boiling in 1.25% H_2SO_4 for 30 minutes. It was filtered, rinsed with 50 ml boiling water and subsequently dried. This was again boiled in a 200ml 1.25% NaOH for 30 minutes, filtered and washed with 25 ml of boiling H_2SO_4, three 50 ml portions of H_2O, and 25 ml of alcohol. The residue was dried for 2 h at 130^{0}C and ashed in a muffle furnace at 600^{0}C for 30 minutes.

In determining NDF, 1 g of sample was weighed into a 600 ml Berzelius flask and 100 ml neutral detergent solution added. This was refluxed for 1 hour and filtered using Gooch crucibles. The residue was washed with hot water acetone, dried overnight at 100^{0}C and weighed to estimate NDF.

Acid detergent fibre was determined by weighing 1 g of sample into a 600 ml Berzelius flask and 100 ml acid detergent solution added. This was refluxed for 1

hour and filtered using glass-sintered crucibles. The residue was washed with hot water and acetone. The residue was oven dried at 100^{0}C overnight and weighed to estimate the ADF.

1.4 Estimation of Lignin

The ADF residue was used in estimating lignin as acid detergent lignin (ADL). The residue, '*w*' was transferred into crucibles and a 72% H_2SO_4 added. This was stirred at hourly intervals for three hours with a glass rod. The acid was added, filtered off and the residues washed with hot water until free from acid. The crucibles with residue were dried at 105^{0}C for 24 hours and the weight recorded as, "*a*". This was then ignited in a muffle furnace at 500^{0}C for 3 hours, cooled and weighed as, *b*. ADL was calculated as $(a\text{-}b) / w$ expressed as a percentage.

Where a = dry ADF residue after acid extraction; b = weight of ash;

w = ADF residue

Appendix V: Agronomy Experiment 1

Table 1. Raw data set for wet and dry season grain yield and haulm yield (tha^{-1}) of cowpea cultivars in 2005, 2006 and 2007.

OBS	CULTIVARS	YEAR	SEASON	REP	HY	GY
1	SORONKO	2005	1	1	5.40	2.24
2	IT93K 2309	2005	1	1	14.22	2.09
3	IT86D-716	2005	1	1	4.84	2.36
4	IT93K 2045-29	2005	1	1	14.08	2.03
5	SORONKO	2005	1	2	6.60	2.13
6	IT93K 2309	2005	1	2	13.20	1.92
7	IT86D-716	2005	1	2	6.07	2.23
8	IT93K 2045-29	2005	1	2	12.79	2.24
9	SORONKO	2005	1	3	6.03	1.79
10	IT93K 2309	2005	1	3	15.32	1.67
11	IT86D-716	2005	1	3	6.47	2.60
12	IT93K 2045-29	2005	1	3	9.63	2.08
13	SORONKO	2005	1	4	6.51	1.94
14	IT93K 2309	2005	1	4	14.84	2.23
15	IT86D-716	2005	1	4	3.70	2.52
16	IT93K 2045-29	2005	1	4	11.42	1.96
17	SORONKO	2005	2	1	1.28	0.28
18	IT93K 2309	2005	2	1	2.33	0.42
19	IT86D-716	2005	2	1	1.54	0.25
20	IT93K 2045-29	2005	2	1	0.94	0.47
21	SORONKO	2005	2	2	1.87	0.42
22	IT93K 2309	2005	2	2	1.63	0.46
23	IT86D-716	2005	2	2	0.72	0.37
24	IT93K 2045-29	2005	2	2	1.95	0.42
25	SORONKO	2005	2	3	2.10	0.19
26	IT93K 2309	2005	2	3	2.32	0.37
27	IT86D-716	2005	2	3	1.32	0.26
28	IT93K 2045-29	2005	2	3	3.29	0.28
29	SORONKO	2005	2	4	1.75	0.24
30	IT93K 2309	2005	2	4	1.60	0.23
31	IT86D-716	2005	2	4	1.75	0.20
32	IT93K 2045-29	2005	2	4	1.87	0.18
33	SORONKO	2006	1	1	12.89	2.47
34	IT93K 2309	2006	1	1	15.89	1.89
35	IT86D-716	2006	1	1	9.13	2.49
36	IT93K 2045-29	2006	1	1	14.15	2.18
37	SORONKO	2006	1	2	12.45	2.31
38	IT93K 2309	2006	1	2	16.74	2.06
39	IT86D-716	2006	1	2	8.27	2.30
40	IT93K 2045-29	2006	1	2	14.45	2.53
41	SORONKO	2006	1	3	12.81	2.75
42	IT93K 2309	2006	1	3	13.80	1.90

Table 1 (contd).

OBS	CULTIVARS	YEAR	SEASON	REP	HY	GY
43	IT86D-716	2006	1	3	9.29	2.36
44	IT93K 2045-29	2006	1	3	14.41	1.82
45	SORONKO	2006	1	4	9.09	2.44
46	IT93K 2309	2006	1	4	17.25	1.99
47	IT86D-716	2006	1	4	9.29	2.64
48	IT93K 2045-29	2006	1	4	11.36	1.98
49	SORONKO	2006	2	1	3.98	1.72
50	IT93K 2309	2006	2	1	9.53	2.44
51	IT86D-716	2006	2	1	4.56	1.04
52	IT93K 2045-29	2006	2	1	8.15	2.49
53	SORONKO	2006	2	2	5.87	1.37
54	IT93K 2309	2006	2	2	8.69	1.72
55	IT86D-716	2006	2	2	4.89	1.59
56	IT93K 2045-29	2006	2	2	7.23	1.90
57	SORONKO	2006	2	3	4.71	1.38
58	IT93K 2309	2006	2	3	7.43	1.59
59	IT86D-716	2006	2	3	5.52	1.57
60	IT93K 2045-29	2006	2	3	8.45	2.30
61	SORONKO	2006	2	4	5.06	1.00
62	IT93K 2309	2006	2	4	8.06	2.05
63	IT86D-716	2006	2	4	5.32	1.03
64	IT93K 2045-29	2006	2	4	8.89	1.46
65	SORONKO	2007	1	1	11.38	2.36
66	IT93K 2309	2007	1	1	16.95	2.38
67	IT86D-716	2007	1	1	11.10	2.97
68	IT93K 2045-29	2007	1	1	14.42	2.54
69	SORONKO	2007	1	2	12.16	2.45
70	IT93K 2309	2007	1	2	17.17	2.46
71	IT86D-716	2007	1	2	11.28	2.81
72	IT93K 2045-29	2007	1	2	12.73	2.57
73	SORONKO	2007	1	3	12.31	2.41
74	IT93K 2309	2007	1	3	17.39	2.39
75	IT86D-716	2007	1	3	11.28	2.64
76	IT93K 2045-29	2007	1	3	15.33	2.43
77	SORONKO	2007	1	4	12.33	2.36
78	IT93K 2309	2007	1	4	17.48	2.56
79	IT86D-716	2007	1	4	12.19	2.76
80	IT93K 2045-29	2007	1	4	15.38	2.72
81	SORONKO	2007	2	1	3.06	0.99
82	IT93K 2309	2007	2	1	8.24	1.64
83	IT86D-716	2007	2	1	3.32	0.95
84	IT93K 2045-29	2007	2	1	8.23	1.84
85	SORONKO	2007	2	2	4.46	1.17
86	IT93K 2309	2007	2	2	7.32	1.71

Table 1 (contd)

OBS	CULTIVARS	YEAR	SEASON	REP	HY	GY
88	IT93K 2045-29	2007	2	2	8.83	1.65
89	SORONKO	2007	2	3	4.60	1.23
90	IT93K 2309	2007	2	3	8.97	1.42
91	IT86D-716	2007	2	3	2.83	1.40
92	IT93K 2045-29	2007	2	3	7.49	2.08
93	SORONKO	2007	2	4	3.99	1.31
94	IT93K 2309	2007	2	4	7.08	1.50
95	IT86D-716	2007	2	4	3.43	0.82
96	IT93K 2045-29	2007	2	4	8.31	1.55

Where

OBS Observation Number
REP Replication
GY Grain Yield
HY Haulm Yield

Table 2. **The GLM and Mixed Procedures for seasonal and year effect on grain yields of cowpea cultivars.**

Table 2a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	26	56.465	2.171	49.51	0.0001
Error	69	3.027	0.043		
Corrected Total	95	59.492			

Table 2b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.198	0.066	1.510	0.2190
Cultivar	3	0.470	0.157	3.590	0.0200
Year	2	11.938	5.969	136.080	0.0001
Season	1	33.630	33.630	766.620	0.0001
Cultivar *Season	3	2.925	0.975	22.230	0.0001
Cultivar *Year	6	0.514	0.085	1.950	0.0843
Year*Season	2	5.974	2.987	68.100	0.0001
Cultivar *Year*Season	6	0.8110	0.1351	3.08	0.0099

Table 2c. Least Square Means

Cultivar	Year	Season	Estimate	SE	DF	t Value	Pr > |t|
IT86D-716			1.7225	0.0427	69	40.29	0.0001
IT93K 2045-29			1.8208	0.0427	69	42.59	0.0001
IT93K 2309			1.7121	0.0427	69	40.05	0.0001
SORONKO			1.6229	0.0427	69	37.96	0.0001
	2005		1.2209	0.037	69	32.98	0.0001
	2006		1.9613	0.037	69	52.97	0.0001
	2007		1.9766	0.037	69	53.38	0.0001
		1	2.3115	0.0302	69	76.46	0.0001
		2	1.1277	0.0302	69	37.30	0.0001

Table 3. The GLM and Mixed Procedures for seasonal and year effect on haulm yields of cowpea cultivars

Table 3a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	26	2167.24	83.36	89.18	0.0001
Error	69	64.49	0.93		
Corrected Total	95	2231.73			

Table 3b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.599	0.199	0.21	0.8865
Cultivar	3	414.250	138.083	147.74	0.0001
Year	2	362.727	181.363	194.05	0.0001
Season	1	1238.334	1238.334	1324.92	0.0001
Cultivar *Season	3	63.565	21.188	22.67	0.0001
Cultivar *Year	6	1.953	0.325	0.35	0.9085
Year*Season	2	19.017	9.508	10.17	0.0001
Cultivar *Year*Season	6	66.7865	11.131	11.91	0.0001

Table 3c. Least Square Means

Cultivar	Year	Season	Estimate	SE	DF	t Value	Pr > |t|
IT86D-716			5.9096	0.1973	69	29.95	0.0001
IT93K 2045-29			9.7408	0.1973	69	49.36	0.0001
IT93K 2309			10.9771	0.1973	69	55.62	0.0001
SORONKO			6.7788	0.1973	69	34.35	0.0001
	2005		5.6056	0.1709	69	32.80	0.0001
	2006		9.6128	0.1709	69	56.25	0.0001
	2007		9.8363	0.1709	69	57.55	0.0001
		1	11.9431	0.1395	69	85.59	0.0001
		2	4.7600	0.1395	69	34.11	0.0001

Table 4. The GLM Procedure for 2005 wet season haulm yield

Table 4a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	238.05	39.6760083	19.56	0.0001
Error	9	18.25	2.0286833		
Corrected Total	15	256.31			

Table 4b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.7943	0.2647500	0.13	0.9395
Cultivar	3	237.2618	79.0872667	38.98	0.0001

Table 5. The GLM Procedure for 2005 wet season grain yield

Table 5a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.55218750	0.09203125	2.25	0.1321
Error	9	0.36815625	0.04090625		
Corrected Total	15	0.92034375			

Table 5b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.05016875	0.01672292	0.41	0.7506
Cultivar	3	0.50201875	0.16733958	4.09	0.0435

Table 6 The GLM Procedure for 2005 dry season haulm yield

Table 6a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	2.56770000	0.42795000	1.35	0.3294
Error	9	2.85607500	0.31734167		
Corrected Total	15	5.42377500			

Table 6b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	1.40547500	0.46849167	1.48	0.2855
Cultivar	3	1.16222500	0.38740833	1.22	0.3573

Table 7 **The GLM Procedure for 2005 dry season grain yield**

Table 7a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.12330000	0.02055000	6.06	0.0087
Error	9	0.03050000	0.00338889		
Corrected Total	15	0.15380000			

Table 7b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.09685000	0.03228333	9.53	0.0037
Cultivar	3	0.02645000	0.00881667	2.60	0.1165

Table 8. **The GLM Procedure for 2006 wet season haulm yield**

Table 8a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	106.6646875	17.7774479	7.93	0.0035
Error	9	20.1662062	2.2406896		

Table 8b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	4.1611687	1.3870562	0.62	0.6200
Cultivar	3	102.5035188	34.1678396	15.25	0.0007

Table 9. **The GLM Procedure for 2006 wet season grain yield**

Table 9a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.80423750	0.13403958	2.66	0.0911
Error	9	0.45430625	0.05047847		
Corrected Total	15	1.25854375			

Table 9b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.01731875	0.00577292	0.11	0.9495
Cultivar	3	0.78691875	0.26230625	5.20	0.0235

Table 10. The GLM Procedure for 2006 dry season haulm yield

Table 10a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	44.36625000	7.39437500	10.96	0.0011
Error	9	6.07052500	0.67450278		
Corrected Total	15	50.43677500			

Table 10b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.23072500	0.07690833	0.11	0.9497
Cultivar	3	44.13552500	14.71184167	21.81	0.0002

Table 11. The GLM Procedure for 2006 dry season grain yield

Table 11a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	2.33368750	0.38894792	3.41	0.0486
Error	9	1.02690625	0.11410069		
Corrected Total	15	3.36059375			

Table 11b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.58851875	0.19617292	1.72	0.2322
Cultivar	3	1.74516875	0.58172292	5.10	0.0247

Table 12. The GLM Procedure for 2007 wet season haulm yield

Table 12a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	86.30150000	14.38358333	39.41	0.0001
Error	9	3.28450000	0.36494444		
Corrected Total	15	89.58600000			

Table 12a. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	2.81625000	0.93875000	2.57	0.1190
Cultivar	3	83.48525000	27.82841667	76.25	0.0001

Table 13. The GLM Procedure for 2007 wet season grain yield

Table 13a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.41898750	0.06983125	7.35	0.0045
Error	9	0.08550625	0.00950069		
Corrected Total	15	0.50449375			

Table 13b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.03986875	0.01328958	1.40	0.3054
Cultivar	3	0.37911875	0.12637292	13.30	0.0012

Table 14. The GLM Procedure for 2007 dry season haulm yield

Table 14a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	78.44155000	13.07359167	25.51	0.0001
Error	9	4.61275000	0.51252778		
Corrected Total	15	83.05430000			

Table 14b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.03986875	0.01328958	1.40	0.3054
Cultivar	3	0.37911875	0.12637292	13.30	0.0012

Table 15. The GLM Procedure for 2007 dry season grain yield

Table 15a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.40820000	0.23470000	6.17	0.0081
Error	9	0.34210000	0.03801111		
Corrected Total	15	1.75030000			

Table 15b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.12535000	0.04178333	1.10	0.3986
Cultivar	3	1.28285000	0.42761667	11.25	0.0021

Appendix VI: Agronomy Experiment 2

Table 1. The raw data set of cowpea haulm loss, HL (kg); haulm remaining, HR (kg) and nutrient retention (g/kg) under two storage methods (SM).

OBS	CULTIVAR	WK	BLK	SM	HL	HR	Nutrient retention			
							CP	ADF	NDF	ADIN
1	SORONKO	4	1	Shed	0.780	9.220	0.637	4.934	5.611	0.050
2	IT93K 2309	4	1	Shed	1.880	8.120	0.790	4.527	5.096	0.042
3	IT86D-716	4	1	Shed	0.560	9.440	0.837	4.924	5.790	0.032
4	IT93K 2045-29	4	1	Shed	1.030	8.970	0.687	4.739	5.424	0.032
5	SORONKO	4	1	Roof	0.800	9.200	0.846	4.921	5.665	0.065
6	IT93K 2309	4	1	Roof	2.880	7.120	0.696	3.861	4.474	0.044
7	IT86D-716	4	1	Roof	1.150	8.850	0.843	4.767	5.548	0.044
8	IT93K 2045-29	4	1	Roof	1.800	8.200	0.647	4.395	5.003	0.038
9	SORONKO	4	2	Shed	0.200	9.800	0.677	5.245	5.964	0.053
10	IT93K 2309	4	2	Shed	0.900	9.100	0.886	5.073	5.711	0.047
11	IT86D-716	4	2	Shed	0.580	9.420	0.836	4.913	5.778	0.032
12	IT93K 2045-29	4	2	Shed	0.800	9.200	0.704	4.860	5.563	0.032
13	SORONKO	4	2	Roof	0.900	9.100	0.837	4.868	5.604	0.064
14	IT93K 2309	4	2	Roof	1.300	8.700	0.851	4.717	5.466	0.054
15	IT86D-716	4	2	Roof	0.600	9.400	0.896	5.063	5.892	0.047
16	IT93K 2045-29	4	2	Roof	1.800	8.200	0.647	4.395	5.003	0.038
17	SORONKO	4	3	Shed	1.250	8.750	0.605	4.683	5.325	0.047
18	IT93K 2309	4	3	Shed	0.400	9.600	0.988	5.352	6.024	0.049
19	IT86D-716	4	3	Shed	1.150	8.850	0.862	4.616	5.428	0.030
20	IT93K 2045-29	4	3	Shed	0.600	9.400	0.719	4.966	5.684	0.033
21	SORONKO	4	3	Roof	1.700	8.300	0.763	4.440	5.111	0.059
22	IT93K 2309	4	3	Roof	0.750	9.250	0.904	5.016	5.812	0.057
23	IT86D-716	4	3	Roof	1.300	8.700	0.829	4.686	5.454	0.043
24	IT93K 2045-29	4	3	Roof	0.700	9.300	0.733	4.985	5.674	0.043
25	SORONKO	8	1	Shed	0.350	8.870	0.633	4.756	5.487	0.063
26	IT93K 2309	8	1	Shed	0.100	8.020	0.793	4.393	5.067	0.050
27	IT86D-716	8	1	Shed	0.220	9.220	0.814	4.916	5.726	0.046
28	IT93K 2045-29	8	1	Shed	0.220	8.750	0.679	4.666	5.295	0.041
29	SORONKO	8	1	Roof	0.930	8.270	0.832	4.534	5.195	0.078
30	IT93K 2309	8	1	Roof	1.540	5.580	0.584	3.054	3.547	0.051
31	IT86D-716	8	1	Roof	0.680	8.170	0.815	4.467	5.222	0.071
32	IT93K 2045-29	8	1	Roof	1.000	7.200	0.640	3.952	4.418	0.067
33	SORONKO	8	2	Shed	0.070	9.730	0.694	5.217	6.019	0.069
34	IT93K 2309	8	2	Shed	0.160	8.940	0.884	4.896	5.648	0.055
35	IT86D-716	8	2	Shed	0.270	9.150	0.808	4.878	5.683	0.046

(Table 1 contd.)

OBS	CULTIVAR	WK	BLK	SM	HL	HR	Nutrient retention			
							CP	ADF	NDF	ADIN
36	IT93K 2045-29	8	2	Shed	0.440	8.760	0.680	4.671	5.301	0.041
37	SORONKO	8	2	Roof	0.550	8.550	0.860	4.688	5.371	0.081
38	IT93K 2309	8	2	Roof	0.750	7.950	0.832	4.352	5.053	0.073
39	IT86D-716	8	2	Roof	0.350	9.050	0.903	4.948	5.785	0.078
40	IT93K 2045-29	8	2	Roof	1.000	7.200	0.640	3.952	4.418	0.067
41	SORONKO	8	3	Shed	0.200	8.550	0.610	4.584	5.289	0.060
42	IT93K 2309	8	3	Shed	0.740	8.860	0.876	4.853	5.597	0.055
43	IT86D-716	8	3	Shed	0.290	8.560	0.755	4.564	5.316	0.043
44	IT93K 2045-29	8	3	Shed	0.030	9.370	0.728	4.997	5.670	0.044
45	SORONKO	8	3	Roof	0.950	7.350	0.739	4.030	4.617	0.069
46	IT93K 2309	8	3	Roof	0.470	8.780	0.919	4.806	5.581	0.081
47	IT86D-716	8	3	Roof	0.750	7.950	0.793	4.346	5.082	0.069
48	IT93K 2045-29	8	3	Roof	0.450	8.850	0.786	4.858	5.430	0.082
49	SORONKO	12	1	Shed	0.030	8.840	0.676	4.705	5.475	0.066
50	IT93K 2309	12	1	Shed	0.070	7.950	0.802	4.346	5.142	0.056
51	IT86D-716	12	1	Shed	0.040	9.180	0.813	4.920	5.703	0.053
52	IT93K 2045-29	12	1	Shed	0.050	8.700	0.673	4.789	5.324	0.049
53	SORONKO	12	1	Roof	0.420	7.850	0.833	4.329	4.987	0.080
54	IT93K 2309	12	1	Roof	0.410	5.170	0.602	2.851	3.317	0.052
55	IT86D-716	12	1	Roof	0.340	7.830	0.853	4.284	4.911	0.077
56	IT93K 2045-29	12	1	Roof	0.550	6.650	0.618	3.640	4.161	0.060
57	SORONKO	12	2	Shed	0.060	9.670	0.739	5.147	5.989	0.072
58	IT93K 2309	12	2	Shed	0.040	8.900	0.897	4.865	5.757	0.062
59	IT86D-716	12	2	Shed	0.060	9.090	0.805	4.872	5.647	0.052
60	IT93K 2045-29	12	2	Shed	0.040	8.720	0.674	4.800	5.336	0.049
61	SORONKO	12	2	Roof	0.410	8.140	0.864	4.489	5.171	0.083
62	IT93K 2309	12	2	Roof	0.480	7.470	0.870	4.119	4.792	0.076
63	IT86D-716	12	2	Roof	0.400	8.650	0.942	4.733	5.426	0.085
64	IT93K 2045-29	12	2	Roof	0.460	6.740	0.626	3.689	4.218	0.061
65	SORONKO	12	3	Shed	0.040	8.510	0.651	4.530	5.271	0.063
66	IT93K 2309	12	3	Shed	0.020	8.840	0.891	4.833	5.718	0.062
67	IT86D-716	12	3	Shed	0.050	8.510	0.753	4.561	5.287	0.049
68	IT93K 2045-29	12	3	Shed	0.060	9.310	0.720	5.125	5.697	0.052
69	SORONKO	12	3	Roof	0.370	6.980	0.741	3.849	4.434	0.071
70	IT93K 2309	12	3	Roof	0.400	8.380	0.976	4.621	5.376	0.085
71	IT86D-716	12	3	Roof	0.400	7.550	0.823	4.131	4.736	0.075
72	IT93K 2045-29	12	3	Roof	0.300	8.550	0.794	4.680	5.350	0.077

Table 2. The Mixed procedure of haulm dry matter Loss during storage

Table 2a. Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	13.3	0.73	0.5502
Storage	1	13.3	15.14	0.0018
Week	2	29.1	32.56	0.0001
Variety*Week	6	29.6	0.47	0.8220
Storage*Week	2	29.1	0.41	0.6665
Variety*Storage*Week	9	32.9	0.12	0.9988

Table 2b. Least Squares Means

Effect	Variety	Storage	Estimate	SE	DF	t Value	Pr > |t|
Variety	86D716		0.5106	0.1220	11.5	4.19	0.0014
Variety	93K204	0.6294	0.1220	11.5	5.16	0.0003	
Variety	93K230	0.7383	0.1220	11.5	6.05	0.0001	
Variety	SOR	0.5561	0.1220	11.5	4.56	0.0007	
Storage		Roof	0.8344	0.09021	5.47	9.25	0.0002
Storage		Shed	0.3828	0.09021	5.47	4.24	0.0067

Table 3. The Mixed procedure of haulm remaining during storage

Table 3a. Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	14.3	0.87	0.4773
Storage	1	14.3	10.91	0.0051
Week	2	32.1	78.81	0.0001
Variety*Week	6	32.1	0.42	0.8631
Storage*Week	2	32.1	25.11	0.0001
Variety*Storage*Week	9	34.7	0.23	0.9879

Table 3b. Least Squares Means

Effect	Variety	Storage	Estimate	SE	DF	t Value	Pr > |t|
Variety	86D716		8.7539	0.3012	11.5	29.07	0.0001
Variety	93K204		8.4483	0.3012	11.5	28.05	0.0001
Variety	93K230		8.1517	0.3012	11.5	27.07	0.0001
Variety	SOR		8.6489	0.3012	11.5	28.72	0.0001
Storage		Roof	8.0328	0.2248	5.23	35.73	0.0001
Storage		Shed	8.9686	0.2248	5.23	39.89	0.0001

Table 4. **The Mixed procedure of crude protein remaining in cowpea haulm during storage**

Table 4b. **Type 3 Tests of Fixed Effects**

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	14.4	6.59	0.0050
Storage	1	14.4	1.15	0.3003
Week	2	32.1	6.03	0.0060
Variety*Week	6	32.1	2.17	0.0720
Storage*Week	2	32.1	0.42	0.6610
Variety*Storage*Week	9	34.7	1.66	0.1358

Type 4c. Least Squares Means

Effect	Variety	Storage	Estimate	SE	DF	t Value	Pr > |t|
Variety	86D716		0.8321	0.03095	10.6	26.89	0.0001
Variety	93K204		0.6886	0.03095	10.6	22.25	0.0001
Variety	93K230		0.8358	0.03095	10.6	27.00	0.0001
Variety	SOR		0.7355	0.03095	10.6	23.76	0.0001
Storage		Roof	0.7883	0.02353	4.8	33.50	0.0001
Storage		Shed	0.7577	0.02353	4.8	32.20	0.0001

Table 5. **The Mixed procedure of ADF remaining in cowpea haulm during storage**

Table 5a. **Type 3 Tests of Fixed Effects**

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	14.3	0.43	0.7322
Storage	1	14.3	8.48	0.0112
Week	2	32.1	50.08	0.0001
Variety*Week	6	32.2	1.88	0.1145
Storage*Week	2	32.1	19.49	0.0001
Variety*Storage*Week	9	34.7	0.52	0.8519

Table 5b. **Least Squares Means**

Effect	Variety	Storage	Estimate	SE	DF	t Value	Pr > |t|
Variety	86D716		4.6992	0.1645	11.5	28.57	0.0001
Variety	93K204		4.5644	0.1645	11.5	27.75	0.0001
Variety	93K230		4.4740	0.1645	11.5	27.20	0.0001
Variety	SOR		4.6637	0.1645	11.5	28.35	0.0001
Storage		Roof	4.3754	0.1230	5.19	35.58	0.0001
Storage		Shed	4.8253	0.1230	5.19	39.24	0.0001

Table 6. **The Mixed procedure of NDF remaining in cowpea haulm during storage**

Table 6a. **Type 3 Tests of Fixed Effects**

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	14.3	0.69	0.5738
Storage	1	14.3	8.34	0.0117
Week	2	32.1	47.61	0.0001
Variety*Week	6	32.1	1.28	0.2945
Storage*Week	2	32.1	23.89	0.0001
Variety*Storage*Week	9	34.7	0.40	0.9280

Table 6b. **Least Squares Means**

Effect	Variety	Storage	Estimate	SE	DF	t Value	Pr > |t|
Variety	86D716		5.4673	0.1898	11.5	28.80	0.0001
Variety	93K204		5.1650	0.1898	11.5	27.21	0.0001
Variety	93K230		5.1764	0.1898	11.5	27.27	0.0001
Variety	SOR		5.3658	0.1898	11.5	28.27	0.0001
Storage		Roof	5.0362	0.1419	5.2	35.50	0.0001
Storage		Shed	5.5511	0.1419	5.2	39.13	0.0001

Table 7. **The Mixed procedure of ADIN remaining in cowpea haulm during storage**

Table 7a. **Type 3 Tests of Fixed Effects**

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	14.7	8.58	0.0016
Storage	1	14.7	46.91	0.0001
Week	2	32.5	391.91	0.0001
Variety*Week	6	32.5	8.40	0.0001
Storage*Week	2	32.5	46.89	0.0001
Variety*Storage*Week	9	35.0	7.42	0.0001

Table 7b. **Least Squares Means**

Effect	Variety	Storage	Estimate	SE	DF	t Value	Pr > |t|
Variety	86D716		0.05394	0.002419	12.8	22.30	0.0001
Variety	93K204		0.05037	0.002419	12.8	20.82	0.0001
Variety	93K230		0.05834	0.002419	12.8	24.11	0.0001
Variety	SOR		0.06618	0.002419	12.8	27.35	0.0001
Storage		Roof	0.06517	0.001774	5.83	36.73	0.0001
Storage		Shed	0.04924	0.001774	5.83	27.75	0.0001

Appendix VII: Animal Experiment 1

Table 1: Raw data set of the chemical composition of four cultivars of cowpea

OBS	CULTIVAR	REP	FRAC	CP	ADF	NDF	NDFN	CF	Ash	EE	ADL
1	IT86D-716	1	LVS	313.75	194.89	245.64	18.86	153.66	67.32	32.15	26.88
1	IT93K 2045-29	1	LVS	343.70	204.28	257.85	11.36	121.87	31.77	45.00	22.10
1	IT93K 2309	1	LVS	308.20	185.85	243.13	9.54	148.35	42.04	41.17	21.51
1	SORON	1	LVS	250.08	160.00	190.24	8.32	126.21	50.11	38.36	19.02
1	IT86D-716	1	STM	59.98	555.81	673.85	5.50	349.36	103.21	12.56	51.87
1	IT93K 2045-29	1	STM	100.90	533.21	702.92	5.98	313.42	70.34	19.13	45.67
1	IT93K 2309	1	STM	95.75	562.20	704.10	5.70	314.62	106.47	16.08	48.23
1	SORON	1	STM	72.90	575.81	692.14	5.68	327.83	90.24	15.89	49.04
1	IT86D-716	1	WHL	192.23	356.14	459.65	7.76	320.92	79.93	20.12	36.09
1	IT93K 2045-29	1	WHL	228.88	365.25	466.08	6.08	281.87	51.74	21.27	31.94
1	IT93K 2309	1	WHL	246.93	383.23	512.35	7.08	297.31	77.02	25.89	34.21
1	SORON	1	WHL	151.20	376.48	462.58	6.46	289.84	63.51	21.02	35.23
1	IT86D-716	2	LVS	273.00	190.93	236.33	18.76	157.49	62.13	29.54	26.34
1	IT93K 2045-29	2	LVS	343.00	197.16	261.80	10.72	120.51	30.15	43.86	22.16
1	IT93K 2309	2	LVS	301.88	167.61	237.43	10.08	130.78	50.28	40.87	21.88
1	SORON	2	LVS	226.63	147.93	204.92	8.96	120.24	47.99	39.37	19.83
1	IT86D-716	2	STM	56.00	566.67	677.52	5.04	347.62	110.92	11.73	50.92
1	IT93K 2045-29	2	STM	96.25	527.66	686.52	5.88	309.15	85.17	16.79	45.86
1	IT93K 2309	2	STM	89.25	521.47	667.86	5.60	329.48	93.55	16.68	47.99
1	SORON	2	STM	73.50	564.74	720.44	4.48	321.10	94.72	14.96	48.40
1	IT86D-716	2	WHL	189.88	331.01	474.73	7.84	327.37	70.24	20.34	36.95
1	IT93K 2045-29	2	WHL	233.63	379.29	471.27	6.72	270.52	52.49	21.77	30.52
1	IT93K 2309	2	WHL	198.63	358.51	479.19	7.84	286.75	73.52	26.11	34.75
1	SORON	2	WHL	149.31	369.69	460.84	6.72	297.51	68.23	22.85	35.76
1	IT86D-716	3	LVS	309.75	190.89	241.64	18.76	150.43	68.37	32.42	26.04
1	IT93K 2045-29	3	LVS	339.50	200.08	253.65	10.36	117.95	34.15	46.99	22.44
1	IT93K 2309	3	LVS	304.30	181.95	239.23	10.64	137.78	49.88	43.73	21.84
1	SORON	3	LVS	245.88	155.80	186.04	8.12	129.31	49.17	36.77	19.73
1	IT86D-716	3	STM	56.88	552.71	670.75	5.60	339.37	104.30	13.26	51.85
1	IT93K 2045-29	3	STM	98.00	530.31	700.02	5.88	317.15	81.17	17.17	46.36
1	IT93K 2309	3	STM	92.75	559.20	701.10	5.60	325.48	90.55	16.99	47.25
1	SORON	3	STM	70.00	572.91	689.24	5.88	317.42	97.39	17.03	48.56
1	IT86D-716	3	WHL	188.13	352.04	455.55	7.56	325.36	73.24	21.36	37.23
1	IT93K 2045-29	3	WHL	224.88	361.25	462.08	5.88	273.52	53.18	22.71	30.10
1	IT93K 2309	3	WHL	243.13	379.43	508.55	7.28	296.75	65.52	25.85	34.69
1	SORON	3	WHL	147.00	372.28	458.38	6.16	293.89	75.64	23.23	35.19

Table 2. **The GLM and Mixed Procedures of the chemical composition of four cultivars of cowpea haulm**

Table 2a. **Analysis of variance for crude protein (gkg CP)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	13506.36874	2701.27375	13.48	0.0033
Error	6	1202.60355	200.43392		
Corrected Total	11	14708.97229			

Table 2b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	295.61252	147.80626	0.74	0.5172
Cultivar	3	13210.75623	4403.58541	21.97	0.0012

Table 3. **The GLM and Mixed Procedures acid detergent fibre (g/kg) of four cultivars of cowpea haulm**

Table 3a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1718.238867	343.647773	2.99	0.1075
Error	6	689.571000	114.928500		
Corrected Total	11	2407.809867			

Table 3b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	231.351667	115.675833	1.01	0.4198
Cultivar	3	1486.887200	495.629067	4.31	0.0607

Table 4. **The GLM and Mixed Procedures for neutral detergent fibre (g/kg) of four cultivars of cowpea haulm**

Table 4a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	3100.229742	620.045948	4.25	0.0534
Error	6	874.461150	145.743525		
Corrected Total	11	3974.690892			

Table 4b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	39.617317	19.808658	0.14	0.8755
Cultivar	3	3060.612425	1020.204142	7.00	0.0219

Table 5. The GLM and Mixed Procedures for nitrogen bound to neutral detergent fibre (g/kg) of four cultivars of cowpea haulm

Table 5a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	5.40710000	1.08142000	31.98	0.0003
Error	6	0.20286667	0.03381111		
Corrected Total	11	5.60996667			

Table 5b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	0.69126667	0.34563333	10.22	0.0117
Cultivar	3	4.71583333	1.57194444	46.49	0.0002

Table 6. The GLM and Mixed Procedures for ash free crude fibre (g/kg) of four cultivars of cowpea haulm

Table 6a. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	3764.730208	752.946042	24.90	0.0006
Error	6	181.423283	30.237214		
Corrected Total	11	3946.153492			

Table 6b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	9.598117	4.799058	0.16	0.8567
Cultivar	3	3755.132092	1251.710697	41.40	0.0002

Table 7. **The GLM and Mixed Procedures for ash (g/kg)of four cultivars of cowpea haulm**

Table 7a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	897.475167	179.495033	5.76	0.0273
Error	6	186.983200	31.163867		
Corrected Total	11	1084.458367			

Table 7b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	7.5460667	3.7730333	0.12	0.8881
Cultivar	3	889.9291000	296.6430333	9.52	0.0107

Table 8. **The GLM and Mixed Procedures for ether extract (g/kg)of four cultivars of cowpea haulm**

Table 8a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	49.96675000	9.99335000	33.02	0.0003
Error	6	1.81605000	0.30267500		
Corrected Total	11	51.78280000			

Table 8b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	2.96015000	1.48007500	4.89	0.0550
Cultivar	3	47.00660000	15.66886667	51.77	0.0001

Table 9. **The GLM and Mixed Procedures for ether acid detergent lignin (g/kg) of four cultivars of cowpea haulm**

Table 9a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	57.50088333	11.50017667	24.07	0.0007
Error	6	2.86628333	0.47771389		
Corrected Total	11	60.36716667			

Table 9b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	0.07671667	0.03835833	0.08	0.9238
Cultivar	3	57.42416667	19.14138889	40.07	0.0002

Appendix VIII: Animal Experiment 1 contd.

(a) The NLIN and MIXED Procedures of *In vitro* gas production profile of four cultivars of cowpea haulm

Table 1a. The Iterative phase of 1T93K230 (Rep 1)

Iter	a	b	c	Sum of squares
0	10.0000	30.0000	0.0100	60.3064
1	9.5074	21.8912	0.0144	45.2397
2	9.0299	18.8239	0.0190	40.4928
3	8.1767	16.5846	0.0272	35.7812
4	5.8507	16.3140	0.0488	26.6311
5	3.3100	20.2616	0.0678	0.9369
6	2.6444	21.2703	0.0692	0.2701
7	2.6347	21.2782	0.0692	0.2700
8	2.6346	21.2783	0.0692	0.2700
9	2.6346	21.2783	0.0692	0.2700

Table 1b. The Iterative phase of 1T93K230 (Rep 2)

Iter	a	b	c	Sum of squares
0	10.0000	30.0000	0.0100	58.8908
1	9.4341	22.6880	0.0141	45.4331
2	8.9063	19.9048	0.0182	40.1596
3	7.9744	17.7620	0.0254	34.4053
4	5.4956	17.3651	0.0442	25.7130
5	2.9621	21.0933	0.0606	2.4429
6	2.1897	22.1465	0.0633	1.7152
7	2.1411	22.1829	0.0636	1.7138
8	2.1364	22.1858	0.0637	1.7138
9	2.1357	22.1862	0.0637	1.7138
10	2.1356	22.1863	0.0637	1.7138

Where Iter = Iteration
a = Readily fermentable
b = Potentially fermentable
c = Rate of gas production

Table 1c. The iterative phase of 1T93K230 (Rep 3)

Iter	a	b	c	Sum of Squares
0	10.0000	30.0000	0.0100	60.6226
1	9.4618	22.5891	0.0141	44.7387
2	8.9458	19.6414	0.0183	39.1045
3	8.0316	17.3650	0.0258	33.6346
4	5.6021	16.8640	0.0455	26.2135
5	3.0354	20.6067	0.0634	2.3923
6	2.1634	21.7643	0.0669	1.5469
7	2.0981	21.8153	0.0673	1.5446
8	2.0914	21.8197	0.0674	1.5446
9	2.0905	21.8203	0.0674	1.5446
10	2.0904	21.8204	0.0674	1.5446
11	2.0903	21.8204	0.0674	1.5446

Table 2a. The Analysis of variance for 1T93K230 (Rep 1)

Source	DF	Sum of Squares	Mean Square	F Value	Approx Pr > F
Regression	3	2421.7	807.2	2238.40	0.0001
Residual	4	0.2700	0.0675		
Uncorrected Total	7	2421.9			
Corrected Total	6	302.5			

Table 2b. The Analysis of variance for 1T93K230 (Rep 2)

Source	DF	Sum of Squares	Mean Square	F Value	Approx Pr > F
Regression	3	2408.4	802.8	396.79	0.0001
Residual	4	1.7138	0.4284		
Uncorrected Total	7	2410.1			
Corrected Total	6	341.7			

Table 2c. The Analysis of variance for 1T93K230 (Rep 3)

Source	DF	Sum of Squares	Mean Square	F Value	Approx Pr > F
Regression	3	2374.1	791.4	416.34	0.0001
Residual	4	1.5446	0.3861		
Uncorrected Total	7	2375.6			
Corrected Total	6	323.1			

Table 3a. The NLIN Procedure of 1T93K230 (Rep 1)

Parameter	Estimate	Approx Std Error	Approximate 95%	Confidence Limits
a	2.6346	0.3895	1.5532	3.7160
b	21.2783	0.3760	20.2343	22.3224
c	0.0692	0.00295	0.0610	0.0774

Table 3b. The NLIN Procedure of 1T93K230 (Rep 2)

Parameter	Estimate	Approx Std Error	Approximate 95%	Confidence Limits
a	2.1356	0.9332	-0.4554	4.7267
b	22.1863	0.9006	19.6858	24.6868
c	0.0637	0.00656	0.0455	0.0819

Table 3c. The NLIN Procedure of 1T93K230 (Rep 3)

Parameter	Estimate	Approx Std Error	Approximate 95%	Confidence Limits
a	2.0903	0.9162	-0.4533	4.6340
b	21.8204	0.8844	19.3650	24.2759
c	0.0674	0.00670	0.0488	0.0860

Table 1a. The Iterative phase of IT86D-716 (Rep 1)

Iter	a	b	c	Sum of squares
0	10.0000	20.0000	0.0100	74.3129
1	8.5593	8.3909	0.0201	68.6938
2	6.8930	8.3262	0.0407	58.2407
3	3.3432	13.7293	0.0719	7.6470
4	1.3903	16.8577	0.0699	0.5073
5	1.3776	16.8845	0.0702	0.5042
6	1.3764	16.8853	0.0702	0.5042
7	1.3764	16.8853	0.0702	0.5042

Table 1b. The Iterative phase of IT86D-716 (Rep 2)

Iter	a	b	c	Sum of squares
0	10.0000	20.0000	0.0100	97.8762
1	8.5924	6.9575	0.0207	65.6948
2	7.6659	6.7273	0.0359	58.1047
3	4.6632	10.2003	0.0769	21.4866
4	0.9623	15.7446	0.0922	0.5200
5	0.7211	16.1196	0.0875	0.1722
6	0.7014	16.1387	0.0879	0.1713
7	0.7022	16.1380	0.0879	0.1713
8	0.7022	16.1381	0.0879	0.1713

Table 1c. The Iterative phase of IT86D-716 (Rep 3)

Iter	a	b	c	Sum of Squares
0	10.0000	20.0000	0.0100	92.8476
1	8.5755	7.6539	0.0203	65.6432
2	6.7369	7.2057	0.0467	65.1506
3	2.8831	13.0911	0.0893	9.8973
4	0.6519	16.5523	0.0826	1.2250
5	0.6148	16.6055	0.0842	1.1938
6	0.6136	16.6071	0.0842	1.1938

Table 2a. The Analysis of variance for IT86D-716 (Rep 1)

Source	DF	Sum of squares	Mean Square	F Value	Approx Pr > F
Regression	3	1397.4	465.8	749.92	0.0001
Residual	4	0.5042	0.1261		
Uncorrected Total	7	1397.9			
Corrected Total	6	189.6			

Table 2b. The Analysis of variance for IT86D-716 (Rep 2)

Source	DF	Sum of Squares	Mean Square	F Value	Approx Pr > F
Regression	3	1262.6	420.9	1789.06	0.0001
Residual	4	0.1713	0.0428		
Uncorrected Total	7	1262.7			
Corrected Total	6	153.4			

Table 2c. The Analysis of variance for IT86D-716 (Rep 3)

Source	DF	Sum of Squares	Mean Square	F Value	Approx Pr > F
Regression	3	1299.2	433.1	278.95	0.0001
Residual	4	1.1938	0.2984		
Uncorrected Total	7	1300.4			
Corrected Total	6	167.7			

Table 3a. The NLIN Procedure of IT86D-716 (Rep 1)

Parameter	Estimate	SE	Approx 95%	CL
a	1.3764	0.5371	-0.1148	2.8676
b	16.8853	0.5186	15.4455	18.3252
c	0.0702	0.0052	0.0559	0.0846

Table 3b. The NLIN Procedure of IT86D-716 (Rep 2)

Parameter	Estimate	SE	Approx 95%	CL
a	0.7022	0.3651	-0.3114	1.7158
b	16.1381	0.3527	15.1588	17.1173
c	0.0879	0.0041	0.0767	0.0992

Table 3c. The NLIN Procedure of IT86D-716 (Rep 3)

Parameter	Estimate	SE	Approx 95%	CL
a	0.6136	0.9338	-1.9791	3.2062
b	16.6071	0.9022	14.1022	19.1119
c	0.0842	0.00988	0.0568	0.1116

Table 1a. The NLIN Procedure of IT93K204 (Rep 1)

Iter	a	b	c	Sum of Squares
0	10.0000	20.0000	0.0500	68.8611
1	1.7793	25.4340	0.0797	4.3800
2	0.7562	26.9448	0.0812	3.0185
3	0.7450	26.9539	0.0812	3.0183
4	0.7450	26.9540	0.0812	3.0183

Table 1b. The NLIN Procedure of IT93K204 (Rep 2)

Iter	a	b	c	Sum of Squares
0	10.0000	20.0000	0.0500	50.3249
1	3.1807	24.7327	0.0724	3.8746
2	2.5046	25.6741	0.0734	3.3657
3	2.4971	25.6799	0.0734	3.3657
4	2.4969	25.6800	0.0734	3.3657

Table 1b. The NLIN Procedure of IT93K204 (Rep 3)

Iter	a	b	c	Sum of Squares
0	10.0000	20.0000	0.0500	71.8903
1	1.6661	25.9742	0.0756	1.8759
2	1.0502	26.9828	0.0742	1.0402
3	1.0481	26.9853	0.0743	1.0400
4	1.0481	26.9853	0.0743	1.0400

Table 2a. The Analysis of variance for IT93K204 (Rep 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	3310.5	1103.5	296.73	0.0001
Residual	4	3.0183	0.7546		
Uncorrected Total	7	3313.5			
Corrected Total	6	450.8			

Table 2b. The Analysis of variance for IT93K204 (Rep 2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	3396.5	1132.2	254.52	0.0001
Residual	4	3.3657	0.8414		
Uncorrected Total	7	3399.9			
Corrected Total	6	431.7			

Table 2c. The Analysis of variance for IT93K204 (Rep 3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	3304.3	1101.4	904.26	0.0001
Residual	4	1.0400	0.2600		
Uncorrected Total	7	3305.4			
Corrected Total	6	471.2			

Table 3a. The NLIN Procedure of IT93K204 (Rep 1)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	0.7450	1.4468	-3.2720	4.7619
b	26.9540	1.3978	23.0732	30.8348
c	0.0812	0.00927	0.0554	0.1069

Table 3b. The NLIN Procedure of IT93K204 (Rep 2)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	2.4969	1.4275	-1.4663	6 .4601
b	25.6800	1.3786	21.8523	29.5076
c	0.0734	0.00919	0.0479	0.0989

Table 3c. The NLIN Procedure of IT93K204 (Rep 3)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	1.0481	0.7997	-1.1723	3.2685
b	26.9853	0.7724	24.8407	29.1298
c	0.0743	0.0049	0.0606	0.0880

Table 1a. The Iterative phase of SORONKO (Rep 1)

Iter	a	b	c	Sum of Squares
0	10.0000	20.0000	0.0100	59.1588
1	9.7372	17.0160	0.0128	59.1461
2	9.4732	15.4849	0.0156	56.7373
3	8.9270	13.7985	0.0212	53.4839
4	7.0690	12.6873	0.0396	50.6780
5	3.9937	17.0645	0.0657	7.2976
6	2.0590	19.8460	0.0739	2.0939
7	1.8551	20.0284	0.0748	2.0723
8	1.8382	20.0406	0.0750	2.0721
9	1.8353	20.0427	0.0750	2.0721
10	1.8348	20.0431	0.0750	2.0721
11	1.8347	20.0431	0.0750	2.0721

Table 1b. The Iterative phase of SORONKO (Rep 2)

Iter	a	b	c	Sum of Squares
0	10.0000	20.0000	0.0100	49.2257
1	9.3903	13.7114	0.0156	47.5409
2	8.8795	12.2957	0.0209	43.6834
3	7.0045	11.0649	0.0398	42.9102
4	3.7567	15.3449	0.0687	6.1732
5	1.9540	18.1981	0.0729	0.4790
6	1.9239	18.2389	0.0724	0.4718
7	1.9244	18.2387	0.0724	0.4718

Table 1c. The Iterative phase of SORONKO (Rep 3)

Iter	a	b	c	Sum of Squares
0	10.0000	20.0000	0.0100	49.9413
1	9.7021	17.0300	0.0128	47.5585
2	9.0889	13.8198	0.0185	46.7087
3	8.0887	12.5104	0.0278	41.3789
4	5.4095	14.0078	0.0513	20.9179
5	2.5655	18.3183	0.0687	1.2835
6	1.9521	19.0911	0.0698	0.9353
7	1.9374	19.1018	0.0699	0.9352
8	1.9361	19.1027	0.0699	0.9352
9	1.9359	19.1028	0.0699	0.9352
10	1.9359	19.1028	0.0699	0.9352

Table 2a. The Analysis of variance for SORONKO (Rep 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	2058.3	686.1	249.16	0.0001
Residual	4	2.0721	0.5180		
Uncorrected Total	7	2060.4			
Corrected Total	6	260.2			

Table 2b. The Analysis of variance for SORONKO (Rep 2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	1736.7	578.9	921.80	0.0001
Residual	4	0.4718	0.1179		
Uncorrected Total	7	1737.1			
Corrected Total	6	217.9			

Table 2c. The Analysis of variance for SORONKO (Rep 3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	1865.5	621.8	518.56	0.0001
Residual	4	0.9352	0.2338		
Uncorrected Total	7	1866.4			
Corrected Total	6	243.4			

Table 3a. The NLIN Procedure of SORONKO (Rep 1)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	1.8347	1.1361	-1.3196	4.9890
b	20.0431	1.0974	16.9964	23.0898
c	0.0750	0.00946	0.0488	0.1013

Table 3b. The NLIN Procedure of SORONKO (Rep 2)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	1.9244	0.5298	0.4533	3.3954
b	18.2387	0.5117	16.8181	19.6594
c	0.0724	0.00477	0.0592	0.0857

Table 3c. The NLIN Procedure of SORONKO (Rep 3)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	1.9359	0.7294	-0.0892	3.9609
b	19.1028	0.7043	17.1475	21.0581
c	0.0699	0.00618	0.0528	0.0871

(b) The mixed procedure for readily fermentable fractions of four cultivars of cowpea haulm

Table 1. Least Squares Means

Effect	Cultivar	Estimate	SE	DF	t Value	Pr > |t|
Cultivar	IT93K230	2.2868	0.3088	6	7.40	0.0003
Cultivar	IT86D-71	0.8974	0.3088	6	2.91	0.0271
Cultivar	IT93K204	1.4300	0.3088	6	4.63	0.0036
Cultivar	SORONKO	1.8945	0.3088	6	6.13	0.0009

(c) The mixed procedure for potentially fermentable fractions of four cultivars of cowpea haulm

Table 1. Least Squares Means

Effect	Cultivar	Estimate	SE	DF	t Value	Pr > |t|
Cultivar	IT93K230	21.7617	0.4074	6	53.42	0.0001
Cultivar	IT86D-71	16.5435	0.4074	6	40.61	0.0001
Cultivar	IT93K204	26.5398	0.4074	6	65.15	0.0001
Cultivar	SORONKO	18.8402	0.4074	6	46.25	0.0001

(d) **The mixed procedure for rate of gas production of four cultivars of cowpea haulm**

Table 1. Least Squares Means

Effect	Cultivar	Estimate	SE	DF	t Value	Pr > |t|
Cultivar	1T93K230	0.06677	0.003102	6	21.52	0.0001
Cultivar	IT86D-71	0.08077	0.003102	6	26.03	0.0001
Cultivar	IT93K204	0.07630	0.003102	6	24.59	0.0001
Cultivar	SORONKO	0.07327	0.003102	6	23.62	0.0001

(e) The NLIN and MIXED Procedures of *in situ* dry matter digestibility of four cultivars of cowpea haulm

Table 1a. The Iterative phase of 1T93K230 (Rep 1)

Iter	a	b	c	Sum of Squares
0	20.0000	40.0000	0.1000	151.2000
1	20.5440	43.0224	0.0442	77.9192
2	21.1194	44.6641	0.0498	22.5139
3	20.9325	44.7657	0.0509	22.3517
4	20.9022	44.7643	0.0511	22.3465
5	20.8963	44.7634	0.0511	22.3463
6	20.8951	44.7632	0.0511	22.3463
7	20.8949	44.7632	0.0511	22.3463
8	20.8949	44.7632	0.0511	22.3463

Table 1b. The Iterative phase of 1T93K230 (Rep 2)

Iter	a	b	c	Sum of Squares
0	20.0000	40.0000	0.1000	109.3
1	18.6741	44.5043	0.0630	40.3144
2	19.1145	45.0283	0.0653	31.3029
3	19.0502	45.0461	0.0658	31.2824
4	19.0371	45.0484	0.0659	31.2815
5	19.0344	45.0488	0.0659	31.2814
6	19.0338	45.0489	0.0659	31.2814
7	19.0337	45.0489	0.0659	31.2814

Table 1c. The iterative phase of 1T93K230 (Rep 3)

Iter	a	b	c	Sum of Squares
0	20.0000	40.0000	0.1000	76.7396
1	22.0332	41.5938	0.0592	22.6422
2	22.3236	42.3669	0.0615	13.3288
3	22.2723	42.3813	0.0619	13.3172
4	22.2644	42.3822	0.0620	13.3169
5	22.2630	42.3823	0.0620	13.3169
6	22.2628	42.3823	0.0620	13.3169
7	22.2628	42.3823	0.0620	13.3169

Table 2a. The Analysis of variance for 1T93K230 (Rep 1)

Source	DF	Sum Sq	Mean Sq	F Value	Pr > F
Regression	3	18980.8	6326.9	244.8000	0.0001
Residual	5	22.3463	4.4693		
Uncorrected Total	8	19003.2			
Corrected Total	7	2210.5			

Table 2b. The Analysis of variance for 1T93K230 (Rep 2)

Source	DF	Sum of Sq	Mean Sq	F Value	Pr > F
Regression	3	19168.8	6389.6	177.10	0.0001
Residual	5	31.2814	6.2563		
Uncorrected Total	8	19200.1			
Corrected Total	7	2247.2			

Table 3c. The NLIN Procedure of 1T93K230 (Rep 3)

Source	DF	Sum of Sq	Mean Sq	F Value	Pr > F
Regression	3	19816.8	6605.6	369.11	0.0001
Residual	5	13.3169	2.6634		
Uncorrected Total	8	19830.1			
Corrected Total	7	1979.5			

Table 3a. The NLIN Procedure of 1T93K230 (Rep 1)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	20.8949	1.6593	16.6295	25.1603
b	44.7632	2.0303	39.5443	49.9821
K	0.0511	0.00696	0.0333	0.0690

Table 3b. The NLIN Procedure of 1T93K230 (Rep 2)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	19.0337	2.0615	13.7346	24.3327
b	45.0489	2.3996	38.8805	51.2173
K	0.0659	0.00979	0.0408	0.0911

Table 3c. The NLIN Procedure of 1T93K230 (Rep 3)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	22.2628	1.3291	18.8462	25.6794
b	42.3823	1.5611	38.3694	46.3953
K	0.0620	0.00648	0.0453	0.0786

Table 1a. The Iterative phase of IT86D-716 (Rep 1)

Iter	a	b	c	Sum of Squares
0	20.0000	40.0000	0.0500	69.8419
1	18.8836	44.7714	0.0510	22.3999
2	18.8545	44.7693	0.0511	22.3969
3	18.8518	44.7689	0.0512	22.3969
4	18.8513	44.7688	0.0512	22.3969
5	18.8512	44.7688	0.0512	22.3969

Table 1b. The Iterative phase of IT86D-716 (Rep 2)

Iter	a	b	c	Sum of Squares
0	20.0000	40.0000	0.0500	100.1
1	17.5279	44.5753	0.0627	32.0673
2	17.0284	45.0257	0.0652	31.2963
3	16.9569	45.0467	0.0658	31.2704
4	16.9422	45.0492	0.0659	31.2692
5	16.9390	45.0497	0.0659	31.2692
6	16.9384	45.0498	0.0659	31.2692
7	16.9382	45.0498	0.0659	31.2692
8	16.9382	45.0498	0.0659	31.2692

Table 1c. The Iterative phase of IT86D-716 (Rep 3)

Iter	a	b	c	Sum of Squares
0	20.0000	40.0000	0.1000	88.9436
1	19.9299	41.5927	0.0592	22.6445
2	20.2201	42.3661	0.0615	13.3245
3	20.1687	42.3805	0.0619	13.3129
4	20.1608	42.3814	0.0620	13.3125
5	20.1595	42.3815	0.0620	13.3125
6	20.1592	42.3815	0.0620	13.3125
7	20.1592	42.3816	0.0620	13.3125

Table 2a. The Analysis of variance for IT86D-716 (Rep 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	17520.6	5840.2	244.31	0.0001
Residual	5	22.3969	4.4794		
Uncorrected Total	8	17543.0			
Corrected Total	7	2211.1			

Table 2b. The Analysis of variance for IT86D-716 (Rep 2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	17661.5	5887.2	177.17	0.0001
Residual	5	31.2692	6.2538		
Uncorrected Total	8	17692.8			
Corrected Total	7	2247.3			

Table 2c. The Analysis of variance for IT86D-716 (Rep 3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	18261.6	6087.2	369.22	0.0001
Residual	5	13.3125	2.6625		
Uncorrected Total	8	18274.9			
Corrected Total	7	1979.4			

Table 3a. The NLIN Procedure of IT86D-716 (Rep 1)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	18.8512	1.6613	14.5807	23.1216
b	44.7688	2.0325	39.5441	49.9934
c	0.0512	0.00697	0.0332	0.0691

Table 3b. The NLIN Procedure of IT86D-716 (Rep 2)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	16.9382	2.0611	11.6401	22.2364
b	45.0498	2.3992	38.8826	51.2171
K	0.0659	0.00979	0.0408	0.0911

Table 3c. The NLIN Procedure of IT86D-716 (Rep 3)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	20.1592	1.3289	16.7432	23.5752
b	42.3816	1.5609	38.3693	46.3938
K	0.0620	0.00648	0.0453	0.0786

Table 1a. The NLIN Procedure of IT93K204 (Rep 1)

Iter	a	b	c	Sum of Squares
0	30.0000	40.0000	0.0500	109.3
1	25.3404	41.9763	0.0647	64.0801
2	24.5311	42.6472	0.0707	60.4792
3	24.2747	42.7536	0.0730	60.1168
4	24.1883	42.7793	0.0738	60.0737
5	24.1586	42.7870	0.0741	60.0686
6	24.1485	42.7894	0.0742	60.0680
7	24.1451	42.7903	0.0742	60.0680
8	24.1439	42.7906	0.0742	60.0680
9	24.1435	42.7906	0.0742	60.0680
10	24.1434	42.7907	0.0742	60.0680
11	24.1433	42.7907	0.0742	60.0680

Table 1b. The NLIN Procedure of IT93K204 (Rep 2)

Iter	a	b	c	Sum of Squares
0	30.0000	40.0000	0.0500	102.1
1	24.7135	45.1323	0.0535	40.5247
2	24.6302	45.1577	0.0537	40.5071
3	24.6258	45.1574	0.0538	40.5070
4	24.6251	45.1573	0.0538	40.5070
5	24.6250	45.1573	0.0538	40.5070

Table 1b. The NLIN Procedure of IT93K204 (Rep 3)

Iter	a	b	c	Sum of Squares
0	30.0000	40.0000	0.0500	82.0379
1	24.6334	44.3602	0.0654	31.1155
2	24.0864	45.0048	0.0682	29.9631
3	24.0190	45.0281	0.0687	29.9418
4	24.0068	45.0307	0.0688	29.9411
5	24.0046	45.0311	0.0689	29.9410
6	24.0041	45.0312	0.0689	29.9410
7	24.0040	45.0312	0.0689	29.9410

Table 2a. The Analysis of variance for IT93K204 (Rep 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	22464.3	7488.1	82.53	0.0001
Residual	5	60.0680	12.0136		
Uncorrected Total	8	22524.4			
Corrected Total	7	2043.0			

Table 2b. The Analysis of variance for IT93K204 (Rep 2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	22354.1	7451.4	137.74	0.0001
Residual.	5	40.5070	8.1014		
Uncorrected Total	8	22394.6			
Corrected Total	7	2272.3			

Table 2c. The Analysis of variance for IT93K204 (Rep 3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	23270.1	7756.7	184.41	0.0001
Residual	5	29.9410	5.9882		
Uncorrected Total	8	23300.1			
Corrected Total	7	2238.6			

Table 3a. The NLIN Procedure of IT93K204 (Rep 1)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	24.1433	2.9218	16.6327	31.6539
b	42.7907	3.3541	34.1688	51.4126
c	0.0742	0.0157	0.0339	0.1145

Table 3b. The NLIN Procedure of IT93K204 (Rep 2)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	24.6250	2.2554	18.8274	30.4226
b	45.1573	2.7238	38.1556	52.1590
c	0.0538	0.00960	0.0291	0.0784

Table 3c. The NLIN Procedure of IT93K204 (Rep 3)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	24.0040	2.0337	18.7763	29.2318
b	45.0312	2.3543	38.9795	51.0829
c	0.0689	0.00991	0.0434	0.0944

Table 1a. The Iterative phase of SORONKO (Rep 1)

Iter	a	b	c	Sum of Squares
0	20.0000	40.0000	0.0500	59.8641
1	18.4704	44.7454	0.0511	22.3323
2	18.4387	44.7436	0.0512	22.3289
3	18.4358	44.7431	0.0513	22.3289
4	18.4352	44.7430	0.0513	22.3289
5	18.4351	44.7430	0.0513	22.3289
6	18.4350	44.7430	0.0513	22.3289

Table 1b. The Iterative phase of SORONKO (Rep 2)

Iter	a	b	c	Sum of Squares
0	20.0000	40.0000	0.0500	93.7369
1	17.2960	44.5745	0.0627	32.0655
2	16.7965	45.0251	0.0652	31.2942
3	16.7249	45.0460	0.0658	31.2683
4	16.7102	45.0486	0.0659	31.2671
5	16.7071	45.0491	0.0659	31.2671
6	16.7064	45.0492	0.0659	31.2671
7	16.7063	45.0492	0.0659	31.2671
8	16.7063	45.0492	0.0659	31.2671

Table 1c. The Iterative phase of SORONKO (Rep 3)

Iter	a	b	c	Sum of Squares
0	20.0000	40.0000	0.0500	85.2950
1	20.1458	42.1001	0.0598	13.6984
2	19.8582	42.3701	0.0616	13.3096
3	19.8191	42.3795	0.0619	13.3018
4	19.8126	42.3802	0.0620	13.3016
5	19.8116	42.3803	0.0620	13.3016
6	19.8114	42.3803	0.0620	13.3016
7	19.8113	42.3803	0.0620	13.3016

Table 2a. The Analysis of variance for SORONKO (Rep 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	17229.2	5743.1	244.80	0.0001
Residual	5	22.3289	4.4658		
Uncorrected Total	8	17251.5			
Corrected Total	7	2208.8			

Table 2b. The Analysis of variance for SORONKO (Rep 2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	17498.7	5832.9	177.18	0.0001
Residual	5	31.2671	6.2534		
Uncorrected Total	8	17530.0			
Corrected Total	7	2247.2			

Table 2c. The Analysis of variance for SORONKO (Rep 3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Regression	3	18010.5	6003.5	369.50	0.0001
Residual	5	13.3016	2.6603		
Uncorrected Total	8	18023.8			
Corrected Total	7	1979.3			

Table 3a. The NLIN Procedure of SORONKO (Rep 1)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	18.4350	1.6594	14.1694	22.7007
b	44.7430	2.0290	39.5272	49.9587
K	0.0513	0.00697	0.0333	0.0692

Table 3b. The NLIN Procedure of SORONKO (Rep 2)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	16.7063	2.0610	11.4083	22.0043
b	45.0492	2.3991	38.8822	51.2162
K	0.0659	0.00979	0.0408	0.0911

Table 3c. The NLIN Procedure of SORONKO (Rep 3)

Parameter	Estimate	Std Error	Approx 95%	Confidence Limits
a	19.8113	1.3284	16.3967	23.2259
b	42.3803	1.5602	38.3697	46.3909
K	0.0620	0.00648	0.0453	0.0786

(f) **The mixed procedure for readily soluble material of four cultivars of cowpea haulm**

Table 1. Least Squares Means

Effect	Cultivar	Estimate	SE	DF	t Value	Pr > |t|
Cultivar	1T93K230	20.7305	0.8051	6	25.75	<.0001
Cultivar	IT86D-71	18.6495	0.8051	6	23.16	<.0001
Cultivar	IT93K204	24.2575	0.8051	6	30.13	<.0001
Cultivar	SORONKO	18.3175	0.8051	6	22.75	<.0001

(g) **The mixed procedure for potentially degradable fraction of cultivars of cowpea haulm**

Table 1. Least Squares Means

Effect	Cultivar	Estimate	SE	DF	t Value	Pr > |t|
Cultivar	IT93K230	44.0648	0.8266	6	53.31	<.0001
Cultivar	IT86D-71	44.0667	0.8266	6	53.31	<.0001
Cultivar	IT93K204	44.3264	0.8266	6	53.63	<.0001
Cultivar	SORONKO	44.0575	0.8266	6	53.30	<.0001

(h) **The mixed procedure for rate of degradation of four cultivars of cowpea haulm**

Table 1. Least Squares Means

Effect	Cultivar	Estimate	SE	DF	t Value	Pr > |t|
Cultivar	1T93K230	0.05967	0.004882	6	12.22	<.0001
Cultivar	IT86D-71	0.05970	0.004882	6	12.23	<.0001
Cultivar	IT93K204	0.06563	0.004882	6	13.44	<.0001
Cultivar	SORONKO	0.05973	0.004882	6	12.24	<.0001

Table 1: **Raw data set of cowpea haulm supplement on intake and digestibility of maize stover**

OBS	PERIOD	ANL	CHL	MSINT	CHINT	TOTINT	DMD
1	1	A1	0	10.02	0.00	10.02	25.47
2	1	A2	150	13.83	3.24	17.07	71.16
3	1	A3	300	21.13	10.79	31.93	75.98
4	1	A4	450	16.77	7.12	23.89	74.73
5	2	A4	0	14.05	0.00	14.05	30.43
6	2	A1	150	15.59	4.77	20.36	69.60
7	2	A2	300	16.01	4.39	20.40	74.14
8	2	A3	450	19.73	11.16	30.89	77.06
9	3	A3	0	16.00	0.00	16.00	37.57
10	3	A4	150	18.40	4.52	22.92	67.73
11	3	A1	300	16.65	6.62	23.27	73.28
12	3	A2	450	15.04	5.49	20.53	75.12
13	4	A2	0	13.92	0.00	13.92	32.74
14	4	A3	150	18.18	7.50	25.68	75.37
15	4	A4	300	19.32	6.28	25.60	72.87
16	4	A1	450	16.24	6.98	23.22	75.55

Where ANL = Animal; CHL = Cowpea haulm level; MSINT = Maize stover intake; CHINT = Cowpea haulm intake; TOTINT = Total intake and DMD = Dry matter digestibility

Table 2. **The GLM procedures for cowpea haulm supplement on maize stover intake (g/d)**

Table 2a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	102.0334	11.3370444	7.55	0.0115
Error	6	9.003800	1.5006333		
Corrected Total	15	111.0372			

Table 2b **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERIOD	3	4.6942	1.5648	1.04	0.4391
ANL	3	48.457	16.152	10.76	0.0079
CHL	3	48.882	16.294	10.86	0.0077

Table 2c **Orthogonal contrast**

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
0 V 150	1	18.0300	18.0300	12.01	0.0134
0 V 300	1	45.6968	45.6968	30.45	0.0015
0 V 450	1	23.7705	23.7705	15.84	0.0073
150 V 300	1	6.31901	6.31901	4.21	0.0860
150 V 450	1	0.39605	0.39605	0.26	0.6258
300 V 450	1	3.55111	3.55111	2.37	0.1749

Table 3 **The GLM procedures for cowpea haulm supplement on maize stover intake (g/d)**

Table 3a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	184.2137	20.4682	12.16	0.0033
Error	6	10.0955	1.6826		
Corrected Total	15	194.3092			

Table 3b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERIOD	3	3.2586	1.0862	0.65	0.6136
ANL	3	35.8238	11.9413	7.10	0.0212
CHL	3	145.1312	48.3771	28.75	0.0006

Table 3c. **Orthogonal contrast**

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
0 V 150	1	50.1501	50.1501	29.81	0.0016
0 V 300	1	98.5608	98.5608	58.58	0.0003
0 V 450	1	118.1953	118.1953	70.25	0.0002
150 V 300	1	8.1003	8.1003	4.81	0.0707
150 V 450	1	14.3648	14.3648	8.54	0.0266
300 V 450	1	0.8911	0.8911	0.53	0.4942

Table 4. **The GLM procedures for cowpea haulm supplement on maize stover intake (g/d)**

Table 4a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	512.3632062	56.9292	11.52	0.0038
Error	6	29.6417875	4.94030		
Corrected Total	15	542.0049938			

Table 4b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERIOD	3	5.4343	1.8114	0.37	0.7801
ANL	3	154.8873	51.6291	10.45	0.0085
CHL	3	352.0415	117.3471	23.75	0.0010

Table 4c **Orthogonal contrast**

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
0 V 150	1	128.3202	128.3202	25.97	0.0022
0 V 300	1	278.5980	278.5980	56.39	0.0003
0 V 450	1	247.9765	247.9765	50.19	0.0004
150 V 300	1	28.7661	28.7661	5.82	0.0524
150 V 450	1	19.5313	19.5313	3.95	0.0939
300 V 450	1	0.8911	0.8911	0.18	0.6859

Table 5. **The GLM procedures for cowpea haulm supplement on dry matter digestibility**

Table 5a. **Analysis of variance**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	5422.7003	602.5224	121.30	0.0001
Error	6	29.8021	4.9670		
Corrected Total	15	5452.5024			

Table 5b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERIOD	3	11.3899	3.7966	0.76	0.5541
ANL	3	75.2934	25.0980	5.05	0.0442
CHL	3	5336.0171	1778.6724	358.10	0.0001

Table 5c Orthogonal contrast

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
0 V 150	1	3106.6903	3106.6903	625.46	0.0001
0 V 300	1	3615.0505	3615.0505	727.81	0.0001
0 V 450	1	3883.0078	3883.0078	781.76	0.0001
150 V 300	1	19.2510	19.2510	3.88	0.0965
150 V 450	1	43.2450	43.2450	8.71	0.0256
300 V 450	1	4.7895	4.7895	0.96	0.3640

Table 1. Raw data set of cowpea haulm supplement on maize stover degradation

OBS	TREATMENT	REP	a	b	c
1	Control	1	5.332	47.2768	0.033
2	LEVEL_150	1	6.9601	50.8887	0.0482
3	Level_300	1	5.8979	54.3698	0.0655
4	Level_450	1	8.7553	52.4881	0.0739
5	Control	2	6.9192	48.267	0.0353
6	Level_150	2	10.1864	49.0452	0.049
7	Level_300	2	7.7971	54.8452	0.0596
8	Level_450	2	7.5802	54.8555	0.0776
9	Control	3	6.5056	48.1959	0.0342
10	Level_150	3	7.8624	50.3481	0.0578
11	Level_300	3	8.5438	52.0231	0.0727
12	Level_450	3	9.3113	52.916	0.0678
13	Control	4	5.9354	48.2648	0.0341
14	Level_150	4	8.3301	49.9799	0.0518
15	Level_300	4	7.8225	53.0111	0.0659
16	Level_450	4	8.2337	53.3357	0.0736

Where a = readily soluble
b = potentially degradable
c = rate of degradation

Table 2. **The GLM Procedure of readily soluble fraction of maize stover**

Table 2a. **Analysis of variance**

Source	DF	Sum Squares	Mean Square	F Value	Pr > F
Model	6	18.2447	3.0408	3.62	0.0414
Error	9	7.5648	0.8405		
Corrected Total	15	25.8095			

Table 2b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	4.8921	1.6307	1.94	0.1937
Treatment	3	13.3526	4.4509	5.30	0.0223

Table 2c. **Least Squares Means**

Treatment	a	LSMEAN Number
Control	6.1731	1
Level_150	8.3348	2
Level_300	7.5153	3
Level_450	8.4701	4

Table 2d. **Least Squares Means for effect Treatment**

Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	1	2	3	4
1		0.0087	0.0683	0.0063
2	0.0087		0.2380	0.8392
3	0.0683	0.2380		0.1749
4	0.0063	0.8392	0.1749	

Table 3. **The GLM Procedure of potentially degradable fraction of maize stover**

Table 3a. **Analysis of variance for potentially degradable fraction**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	89.3184	14.8864	14.82	0.0003
Error	9	9.0387	1.0045		
Corrected Total	15	98.3570			

Table 3b. **Type III model Analysis of variance**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	1.62927428	0.54309143	0.54	0.6663
Treatment	3	87.68910497	29.22970166	29.10	0.0001

Table 3b. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	1.62927428	0.54309143	0.54	0.6663
Treatment	3	87.68910497	29.22970166	29.10	0.0001

Table 3c. Least Squares Means

Treatment	b	LS MEAN Number
Control	48.0011250	1
Level_150	50.0654750	2
Level_300	53.5623000	3
Level_450	53.3988250	4

Table 3d. Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	1	2	3	4
1		0.0172	0.0001	0.0001
2	0.0172		0.0008	0.0011
3	0.0001	0.0008		0.8227
4	0.0001	0.0011	0.8227	

Table 4a. The GLM Procedure for rate of degradation of maize stover

Table 4b. Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.0036	0.0006	31.15	0.0001
Error	9	0.0002	0.0000		
Corrected Total	15	0.0038			

Table 4c. Type III model Analysis of variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.0000	0.0000	0.38	0.7683
Treatment	3	0.0036	0.0012	61.92	0.0001

Table 3c. Least Squares Means

Treatment	k	LSMEAN Number
Control	0.03415000	1
Level_150	0.05170000	2
Level_300	0.06592500	3
Level_450	0.07322500	4

Table 3d. Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	1	2	3	4
1		0.0003	0.0001	0.0001
2	0.0003		0.0013	0.0001
3	0.0001	0.0013		0.0428
4	0.0001	0.0001	0.0428	