

UNIVERSITY FOR DEVELOPMENT STUDIES

**EFFECT OF COWPEA VARIETY AND PHOSPHATE FERTILIZER
RATE ON NUTRITIVE VALUE OF COWPEA HAULMS FED TO
DJALLONKÉ SHEEP**

BY

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[UDS/MAN/0010/13]

**THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE,
FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT
STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN ANIMAL
SCIENCE (ANIMAL NUTRITION)**

AUGUST, 2016



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

Two experiments were conducted to determine the effects of cowpea variety (V) and phosphate (P) fertilizer application rate on nutritive value of cowpea haulms fed to Djallonké sheep. In Experiment 1, the effects of three levels of phosphate fertilizer application rate (30, 60 and 90 kg P₂O₅/ha) on the *in vitro* gas production characteristics, concentration of digestible organic matter (DOM), crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) of haulms from five cowpea varieties (V) (Zaayura-SARC 4-75, Songotra-IT97K-499-35, Hewale-IT93K-192-4, IT- IT99K-573-1-1 and Asomdwe-IT94K-410-2) were studied using a 3 x 5 factorial treatment arrangements in a Completely Randomized Design. In Experiment 2, Djallonké rams grazing natural pasture were randomly assigned to haulms of two V (Zaayura-SARC 4-75 and Hewale-IT93K-192-4) of cowpea and two P fertilizer rates (30 and 90 kg P₂O₅/ha) in a 2 x 2 factorial treatment arrangement, to determine the (1) intake, (2) growth performance, (3) blood profile and (4) carcass characteristics of the rams. In Experiment 1, there was a significant V x P interaction for CP, NDF and ADF. The CP for Zaayura-SARC 4-75 and Asomdwe-IT94K-410-2 increased (P<0.044) as the P rates increased from 30 to 60 kg P₂O/ha but there was a declined, which was significant (P<0.044) for IT- IT99K-573-1-1 and similar (P>0.05) for the other varieties. The CP at 90 kg P₂O₅/ha for all V was lower (P=0.119) than that at 30 kg P₂O₅/ha. The NDF for Songotra-IT97K-499-35 and Asomdwe-IT94K-410-2 increased (P= 0.032) as the P rates increased from 30 to 90 kg P₂O₅/ha whilst the other V increased from 30 to 60 kg P₂O₅/ha and declined (P= 0.032) at 90 kg



P₂O₅/ha. Whilst all V declined in ADF with increase in P rates, Songotra-IT97K-499-35 and Asomdwe-IT94K-410-2 increased (P= 0.031). There was a significant V effect on DOM with the highest reported in Zaayura-SARC 4-75 (43.44%). The *in vitro* gas production characteristics did not differ (P>0.05) by interaction (V x P rates) or main effects (V or P). The V x P rates interaction was significant (P<0.05) for DM intake. Whilst intake increased (P=0.003) as P rate increased for Zaayura-SARC 4-75, there was a decline in Hewale-IT93K-192-4. There was a significant (P<0.05) V x P interaction for the blood urea nitrogen (BUN) and a V effect on globulin. There was a significant (P= 0.039) V x P rate on carcass length whilst live weight at slaughter, dressed weight, chuck, leg, loin, rib and flank and shoulder all differed by P rates only. Except for liver and lungs which differed (P= 0.029; P= 0.048) by P rates among the non-carcass parameters, the others were not affected (P>0.05) by the treatments. The CP, NDF and ADF of the V responded differently to the increasing P rates. The feed intake, carcass and non-carcass characteristics as well as BUN were also affected (P<0.05) by the treatments. It can be concluded that nutrient concentrations of cowpea haulms were influenced by different P rates and varieties with favorable effects on growth, haematology and carcass composition of lambs. Varieties Zaayura-SARC 4-75 and Hewale-IT93K-192-4 at P rates at 90 kg/ha are recommended to enhance growth performance and carcass yield of the Djallonké lambs.



ACKNOWLEDGEMENTS

I am very grateful to the Almighty God for his protection and guidance from the beginning of the course to the successful completion of this thesis. I owe him an unending gratitude for bringing me this far. My profound gratitude goes to Dr. Terry Ansah and Prof. Herbert K. Dei, my project supervisors for whose efforts both spiritually and physically, patience, constructive criticisms and suggestions have brought the study to a successful end. I say God shower his blessings on them and their families that they may execute such a fatherly care on those students that may find themselves under their care in all aspects of life in the near future. My singular gratitude goes to Dr. Asamoah Larbi of IITA, Africa RISING Project. I thank all lecturers in the Department of Animal Science for their support and encouragement.

I would like to thank Mr. Cudjoe Shadrack, Mr. Aminu Mumuni, Mr. Bawa Abdul-Aziz and Mr. Abu Alexander for the help they provided me during my data collection. To Mr. Samuel Addy and Mr. Ebenezer Nattey, I cannot complete this work without acknowledging the assistance you offered me during the blood collection. To UDS Meat Unit workers, I say may God bless you all for your effort towards the success of this work.

My special thanks go to IITA (Africa RISING project) for sponsoring this research work.

My final thanks go to my parents, Mr. and Mrs. Robert Alagma Akolgo and uncle Mr. John Azure Akolgo for their care and support; and may God bless you all.



DEDICATION

This thesis is dedicated to my beloved parents, Madam Asibi Akolgo and my father Mr. Robert Alagma Akolgo for their unending prayers and support in my education.



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LIST OF ABBREVIATIONS

ADF: Acid detergent fibre

ANOVA: Analysis of variance

AOAC: Association of Analytical Chemists

ADWG: Average daily weight gain

ADG: Average daily gain

BUN: Blood Urea Nitrogen

CP: crude protein

CRI: Crop Research Institute

CSIR: Council for Scientific and Industrial Research

DM: Dry Matter

DMD: Dry Matter Digestibility

DMI: Dry Matter Intake

FAO: Food and Agriculture Organization

H₂SO₄: Sulphuric Acid

HCL: Hydrochloric Acid

IITA: International Institute of Tropical Agriculture



IVDMD: *In vitro* Dry Matter Digestibility

IVOMD: *In vitro* Organic Matter Digestibility

NDF: Neutral Detergent fibre

NaOH: Sodium Hydroxide



CHAPTER ONE

1.0 INTRODUCTION

Availability and quality of feed all year round is a major constraint to small-holder ruminant production in Ghana. Ruminants survive on crop residues and unimproved sward deficient in nitrogen, minerals and energy which affects feed intake, feed utilization and animal productivity (Antwi *et al.*, 2014). The deficiencies in these roughages can be overcome partly by nitrogen supplementation.

Leguminous fodders are promising and cheap source of nitrogen for use by smallholder livestock farmers. Cowpea haulms have been shown to increase microbial nitrogen supply in calves fed a basal diet of teff straw (Abule *et al.*, 1995). Intake of maize stover, degradation and ammonia concentration in ewes were improved when cowpea haulms were offered as supplement (Chakeredza *et al.*, 2002).

The cultivation of cowpea as food is very common among crop farmers in most tropical countries including Ghana. Several breed improvement attempts have been made on cowpea (CSIR-SARI, 2012). Breeding and agronomic studies in Nigeria have identified some promising dual-purpose varieties that call for further nutritional investigations to ensure their proper characterization (Singh *et al.*, 2003). Such varieties have been bred for various traits such as improved grain, better pest resistance and drought tolerance, and their straws could be sold to account for between 30 and 50% of grain incomes (Singh and Tarawali, 1997). This has always been at the neglect of cowpea fodder quality. There is the need to



do more advance cowpea breeding programmes to select for fodder quality since most small holder farmers rely on crop residues for ruminant feeding in times of feed shortage.

Application of phosphate (P) fertilizer has been shown to increase grain yields Turk *et al.* (2007), but there is limited information on its effect on the feed value of the haulms. Phosphate fertilization has been found to affect crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) quality of fodder from leguminous crops (Thomson *et al.*, 1992; Turk *et al.*, 2007; Ylmaz, 2008; Larbi *et al.*, 2010).

The basic information required about any feed resource for possible use as animal feed is its chemical composition. Information on the DM, CP, NDF and ADF are some of the attributes required to select a particular feed for its feeding potential. However, chemical assessment of feed for its nutrient content fails to provide information on feed degradation characteristics which determines its utilization and in turn the performance of animals (Blummel *et al.*, 1997). *In situ* nylon bag Orskov *et al.* (1980) and *in vitro* Menke and Steingass (1988) techniques have been used extensively for measuring ruminal degradation of feedstuff and predicting digestible organic matter because of their high degree of correlation with *in vivo* digestibility values (AOAC, 1990).

The main objective of this study, therefore sought to investigate the effect of phosphate fertilizer application rates and cowpea variety on *in vitro* digestibility and growth performance of Djallonké sheep.



1.1 Specific Objectives

The specific objectives were to determine:

1. the nitrogen, NDF and ADF composition of the cowpea haulms as affected by 5 improved cowpea varieties and 3 Phosphate fertilizer application rates (30, 60 and 90 kg/ha).
2. the effect of variety and rates of Phosphate fertilizer application on *in vitro* gas production and organic matter digestibility of the cowpea haulms.
3. the effect of supplementing Djallonké sheep with the cowpea haulms on growth, carcass and blood metabolites concentration.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Ruminant Feed Resource in the Tropics

There are many feed resources in the tropics that can be used for feeding ruminant livestock. In the tropics, small ruminants are almost entirely dependent on grazing on natural pastures, with its extreme seasonal variation in quantity and quality. Most farmers practice supplementary feeding, using crop residues in the dry season. In the tropics, animals are fed on crop residues in the form of groundnut tops, maize cobs, by-products from grain winnowing, cowpea pods and peels of plantain and cassava (Ansah *et al.*, 2014).

2.1.1 Pastures

A pasture is an area of land covered with forage crops usually grass and legumes, used for grazing (Oppong-Anane *et al.*, 2008). Forage crops are grasses or legumes grown mainly for feeding ruminant livestock. Quality pastures are the powerhouse of any livestock enterprise. Healthy pastures mean healthy animals, an important goal for landholders of any scale. In Ghana, grasses used for establishing pasture include: Guinea grass (*Panicum maximum*), Bahama grass (*Andropogon gayanus*), elephant grass (*Pennisetum purpureum*), giant star grass (*Cynodon plectostachyus*) and carpet grass (*Axonopus compressus*) (Addo, 2007). Leguminous plants suitable for pastures include: centrosema (*Centrosema pubescens*), stylo (*Stylosanthes gracilis*), tropical Kudzu (*Pueraria phaseoloides*) and pigeon pea (*Cajanus cajan*) (Addo, 2007). Good pasture management is crucial in animal production since differences between a well-managed and



poorly-managed forage programme often show up in animal performance, milk production, conception rates and fewer days of feeding stored forage (Arseneau, 2010).

There are two main types of pastures namely natural/native pastures and artificial/man-made/cultivated pastures. Natural pasture/rangeland is an area of land covered with forage crops with little or no human management. The forage species may be annual or perennial. Overgrazing is common as no proper maintenance is done. The nutritive value is usually low. The forage species are those that can tolerate fire and are more adapted to the environment. According to Oppong-Anane *et al.* (2008) small ruminants (sheep and goats) are almost entirely dependent on the grazing of natural pastures and rangeland, comprising of Savannah woodlands and unimproved pastures which constitute 45% of the total land area (MOFA, 2011), with extreme seasonal variation in quantity and quality. Table 1 shows the main sources of feed for sheep and goats in the various ecological zones of Ghana.



**Table 1: Main Feed Sources for Sheep and Goats by Ecological Zones
(Percentage of Respondent)**

Feed Sources	Sudan Savannah		Guinea Savannah		Derived Savannah		Forest		Coastal Savannah	
	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat
Natural pasture	100	100	64.1	71.4	71.7	52	38.1	39.7	46	36
Shrubs	-	-	6.1	3.6	11.5	11.4	8.2	6.3	32.3	8.7
Wheat bran	-	-	2	1.8	3.8	2.9	-	-	3	4.3
Rice bran	-	-	10.2	5.4	2.4	2.4	-	1.6	2.3	2.2
Maize bran	-	-	2	5.2	1.3	17.1	22.2	12.8	1.2	1
Pito mash	-	-	2	3.6	-	2.9	-	-	-	-
Cassava peels*	-	-	10.4	3.6	5.4	5.7	26	30	15.2	47.8
Cassava leaves*	-	-	3.2	5.4	3.9	2.9	2.8	4.8	-	-
Plantain peels	-	-	-	-	-	27	27	4.8	-	-

Source: Oppong-Anane *et al.* (2008). Cassava as animal feed in Ghana: Past, Present and Future (2013). Dash sign (-) indicates feed items not being major feed sources.

From Table 1, Natural pasture is the main source of feed for rearing small ruminants (sheep and goats) in all the ecological zones of Ghana. Natural pastures can be improved by the application of manure or the introduction of legumes Oppong-Anane *et al.* (2008) to improve the nitrogen status of the soil.

Man-made pastures, unlike natural pastures, are planted and taken care of by man. Management practices like weed control, fertilizer application, irrigation, reseeding, rotational grazing and using the correct stocking rate during grazing are adopted.



Ghana has about 8,500 ha of sown pastures according to Oppong-Anane (2006) including rangelands over-sown with *Stylosanthes hamata* and *Andropogon gayanus* as well as forages introduced into plantation crops. Although the rate of establishing pastures in the country has generally been low, the role of sown pastures in supplementing grazing from natural pastures, and in particular during the dry season, is well acknowledged by both commercial and smallholder farmers.

2.1.2 Crop Residues and Agro-industrial By-products

Millions of tonnes of cereal straws are produced annually on agricultural fields in Ghana. Oppong-Anane (2006) reported that, about 8,000,000 metric tonnes of cereal stalks and 3,500,000 metric tonnes of residues from roots and tubers are produced annually in Ghana. Of these, only a small fraction of the available crop residues is actually fed to livestock. Assessing the major crop residues and agro-by-products generated and their estimates and how they can be used to bridge the feed shortages in the dry season is of great importance.

A study carried out by Issaka (2014) in the Kumbungu district of the northern region revealed that a total average of 821.6 kg of crop residues was produced from maize, rice and millet. Crop residues therefore assume great importance in ameliorating the feed deficit in animal nutrition in Ghana. However, some crop residues have limited feeding value such as bulkiness, high fibre, poor degradability in the rumen, low nitrogen and minerals resulting in very low intakes; alternatively, could be used as fuel wood and thatch (Issaka, 2014; Ansah *et al.*, 2014; Osuji *et al.*, 1995). In addition, crop residues generate low levels of



ammonia (NH₃) in the rumen upon fermentation (Orskov, 1995). However, better utilization or improvement of the feeding value of crop residues can be achieved by physical and chemical treatments Liu and Orsko (2000) or supplementation with conventional feedstuffs such as oilseed cakes (Selim *et al.*, 2004).

Different agro-industrial by-products and/or crop residues have been used for feeding ruminants over the years. Obese (1998) fed soya bean cake to Djallonke sheep as supplement with other by-products. The treatments were 200 g of cassava peels and 100 g of soya bean cake (T1), 200 g of cassava peels and 100 g of groundnut haulms (T2) and no supplement (T3) as control under on-station trial conditions. The result obtained indicated that the average daily supplementary feed intake did not differ significantly between T1 and T2. However, T1 had the highest mean weight gain (2.96 kg) while T3 had the lowest (0.96 kg). The average daily weight gains were 48 g, 32 g and 16 g for T1, T2 and T3 respectively. On – farm studies of the same treatments showed similar trend but higher values were obtained for the soya bean cake supplemented sheep, with an average daily weight gain (55.56 g/day) significantly higher than that of the control group of 22.2 g/day (Obese, 1998). However, the soya bean cake is more costly than other by-products such as groundnut haulms and cassava peels (Karbo *et al*, 2002). Baiden *et al.* (2007) reported that dry matter intake, daily weight gains and feed conversion efficiency (FCE) were not influenced in sheep and goats when fed different levels of cassava pulp as a replacement for cassava peels. The treatment diets were; 0%, 15%, and 30% cassava pulp. However, total dry matter intake was significantly higher in sheep than in goats.



Other by-products like rice straw (Otchere *et al*, 1977; Karbo *et al*, 2002) and cocoa husk (Otchere *et al*, 1986), cassava and groundnut haulms (Addah, 1999) to feed ruminants have been recommended as good supplementary feed for ruminants.

2.2 Importance of Crop Residues and Agro-industrial By-products

In the savanna zone of Ghana, natural forages become scarce in the dry season due to outbreak of bush fires and the small dried fodder left also loses its nutritive value. This makes the animals lose body weight during the period and creates a cyclic body weight gain and weight loss in the rainy and dry seasons respectively (Otchere *et al.*, 1986). To break this cycle, animal scientists over the years have identified feed supplementation, which has proved successful particularly with sheep. However, the use of staple cereal grains leads to competition between humans and animals and increases the cost of supplementation making it unprofitable and unsustainable in livestock production (Otchere *et al.*, 1986; Croston and Pollot, 1994; Karbo *et al.*, 2002). Indeed, there is the need to sustain supplementation by exploring cheaper sources of supplementary feed.

Crop residues make up a major component of livestock diets in mixed crop–livestock systems and therefore, improving the use and nutritional quality of crop residues is important to enhancing farm productivity and profitability (Ansah *et al.*, 2014). Ranjhan (2001) stated that crop residues (straws and stovers) and agro-industrial by-products alongside with grazing and scavenging will remain important sources of feed ingredients for livestock production. Related to this, crop residues such as groundnut haulms, potato and cassava peels and leaves have



been recommended as good supplementary feedstuffs that can be used in the dry season to supplement the dry and fibrous fodder (Koney, 2004). Much work has been done with the use of crop residues and some industrial by-products as supplementary feed for sheep in particular. Among these are; rice straw (Otchere *et al.*, 1977; Karbo *et al.*, 2002), cotton seed (Dzoagbe, 1998; Avornyo *et al.*, 2001), pigeon pea waste (Agbolosu, 1997) and Soyabean cake (Obese, 1998) pito mash, shea nut cake (Ansah *et al.*, 2006), cassava and groundnut haulms; Addah, 1999). These have been recommended as feeds for dry season supplementation in Ghana (Karbo *et al.*, 2002).

Cowpea is valued for its potential to produce high levels of fodder for livestock in addition to grain for people in the tropics. A number of studies have been conducted to evaluate additions of cowpea fodder as a supplement to poor quality hay and stover. Cowpea haulm addition improves nutrient supply and growth of livestock over the use of low quality forages alone but degree of weight change varies relative to total nutrient supply (Ngwa and Tawah 2002; Baloyi *et al.*, 2006; Baloyi *et al.*, 2008). It should be noted that only a limited number of studies report the specific variety of cowpea used and animal response has been reported to differ with variety and its associated forage quality (Anele *et al.*, 2010). Singh *et al.* (2003) reported higher weight gain in rams supplemented with the cowpea haulms of variety IT90K-277-2 compared to Akinlade *et al.* (2005) who reported increased milk yield in cows supplemented with cowpea haulms of variety IT96D-716. Residues of cereal crops are generally nutritionally inadequate to produce high yields of meat and milk. The greater nutritional quality of legume residues



allows them to be used as a supplement to livestock diets fed on cereal stover and other low-quality forage as basal diet. One benefit of the use of cowpea and other legume fodders as a supplement is the provision of nitrogen to the rumen microbes, allowing them to improve utilization of the low quality forage. Energy intake is improved by both the addition of a higher energy feed (cowpea) and by increasing the availability of energy through increased digestibility of the lower quality forage. At some level of supplementation, nitrogen becomes surplus to available carbohydrates for microbial growth and additional nitrogen may be wasted. Therefore, it is important to optimize ruminant diets to maximize digestibility with minimum nitrogen wastage. An example of this diet phenomenon is found in the study of Koralagama *et al.* (2008) who fed either 150 or 300 g/d haulms from either a forage- or dual purpose-type cowpea to Ethiopian sheep fed a basal diet of maize stover. Dietary nitrogen was increased by cowpea haulm addition and higher levels of cowpea feeding resulted in higher nitrogen intakes. Total feed intake increased with increasing levels of cowpea supplementation but, while diet digestibility was greater for diets containing cowpea haulms, it did not differ between the levels or types of cowpea. The results of the study indicated that nitrogen level for the lower levels of cowpea supplementation likely matched the needs of the rumen microbes for the type of carbohydrate found (fiber) in these diets. This is also supported by increased urinary nitrogen excretion in sheep fed cowpea at 300 g/d compared to 150 g/day, indicating that some nitrogen was likely leaving the rumen as ammonia nitrogen



rather than being incorporated into microbial cells. Sheep in these studies gained about between 32 and 51 g/d when supplemented with cowpea.

2.3 Quality and quantity of Feed Resources

The productivity of livestock depends on the availability, accessibility and the amount of feed resources. Ruminants in Ghana are mainly produced on the open range (Oppong-Anane, 2011). Herbage growth depends on the natural weather conditions. During the rainy season (March to August, September to December) in the forest belt and a single peak rainfall (May to September) in the northern savannah areas, there is fast growth of edible herbage which is nutritionally adequate and accessible to livestock. The crude protein content (herbage) is about 8-12% of dry matter, but drops to 2% in the dry season (Oppong-Anane, 2006). As herbage matures, there is rapid lignification and a reduction of the crude protein content resulting in depressed (1) voluntary intake and (2) digestibility (Ansah *et al.*, 2010; Gatenby, 1991).

Also in the dry season, most forage plants dry out and leaf fall increases leaving feed materials with a high stem to leaf ratio. Bush fires destroy a good quantity of the grazing materials during the dry season (AIS Technical Bulletin, 1995).

Gatenby (1991) stated that in the dry season when there is prolonged period of below average rainfall, there is to shortage of feed, which compells animals to feed on the vegetation that they would normally reject leading to animals having poor conception rate and offsprings experience stunted growth.

The animals suffer severe nutritional stress in the dry season when the natural pasture is of low nutritional value and usually in short supply (Valizadeh and



Sobhanirad, 2009). During this time of the year, animals waste a lot of energy as they have to walk long distance in search of food and water. Shortage of water leads to slow rate of growth and low milk production, all these lead to high economic losses to the smallholder farmer (Chesworth, 1992).

According to Agbolosu (1997) in the rainy season, there is usually plenty of green forage for livestock feeding, but small ruminants suffer the consequences associated with tethering.

Tethering is common because, during this period, there is an inevitable conflict between livestock rearing and crop farming, as such animals suffer from lack of adequate feed in the midst of the abundance. During the dry season, there is lack of adequate water and good quality feed and also some quantity of grazing material is destroyed by bush fires (AIS, 1995).

Observation made by AIS (1995) showed that, animals gain weight during the rainy season only to lose weight during the dry season. Live weight losses of up to 11% for cattle Rose (1961) and 15% for sheep Otchere *et al.* (1997) have been reported. AIS (1995) also reported that, little or no deliberate effort is made by most farmers at conserving feed or improving its quality for traditional feeding to their animals in the dry season. As a result of lack of adequate feed during the dry season, animals usually perform poorly in terms of production, weight gain and reproduction.

Feeding of ruminant livestock is aimed at ensuring maximum requirement for optimum production (Ranjhan, 2001). Therefore, there is the need to exploit ways of dealing with the feed situation to help boost livestock production in the country



especially in the northern region. Some of the measures include; conservation and preservation of forages or fodder either from an excess natural growth or from crop residues such as agro-industrial by-products (AIS, 1995). Practicing of supplementary feeding involves deliberately giving additional feed to animals to compensate for the low quality and quantity pastures (Wright *et al.*, 1985). If small ruminant farmers are expecting high reproductive rates, the nutrients needed for this must be given through supplementary feeding, since the objective of these supplements is to ensure additional supply of nutritional elements to allow them to develop to target levels (Chenost and Kayouli, 1997). For example, cotton seed can be used as a supplement to increase protein and energy density in the diets of ruminants while reducing the cost of production (Solaiman, 2007).

Improvement of the quality of cereal crop residues such as maize stalks, rice straw and maize husks is done by treating crop residues with fertilizer grade urea which naturally contains about 46% nitrogen. The 4% weight by weight (W/W) treatment has been widely recommended (AIS, 1995). However, its practice is currently declining because of the high price of urea and its associated scarcity. The practice of combined feeding with relatively high nitrogen content such as leguminous crop residues (groundnut tops and cowpea vines etc) and agro-industrial by-product (cotton seed and rice straw) is gaining significance in the feeding of livestock in the dry season which help boost production (Issaka, 2014).



2.4 Effect of Dietary Supplementation on Dry Matter Intake and Nutrient Digestibility of Small Ruminants

Feed supplementation has been identified as a requirement for a higher turnover in livestock production. In General, the response of sheep and goats to any supplementary feeding depends on the qualities of the natural pasture and the feed supplement (Wright *et al.*, 1985). The critical period for supplementation in most parts of Ghana is between January and April when forages and water are in acute shortage (Amoako, 2008). Supplementing poor quality diet to a protein level of at least 7% will increase feed intake and animal productivity (Minson and Milford, 1967).

According to Crowder and Chedda (1982) supplementation with energy or nitrogen during long periods of nutritional inadequacies is of considerable importance for animal survival, it results in improved body status of animals either by minimizing live weight loss or by increasing body weight during periods of stress. Supplementary feeding of livestock with readily available agricultural by-products has been identified as a pre-requisite for higher turnover in ruminant livestock production (Wright *et al.*, 1985).

Supplementation is the main feeding system practiced by farmers during the dry season using crop residues, urea treated straw, agro-industrial by-products, browse plants and forage tree legumes (Ansah *et al.*, 2010; Issaka, 2014). The practice can ease feeding problems. However, these methods which are employed do not address the problem to a considerable extent. This is because, there is scarcity of labour during periods when the feed resources have to be gathered and the poor



nutritive value of range grasses particularly in protein and inadequate supply of feedstuffs throughout the dry season because of bush burning, lack of storage facilities and use of some crop residues as fuel wood (Ansah *et al.*, 2010; Issaka, 2014). One way of improving the quality of an animal's diet is to supplement it with other resources which are richer in energy and protein and/or superior in digestibility or intake (Renard, 1997). Feeding of energy and protein supplement is known to enhance the utilization of poor quality feeds like crop residues by maximizing roughage degradation and optimizing rumen microbial protein synthesis (Anderson, 1978).

According to Njwe and Olubajo (1992) the intake of forage or straws is improved by the intake of concentrate supplement. Dry matter intake and digestibility of grass was increased when soya bean meal was offered as a supplement in sheep. The improved intake and digestibility was attributed to the increased supply of degradable protein to the rumen microbes (Jamie *et al.*, 2009). An increased intake of cell content was recorded when the concentrate levels in the diet of goats were increased (Njwe and Olubajo, 1992). Related to the concentrate intake, an increase in the level of groundnut cake and cassava flour in goats ration led to an increased live weight gain. Goats that were kept on 0 to 50 g concentrate supplement lost weight due to inadequate energy and protein intake (Njwe and Olubajo, 1992). The DM intake of growing goats was reported as 334.25 g and 337.00 g when the goats were fed maize stovers supplemented with different browse leaf meal-based concentrate and cotton seed-based concentrate respectively (Ndemanisho *et al.*, 2007). In similar studies Njwe and Godwe (1988) recorded DM intake of 590.20



g/d, 699.53 g/d and 701.38 g/d for West African Dwarf sheep. The three treatment diets were; (fresh Napier grass with no supplement), (fresh napier grass supplemented with soyabean meal) and (NaOH treated soya bean pods with soyabean meal).

Jamie *et al.* (2009) reported an apparent DM digestibility of 65.2% when lambs were supplemented with different crop residues and fed fresh Bahia grass as basal diets. When maize stovers were fed to growing goats and supplemented with browse leaf meal - based concentrate and compared with cotton seed cake based concentrate, the feed intake and apparent digestibility values recorded are shown in Table 2. The digestibility of dry matter, crude protein and energy improved (Ndemanisho *et al.*, 2007). Njwe and Olubajo (1992) reported the following digestibility values in West African Dwarf Goats fed fresh guatemala grass and supplemented with cassava flour or groundnut cake; 69.90, 71.99 and 68.49% for DM, CP and energy respectively for groundnut cake supplementation and 69.90, 69.44 and 67.78% respectively, for cassava flour supplementation.



Table 2: Intake and Apparent Digestibility of Growing Goats Fed Maize Stovers and Supplemented with Different Browse Leaf Meals Compared to Whole Cotton Seed

Parameter	Treatments				
Intake g/d	LBC	ABC	MBC	GBC	CBC
DM	347	335	331	324	337
OM	316	312	305	297	316
CP	41.1	39.6	39.1	38.3	39.8
NDF	206	243	220	221	172
ADF	141	148	135	136	115
Digestibility					
DM	0.63	0.62	0.62	0.69	0.68
OM	0.66	0.65	0.66	0.71	0.71
CP	0.68	0.73	0.71	0.79	0.79
NDF	0.62	0.66	0.65	0.66	0.60
ADF	0.57	0.54	0.53	0.57	0.49
FCR	12.8	13.7	12.8	12.1	9.5

Treatment: LBC; leucaena- based concentrate, ABC; Albizia based –concentrate, MBC; Moringa-based concentrate, GBC; Gliricidia based concentrate. CBC; cotton seed- based concentrate. Source: Ndemanisho *et al.* (2007).

According to Lee (2008) forage with DM digestibility of 60 to 69% is considered as high quality forage in terms of energy supply. About 70% of the energy in these kinds of forages is degraded in the rumen by microorganisms and 30% escape to small intestines and digested by enzymes for absorption.

For all feedstuffs, ruminant animals need about 65 to 68% of the protein to be rumen degradable for adequate rumen functions and the synthesis of microbial protein (McDonalds *et al.*, 1995). If more protein is degraded in the rumen, the animal gets less bypass protein for absorption in the small intestines, and the ruminant is denied of certain essential amino acids such as lysine and methionine that are deficient in microbial protein (Lee, 2008). Also, much of the rumen degraded protein is absorbed in the form of ammonia and excreted via the urine which is a waste of protein. It is therefore necessary to have bypass protein in



every feedstuff fed to ruminants (Lee, 2008). Sewell (1993) stated that supplementation of ruminants (cattle) should be limited to 0.5 - 1% of the animal's body weight. The digestibility values of West African Dwarf sheep fed (fresh Napier grass without soya supplement), (fresh napier grass supplemented with soyabean meal) and (NaOH treated soyabean pods with soyabean meal) are shown in Table 3. Generally, the tested diets were fairly digested. The live weights gained were 41 g/d, 77 g/d and 79 g/d (Njwe and Godwe, 1988).

Table 3: Digestibility of Dry Matter and other Nutrients by West African Dwarf Sheep Supplemented with Soya Bean Meal

Nutrients %	Treatments		
	FNG	FNGS	NaOHTS
Dry matter	68.89±4.23	69.67±1.06	66.70±3.6
Crude protein	74.88±2.72	83.04±0.43	79.70±1.09
Organic matter	72.53±4.69	73.34±1.229	67.77±2.15
Crude fibre	75.29±3.82	65.36±3.66	62.13±1.58
Ether extract	59.18±5.12	83.89±2.66	83.85±2.73
Nitrogen free extract	70.13±4.78	69.18±1.98	60.89±2.51

Treatments: FNG (fresh Napier grass without soya supplement), FNGS (fresh napier grass supplemented with soyabean meal) and NaOHTS (NaOH treated soyabean pods with soyabean meal). Source: Njwe and Godwe (1988)

2.5 Weight Gain of Djallonke Rams Fed Rice Straw and Groundnut Haulms with Concentrates as Supplement

According to Konlan *et al.* (2012) animals fed diets containing T1 (62.5% rice straw and 37.5% groundnut haulms), T2 (T1 plus 11.5% shea nut cake in a concentrate supplement) and T3 (T1 plus 23% shea nut cake (SNC) in a concentrate supplement) recorded an increasing trend in ADG. Animals on T3 had the highest ADG of 37.74 g/day which was significantly higher than what animals on T2 and T1 obtained. The gain per day of T2 animals were also significantly



higher than that of T1. Animals on T3 converted the feed more efficiently than the rest as indicated in Table 4. The substitution rate increased from 0.13 to 0.45 as the SNC inclusion level increased from 11.5% to 23% in the treatment diets.

Table 4: Growth Performance of Djallonké Rams Fed Rice Straw and Groundnut Haulms with Graded Levels of Shea Nut Cake in a Concentrate Supplement

Parameter	Treatment diets		
	T1	T2	T3
Concentrate intake g/d	-	154.07	152.61
Basal diet intake g/d	439.12	418.87	370.17
Total dry matter intake g/d	439.12	572.94	522.79
Substitution rate (SR)	-	0.13	0.45
Average daily gain (g)	20.88	31.19	37.74
Weight gain / feeding period (g)	292.3	436.7	528.3
FCR (Feed /gain)	21.03	18.34	13.85
TDMI (g/kg body weight)	26.84	33.74	30.29

FCR; Feed Conversion Ratio, TDMI; Total Dry Matter Intake.

Source: Konlan *et al.* (2012)

The ADG values obtained are far below what was reported by Esien (2003) and Issaka (2006) but agrees with the Djallonké growth rate values reported by Obese (1998) and close to the findings of Ngwa and Tawa (1991) who supplemented Djallonké sheep with rice straw, groundnut haulms, cotton seed and cowpea vines and recorded an average daily weight gain of 29.24 g, 48.98 g, 52.4 g and 49.19 g/d respectively. The differences in the growth rate values are attributable to differences in nutrient composition of the basal diet offered by McDonalds *et al.* (1995), since the straw offered in this study had less nutritive value and low digestible components compared to fresh pasture grass basal diet by Esien (2003), Issaka (2006) and Ndemanisho *et al.* (2007).

Etele and Larbi (2011) reported that, bodyweight changes of West African Dwarf (WAD) sheep maintained on straw left after harvesting the grains of cowpea were



different among the varieties. The bodyweight changes ranged from the lowest for IT86D-716 to the highest for IT81D-994 with a trend of IT86D-716 < IT89KD-391 < IT86D-719 < IT81D-994 observed for bodyweight changes, in an increasing order.

Table 5: Grain Yields and Average Daily Gain of West African Dwarf Rams Fed Straw from Four Dual-Purpose Cowpea Varieties

Parameter	IT89KD-391	IT86D-716	IT86D-719	IT81D-994	Mean
Initial bodyweight (kg)	19.7	20.1	20.2	20.3	20.1
Ending bodyweight (kg)	20.3	20.3	21.4	23.6	21.4
Average daily gain (g/ d)	10	3	20	55	22.1
Dry matter intake (g/d)	612	590	815	920	734
Feed conversion ratio	61.2	196.6	40.7	16.7	78.8

Source: Etele and Larbi (2011).

Table 5 indicates that animals on variety IT81D-994 had higher daily live-weight gains compared to the other three varieties. The strong varietal differences among the cowpea varieties, in terms of bodyweight changes and grain yields, could be very useful in developing better varieties in cowpea breeding programmes (Becker and Einfeldt, 1995).

2.6 Haematology and Serum Metabolites of Sheep

An analysis of the blood of a number of ruminant livestock was done in an animal health care laboratory at Daya, India Pampori (2003) and the normal ranges and



means of the haematological profile of ruminant livestock were reported as shown in Table 6.

Table 6: Normal Heamatological Profile of Ruminant Animals

Parameters	Animal					
	Cattle	Average	Sheep	Average	Goat	Average
Hb %	8-14	11	8-16	12	8-14	11
PCV %	24-38	32	26-36	30	24-36	30
RBC (106/C)	5-9	7	5-11	8	10-18	13
WBC (103/C)	4-10	7	4-10	8	4-13	8
Glucose mg/dl	35-55	45	35-74	54.5	45-60	52.5
Serum total cholesterol mg/dl	50-130	90	100-150	125	55-200	125
Plasma protein g/dl	6.7-7.46	7.1	6.0-7.9	6.95	6.4-7.9	7.15
Plasma albumin g/dl	3.0-3.55	3.29	2.7-3.9	3.3	2.7-3.9	3.3
Plasma globulin g/dl	3.0-3.48	3.24	3.5-5.7	4.6	2.7-4.1	3.4

Hb; Haemoglobin, PCV; Packed Cell volume, RBC; Red Blood Cells, WBC; White Blood Cells.

Source: Pampori (2003).

Low albumin suggests poor clotting ability of blood and hence poor prevention of hemorrhage (Robert *et al.*, 2000). A decrease in serum globulin is an indication of reduction in disease-fighting ability of the animal's body system. This could lead to high mortality when there is an infection or outbreak of disease in the flock (Iheukwumere *et al.*, 2005). The amount of cholesterol in serum is associated with the quantity and quality of protein supplied in the diet (Esonu *et al.*, 2001). Iheukwumere *et al.* (2008) reported changes in the immune status and serum metabolites values of West African Dwarf (WAD) goats on concentrate supplementation or FSH+LH (pergonal) treatment. The change in values however, fell within the normal ranges for adult WAD goats.



Animals on high plane of nutrition have high haemoglobin and packed cell volume (PCV) count than similar animals fed poor diet (Olayimi *et al.*, 2000). On the other hand, high white blood cells count is attributed to parasite and bacterial infection among animals (Egbe-Nwiye *et al.*, 2000).

White blood cells (WBCs) are the soldiers of the body, their high count rate in the blood stream may be to increase or complement the immune system of the animal (Coles, 1980). Baiden *et al.* (2007) fed sheep and goats with different levels of cassava pulp (residue left after starch extraction) and reported the haematological profile as indicated in Table 7. They recorded a significant change in the haematology of sheep fed the pulp compared to the control. The changes were attributed to the pulp. However, there was no significant change in goats fed the pulp. The PCV was significantly higher in sheep on 15% and 30% pulp diets compared to those on the control diet. The same trend was also found in goats. Total white blood cell (WBCs) counts and total red blood cell (RBCs) counts were significantly higher in goats than in sheep. Haemoglobin (Hb) values were significantly higher in sheep on the 15% and 30% pulp diets compared to goats. Diet and animal species had no statistical effect on the WBC differential counts.



Table 7: Haematological Values of Sheep and Goats on Various Diets

Parameter	0% Pulp		15% Pulp		30% Pulp	
	Goat	Sheep	Goat	Sheep	Goat	Sheep
PCV%	29.5	28.5	29.8	34.3	32.0	34.5
WBCs x 10 ³ /μl	15.3	6.94	14.3	7.09	14.2	10.5
RBCs x 10 ⁶ /μl	14.0	8.23	13.7	10.8	14.6	11.1
Hb g/100ml	9.50	9.00	9.45	11.8	9.91	11.4
<i>Percentage distribution of leucocytes</i>						
Lymphocytes	57	57	52	49	56	46
Neutrophils	41	43	46	49	41	52
Eosinophils	2	0	2	2	2	3
Basophils	0	0	0	0	0	0
Monocytes	1	0	1	1	1	0

PCV = Packed cell volume; RBC = Total red blood cell count; WBC = Total white blood cell count Hb = Haemoglobin values; SEM = Standard error of the mean Source: Baiden *et al.* (2007).

The leucocytes in the body work as part of the body immune system and react to foreign substances (David, 2008). The components that make up leucocytes are neutrophils, eosinophils, basophils, monocytes and lymphocytes. The functions of the individual leucocytes are described as follows; neutrophils; these are more in the blood stream and have the main function of breaking down bacterial cells in the body with its enzymes stored in grains located in the cytoplasm to prevent further multiplication and infection of the body by the bacteria. The neutrophil cells die soon after phagocytosis due to the depletion of their glycogen reserves. Eosinophils stain red when microscopic slide is stained with acidic stain, the number of eosinophils increases in the blood in the presence of allergens or parasites in the animal's body. Basophils are the least of the leucocytes that stain for viewing when treated with basic stain on a slide. These cells are called to action at the beginning of inflammation in response to an injury or irritation in any





part of the body, and it is characterized by redness, warmth, swelling and pain in that place. During these processes basophils migrate from the blood stream to the site of the inflammation. Monocytes are the largest leucocytes that help devour foreign microorganism in the body and also digest dead body cells and remove unwanted cellular materials from the body. An increase in monocytes number in an animal's body is an indication of evasion of foreign microorganisms in the body or high rate of body cells death (David, 2008). Lymphocytes use the blood to travel round the body but can wander freely in other types of tissues using the lymphatic channels. An increase in lymphocytes number is in response to viral, parasitic and bacterial infection of the animal's body (Coles, 1980). Ekenyem and Madubuike (2007) reported significant differences in haematological parameters in pigs fed varying levels of *Ipomoea asarifolia* leaf meal compared to the control. The white blood cells differential significantly differed between treatments and increased as the level of the leaf meal increased in the diet and later fluctuated within the normal range. It therefore registered no deleterious effects on the haematology and serum biochemistry of the pigs. Serum cholesterol did not show any significant difference assuring the safety of the leaf meal in pigs' diet at the inclusion level of 5, 10, and 15%. Gangapadhyah (1981) observed a depression in plasma protein concentration in Milch Murrah buffaloes on replacement of concentrate mixture with 15 and 20 parts of neem seed cake and Verma *et al.* (1995) reported no effects in the level of serum total protein in growing goats on replacement of concentrate with 15 and 25 parts of water washed neem seed cake. Blood glucose levels in ruminants are considerably lower than that of non-

ruminants. They are also relatively insensitive to insulin secretion (Annison and white, 1961). The blood glucose level is expected to increase with feeding because propionate, the precursor for gluconeogenesis is produced in the rumen and absorbed after feeding (Bassett, 1975; Sano *et al.*, 1999). Glucose is required for maintenance of nerve tissues, retina, germ and epithelial cells as well as synthesis of lactose in the animals' body (Bolukbasi, 1989). Trinders (1969) reported mean glucose levels of $51.18 \text{ g/dl} \pm 3.24$, $50.33 \text{ g/dl} \pm 2.43$ and $46.89 \text{ g/dl} \pm 5.32$ when lambs were fed with straw alone, straw + Urea Molasses Mineral Block (UMMB), and straw + UMBB + Barley respectively for 30 days. The measurements of serum cholesterol levels are useful in the evaluation of the risk of coronary arterial occlusion, atherosclerosis, liver function, intestinal absorption, thyroid function and adrenal diseases (Harper *et al.*, 1979).

2.7 Growth Performance of Djallonké Sheep

Growth and nutrition moves together in that, when an animal is poorly nourished, growth will be retarded. Live weight of Djallonké sheep from birth to 5 months of age appears to be of the order 40-50 g/day Rombant and Van Vaenderen (1976) but can be high as 80 g/day if the diet is adequately supplemented (Berger, 1983). When young animals are poorly fed, they grow poorly than matured ones.

Ngwa and Tawah (1991) observed that, Djallonké sheep averaging 22 kg live weight at 15 months of age recorded daily weight gain of 29.0 g, 48.9 g, 52.4 g and 49.2g for rice straw, groundnut haulms, cotton seed and cowpea vines respectively. Karbo *et al.* (1997) reported that, feeding Sahelian and Djallonké crosses with cassava peels and pigeon pea waste as the basal diet had an average



daily weight gains of 130 g and 87.9 g respectively. Health and Segun (1985) stated that, the growth rate of young animals is markedly affected when the nutritive value is low. Growth rate of lambs depend on sex of the lamb, the year and month of birth (Tuah and Abu, 1988). The breed of lambs also has marked influence on its growth rate (Ansaayiri, 1996). An overall growth rate of 112 g/day for Djallonké lamb over a period of 80 days has been reported with concentrate diets. Males registered a growth rate of 72.2 g/day while females had 66.9 g/day over a period of 80 days on the same concentrate. Ram lambs grow faster than ewe lamb whether the diet is restricted or not (Ansaayiri, 1996). The most important determinants are the feeding level, genotype, sex, health and management (Mike, 1996).

2.8 Cowpea Production

One of the most important food and forage legumes in the semi-arid tropics that include parts of Asia, Africa, Southern Europe, Southern America and Central America is cowpea (*Vigna unguiculata* (L.) Walp) (Singh, 2005; Timko *et al.*, 2007).

Allen (1983) stated that cowpea was introduced from Africa to Indian sub-continent approximately 2000 to 3500 years ago. One view also postulates that the Transvaal region of the Republic of South Africa was the centre of speciation of cowpea due to the presence of primitive wild species (Padulosi and Ng, 1997). Ng and Marachel (1985) also reported that the centre where maximum diversity of cultivated cowpea is found is in West Africa encompassing the Savannah regions of Nigeria, Southern Niger, parts of Burkina Faso, Northern Benin, Togo and



South-eastern parts of Cameroon. Presently, cowpea is cultivated throughout the Tropics and Sub-tropics of the world.

Cowpea has been considered as a vital leguminous crop largely in the tropical region of the world (Blade *et al.*, 1997). It is also considered as one of the most important pulse crops, providing a significant proportion of the dietary protein of people and plays a pivotal role in terms of nutrition in developing countries of the tropics and subtropics particularly in Sub-Saharan Africa (Rachie, 1985). In Ghana, cowpea is the most important legume on the basis of area cultivated. The bulk of production of cowpea comes from the Northern part of the country although it can be grown in all the agro-ecological zones of the country (CSIR-SARI, 2012).

Sub-Saharan Africa accounts for 84% of the World's cowpea grain production. Nigeria produces more than 45% followed by Niger which is nearly 15% of the world's cowpea grains of 6.7 million metric tons produced each year covering an area of about 14.5 million hectares (MoFA, 2010). In Ghana, 143,000 MT of cowpea is produced annually on about 156,000ha making Ghana the fifth highest producer in Africa (MoFA, 2010). It has been projected that the rate of growth for the period between 2010 and 2020 would be 11.1% for cowpea (MoFA, 2010). Cowpea tolerates warm weather and requires less rainfall than most crops. It is particularly tolerant to drought during vegetative growth. Because the crop requires dry weather for harvesting, the bulk of production is in the dry savannah regions. Heavy rainfall encourages excessive vegetative growth and disease incidence becomes high. Though cowpea is grown on a range of soil types, they



are best adapted to well-drained sandy loamy soils. Cowpea is sensitive to water logging conditions that commonly occur when cultivated on heavy clay soils (SARI, 2012). Depending on the cultivar and the environment, cowpea may take from about 60 to 240 days to produce mature seeds.

2.8.1 Importance of Cowpea

Cowpea is a major source of livelihood to many millions of people in less developed countries of the tropics. Cowpea is of great nutritional value and provides high amounts of dietary fibre, protein and mineral elements (Ayodele and Yawla, 2004; Mullen, 2005). Cowpea grains are valued for their carbohydrates and protein levels which have been reported as 60% and 25% respectively (Rathore, 2005). Cowpea is the most important indigenous food legume crop in Africa (Geonaga *et al.*, 2008). Cowpea is consumed in many different forms which comes with many local variations in their preparations, most frequently they are cooked together with vegetables, spices and palm oil to produce a thick bean soup which is accompanied by a basic staple food such as cassava, yam and plantain (Valenzuela and Smith, 2002). The protein in cowpea seed is rich in amino acids (lysine and tryptophan) compared to other cereal grains; however, it is deficient in methionine and cysteine when compared to animal protein (Rathore, 2005).

Cowpea can be used at all stages as a vegetable crop. The tender green leaves are an important food source in Africa and prepared as a pot herb like spinach. Immature snapped pods are used in the same way as snap beans, often being mixed with other foods (Valenzuela and Smith, 2002). The seed or grain as it is





sometimes referred to is the most important part of the cowpea plant for human consumption. The seeds are most often harvested and dried for storage and consumption at a later time, either after cooking whole or after being milled into a flour product and used in various recipes (Nielsen *et al.*, 1997; Ahenkora *et al.*, 1998). In Ghana, cowpea is used for various kinds of dishes which include: “waakye” ie beans cooked with rice, cooked beans with gari, in the north cowpea is used for variety of dishes such as “koose”, “obkore” and “cable” (MoFA, 2010). In addition to human consumption, cowpea leaves and stems (stovers) are also an important source of high-quality hay for livestock feed (Tarawali *et al.*, 1997). The haulms are used to feed livestock particularly during the dry season (Blade *et al.*, 1997).

The symbiotic fixation of atmospheric nitrogen into the soil by the help of root nodule bacteria *Bradyrhizobium* spp. is another important feature of cowpea. Many experimental findings indicate that soil nitrogen levels increased following cultivation of cowpea (Singh and Tarawali, 1997). Some cowpea varieties also help prevent the germination of *Striga hermonthica*, a devastating parasitic weed that occurs in most cereal farms in Africa (Singh and Tarawali, 1997). The above ground parts as well as the roots serve as organic matter and nutrient source for the soil after harvesting the grains (Duke, 1978). Cowpea is grown for soil improvement as it is a good green manure crop when intercropped with cereals and used as a cover crop. It is also rotated with cereal crops to gain maximum benefits from the improved soil conditions resulting from the cowpea crop (Singh and Rachie, 1985).

It is also a valuable component of farming systems in areas where soil fertility is low. This is because cowpea has a high rate of nitrogen fixation Elawad and Hall (1987), forms effective symbiosis with mycorrhizae Kwapata and Hall (1985) and has the ability to better tolerate a wide range of soil pH when compared to other grain legumes (Fery, 1990).

2.8.2 Cowpea Variety Improvement Attempts

Conventional breeding of cowpea has been in place since the 1970s though full-fledged research undertakings started at the Council for Scientific and Industrial Research- Savannah Agriculture Research Institute (CSIR-SARI) in 1981, using local diversity and elite lines from International Institute of Tropical Agriculture (IITA) collection (CSIR-SARI, 2012). The germplasm pool in the early breeding program emphasized on higher grain yields and earliness. Hence varieties that were introduced or developed were early maturing with improved yields over the local types. Recently these have been replaced by other important traits such as field and storage pests, seed coat color, cooking ability, intercropping, and drought and *Striga* tolerance (CSIR-SARI, 2012). Variety development is a well-established scheme in research, where on average each variety has taken 6 to 8 years in its development (ICRISAT, CIAT and IITA, 2012).

Table 8 gives the characteristic features of the cowpeas varieties that have been developed by the Ghanaian research system.



Table 8: Characteristic Features of Common Cowpea Varieties Developed by the Ghanaian Research System

Varieties	Year of release	Source of Material	Genetic background (parentage, pedigree, ancestry)	Yield Potential kg/ha	Growth Habit	Maturity date	Characteristics
Zaayura	2008	SARI Cross	SARC 4-75	600	Erect	-	Resistant to aphids, high fodder and grain yields
Songotra	2008	IITA	IT97K-499-35	600	-	69-75 days	High grain yield, Striga resistant, high yielding
Hewale*	NA	IITA	IT93K-192-4*	3130	Semi-erect	64-72 days	-
IT*	NA	IITA	IT99K-573-1-1*	350	Erect	-	Grain yield, early maturing, combined resistance to Striga and Alectra
Asomdwe*	NA	IITA	IT94K-410-2*	2863	Semi-erect	65-72	-

Source: Crops Research Institute (2010). *NA= Not Officially Released.





From 2005 to 2007, fourteen advanced breeding cowpea lines developed by SARI and the University of California, Riverside were tested in a participatory multi-location trials following a mother-baby trial methodology (De Groote *et al.*, 2002). To assess the adaptation of the new varieties to farmer's conditions, 14 advanced breeding lines and check cultivar "Apagbaala" were tested across the predominant cowpea growing belts in the Upper West, Upper East and Northern Regions of Ghana. Evaluations were done in 2006 and 2007. In all 126 farmers were involved in over 12 districts. Each farmer tested 3 varieties in addition to his currently used variety.

Six of the lines were submitted to the National Variety Release Committee of Ghana (NVRC) for assessment. In 2008, four lines with potential to tolerate major field pest were officially released by the NVRC for cultivation. Currently, these cultivars have assumed prominence among varieties used by cowpea seed producing companies and institutions in Northern Ghana.

The results indicated that there were wide variations in the yield of each genotype among farmers in a community, district and across the 3 regions. The performance of the six proposed varieties was generally higher than "Apagbaala" and the farmers' varieties. In general farmers appreciated the new varieties for their large white seeds, earliness to mature and resistance to *Striga gesnerioides*. In almost all farms visited, farmers rated the new varieties better than the traditional varieties. In 2008, four of the six varieties proposed for release were accepted by the National Variety Release Committee. They were released as Songotra (IT97K-

499-35), Bawutawuta (IT95K-193-2), Zaayura (SARC4-75), and Padi Tuya (SARC 3-122-2).

Despite all these efforts made in breeding for the best cowpea variety, there is the need to do more advance cowpea breeding programmes to select for fodder quality since most small holder farmers rely on crop residues for ruminant feeding in times of feed shortage.

2.8.3 Factors Affecting Cowpea Production

Despite all the beneficial uses of cowpea, there are numerous constraints that impede the optimal utilisation of the crop. Diseases induced by various pathogenic groups, including fungi, bacteria, viruses, nematodes and parasitic flowering plants, are considered as important constraints to cowpea production (Emechebe and Lagoke, 2002). Cowpea Production is limited due to several abiotic and biotic constraints. Prominent among the biotic stresses are insects which attack the crop from the vegetative stage to flowering, podding and storage. These include aphids, pod borers (*Maruca vitrata*), pod-sucking bugs (*Clavigralla tomentosicollis*) and flower thrips (Jackai and Adalla, 1997).

Similarly, after harvesting when the seeds are stored, seed deterioration can occur as a result of physical (temperature, humidity), biological (fungi, bacteria, insects, rodents) and technical (method and duration of storage) factors (Appert, 1987). When seeds are stored in conditions where high relative humidities and high temperatures prevail, certain fungi produce mycotoxins. These mycotoxins when ingested after consuming infected seed, can lead to adverse health conditions for both animals and humans (Moss, 1996).



Attempts at crossing wild *Vigna* species with cowpea in order to breed for insect resistance have been futile (Fatokun, 2000; Singh *et al.*, 2000). The use of chemical pesticides for the control of insects is associated with several risks and damage to human health and environment.

Genetic transformation offers new possibilities of transferring useful genes from other sources into cowpea to address these yield constraints.

However, the use of gene technology in cowpea improvement has been hindered by the lack of an efficient transformation technique (Popelka *et al.*, 2004).

2.9 Effect of Phosphate Fertilizer and Variety on Grain Yield and Fodder

Quality

Phosphorus is an essential macro nutrient for plant growth and it is limiting crop production in many regions of the world. Deficiency in this macro nutrient leads to stunted and sticky looking plants that produce lower quality fruits (Gascho and Davis, 1997).

Nutrient management is an important component of cropping systems. Phosphorus nutrition is essential for improving productivity of smallholder agriculture in Sub-Saharan Africa (Snapp, 1998). Lucius (2001) showed that leguminous crops require abundant supply of phosphorus, calcium and potassium compounds, because it is needed for symbiosis with the nitrogen fixing rhizobacteria and for seed quality. On global scale, phosphorus is probably the most deficient element for legume production (Gascho and Davis, 1997). Application of 90 kg P/ha in poor alluvial and sandy soil increased groundnuts yield by 38% (Tran, 2003).



In general, cowpea production is relatively less sensitive to soil fertility than other crops, mainly because cowpea is very efficient in obtaining nutrient from the soil and exploiting residual fertilizer from previous crop in rotation (Yayock *et al.*, 1988). Lack of phosphorus fertilization of cowpea is considered as one limiting yield factor common to several soil types (Tran, 2003).

A research conducted by John (2010) revealed that, phosphorus fertilization of groundnuts influenced the number of pods per plant, seed yields, number of branches per plant and haulm biomass. Phosphorus rates up to 40 kg P/ha increases seed yield of groundnuts and then declined as the phosphorus level was increased to 50 kg P/ha.

A study conducted by Turk *et al.* (2011) to determine the effects of five phosphorus rates (0, 30, 60, 90 and 120 kg/ha) and three harvesting stages (beginning of flowering, full flowering, and seed filling) on forage yield and quality of sainfoin with respect to dry matter (DM) yield, crude protein (CP), N, P, K, Ca, Mg, acid detergent fibre (ADF) and neutral detergent fibre (NDF) showed that; Phosphorus rates and harvesting stages significantly affected most of the components determined in sainfoin (Table 9).



Table 9: Dry Matter Yield and Chemical Composition of Sainfoin as Affected by Phosphorus Fertilization and Harvesting Stages

	DM t ha ⁻¹	CP%	N%	P%	K%	Ca%	Mg%	Tetany K/Ca+Mg	ADF %	NDF %
Phosphorus doses, kg ha⁻¹										
0	3.90	15.67	2.51	0.17	2.47	1.52	0.26	1.48	37.02	48.86
30	4.90	18.64	2.98	0.22	2.21	1.59	0.35	1.22	32.18	42.48
60	5.95	19.35	3.10	0.25	2.01	1.82	0.45	0.92	30.37	40.09
90	7.61	19.79	3.17	0.33	1.67	1.99	0.56	0.66	28.71	37.89
120	6.75	18.56	2.97	0.37	1.58	1.64	0.47	0.78	28.30	37.35
LSD (5%)	0.27	0.41	0.07	0.016	0.05	0.152	0.048	0.063	0.62	0.82
Harvesting stages										
BF	5.09	19.85	3.18	0.30	2.18	1.28	0.46	1.35	28.62	37.78
FF	5.90	18.24	2.92	0.26	1.97	1.66	0.42	0.96	31.50	41.58
SF	6.47	17.13	2.74	0.23	1.82	2.20	0.37	0.72	33.82	44.65
LSD (5%)	0.21	0.32	0.051	0.012	0.04	0.037	0.04	0.05	0.48	0.63

BF: Beginning of Flowering, FF: Full Flowering, SF: Seed Filling, LSD: Least Significant Difference Test. Source: Turk *et al.* (2011).



Phosphorus applications had significant effects on DM yield, CP, P, K, Ca, Mg, tetany ratio, ADF and NDF contents. The highest DM yield was obtained from 90 kg/ha P rates (7.61 t/ha), while the lowest DM yield (3.90 t/ha) was obtained from the control plot (Table 9). Increasing P rate resulted in an increase in CP contents. The highest CP (19.79%) content was obtained from 90 kg/ha P treatment. However, application of more than 90 kg/ha of P decreased the CP contents. Phosphorus applications decreased K, ADF and NDF contents while they increased P, Ca and Mg contents.

The effects of harvesting stages on DM yield, CP, P, K, Ca, Mg, ADF and NDF contents of sainfoin were highly significant. The DM yield, Ca, ADF and NDF contents increased, while CP, P, K, Mg and tetany ratio decreased with advancing stages (Table 9). Phosphorus levels significantly increased forage yield and quality components of sainfoin.

Bell *et al.* (2001) reported that P is the most important fertilizer nutrient required for growing narbon vetch, and was ranked the next most important. Crude protein and N content of sainfoin increased as phosphorus fertilization increased. These confirm the results of other researchers who found positive effects of P on crude protein content of rangelands (Miskovic *et al.*, 1977; Celik, 1980; Comakli and Tas, 1996). Phosphorus treatments significantly increased P, Ca and Mg, while it decreased K, ADF and NDF content. Comakli and Tas (1996) found that P fertilization increased P, Ca and Mg contents and decreased K content of some vetch species. In this study, the DM yield significantly increased at advanced harvest stages. As plants begin to concentrate DM in pods and seeds, an enhanced forage yield with advancing maturity is consistent with results of several researchers (Munoz *et al.*, 1983; Hintz *et al.*, 1992; Osborne and Riedell, 2006). Crude

protein, P, K and Mg contents decreased with advancing stages, while Ca, ADF and NDF contents increased in the present study.

Besides CP, most minerals also decline with advancing plant development, including K and P (Rauzi *et al.*, 1969). Maturity stage at harvest is the most important factor of determining forage quality. Forage quality declines with advance maturity because of decrease in P, Ca, Mg and K content as a result of delay in cutting (Blaser *et al.*, 1986; Tan and Serin, 1996; Georgieva and Kertikov, 2006). Tan *et al.* (2003) reported that the contents of K, Mg, Ca and P decreased from 29.31– 22.04, 3.48–2.85, 12.83–11.58 and 1.50–1.19 g/kg respectively as the age of the plant increased. The changes in element content with maturity are related to the increasing stem to leaf ratio. Leaves are richer in mineral nutrients than stems Tan *et al.* (1997) and the proportion of leaves declines at maturity because of senescence of the lower leaves or damage by diseases (Albrecht and Marvin, 1995). According to the results of Turk *et al.* (2011) 90 kg/ha phosphorous fertilizer application and harvesting at the beginning of flowering are recommended for high herbage quality in sainfoin.

A field experiment conducted at Choudhary Charan Singh Haryana Agricultural University (C.C.S. HAU), regional research station, Bawal by Kumar *et al.* (2012) to evaluate the effect of phosphorus and sulphur fertilization on fodder yield and quality of cowpea (*Vigna unguiculata*) showed that, there was significant increase in green and dry fodder yield with increase in sulphur levels from 0 to 40 kg/ha. Similarly, with increase in P₂O₅ level from 0 to 60 kg/ha, there was a significant increase in green and dry fodder yield of cowpea. Application of 60 kg/ha P₂O₅ with 40 kg/ha sulphur resulted in maximum green and dry fodder yield of cowpea as compared to other treatment combinations. Crude protein, ether



extract and ash content were increased with each increment of P_2O_5 and sulphur levels while crude fibre and nitrogen free extract showed reversed trend.

2.10 Forage Evaluation Techniques

Information on the kinetics of forage digestion is important as rate and extent of digestion of feeds in the rumen to a large extent determine voluntary intake (Hovell *et al.*, 1986; Orskov *et al.*, 1988). The microbial degradation of forages in the rumen is a complex process involving complex interactions, both between the microorganisms present (bacteria, protozoa and fungi) and between the microbial population and the host (Czerkawski, 1986). Several indirect methods have been used to estimate the extent of forage digestibility. The two-stage *in vitro* technique of Tilley and Terry (1963) has been widely used in predicting forage digestibility for ruminants and for screening large numbers of forages in plant breeding programs. However, the method provides no information on digestion kinetics and measures only an end-point of digestion after 48 h. Grant and Mertens (1992) showed that the method could be modified by using only the first stage of incubation to measure *in vitro* dry matter (DM) degradation pattern with time. However, this involved destructive sampling of the contents of digestion tubes thereby limiting the number of samples that could be tested at a given time.

The Dacron bag method, in which nylon bags are suspended in the rumen of fistulated animals and removed sequentially for DM determination, can serve as a tool in supplying information on rate and extent of DM disappearance of feeds (Orskov and McDonald, 1979). However, due to cost and the difficulty of maintaining large numbers of fistulated animals, the method is not convenient for concurrent evaluation of large numbers of samples. Given that *in situ* DM disappearance measures reflect the rumen environment





involving diet and animal differences, they are therefore inherently more variable than the corresponding *in vitro* measurements of digestion (Noeck, 1985). The accuracy of the Dacron bag method is also influenced by certain technical aspects such as amount of sample in relation to bag size, bag pore size, sample particle size, the washing procedure for bags after removal from the rumen and the basal diet of the fistulated animals (Noeck, 1985; Uden *et al.*, 1974 and Van der Koelen *et al.*, 1992). Thus, for these reasons it is often difficult to make comparisons of results from different works or research centers.

The principle of determining potential rumen degradability/fermentability of a feed by measuring gas produced from a batch culture was first developed by McBee (1953) and Hungate (1966). Trei *et al.* (1970) adapted the earlier techniques by attaching a water displacement manometer to each vessel to measure the gas produced. Similarly, Jouany and Thivend (1986) and Beuvink and Spoelstra (1992) used inverted measuring cylinders to determine the volume of water displaced. Beuvink *et al.* (1992) then automated this water displacement technique.

Direct displacement of a plunger by fermenting a feedstuff within a glass syringe was developed by Czerkawski and Breckenridge (1975) and was the basis of the 'Hohenheim Gas Test' later developed by Menke *et al.* (1979). Blummel and Orskov (1993) modified the technique by incubating syringes in a water bath rather than a rotating incubator.

It has been shown that measurement of rate of gas production during *in vitro* fermentation of forages with microbial rumen inoculum can be used to assess fermentation kinetics (Theodorou *et al.*, 1991; Beuvink and Kogut, 1993; Blummel and Orskov, 1993 and Khazaal *et al.*, 1993). Theodorou *et al.* (1991, 1994) developed the pressure transducer technique (PTT) for measuring *in vitro* gas production of forages. The procedure is

inexpensive and can handle large numbers of samples. In order to determine the precision of the results obtained, factors that may affect the kinetics of gas production such as the effect of inoculum, changes in atmospheric pressure, effect of sample size and preparation need to be investigated.

2.11 Sources of Rumen Fluid for *In vitro* Gas Production

A considerable amount of research has investigated the use of alternatives to rumen fluid as a source of inoculum. The necessity for fistulated animals to provide this inoculum raises a number of practical problems, e.g. surgical facilities, constant care to avoid infections and costs associated with the long-term maintenance of these animals (Mauricio *et al.*, 2001). Several studies, reviewed by Omed *et al.* (2000) have demonstrated faeces to have high potential as an alternative inoculum for *in vitro* digestibility techniques. The successful use of a liquid suspension of faeces has been reported from sheep Varadyova *et al.* (2005), cattle Holden (1999), Mabjeesh *et al.* (2000) and recently from horses Lattimer *et al.* (2007) and Murray *et al.* (2008) to estimate digestibility of a range of feeds. In part, this has been to devise more appropriate inocula or studying hindgut fermentation in monogastric species. Thus caecal fluid from broilers Williams *et al.* (1997), pigs Williams *et al.* (1998) and ponies Lowman *et al.* (1996) has been used in avian, porcine and equine nutrition, respectively. In ruminant nutrition, the driving force has either been to reduce use of surgically modified animals and/or to provide a more standardised inoculum than is possible with variable rumen fluid. Several researches have come out with the following (faeces, bacterial cultures, continuous culture and cell-free enzymes), as alternative sources of rumen fluid for *in vitro* studies. This will be reviewed in the next session.



2.11.1 Faeces

Use of faeces as an inoculum would overcome the need for surgically modified animals, although the effect of the donor animal's diet might still have an impact on faecal microbial activity, although presumably this would be much less than its impact on rumen fluid. In ruminants, the faecal microbial population differs from that of rumen fluid, as it has been influenced by gastric digestion and the contributions of the caecum to the microbial population. The study of monogastric hindgut digestion would be easier to simulate using monogastric faeces as a replacement for caecal digesta. Lowman *et al.* (1996) observed similar results when using faeces or caecal fluid from ponies. Machboeuf *et al.* (1998) also observed that caecal fluid and faeces from horses produced similar potential gas volume from 54 forages. However, faecal inoculum resulted in faster rates of fermentation with some forages. It was thought that this was because faecal inoculum had a higher density of microflora compared with caecal digesta. For pigs, Bauer *et al.* (2004) showed that faeces were highly representative of microbial activity of digesta from the rest of the large intestine, although there were small differences with caecal contents. Endpoint measures, such as gas production or digestibility after 24 h or more, from faecal inocula are well related to estimates using rumen fluid inocula (Cone *et al.*, 2002; Zhao and Chen, 2004) or estimates of *in vivo* digestibility in horses (Lowman *et al.*, 1999). However, the rate of gas production is usually lower with faeces compared with rumen fluid Cone *et al.* (2002), making estimations of dynamic parameters of the gas production profile difficult. If the diet of the donor animal is highly fibrous, such that the microbial activity of the rumen is low, then differences between rumen fluid and faeces are much smaller Mauricio *et al.* (1999), but when characterising feeds that will be fed to highly productive animals, such inocula



are of limited value. Microbial activity of faecal inocula is generally much lower than that of rumen fluid inocula, and apart from the drastic changes to the diet as in the work of Mauricio *et al.* (1999) it is not clear how activity of faecal inocula might be increased. However, a mathematical means of adjusting gas production profile from faecal inocula to those produced by rumen fluid inocula has been proposed (Dhanao *et al.*, 2004). While this approach needs development, it does offer a means whereby dynamics of feed fermentation in ruminants might be estimated without relying on surgically modified animals.

2.11.2 Bacterial Cultures

Bacterial cultures could in theory, be used to produce standard inoculum without the need for experimental animals, provided such a culture could be developed with a sufficiently wide range of activity, and that conditions could be maintained to reduce variation in its activity. Luchini *et al.* (1996) compared freeze-dried and frozen rumen bacteria with fresh cultures, and observed initial differences in fermentation pattern, although there were no differences at later times. Rates of protein degradation were similar for fresh and frozen bacteria, but much lower for freeze-dried bacteria. They concluded that if frozen bacteria were incubated overnight, they could be used as an alternative inoculum, but that freeze-dried bacteria were not a viable option. Rymer *et al.* (2000) investigated the use of a bacterial culture made from mixing pure cultures of *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Selenomonas ruminantium*, *Butyrivibrio fibrisolvens*, *Prevotella ruminicola* and *Streptococcus bovis*, and compared gas production profile of several feeds produced by use of this inoculum with gas production profile produced by rumen fluid inoculum. Although there were differences in the shape of the gas production profile, with the bacterial culture producing gas at a much lower rate and in two



distinct phases, there were good mathematical relationships between gas production profile parameters of the inocula. Much would need to be done, and it seems likely that many more bacterial species would need to be included in the mixture, before such an approach could be used to replace rumen fluid. However, it could produce an inoculum that would eliminate the need for surgically modified animals, and it would be a defined inoculum, in contrast to rumen fluid or faeces, with its specificity and activity adjustable by addition or removal of any bacterial species.

2.11.3 Continuous Culture (*in vitro*) Effluent

Barbi *et al.* (1993) investigated the use of effluent from a simulated rumen fermentation (i.e., Rusitec) to replace rumen fluid. The microbial activity of effluent was much lower than that observed for rumen fluid, and so Barbi *et al.* (1994) tried to increase this activity by increasing feed input and decreasing particle size of the feed that was fed to the Rusitec, and by infusing glucose. These interventions did not increase microbial activity, but addition of hay to the effluent flask did increase the microbial concentration. Van Kessel and Russell (1996) were able to maintain a mixed culture of predominantly ruminal bacteria in continuous culture for up to four months, but methanogens did not persist and *Selenomonas ruminantium* had to be added. Variation in Rusitec effluent fluid between days was also found to be similar to that in rumen fluid (Barbi *et al.*, 1995).

2.11.4 Cell-free Enzymes

Use of a cocktail of cell-free enzymes could overcome the need to maintain experimental animals, and provide a more consistent inoculum source. To date, relatively little has been done to develop means of predicting fermentation dynamics Broderick (1998), and it could be that for this approach to be viable, at least in the context of the gas production technique,



the enzymes used would need to be those associated with fermentation, as well as degradation, pathways as any gas produced would be indirect rather than a combination of direct and indirect.

2.11.5 Rumen Fluid from Slaughter Houses

Chemical composition of pasture vegetation is crucial, particularly in combination with *in vitro* digestibility, to evaluate the nutritive value of browse species which are not known previously (Laudadio *et al.*, 2009). Consequently, a detailed survey of browse species is important to identify the better shrub species for ruminants, in terms of nutrient content and digestibility. *In vitro* digestion techniques using rumen liquor as a microbial inoculum Tilley and Terry (1963) have proved useful in assessing the relative digestibility of many feeds (Minson, 1990).

The search for a better labor efficiency technique has led to the development of the DaisyII apparatus (ANKOM® Technology Corp., Fairport, NY), which allows simultaneous incubation of different feedstuffs in sealed polyester bags in the same incubation vessel. Results previously showed that the use of rumen fluid or faecal liquor inoculum Lattimer *et al.* (2007), Tufarelli *et al.* (2010) with a closed-system fermentation apparatus (DaisyII Incubator) yielded valid *in vitro* estimates of DM and fibre digestibility of forages and grains. Furthermore, this technique allows the estimation of *in vitro* digestibility of a large number of samples simultaneously, in addition to recovery of the residue for the final prediction of *in vitro* digestibility of feeds.

In recent times, some researchers have proposed the use of rumen fluid from slaughtered animals in place of the fistulated ones (Mohammed and Chaudhry, 2008; Chaudhry and Mohammed, 2011; 2012). The results from the use of rumen fluid from slaughtered



animals are highly correlated with Tilley and Terry *In vitro* Organic Matter digestibility (IVOMD) ($R^2=0.73$) and ME ($R^2=0.92$). Although slaughtered cattle may be used as an alternative source to obtain rumen fluid, obtaining rumen fluid from slaughtered animals needs transport man-hours and time adjustment between researchers and abattoirs (Chaudhry, 2008). Therefore, it would help if the rumen fluid from slaughtered animals could be preserved in sufficient quantities for subsequent incubation with various feeds to estimate their *in vitro* degradation on regular basis.

2.12 Factors Affecting *In vitro* Gas Production

2.12.1 Effect of Inoculum

The husbandry conditions of the donor animals, their diet and the timing of inoculum collection may all have effects on consistency of results between runs. This is true even for animals fed the same diet and living under the same conditions. It seems clear that samples should be collected from several animals, and then combined, to reduce variation Williams (2000) because variability among animals, as assessed by the *in situ* technique, can be higher than between day variation within animals (Mehrez and Orskov, 1977). However, there are other factors affecting the inoculum such as effect of donor species, inoculum concentration and the sources of inoculum.

2.12.2 Changes in Atmospheric Pressure

Pell *et al.* (1998) noted that in the course of *in vitro* incubation, atmospheric pressure can change. It was noted that during summer thunderstorms at Cornell University in Ithaca (NY, USA), the change in pressure could be as much as 0.4 kPa. For systems that use pressure transducers, this could be a problem as pressure in the fermentation vessel is



measured relative to atmospheric pressure. In the system of Cone *et al.* (1996) the pressure accumulation that is required to cause the valve to open is only 0.25 kPa. Therefore, during a storm Pell *et al.* (1998) suggested that valve opening could be delayed as a low-pressure weather front passes and accelerated as atmospheric pressure rises at the end of a storm.

Theodorou *et al.* (1994) also observed that the volume of gas produced, with respect to a change in atmospheric pressure, was affected by altitude, and that this needed to be considered when comparing results between laboratories. Pell and Schofield (1993) and Pell *et al.* (1998) postulated that changes in atmospheric pressure could be corrected in unvented systems by use of blanks. However, in vented systems, this is only possible if blank fermentations produce enough gas to cause regular valve openings, usually during the first 6 h of fermentation (Pell *et al.*, 1998). They advised that atmospheric pressure changes be recorded during incubation. Later, Schofield (2000) suggested that it was possible to allow for changes in atmospheric pressure by recording results relative to a reference pressure. Schofield (2000), however concluded that most changes in atmospheric pressure are not sufficient to have much effect on gas production profile.

2.12.3 Effect of Sample Size and Preparation

Schoner (1981) and Theodorou *et al.* (1994) observed that increasing the amount of substrate resulted in a linear increase in total gas volume, but the rate of gas production was not affected. The sample weight used by different research groups ranged from 100 to 1250mg (Getachew *et al.*, 1998). Small sample weights used by Pell and Schofield (1993) may make the system more prone to experimental errors in weighing of heterogeneous samples. However, where large sample weights are used, it is essential that the system is



capable of buffering the acid produced, and that the accumulated pressure is not so high that it affects the gas production profile.

Using cereals as substrates resulted in particle size having no effect on gas production profile as reported by Trei *et al.* (1970) and Lowman *et al.* (2002) provided that the grain kernel had been cut (Lowman *et al.*, 2002). With highly soluble feeds, it would appear that as long as the feed has undergone some abrasion, its particle size does not affect estimates of gas production rate.

With fibrous and more slowly degraded feeds, gas production rate increases as particle size decreases (Menke and Steingass, 1988; Lowman *et al.*, 2002) and it seems likely that this is a consequence of increased surface area as a result of grinding, thereby allowing better microbial access. However, this presents a problem when using the *in vitro* gas production profile to predict kinetics of degradation and fermentation *in vivo*, as substrate particles are continually changing shape, size and composition in the gut (Lowman *et al.*, 2002). Although adoption of a standardised approach to sample preparation may be possible to enable comparison between independently produced gas production data of different feeds, it seems unlikely that gas production profile will represent kinetics of plant biomass as it is digested in the rumen (Lowman *et al.*, 2002) or in the hindgut of monogastric animals, although in the case of the latter, processes of mastication will not further disturb the physical nature of the feed.

Menke and Steingass (1988) showed that drying silage at 60 or 105°C had no effect on total gas volume produced, but it did affect gas production profile. However, there was no difference between the gas production profile of maize meal or maize residues when they were freeze-dried or dried at 60°C. In contrast, Cone (1998) observed that freeze-drying



enhanced gas production of substrates compared with oven-drying. He also noted that fresh silage samples had a different gas production profile compared with their dried counterparts. Sanderson *et al.* (1997) observed that rate of fermentation of silage *in vitro* was grossly overestimated if a ground and freeze-dried sample was used, rather than a fresh or chopped sample, although effects of drying were confounded by differences in particle size of samples.

2.12.4 Effect of Agitation of the Medium

There is wide variation among systems in agitation of bottles during incubation for example, the automated system of Cone *et al.* (1996) uses a shaking water bath which is continually agitated, while the system of Pell and Schofield (1993) uses a magnetic stir bar that intermittently stirs the medium. The manual pressure transducer technique of Theodorou *et al.* (1994) in contrast, permits shaking only when a measurement is made.

Wilkins (1974) vigorously agitated vessels at 200 oscillations/min, which was four times faster than that of Cone *et al.* (1996) and more vigorous than the intermittent stirring of Pell and Schofield (1993). Results of Wilkins (1974) showed no effect of agitation on lag time, but he did observe that shaking increased rate of gas production. Conversely, Stevenson *et al.* (1997) observed no change in the pattern of fermentation, or in yield of microbial biomass, when the medium was agitated. However, Pell and Schofield (1993) observed that the coefficient of variation of the gas production profile increased if the incubation medium was not agitated.

Pell and Schofield (1993) suggested that the CO₂ tended to form supersaturated solutions if it was not shaken or stirred. In a study by Rymer *et al.* (1998b), volume of gas produced by adding propionic acid to a medium increased 58% when shaking speed was increased from



0 to 45 oscillations/min, although there was no further increase in volume of gas produced when shaking speed was increased to 60 oscillations/min. In a subsequent experiment using a manual pressure transducer technique, there was no effect of agitation (Rymer *et al.*, 1998a). A comparison between laboratories in which the automated system of Cone *et al.* (1996) that agitates the medium, was compared with the automated system of Davies *et al.* (2000) which does not, found no consistent differences between apparatus, and data between apparatus were well correlated (Rymer *et al.*, 2005) suggesting that agitating the medium has relatively little effect on reproducibility of results within and between laboratories, though a consistent approach within experiment is advisable.

2.12.5 Effect of Medium Composition

There is considerable variation in the composition of the medium used between gas production techniques. A distinction should be made between ‘medium’ (i.e., a solution containing a number of components including buffering agents, trace elements, true protein and reducing agents) and the ‘buffer’ that is a component of the medium (Williams, 1998).

The bicarbonate component of the buffer complicates interpretation of the gas production profile, because of indirect gas that is produced by the reaction between bicarbonate ions and fermentation acids. However, it is an important component of the rumen buffering system, and is usually included in all media to simulate rumen conditions more closely. However, Omed *et al.* (1998) suggested that phosphate could completely replace bicarbonate in the buffer, although microorganisms require both bicarbonate and phosphate for growth (Williams, 1998). Phosphates also increase fibre digestibility to some extent and so phosphate buffers might also alter the gas production profile (Kennedy *et al.*, 2000).





Rymer *et al.* (1998b) examined the composition of a variety of media used in gas production techniques, with the objective of determining effects of various media compositions on the volume of indirect gas produced. The media of Goering and van Soest (1970), Theodorou (1993), Steingass (1983) and Huntington *et al.* (1998) were compared (Table 10). Aliquots of propionic acid (2mmol) were sequentially added to each medium until 12mmol acid had been added, and the volume of gas produced was measured. Although the Goering and van Soest (1970) medium produced more gas, compared with the other media, the difference was very small (14.8 ml/mmol acid compared with 13.7, 14.1 and 13.7 ml/mmol acid) for the Theodorou (1993), Steingass (1983) and Huntington *et al.* (1998) media, respectively.

There was no difference between media in initial pH, but there were large differences in the final pH, with the media of Steingass (1983) and Goering and van Soest (1970) maintaining a pH above 5.5, while others had a pH of 5.42 (Theodorou, 1993) and 5.29 (Huntington *et al.*, 1998). In a study reported by Fakhri *et al.* (1997) the pH at 72 h of incubation of wheat and maize grains was much lower with the Steingass (1983) medium, but there was little difference between the media of Goering and van Soest (1970) and Theodorou (1993).

The bicarbonate ion concentration is lower in the Theodorou (1993) and Huntington *et al.* (1998) media (Table 10) compared with those of Goering and van Soest (1970) and Steingass (1983) which could explain the low pH observed by Rymer *et al.* (1998b) with the first two media.

Table 10: Composition of the Media (g/l) Used in Various Gas Production Techniques

Component	Medium			
	Menke and Steingass (1988)	Steingass (1983)	Goering and van Soest (1970)	Theodorou (1993)
CaCl ₂ ·2H ₂ O	0.013	0.017	0.016	0.015
MnCl ₂ ·4H ₂ O	9.7×10 ⁻³	0.015	0.012	0.011
CoCl ₃ ·6H ₂ O	0.97×10 ⁻³	0.002	1.25×10 ⁻³	1.11×10 ⁻³
FeCl ₃ ·6H ₂ O	0.77×10 ⁻³	0.012	9.96×10 ⁻³	8.84×10 ⁻³
Na ₂ HPO ₄	1.09	1.43	1.42	2.09
KH ₂ PO ₄	1.19	1.55	1.55	1.37
MgSO ₄ ·7H ₂ O	0.12	0.15	0.15	0.13
NaHCO ₃	6.71	8.75	8.74	7.73
(NH ₄)HCO ₃	0.77	1.00	1.00	0.88
Resazurin	9.87×10 ⁻⁴	0.125	1.25×10 ⁻³	1.11×10 ⁻³
Na ₂ S	0.23	0.52	0.016	2.79×10 ⁻⁴
Trypticase	1.00		2.50	2.21
Cysteine hydrochloride			0.016	2.79×10 ⁻⁴

Source: Rymer *et al.* (1998b)

2.12.6 Use of Blanks

Fermentation vessels containing identical components to all other vessels, with exclusion of any substrate, are termed 'blank' incubations and are routinely used during gas production runs. Blanks can be used to correct for changes in atmospheric pressure Pell and Schofield (1993); Cone *et al.* (1996) as discussed earlier, as well as to correct for residual fermentable OM that is included with the inoculum. However, Cone (1998) noted that



blank incubations do not produce gas at the same rate as the sample because the blanks begin microbial turnover sooner. Calculated dynamics of fermentation are therefore incorrect if the cumulative volume of gas from the blank is simply deducted from the cumulative volume of gas from the sample at each time interval. Williams (2000) suggested that blank results should always be reported in tables of results, but not subtracted from sample results. Blank incubations also provide confirmation of activity of the initial inoculum and, for end point measures, it is a useful correction for contribution of OM in the inoculum.

2.12.7 Effect of Apparatus

Rymer and Givens (1997) compared three types of apparatus using the same methodology. High temperature dried grass, soybean meal and straw were the substrates in an automated pressure transducer Cone *et al.* (1996), a manual pressure transducer Theodorou *et al.* (1994) and flexible plastic bottles. In general, the automated pressure transducer produced more gas than the manual methods, but the combined fractional rates of gas production were similar for the automated technique and the flexible plastic bottles, but lower for the manual pressure transducer. In contrast, a comparison of the manual pressure transducer Theodorou *et al.* (1994) with the automated pressure transducers of Cone *et al.* (1996) and Davies *et al.* (2000) indicated that the manual pressure transducer produced more gas, but at a slower rate, than automated transducers (Rymer *et al.*, 2005). Despite large differences between apparatus used, and interactions of the laboratories involved, there were good mathematical relationships between gas production profile from the different apparatus, suggesting that gas production profile produced by one apparatus can be corrected to standardised apparatus.



2.13 Inferences from Literature Review

- I. Crop residues and agro-by-products assume great importance in ameliorating the feed deficit in animal nutrition in Ghana.
- II. Cowpea haulm as a co-product to grain production when used as a supplement may be economical to farmers.
- III. Lack of phosphate fertilization of cowpea is considered as one limiting yield factor.
- IV. Increasing rates of P fertilization improved CP concentration of sainfoin but little is known about its effect on cowpea.



CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The chemical analyses and *in vitro* gas study were conducted at the Forage Evaluation Unit (FEU) of the Agricultural Sub-Sector Improvement Project (AgSIP) Laboratory whilst the growth trial was undertaken at the Livestock Unit of the Faculty of Agriculture (FOA) both at the University for Development Studies (UDS). The FOA is located at Nyankpala in the Tolon District of Northern Region (Ghana) and about 18 km from Tamale.

Nyankpala is located at longitude $0^{\circ} 58^{\circ} 42^{11}$ W and latitude $9^{\circ} 25^{\circ} 41^{11}$ N and at a height of 183 m above sea level and in the dry savanna ecological zone of Ghana (SARI, 2007). It has a unimodal rainfall pattern that begins in late April and ends in October. The mean annual rainfall is 1043 mm. Temperatures generally fluctuate between 15°C (minimum) and 42°C (maximum) with a mean annual temperature of 28.5°C . The mean annual day time relative humidity is 54% (SARI, 2007). The area experiences dry cold harmattan winds from November to February and a period of warm dry conditions from March to mid-April. The dry season therefore stretches from November to late April.

3.2 Cowpea Cultivation Information and Management

Five varieties (Zaayura-SARC 4-75, Songotra-IT97K-499-35, Hewale-IT93K-192-4, IT-IT99K-573-1-1 and Asomdwe-IT94K-410-2) of cowpea were cultivated and fertilized with three phosphate rates (30 kg/ha, 60 kg/ha and 90 kg/ha).

The cowpea varieties were cultivated at the IITA-Africa RISING community fields in Savelugu Districts of Ghana. The five varieties were cultivated on an area of 1500 m^2 with



each variety occupying a total area of 300 m². The intra and inter row spacing was 75 cm × 20 cm. The phosphate fertilizer (P₂O₅) was applied 14 days after planting. Cultural practices were applied where necessary.

They were harvested at flowering stage and air dried for 3 days after which they were packed and stored under shade for the trial.

3.3 Sample Processing for Chemical Analysis

About 1 kg of cowpea haulm from each variety was harvested and taken to the laboratory. The samples were dried under shade, milled using a hammer mill (Brabender, Germany) and subsequently sieved through 1 mm sieve screen and packaged for further analysis. The milled samples were oven dried overnight at 60°C prior to the chemical analysis.

3.3.1 Experimental Design

The experimental design used for the chemical analyses was the 5*3 in a completely randomized arrangement. The factors were the five varieties of cowpea (Zaayura-SARC 4-75, Songotra-IT97K-499-35, Hewale-IT93K-192-4, IT- IT99K-573-1-1 and Asomdwe-IT94K-410-2) and the three phosphate fertilizer rate (30 kg/ha, 60 kg/ha and 90 kg/ha). The samples were replicated 4 times for NDF and ADF and 3 times for nitrogen.


3.4 Chemical Analysis Parameters

3.4.1 Crude Protein

The crude protein content of the samples was determined according to the method of AOAC (2000). Each sample was replicated three times. Approximately 1 g of each oven dried sample was weighed into filter paper and placed in Kjeldahl digestion tubes. Blank determination was done by digesting filter paper in each set of digestion. Approximately



15 ml of concentrated sulphuric acid (H_2SO_4) and two kjeldahl tabs were added to the content of each digestion tubes. The Kjeldahl tabs contained potassium sulphate (K_2SO_4) and copper sulphate (CuSO_4) which increases the boiling point and act as a catalyst respectively. The tubes were mounted on Kjeldahl digestion block with fume exhaust set (J.P. Selecta RAT 2, Spain) and heated gradually to 420°C and maintained for three hours. The tubes were removed and allowed to cool to room temperature after which, 50 ml of distilled water was added and distilled using an automated Kjeldahl distillation apparatus (J.P. Selecta, s.a, Pro-Nitro II). The apparatus draws 50 ml of previously prepared 35% sodium hydroxide (35% NaOH) into the digestion tubes and 25 ml of 4% Boric acid (4% H_3BO_3) into a 25 ml erlynmeyer flask to trap the librated ammonia during the distillation period of 9 minutes per sample. The distillate was collected and titrated against 0.1N HCL (hydrochloric acid). The average titre values were recorded and used to calculate the percentage nitrogen (%N) and subsequently the percentage crude protein. The CP (%) was calculated using the formulae:


$$\% \text{ Nitrogen} = \frac{(T-B) \times N \times 1.4}{\text{weight of sample (g)}}$$

$$\% \text{ Crude protein} = \% \text{ Nitrogen} \times 6.25$$

Where:

T – Sample titre value

B – Blank titre value

N – Concentration of HCL

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

The NDF and ADF were determined exclusive of residual ash by sodium sulfite and α -amylase following the procedure of Van Soest *et al.* (1991) and was run on the Ankom²⁰⁰ fiber analyser. Each treatment was replicated 4 times for the NDF and ADF.

About 0.45-0.55 g of each sample was weighed directly into filter bags (Ankom F57) and labeled. The filter bags were then sealed within 4 mm of the top with an electronic heat sealer. One blank filter bag was included in each run to determine blank bag correction. The bags with samples were then placed on the bag suspender and inserted into the Ankom fiber analyser vessel with a bag suspender weight on top to keep it submerged. NDF and ADF solutions were then added respectively.

NDF solution was prepared by dissolving 30.0 g Sodium dodecyl sulfate, USP; 18.61 g Ethylenediaminetetraacetic disodium salt, dehydrate; 6.81 g Sodium borate; 4.56 g Sodium phosphate dibasic, anhydrous; and 10.0 ml Triethylene glycol, in 1 L distilled water; Whilst that of ADF was prepared by dissolving 20.0 g Cetyl trimethylammonium bromide (CTAB) in 1 L of 1.00 N H_2SO_4 .

For NDF, two litres of NDF solution was added to every 24 sample bags in the fiber analyser vessel. 20 g (0.5 g/50 mL) of sodium sulfite and 4.0 mL of alpha-amylase were added to the solution in the vessel. The fiber analyser was then allowed to run for 75 minutes. After 75 minutes, the solution in the vessel was exhausted and the content rinsed with 2 L of hot water (70-90°C). Rinsing was repeated three times for 5 minutes and 4.0 ml of alpha-amylase added to the first and second rinses. After rinsing, the samples were placed in acetone for 3-5 minutes after which they were oven dried at 102°C for 2 h and



weights recorded. The weight after oven drying was recorded and used to compute the NDF content using the following equation and converted to dry matter basis.

$$\text{NDF (g/kg)} = \frac{(W3 - (W1 \times C1))}{W2} \times 1000$$

Where: W1 = Bag tare weight

W2 = Sample weight

W3 = Dried weight of bag with fibre after extraction

C1 = Blank bag correction factor (running average of final oven – dried weight divided by original weight).

For ADF, the procedure was the same as that of the NDF except that for ADF the fiber analyser was allowed to run for only 60 minutes and also sodium sulfite and alpha-amylase was not added. ADF was also computed using the equation:

$$\text{ADF (g/kg)} = \frac{(W3 - (W1 \times C1))}{W2} \times 1000$$

Where: W1 = Bag tare weight

W2 = Sample weight

W3 = Dried weight of bag with fibre after extraction

C1 = Blank bag correction factor (running average of final oven – dried weight divided by original weight).

3.4.3 Ash

Ash was determined according to the procedure of AOAC (2000). Approximately 2 g of dried sample was weighed into a known weight of pre dried crucibles. The crucibles containing the samples were placed in a muffle furnace and heated to 550°C for 4 h. The



crucibles were then cooled in a desiccator and its weight taken. The ash content was calculated as:

$$\text{Ash (g/kg DM)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 1000$$

3.5 *In vitro* Gas Experiment

3.5.1 Processing of Samples

About 1 kg of cowpea haulm from each variety were harvested and taken to the laboratory. The samples were initially airdried and milled using a hammer mill (Brabender, Germany) and sieved through a 1 mm sieve screen and oven dried over night at 60°C prior to incubation for the *in vitro* gas analyses.

3.5.2 Experimental Design

The experimental design for the *in vitro* gas studies was a 5*3 factorial in a randomized complete block design. Each treatment was replicated 2 times in each period. The factors were the five cowpea varieties and the three phosphate rates. There were 4 incubation periods in all and each period lasted for 48 h.

3.5.3 *In vitro* Gas Production

The *in vitro* gas production technique of Theodorou *et al*, (1991) was adopted and slightly modified by incubating the samples in a McDougall's buffered rumen fluid. Approximately 200 mg of oven dried samples from each treatment was weighed into 50 ml test tubes. The McDougall's buffer was prepared a day before the incubation and was prepared from



solutions A and B. Solution A was made by dissolving 19.60 g NaHCO₃, 9.28 g Na₂HPO₄·2H₂O, 1.14 g KCl, 0.94 g NaCl and 0.26 g of MgCl·6H₂O in 2 L of distilled water. Solution B was made by dissolving 2.65 g of CaCl₂·2H₂O in 50 mL of distilled water. Complete salivary buffer was prepared by adding 2 ml of solution B to solution A, which was then warmed to 39°C with continuous stirring and flushing with carbon dioxide (CO₂).

Rumen fluid was obtained from 3 different cows at the Tamale Abattoir. The rumen fluid was collected from the rumen after the animals have been slaughtered and rumen taken out. The rumen fluid was collected into a thermos flask that had been pre-warmed to a temperature of 39°C. The rumen fluid was squeezed through a four layer of cheesecloth into a 2 L beaker and mixed with the buffer in a ratio of 4:1 (4 parts of buffer to 1 part of rumen fluid) to form the media. The media was flushed continuously with CO₂. Approximately 30 ml of the media was dispensed into the test tube containing the samples with the help of a 50 ml test tube.

The test tubes and the samples were placed in a water bath at a temperature of 39°C. The gas production was measured using a digital manometer at 3, 6, 12, 24 and 48 h.

The gas readings were then fitted to the exponential curve of Orskov and McDonald (1979) without an intercept using sigmaPlot 10th edition (Systat Software Inc. 2006). The degradation parameters (b and c) were derived from the exponential model.

$$Y = b (1 - e^{-ct})$$

Where Y = gas volume at time t (ml)

b = asymptotic gas production (%)

t = time (h)



c = fractional rate of gas production (ml/h)

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

Where,

GP= gas production (ml/200 mg DM at 24h)

CP= Crude protein (g/kg DM).

3.6 Lamb Growth Study

Two varieties of the cowpea (Zaayura-SARC 4-75 and Hewale-IT93K-192-4) at phosphate rates 30 and 90 kg P₂O₅/ha were selected for the growth study. These were selected based on their slightly superior *in vitro* digestibility.

The feeding trial was conducted at the livestock unit of the Department of Animal Science. Cowpea haulms were fed to about one year old rams with an average initial weight of 18 kg over a period of 8 weeks.

3.6.1 Experimental Design

The 2*2 factorial in a completely randomized design was used for the lamb growth experiment. The factors were the two varieties of cowpea (Zaayura-SARC 4-75 and Hewale-IT93K-192-4) and two phosphate fertilizer rates (30 kg/ha and 90 kg/ha). There were 5 replicates (lambs) per treatments.

3.6.2 Experimental Animals and their Management

A total of 20 Djallonké rams (8-12 months old) purchased from different livestock markets in the Northern Region were used for the study. The animals were fitted with ear tags for



easy identification. There were four treatments in all with each treatment having five replicates. The animals were housed semi-intensively.

The animals were first quarantined for 30 days during which they were treated against internal parasites by administering 2-3 ml of multibendazole oral suspension per 10kg body weight and 1ml of oxytetracycline 200 mg per 10 kg body weight intramuscularly to check secondary bacterial infection. The animals were also dipped into a solution of acaricide to control ecto parasites.

Each animal was randomly assigned to a pen and fed individually. The floor of the pen was made of concrete and the divisions were done with wood. The area of each pen was 1.5×3 m with a height of 3.0 m. The wall was made of concrete blocks and roofed with aluminum sheets. There were ventilation holes for free movement of air in and out of each pen. The animals were allowed three days to adjust to the experimental pens and the feed supplement. The animals were offered the cowpea haulms *ad libitum* as supplements in wooden feeding troughs at 7:00 am each morning. They were released for grazing on natural pastures at 10:00 am and returned to the cages at 5:00 pm each day. Water was also given *ad libitum* in plastic watering bowls. The feeding trial lasted for 56 days.

3.7 Data Collection on Lamb Growth Studies

3.7.1 Daily Dry Matter Intake

The feed consumed per animal was obtained by subtracting feed leftover at the end of the week from the total feed supplied for the week. Daily sub-samples of the feed were taken, oven dried and stored. This was bulked together after the experiment and subsample (200 g) taken for oven drying. Total feed intake for each animal was obtained by adding all the intakes for the whole experimental period per animal. This was then divided by the number



of days the experiment lasted to obtain daily intake per head. This was expressed on dry matter basis to get the daily dry matter feed intake.

3.7.2 Average Daily Weight

Average daily weight was obtained by taking the final weight at the end of the experiment and dividing it by the number of days the experiment lasted to obtain the average daily weight.

3.7.3 Average Daily Weight Gain

Each animal was weighed using a hanging weighing scale (Camry hanging scale, ISO9001:2008, China) at the beginning of the experiment and at the end of every week. The initial live weight per animal was subtracted from the final live weight per animal at the end of the experiment. This was then divided by the number of days the experiment lasted to obtain the average daily weight gain.

3.7.4 Final Weight Gain

The initial live weight per animal was subtracted from the final live weight per animal at the end of the experiment to obtain final live weight gain per animal.

3.8 Blood Sampling

At the end of the growth performance experiment, blood samples were taken from the animals. During the sample collection, the animals were restrained and 10 ml disposable syringes and needles were used to draw about 5 ml of blood from the jugular vein of each sheep. The collected blood was emptied into twenty-five labeled test tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) to prevent the blood from clotting. This was



used for packed cell volume (PVC), haemoglobin concentration (Hb), and total white blood cells (WBCs) analysis at the UDS clinic laboratory.

About 5 ml of the blood was also drawn and emptied into another set of 25 plain test tubes for biochemical analysis at the Tamale Teaching Hospital (TTH) laboratory. The blood was centrifuged at a speed of 500 rpm to separate the serum which was stored at 4°C for analysis.

3.8.1 Packed Cell Volume (PVC)

Blood samples were placed in capillary tubes which were arranged in a capillary centrifuge. The samples were centrifuged at 10,000 rpm for five minutes. This separated the blood into layers. The PCV was determined by measuring the packed red cell column on a micro haematocrit ruler.

3.8.2 Haemoglobin (Hb)

This is the iron-containing oxygen transport metalloprotein in the RBC of all vertebrate with the exception of the fish family as well as the tissues of some invertebrates (Maton *et al.*, 1993). The estimation of haemoglobin levels was done by adding 20µl of blood sample to 5 mls of Drabkins solution. Drabkins solution haemolyses the red blood cells, releasing haemoglobin pigment into the solution. The spectrophotometer (CECIL, CE 1011) was used to estimate the haemoglobin content at wave length of 540 nm.

3.8.3 Total White Blood Cell (WBCs)

Blood sample was taken and diluted with the WBC diluting fluid (Turks solution). This solution destroyed all the RBCs and stained WBCs for easier identification during counting. The dilution factor was 1 in 20 and allowed at least 10 minutes for reaction. The



solution was then transferred into an improved Neubauer counting chamber (Superior Marienfeld) and the WBCs counted using $\times 10$ objective lens of a microscope and the total count was estimated by calculation.

3.8.4 Albumin

The method used for this assay is based on that of Doumas *et al.* (1971) where at a controlled pH, bromocresol green (BCG) forms a coloured complex with albumin. The intensity of the colour at 630 nm is directly proportional to the albumin content.

3.8.5 Glucose

The extracted serum was analysed for glucose following the method of Amidu *et al.* (2013) using the BT 3000 Random Access Chemistry analyzer.

Glucose oxidase catalyses the oxidation of glucose to give hydrogen peroxide (H_2O_2) and gluconic acid. In the presence of peroxidase, the hydrogen peroxide is broken down and the oxygen released reacts with 4-aminoantipyrine and phenol to give a pink colour. The intensity of the colour is directly proportional to the amount of glucose.

3.8.6 Total Protein

Estimation of total protein in this study is based on the modifications of Gornall *et al.* (1949). Protein in serum forms a blue coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the violet colour is proportional to the amount of proteins present when compared to a solution with known protein concentration.

3.8.7 Globulin

The globulin level was determined as the difference between total protein and albumin.



3.8.8 Urea

Blood urea nitrogen was analysed using the procedures of Fawcett and Scott (1960); Chaney and Marbach (1962).

Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with ketoglutarate in the presence of NADH to yield glutamate. An equimolar quantity of NADH undergoes oxidation during the reaction resulting in the decrease in absorbance that is directly proportional to the amount of urea nitrogen concentration in the sample.

3.9 Carcass Characteristics

At the end of the feeding trial, three animals were selected from each treatment group for carcass evaluation. Prior to slaughtering, the animals were starved over night to clear the gut and live weights were recorded. Slaughtering was done at the Meat Unit of the University for Development Studies, Nyankpala. The animals were slaughtered by cutting the jugular vein with a sharp knife. Immediately after slaughter, the skin, head, feet and testis (external organs) were removed and weighed. The dressed weight, dressing percentage and carcass length were taken together with the slaughter weight and were referred to as carcass parameters.

Evisceration of the carcass was carried out and the internal organs were carefully removed, weighed separately and their corresponding weights recorded. The internal organs (non-carcass characteristics) measured included empty digestive tracts, liver, kidney, lungs, heart and spleen. Hot carcass weight was taken after all the internal and external organs have been removed. The carcass was then divided into primal cuts (neck, shoulder, leg, rib and flank, back, chuck and loin)



3.9.1 Live Weight

This was the weight of the animals just before sticking and bleeding. The weights were taken using a digital scale (Jadever JPS-1050).

3.9.2 Carcass Weight/ Dressed Weight

This was the weight of the animals after sticking, bleeding, skinning, removal of head, feet, testis and viscera. Each carcass weight was taken using a digital scale (Jadever JPS-1050). This represented the hot carcass weight.

3.9.3 Carcass Dressing Percentage (%)

The carcass dressing percentage was calculated as follows:

$$\text{Carcass Dressing (\%)} = \frac{\text{Dressed Carcass Weight}}{\text{Live Weight}} \times 100$$

3.9.4 Carcass Length

Carcass lengths were measured using a tape measure from the pelvic bone to the anterior edge of the first rib.

3.9.5 Evaluation of Weights of Primal Cuts and Internal Organs

The weights of the various Primal cuts as well as other visceral were taken using a digital scale (Jadever JPS-1050).

3.10 Statistical Analysis

The data from the chemical analysis and the *in vitro* gas experiment were analysed using a 2-way analysis of variance (ANOVA) of GenStat version 11.1 (Payne *et al.*, 2008). In the *in vitro gas* study, period was used as a block.

The data from the Sheep growth study was analysed using analysis of variance (ANOVA) of GenStat version 12.1 Payne *et al.* (2008) with the initial weight of the rams used as covariates for the live weight parameters. The differences in means were separated using the Fisher's least significant difference test at 5%.



CHAPTER FOUR

4.0 RESULTS

4.1 Chemical Composition of Cowpea Varieties

The results on the chemical composition are shown in table 11.

Table 11: Chemical Composition of haulms of Cowpea Varieties at Different Phosphate Rates (g/kg DM)

Item	Variety (V)	Phosphate (P) fertilizer rate (kg P ₂ O ₅ /ha)			Mean
		30	60	90	
CP	Zaayura	153 ^c	166 ^d	149 ^c	156.0
	Songotra	138 ^{abc}	129 ^a	133 ^{ab}	133.2
	Hewale	146 ^{bc}	140 ^{abc}	143 ^{bc}	143.0
	I.T	145 ^{bc}	126 ^a	126 ^a	132.1
	Asomdwe	145 ^{bc}	150 ^c	145 ^{bc}	146.7
	<i>Mean</i>	<i>145.3</i>	<i>142.0</i>	<i>139.3</i>	
<i>SED (V x P)</i>	<i>6.34</i>				
<i>P-value</i>					
<i>V</i>	<i>0.001</i>				
<i>P</i>	<i>0.119</i>				
<i>V x P</i>	<i>0.044</i>				
NDF	Zaayura	478 ^{ab}	481 ^{ab}	462 ^a	473.8
	Songotra	489 ^{abc}	545 ^{defg}	554 ^{eg}	529.3
	Hewale	503 ^{abcd}	508 ^{abcdef}	460 ^a	490.1
	I.T	491 ^{abc}	512 ^{bcdefg}	507 ^{abcde}	503.5
	Asomdwe	480 ^{ab}	518 ^{bcdefg}	533 ^{cdefg}	510.4
	<i>Mean</i>	<i>488</i>	<i>512</i>	<i>503</i>	
<i>SED (V x P)</i>	<i>20.3</i>				
<i>P-value</i>					
<i>V</i>	<i>0.001</i>				
<i>P</i>	<i>0.032</i>				
<i>V x P</i>	<i>0.020</i>				
ADF	Zaayura	293 ^a	282 ^a	290 ^{ab}	288.3
	Songotra	320 ^{bcd}	348 ^d	352 ^d	339.5
	Hewale	350 ^d	341 ^{cd}	302 ^{ab}	331.0
	I.T	317 ^{abcd}	306 ^{abc}	303 ^{ab}	308.9
	Asomdwe	308 ^{abc}	310 ^{abc}	322 ^{bcd}	313.5
	<i>Mean</i>	<i>317</i>	<i>317</i>	<i>313</i>	
<i>SED (V x P)</i>	<i>15.43</i>				
<i>P-value</i>					
<i>V</i>	<i>0.001</i>				
<i>P</i>	<i>0.031</i>				
<i>V x P</i>	<i>0.038</i>				

CP: Crude protein, NDF: Neutral detergent fibre; ADF: Acid detergent fibre, V: Variety, P: Phosphate fertilizer rate. Means with the same letters in a column are not significantly different ($P > 0.05$). SED: Standard error of difference.





4.1.1 Crude Protein

There was a significant effect of variety by phosphate (V x P) rate interaction for crude protein ($P < 0.05$). The highest crude protein was recorded in Zaayura-SARC 4-75 at 60 kg P_2O_5 /ha level application with the least recorded in IT- IT99K-573-1-1 at 60 and 90 kg P_2O_5 /ha level application. The crude protein did not differ between varieties Zaayura-SARC 4-75, Hewale-IT93K-192-4 and Asomdwe-IT94K-410-2 at 30 and 90 kg P_2O_5 /ha rate application. The results showed a decreasing trend in crude protein as phosphate rates increased from 30 to 90 kg P_2O_5 /ha fertilizer rate application (Table 11).

4.1.2 Neutral Detergent Fibre

Variety by phosphate (V x P) rate interaction for NDF was significant ($P < 0.05$). The highest NDF was obtained in Songotra-IT97K-499-35 at 90 kg P_2O_5 /ha level application with the least reported in Hewale-IT93K-192-4 at 90 kg P_2O_5 /ha rate application. The NDF at 30 kg P_2O_5 /ha rate application was higher for varieties Zaayura-SARC 4-75 and Hewale-IT93K-192-4 than the 90 kg P_2O_5 /ha rate application for the same varieties. The results showed a decreasing trend in NDF in varieties Zaayura-SARC 4-75 and Hewale-IT93K-192-4 as phosphate rates increased from 30 to 90 kg P_2O_5 /ha fertilizer rate application (Table 11).

4.1.3 Acid Detergent Fibre

Variety by phosphate (V x P) level interaction for ADF differed ($P < 0.05$). The ADF was in the range of 282 g/kg DM and 352 g/kg DM for Zaayura-SARC 4-75 at 60 kg P_2O_5 /ha rate application and Songotra-IT97K-499-35 at 90 kg P_2O_5 /ha rate application respectively. The ADF at 30 kg P_2O_5 /ha was higher ($P = 0.031$) for varieties Zaayura-SARC 4-75, Hewale-IT93K-192-4 and IT- IT99K-573-1-1 than the 90 kg P_2O_5 /ha rate application for the same

varieties. The results showed a decreasing trend in ADFom in varieties Zaayura-SARC 4-75, Hewale-IT93K-192-4 and IT- IT99K-573-1-1 as phosphate rates increased from 30 to 90 kg P₂O₅/ha fertilizer rate application (Table 11)

4.2 In Vitro Gas Study

The results of the *in vitro* gas study as affected by cowpea variety and phosphate fertilizer rate application are presented in table 12.

Table 12: Effects of Cowpea Variety and Phosphate Fertilizer Rate on *In vitro* Digestible Organic Matter (DOM), Kinetics and Gas Production

Item	Variety	Phosphate fertilizer rate			Mean
		30	60	90	
DOM	Zaayura	43.61	44.24	42.46	43.44 ^c
	Songotra	41.58	43.02	41.63	42.05 ^{ab}
	Hewale	41.89	43.84	42.02	42.58 ^{bc}
	I.T	44.28	43.31	42.58	43.39 ^c
	Asomdwe	41.75	40.43	41.14	41.11 ^a
	Mean	42.61	42.97	41.97	
<i>SED</i> (V)	0.588				
<i>P</i> -Value					
<i>V</i>	< .001				
<i>P</i>	0.087				
<i>V</i> x <i>P</i>	0.290				
B	Zaayura	19.15	17.40	19.10	18.55
	Songotra	18.84	19.29	17.22	18.45
	Hewale	18.78	19.50	18.31	18.86
	I.T	19.10	19.42	18.91	19.14
	Asomdwe	18.44	17.25	17.69	17.79
	Mean	18.86	18.57	18.25	
<i>SED</i> (V)	0.591				
<i>P</i> -Value					
<i>V</i>	0.217				
<i>P</i>	0.405				
<i>V</i> x <i>P</i>	0.310				
c	Zaayura	0.0672	0.0694	0.0653	0.0673
	Songotra	0.0640	0.0707	0.0678	0.0675
	Hewale	0.0620	0.0671	0.0671	0.0654
	I.T	0.0665	0.0662	0.0644	0.0657
	Asomdwe	0.0611	0.0618	0.0705	0.0644
	Mean	0.0642	0.0670	0.0670	
<i>SED</i> (V)	0.00317				
<i>P</i> -Value					



<i>V</i>	0.855				
<i>P</i>	0.410				
<i>V x P</i>	0.740				
IVGP 48h	Zaayura	18.37	16.83	18.33	17.84
	Songotra	16.38	18.80	16.78	17.32
	Hewale	16.64	18.81	17.68	17.71
	I.T	18.36	18.71	17.98	18.35
	Asomdwe	17.36	15.61	17.15	16.71
	<i>Mean</i>	17.42	17.75	17.58	
<i>SED</i> (<i>v</i>)	0.736				
<i>P-Value</i>					
<i>V</i>	0.241				
<i>P</i>	0.849				
<i>V x P</i>	0.209				

DOM: Digestible organic matter, b: Potential degradability, c: Rate of degradation, V: Variety, P: Phosphate fertilizer rate. SED: Standard error of difference.

4.2.1 *In Vitro* Gas Production Parameters

In the *in vitro* gas study (Table 12), V x P interaction was not significant ($P > 0.05$) for all the parameters measured and calculated. There was however a significant ($P < 0.05$) varietal effect on mean digestible organic matter (DOM). The digestible organic matter was in the range of 41.11% to 43.44% for variety Asomdwe-IT94K-410-2 and Zaayura-SARC 4-75 respectively. The potentially degradable dry matter (b%) was in the range of 17.22 to 19.50 for variety Songotra-IT97K-499-35 at 90 kg P_2O_5 /ha rate application and Hewale-IT93K-192-4 at 60 kg P_2O_5 /ha rate application ($P > 0.05$). The rate of degradation (c) of the potentially degradable dry matter (b) did not differ ($P > 0.05$) among the factors. It was in the range of 0.0611% to 0.0707% for Asomdwe-IT94K-410-2 at 30 kg P_2O_5 /ha rate application and Songotra-IT97K-499-35 at 60 kg P_2O_5 /ha rate application. The highest *in vitro* gas production (IVGP/200 mg DM) at 48 h was recorded in Hewale-IT93K-192-4 at 60 kg P_2O_5 /ha rate application with the least obtained in Asomdwe-IT94K-410-2 at 60 kg P_2O_5 /ha rate application. *In vitro* gas production curves for the various cowpea varieties and phosphate rates can be found in appendix 3.



4.3 Growth performance

Results of the effect of cowpea haulms at different phosphate rates on growth of Djallonké sheep is shown in table 13.

Table 13: Effect of Cowpea Haulm at Different Phosphate (P₂O₅) Rates on Growth of Djallonké Lambs

Intake and weight gain	Zaayura		Hewale		SED	P-value		
	30	90	30	90	V x P	V	P	V x P
IW (g)	14.78	17.90	16.00	15.70	1.430	0.634	0.182	0.110
DMI (g/h/d)	125.5 ^a	143.9 ^b	145.1 ^b	127.7 ^a	6.89	0.621	0.730	0.003
ADWG (g)	35.5	45.1	44.6	49.7	7.62	0.185	0.213	0.694
ADW (g)	322.3	333.3	321.1	336.9	9.57	0.821	0.061	0.734
FWG (g)	1990	2523	2498	2781	426.7	0.185	0.212	0.694

ADWG: Average daily weight gain, ADW: Average daily weight, IW: Initial weight, FWG: Final weight gain, DMI: Dry matter intake, V: Variety, P: Phosphorus fertilizer, SED- Standard Error of Difference, P= probability, Means with the same letters are not significantly different (P > 0.05).

4.3.1 Dry Matter Intake

The results of the dry matter intake (DMI) showed a V x P rate interaction (P<0.05) (Table 13). The highest DM intake was recorded in Hewale-IT93K-192-4 at 30 kg P₂O₅/ha rate application with the least recorded in Zaayura-SARC 4-75 at 30 kg P₂O₅/ha rate application. The DM intake for Zaayura-SARC 4-75 at 90 kg P₂O₅/ha rate application was higher (P=0.003) than that of the same Zaayura-SARC 4-75 at 30 kg P₂O₅/ha rate application. The reverse was however recorded in Hewale-IT93K-192-4 with the animals taking in more (P=0.003) of the 30 kg P₂O₅/ha rate application than the 90 kg P₂O₅/ha rate application.



4.3.2 Average Daily Weight Gain

There was no significant ($P>0.05$) difference in average daily weight gain (ADWG) (Table 13). However, animals that fed on the 90 kg P_2O_5 /ha rate application had slightly higher weights than those that fed the 30 kg P_2O_5 /ha rate application for all varieties.

4.3.3 Average Daily Weight

Average daily weight was similar ($P>0.05$) between animals that were fed Zaayura-SARC 4-75 and Hewale-IT93K-192-4. However, animals that were fed the 90 kg P_2O_5 /ha rate application had slightly higher weights than those that were fed the 30 kg P_2O_5 /ha rate application for all varieties (Table 13).

4.3.4 Final Weight Gain

There was no significant ($P>0.05$) difference in final weight gain between animals that fed Zaayura-SARC 4-75 and Hewale-IT93K-192-4. However, animals that fed the 90 kg P_2O_5 /ha rate application tended to have higher final weight gains than those that fed the 30 kg P_2O_5 /ha rate application for all varieties (Table 13).

4.4 Blood Profile

Effect of cowpea haulm at different phosphate rates on haematology and blood biochemistry of Djallonké sheep is shown in table 14.

Table 14: Effect of Cowpea Haulm at Different Phosphate Rates on Haematology and Blood Biochemistry of Djallonké Sheep

Blood Profile	Zaayura		Hewale		SED V x P	P- value		
	30	90	30	90		V	P	V x P
Hb (g/dl)	10.35	10.19	10.29	9.85	0.876	0.731	0.612	0.827
PCV (%)	31.04	30.60	30.83	29.53	2.619	0.720	0.624	0.826

WBC Total X10 ⁹ L	5.89	5.73	6.47	5.75	0.790	0.584	0.410	0.635
Albumin (g/l)	26.16	24.92	24.27	24.04	1.038	0.079	0.331	0.504
Globulin (g/l)	12.82 ^a	11.32 ^a	15.13 ^b	16.12 ^b	1.982	0.023	0.859	0.387
Glucose (mmol/l)	0.58	0.48	0.78	0.64	0.149	0.113	0.283	0.871
Total protein (g/l)	38.98	36.24	39.40	40.16	1.883	0.124	0.469	0.209
Blood urea nitrogen (mmol/l)	10.88 ^a	10.30 ^a	9.03 ^a	12.16 ^b	1.108	0.997	0.124	0.032

V: Variety, P: Phosphate fertilizer, Hb: Haemoglobin, PCV: Packed cell volume, WBC: White blood cells, SED- Standard Error of Difference, P= probability, Means with the same letters are not significantly different ($P > 0.05$).

4.4.1 Haematology and Blood Biochemistry

The haematological values did not vary significantly for most of the parameters.

There was a significant ($P < 0.05$) effect of variety on globulin with the highest recorded in Hewale-IT93K-192-4. There was also a significant V x P rate effect ($P < 0.05$) on blood urea nitrogen with animals on Hewale-IT93K-192-4 at 90 kg P₂O₅/ha rate application having more blood urea nitrogen than the others (Table 14).

4.5 Carcass Characteristics

The effect of cowpea haulm at different phosphate rates on carcass parameters of Djallonké sheep is shown in table 15.

Table 15: Effect of Cowpea Haulm at Different Phosphate Rates on Carcass Parameters and Primal Cuts of Djallonké Sheep

Carcass Parameters	Zaayura		Hewale		SED V x P	P- value		
	30	90	30	90		V	P	V x P
Live Weight (kg)	14.21 ^c	19.75 ^a	16.09 ^b	18.47 ^a	1.328	0.758	0.003	0.131
Dressed Weight (kg)	5.55 ^c	7.69 ^a	6.17 ^b	7.09 ^a	0.453	0.976	0.001	0.097



Dressing %	39.32	38.92	38.48	38.36	1.775	0.592	0.841	0.915
Carcass Length (m)	0.43 ^c	0.55 ^a	0.50 ^b	0.53 ^a	0.024	0.132	0.003	0.039
Primal cuts (Kg)								
Back	0.31	0.41	0.38	0.37	0.045	0.612	0.208	0.111
Chuck	0.35 ^c	0.54 ^a	0.40 ^b	0.56 ^a	0.058	0.439	0.003	0.753
Leg	0.81 ^c	1.18 ^a	0.94 ^b	1.05 ^a	0.080	0.955	0.003	0.051
Loin	0.29 ^c	0.41 ^a	0.35 ^b	0.40 ^a	0.039	0.424	0.017	0.310
Neck	0.46	0.73	0.48	0.47	0.103	0.147	0.112	0.097
Rib and Flank	0.42 ^b	0.61 ^a	0.55 ^a	0.63 ^a	0.054	0.089	0.008	0.197
Shoulder	0.49 ^c	0.71 ^a	0.57 ^b	0.67 ^a	0.054	0.617	0.004	0.157

V: Variety, P: Phosphorus fertilizer, DW: Dressed weight, LW: Live weight, SED- Standard Error of Difference, P= probability, Means with the same letters are not significantly different ($P > 0.05$).

4.5.1 Carcass parameters

Apart from dressing percentage, there were significant differences ($P < 0.05$) in live weight, dressed weight and carcass length between animals fed Zaayura-SARC 4-75 and Hewale-IT93K-192-4 at 30 and 90 kg P_2O_5 /ha rate application (Table 15).

Live weight and dressed weight differed ($P < 0.05$) between the phosphate rates. There was an increasing trend in weights as phosphate fertilizer application rates increased from 30 to 90 kg P_2O_5 /ha. There was a significant V x P rate interaction ($P < 0.05$) for carcass length with animals on Zaayura at 90 kg P_2O_5 /ha rates having the highest carcass lengths. There was no varietal difference ($P > 0.05$) for all the carcass parameters measured and calculated (Table 15).

4.5.2. Primal Cuts

Apart from back weight and neck weight, there were significant ($P < 0.05$) differences in chuck weight, leg weight, loin weight, rib and flank weight and shoulder weight between animals fed Zaayura-SARC 4-75 and Hewale-IT93K-192-4 at 30 and 90 kg P_2O_5 /ha rate application (Table 15).



Chuck weight, loin weight, rib and flank weight and shoulder weight differed ($P < 0.05$) between the phosphate rates. There was an increasing trend in weights as phosphate fertilizer application rates increased from 30 to 90 kg P_2O_5 /ha. There was a significant V x P rate interaction ($P < 0.05$) for leg weight with animals on Zaayura-SARC 4-75 at 90 kg P_2O_5 /ha rates having the highest leg weights. There was no varietal difference ($P > 0.05$) for all the primal cuts measured (Table 15).

4.5.3 Internal and External Organs Parameters

The effect of cowpea haulm at different phosphate rates on internal and external organs of Djallonké sheep is shown in table 16.

Table 16: Effect of Cowpea Haulm at Different Phosphate Rates on Internal and External Organs of Djallonké Sheep

Internal organs (kg)	Zaayura		Hewale		SED V x P	P-value		
	30	90	30	90		V	P	V x P
Liver	0.27 ^a	0.39 ^b	0.30 ^a	0.36 ^a	0.048	0.924	0.029	0.403
Lungs	0.19 ^a	0.27 ^b	0.27 ^b	0.28 ^b	0.028	0.048	0.048	0.134
Empty digestive tract	0.57	0.72	0.69	0.74	0.084	0.252	0.130	0.395
Heart	0.07	0.08	0.32	0.07	0.170	0.349	0.349	0.324
Spleen	0.04	0.05	0.05	0.04	0.009	0.803	0.803	0.461
Kidney	0.07	0.07	0.07	0.08	0.008	0.580	0.122	0.580
External organs (Kg)								
Testis	0.30	0.34	0.33	0.35	0.049	0.523	0.416	0.782
Skin	1.05 ^a	1.39 ^b	1.33 ^b	1.31 ^b	0.115	0.969	0.014	0.373
Head	1.19 ^a	1.65 ^b	1.29 ^c	1.45 ^d	0.018	0.329	0.001	0.028

V: Variety, P: Phosphorus fertilizer, SED- Standard Error of Difference, P= probability, Means with the same letters are not significantly different ($P > 0.05$).



4.5.3.1 Internal Organs

Apart from liver weight and lungs weight there were no significant differences ($P>0.05$) in all the internal organ parameters measured between animals fed Zaayura-SARC 4-75 and Hewale-IT93K-192-4 at 30 and 90 kg P_2O_5 /ha rate application (Table 16).

There was however a significant ($P<0.05$) effect of phosphate rates on liver weight and lungs weight. There was an increasing trend in weights as phosphate fertilizer application rates increased from 30 to 90 kg P_2O_5 /ha.

There was also a varietal difference ($P<0.05$) on lung weights with the highest recorded in Hewale-IT93K-192-4 (Table 16).

4.5.3.2 External Organs

Apart from testis weight, there were significant differences ($P<0.05$) in skin weight and head weight between animals fed Zaayura-SARC 4-75 and Hewale-IT93K-192-4 at 30 and 90 kg P_2O_5 /ha rate application (Table 16).

Skin weight differed ($P<0.05$) between the phosphate rates. There was an increasing trend in weights as phosphate fertilizer application rates increased from 30 to 90 kg P_2O_5 /ha. There was a significant ($P<0.05$) V x P rate interaction for head weight with animals on Zaayura-SARC 4-75 at 90 kg P_2O_5 /ha rates having the highest head weights. There was no varietal difference ($P>0.05$) for all the external organs measured (Table 16).



CHAPTER FIVE

5.0 DISCUSSION

5.1 Chemical Composition of haulms of Cowpea Varieties

In terms of ruminant production, the quality of forage is more important than the quantity. Forage quality is determined by the content of different nutrients such as minerals, crude protein and fiber component (Mahmut *et al.*, 2010). The concentration of chemical components in forage varies depending on many variables such as species Ramirez *et al.* (2004), harvesting date Ansah *et al.* (2010); Ball *et al.* (2001), fertilization Turk *et al.* (2007), soil properties and other environmental variables Kulik (2009).

In this study, phosphate fertilizer application significantly had a positive effect on the crude protein content of the cowpea varieties (Table 11). Fertilization is the most effective and practical method of increasing forage production in a natural meadow. Fertilization enhances not only dry matter production, but also affects the chemical content of the forage (Bell *et al.*, 2001; Turk *et al.*, 2007). Phosphate fertilizer enhances nitrogen uptake by plant, and crude protein content may increase in forage harvested from plots where phosphate is applied because nitrogen is the main component of the protein (Benedycka *et al.*, 1992).

The crude protein content is an important indication of nutritional quality since the cultivars are intended to be used as supplements for poor quality crop residues.

The differences observed in the varieties at different phosphate rates suggest the varieties could be selected for different rates of fertilizer application. Bell *et al.* (2001) reported differences in dry matter yield and chemical composition of forage legume at different rates



of phosphate application. Turk *et al.* (2007) reported an increase in crude protein and decrease in NDF and ADF with increasing phosphate level application.

However, the increasing level of phosphate did not correspond to an increase in crude protein in all the varieties (Table 11). This finding differs from what has been reported earlier (Miskovic *et al.*, 1977; Celik, 1980; Comakli and Tas, 1996; Turk *et al.*, 2011). The differences in CP values from the reported data may be as a result of genetic improvement of the cultivars and inherent genetic characteristics (Badve *et al.*, 1994; Subba Rao *et al.*, 1994; Singh and Schiere, 1995). 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) could be other reasons (Harika and Sharma, 1994; Walli *et al.*, 1994). The lower levels (30 and 60 kg P₂O₅/ha) application resulted in much higher CP in all varieties than the 90 kg P₂O₅/ha application. The CP levels reported in the present study were above the minimum of 80 g/kg DM necessary for adequate functioning of rumen microbes Orskov and McDonald (1979) and could therefore be used as supplemental CP to ruminants fed low quality cereal straw.

Even though decreasing levels of NDF and ADF have been reported with increasing phosphate rates by Turk *et al.* (2007), it did not reflect in this study. Different varieties showed different trends in aNDFom and ADFom at different phosphate rates. In contrast to Asomdwe-IT94K-410-2 and Songotra-IT97K-499-35 where aNDFom increased with increase in P rates, Hewale-IT93K-192-4 declined. Varieties IT- IT99K-573-1-1 and Zaayura-SARC 4-75 increased from P rates 30 kg P₂O₅/ha to 60 kg P₂O₅/ha but decline at P rate 90 kg P₂O₅/ha. Whereas the ADFom for Songotra-IT97K-499-35 and Asomdwe-IT94K-410- increased with increasing P rates, IT- IT99K-573-1-1 and Hewale-IT93K-192-



4 declined. Cellulose and hemicellulose which form a major fraction of the cell wall carbohydrates are major components of aNDFom and ADFom and are usually found to increase in vegetative plant parts with advance plant maturity.

The five cowpea varieties studied showed marked differences in all the parameters investigated which is attributable to possible differences in their cell wall structures as they were bred for improved pest's resistance and drought tolerance amongst others (Singh and Tarawali, 1997; CRI, 2010). This could imply that different rates of phosphate fertilizer could alter the maturity periods of the different cowpea varieties.

The highest aNDFom and ADFom contents recorded for variety Songotra-IT97K-499-35 at 90 kg P₂O₅/ha application could be a reflection of its higher insect pest resistance.

5.2 *In Vitro* Gas Study

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

The differences in digestible organic matter (DOM) among varieties could be explained by the differences in CP, aNDFom and ADFom and this agrees with findings of Larbi *et al.* (2010) who found differences in *in vitro* organic matter digestibility (IVOMD) of haulms from different cowpea accessions. The high CP content may have supplied the needed degradable protein for cellulolytic microbes to digest the available organic matter. It is accepted that forage degradation in the rumen is affected mainly by the cell wall content and its lignification, as lignin is an indigestible fraction and acts as a barrier, limiting the



access of microbial enzymes to the structural polysaccharides of the cell wall. Ammar (2002) reported that NDF, ADF and ADL levels were negatively correlated with *in vitro* digestibility.

Nsahlai *et al.* (1994) and Larbi *et al.* (1998) reported that there was a positive correlation between CP and the rate of gas production, and negative correlations between NDF and ADF with the rate and extent of gas production.

The variations in the digestible organic matter among the cowpea varieties may also be related to differences in physical structure, such as the distribution of lignified cells within the tissues (Ramanzin *et al.*, 1991). 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.

Thus the high DOM of variety Zaayura-SARC 4-75 (Table 12) among the varieties is a reflection of its low contents of lignin and crude fibre but high CP.

Other factors known to affect the composition and digestibility of straw are variety and cultivar Mould *et al.* (2001), Kafilzadeh and Maleki (2011), environmental and seasonal effects Mathison *et al.* (1999) and proportion of morphological fractions (stem, leaf and seed ratios) of the straw (Agbagla-Dohnani *et al.*, 2001).

5.3 Growth performance

The existence of new cowpea varieties may suggest possible differences in fodder quality that could have either positive or negative correlation with other traits, such as intake and body weight.



These differences in fibre components are expected to influence voluntary intake and apparent digestibility of the varieties. Dry matter intakes (DMI) were within reported ranges for cowpea crop residues reported by Koralagama *et al.* (2008).

Higher DMI ($P < 0.05$) was obtained in Zaayura-SARC 4-75 at higher (90 kg P_2O_5 /ha) rates than the lower phosphate rates and might be attributed to the high ADF reported in Zaayura-SARC 4-75 at 30 kg P_2O_5 /ha. Higher ADF has been found to reduce voluntary dry matter intake (Riaz *et al.*, 2014). The DMI was however high in animals supplemented with Hewale-IT93K-192-4 at 30 kg P_2O_5 /ha than 90 kg P_2O_5 /ha despite the high ADF at 30 kg P_2O_5 /ha. This could be attributed to the fact that Hewale-IT93K-192-4 at 30 kg P_2O_5 /ha might have been palatable to the animals thereby causing them to eat more. The differences in DMI among the varieties are related to their digestibility, which agrees with the observations by Allen (1996) that differences in fibre digestibility also affect DMI. Knowledge of their respective voluntary intake and digestibility is important in selecting the varieties that would be suitable for crop-livestock farming systems.

The strong varietal differences among the cowpea varieties, in terms of dry matter intake (DMI) could be very useful in developing better varieties in cowpea breeding programmes (Becker and Einfeldt, 1995).

Differences in the proportions could also affect the quality of the varieties since those with high leaf-to-stem ratios are expected to be of better fodder quality in terms of high CP, low fibre and lignin contents than those with lower leaf-to-stem ratios (Alhassan *et al.*, 1987; Devendra, 1997). Using the cowpea haulms as supplement to poor quality roughage in the dry season is also a promising option both in the management of the feed budget and to ensure efficient utilization of available feed resources.



5.4 Haematology and Blood Biochemistry

Blood is an important index of physiological, pathological and nutritional status of an organism (Ewuola *et al.*, 2004). Aletor (1989) indicated that the blood variables most consistently affected by dietary influence includes RBC, PCV and plasma protein.

The significance of haematological profile is to provide valuable information on the physiological responsiveness of the animal to its internal and external environment, which includes feed and feeding (Kral and Suchy, 2000; Esonu *et al.*, 2001), which might affect the circulation of blood component. The haematological and blood biochemical parameters measured for all treatment groups (Table 14) in this study did not differ except for globulin and blood urea nitrogen (BUN). The range of globulin concentration in the blood depends on total protein and albumin level in the blood. Globulin concentration is done mathematically by subtracting albumin value from that of total protein. Globulin concentration could give an indication of the immune response of an organism (Ismail *et al.*, 2002). The globulin concentrations reported in this study were enough to support the immune system of the lambs. According to Shrikhande *et al.* (2008) the variation in serum globulin may be due to various physiological, management and genetic factors. The values in this study were lower than 37-40 g/l reported by Konlan *et al.* (2012). This difference might be due to the difference in management and genetic factors. Higher BUN ($P < 0.05$) was reported in this study for Hewale-IT93K-192-4 at higher rates indicating a higher protein degradation in the rumen. The Hb reported fell within the range of 8-16 g/dl reported for normal sheep (Pampori, 2003). The WBC and PCV were also in line with the range reported for normal sheep. The normal PCV and WBC ranges reported by Pampori (2003) for sheep were 26-36% and 4-10 L respectively.



5.5 Carcass Parameters

The higher carcass parameters reported in animals supplemented with haulm at 90 kg P₂O₅/ha is an indication that the CP in the cowpea haulms for this phosphate rate matched with the needs of rumen microbes for the type of fermentable carbohydrate found in the diets. Even though lower CP was obtained in 90 kg P₂O₅/ha compared to 30 kg P₂O₅/ha, there was a slightly lower BUN concentration reported in the animals on the 30 kg P₂O₅/ha suggesting some limitation on the ability of rumen microbes to effectively degrade the CP in the 30 kg P₂O₅/ha compared to the 90 kg P₂O₅/ha. The differences observed in both internal and external organs might be attributed to similar effects. Ledger (1965) reported that as animals grow their organs increase in weight but in proportion to their live weight however, the results of this study did not conform to it. The dressing percentage reported in this present study is in line with what was reported by Ansah *et al.* (2015).



CHAPTER SIX

6.0 Conclusions and Recommendations

6.1 Conclusions

Based on the study, it can be concluded that:

- The CP levels of the haulms of all the varieties were adequate for ruminant supplementation.
- Phosphate applications rate at 30 and 60 kg P_2O_5 /ha were adequate for improved chemical composition whilst phosphate rate at 90 kg P_2O_5 /ha improved carcass characteristics.
- The growth indices of sheep, carcass and blood profile were affected differently by cowpea variety and rates of phosphate fertilizer application.
- Hewale-IT93K-192-4 at 30 kg P_2O_5 /ha showed a higher potential for growth and therefore can be used as quality fodder for sheep.
- Cowpea variety and rates of phosphate fertilizer application had no adverse effect on *in vitro* gas production parameters except for digestible organic matter (DOM) which was above 40% for all varieties.

6.2 Recommendations

- Ruminant farmers when selecting and cultivating dual purpose cowpea should use phosphate fertilizer rates up to 60 kg P_2O_5 /ha to improve the quality of fodder for sheep but could use 90 kg P_2O_5 /ha if the intention is to improve carcass characteristics.



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APPENDIX

AP.1 ANOVA Tables for chemical composition of cowpea varieties

Variate: CP g/kg					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	4	3534.07	883.52	14.64	<.001
Phosphate Level	2	275.66	137.83	2.28	0.119
Variety X Phosphate Level	8	1127.78	140.97	2.34	0.044
Residuals	30	1811.00	60.37		
Total	44	6748.51			

Variate: NDF g/kg					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	4	21000.5	5250.1	6.37	<.001
Phosphate Level	2	6146.5	3073.3	3.73	0.032
Variety X Phosphate Level	8	17169.6	2146.2	2.60	0.020
Residuals	45	37086.8	824.2		
Total	59	81408.4			

Variate: ADF g/kg					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	4	19177.5	4794.4	10.07	<.001
Phosphate Level	2	177.5	88.7	0.19	0.031
Variety X Phosphate Level	8	8712.5	1089.1	2.29	0.038
Residuals	45	21431.0	476.2		
Total	59	49498.5			



AP.2 ANOVA Tables for *in vitro* organic matter digestibility and gas production

Variate: DOM					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Run stratum	3	270.361	90.120	21.73	
Run. *Units* Stratum					
Variety	4	90.982	22.745	5.48	<.001
Phosphate Level	2	20.778	10.389	2.51	0.087
Variety X Phosphate Level	8	40.746	5.093	1.23	0.290
Residuals	102	423.024	4.147		
Total	119	845.892			

Variate: b					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Run stratum	3	286.375	95.458	22.81	
Run. *Units* Stratum					
Variety	4	24.618	6.154	1.47	0.217
Phosphate Level	2	7.628	3.814	0.91	0.405
Variety X Phosphate Level	8	39.971	4.996	1.19	0.310
Residuals	102	426.857	4.185		
Total	119	785.449			

Variate: c					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Run stratum	3	0.0046628	0.0015543	12.86	
Run. *Units* Stratum					
Variety	4	0.0001609	0.0000402	0.33	0.855
Phosphate Level	2	0.0002177	0.0001089	0.90	0.410
Variety X Phosphate Level	8	0.0006217	0.0000777	0.64	0.740
Residuals	102	0.0123311	0.0001209		
Total	119	0.0179942			



Variate: <i>in vitro</i> gas at 48hours					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Run stratum	3	496.529	165.510	25.44	
Run. *Units* Stratum					
Variety	4	36.282	9.071	1.39	0.241
Phosphate Level	2	2.140	1.070	0.16	0.849
Variety X Phosphate Level	8	72.472	9.059	1.39	0.209
Residuals	102	663.681	6.507		
Total	119	1271.104			

AP.3 *In vitro* gas production curves for the various varieties of cowpea

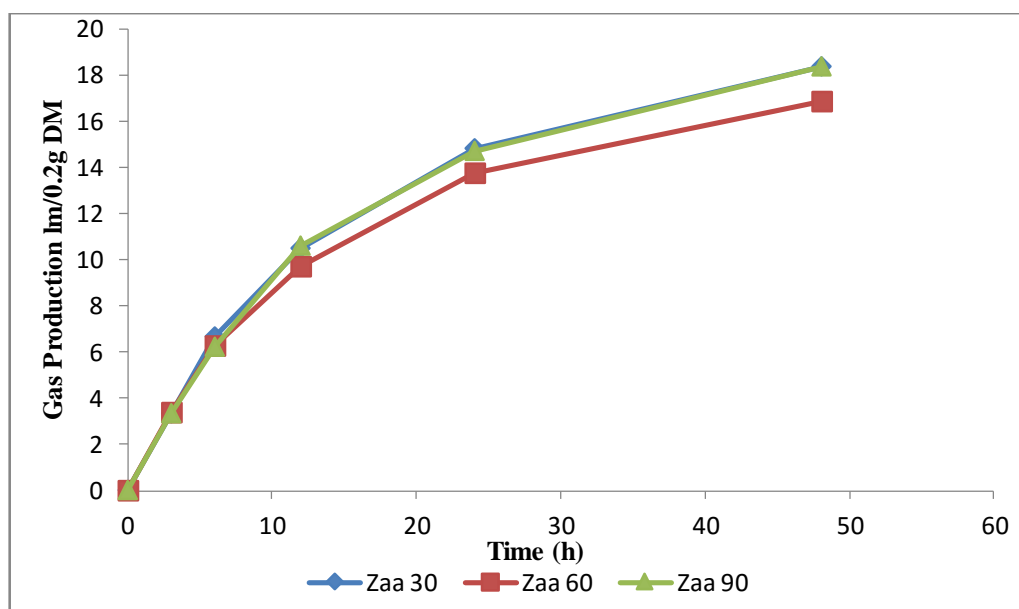


Figure 1: Cumulative *in vitro* gas production for Zaayura (Zaa)

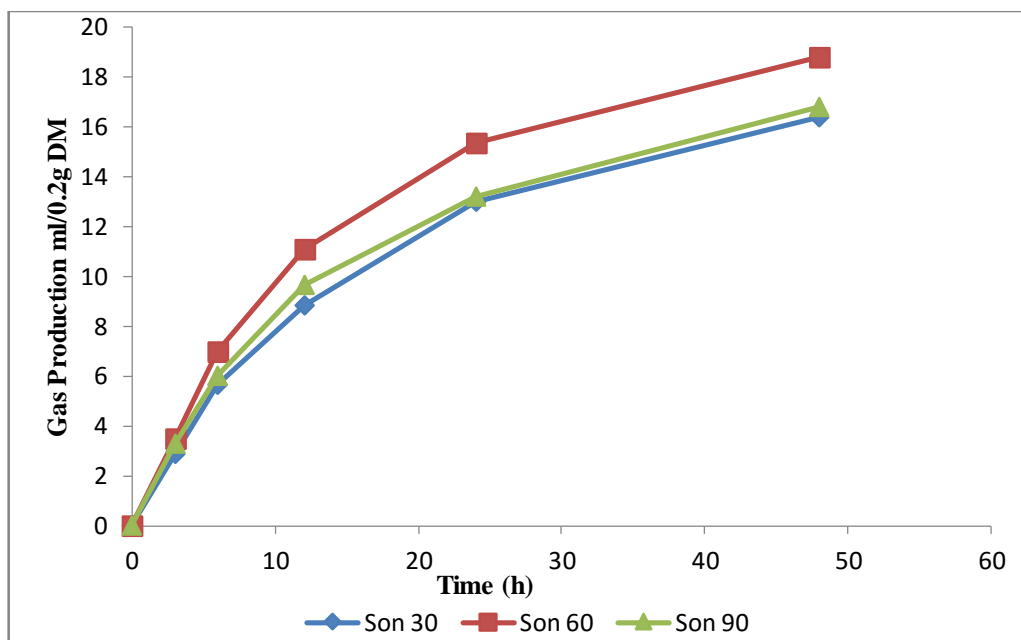


Figure 2: Cumulative *in vitro* gas production for Songotra (Son)

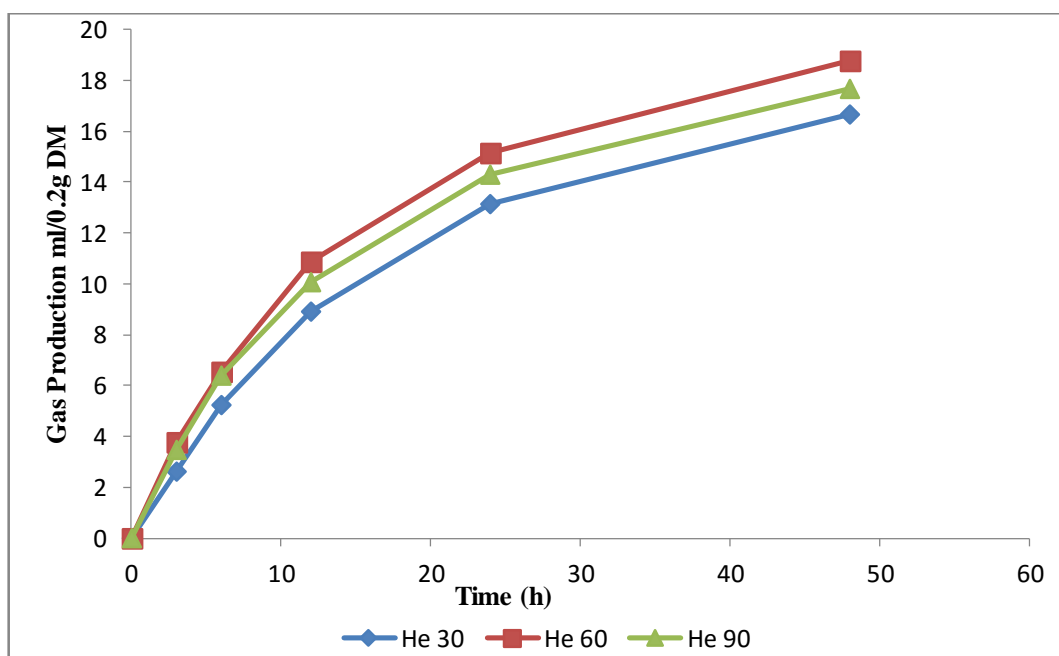
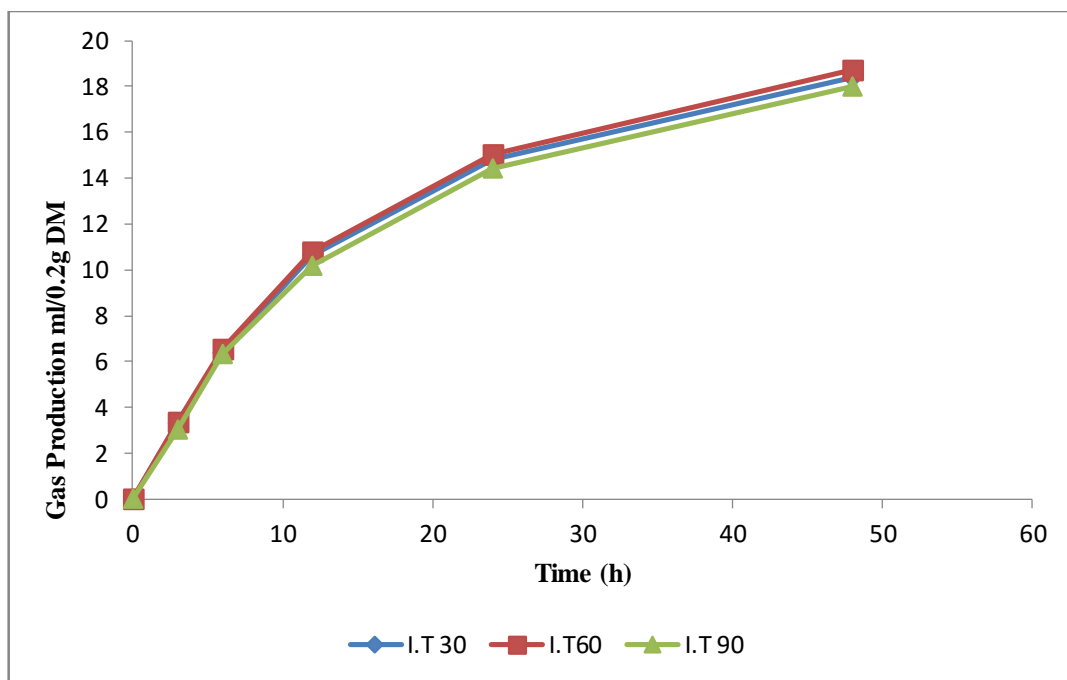


Figure 3: Cumulative *in vitro* gas production for Hewale (H)





Figure

4: Cumulative *in vitro* gas production for I.T

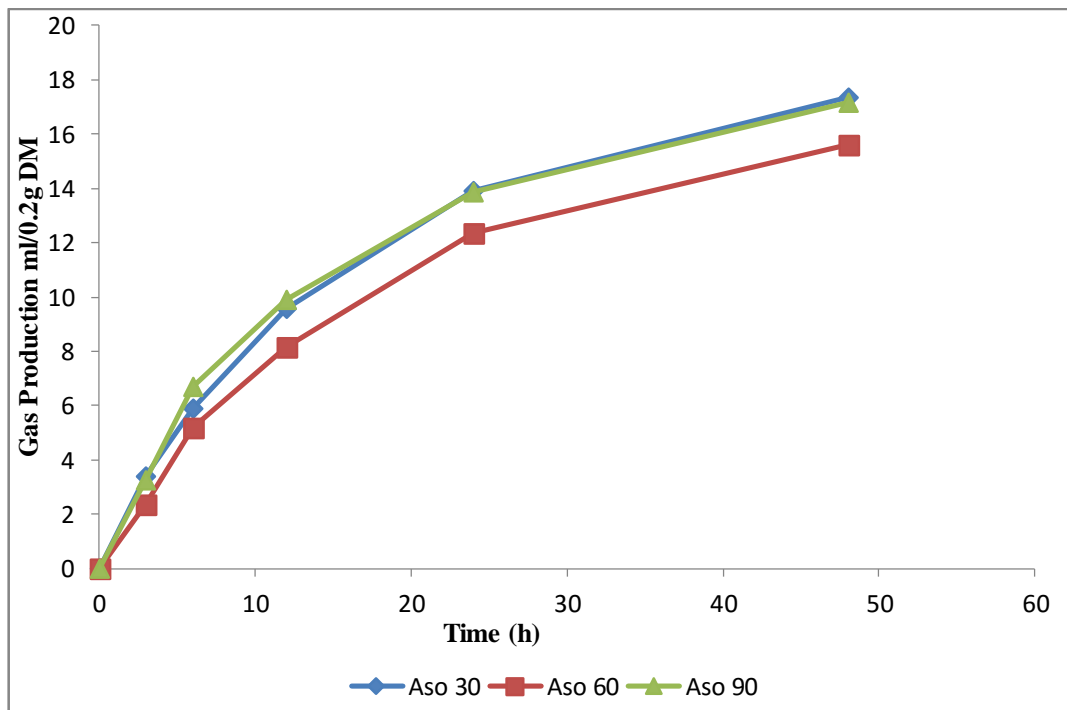


Figure 5: Cumulative *in vitro* gas production for Asomdwe (Aso)



AP.4 ANOVA Tables for growth

Variate: Initial weight (g)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	1.201	1.201	0.23	0.634
Phosphate Level	1	9.941	9.941	1.95	0.182
Variety X Phosphate Level	1	14.620	14.620	2.86	0.110
Residuals	16	81.768	5.111		
Total	19	107.529			

Variate: Dry matter intake (g)						
Covariate: Initial weight (kg)						
Source of variation	d.f.	s.s.	m.s.	v.r.	Cov.ef.	F pr.
Variety	1	27.4	27.4	0.26	0.99	0.621
Phosphate Level	1	3.3	13.3	0.12	0.89	0.730
Variety X Phosphate Level	1	1356.0	1356.0	12.61	0.85	0.003
Covariate	1	13.4	13.4	0.12		0.729
Residuals	15	1612.8	107.5		0.95	
Total	19	3120.1				

Variate: Average daily weight gain (g)						
Covariate: Initial weight (kg)						
Source of variation	d.f.	s.s.	m.s.	v.r.	Cov.ef.	F pr.
Variety	1	253.6	253.6	1.93	0.99	0.185
Phosphate Level	1	222.4	222.4	1.69	0.89	0.213
Variety X Phosphate Level	1	21.2	21.2	0.16	0.85	0.694
Covariate	1	74.7	74.7	0.57		0.462
Residuals	15	1969.7	131.3		0.97	
Total	19	2694.5				



Variate: Average daily weight (g)						
Covariate: Initial weight (kg)						
Source of variation	d.f.	s.s.	m.s.	v.r.	Cov.ef.	F pr.
Variety	1	11.0	11.0	0.05	0.99	0.821
Phosphate Level	1	851.5	851.5	4.11	0.89	0.061
Variety X Phosphate Level	1	24.9	24.9	0.12	0.85	0.734
Covariate	1	27513.6	27513.6	132.88		<.001
Residuals	15	3105.9	207.1		9.24	
Total	19	42826.1				

Variate: Final weight gain (g)						
Covariate: Initial weight (kg)						
Source of variation	d.f.	s.s.	m.s.	v.r.	Cov.ef.	F pr.
Variety	1	794216.	794216.	1.93	0.99	0.185
Phosphate Level	1	698448.	698448.	1.70	0.89	0.212
Variety X Phosphate Level	1	66198.	66198.	0.16	0.85	0.694
Covariate	1	233978.	233978.	0.57		0.463
Residuals	15	6177619.	411841.		0.97	
Total	19	8450191.				

AP.5 ANOVA Tables for haematology and blood biochemistry

Variate: Haemoglobin (g/dl)						
Covariate: Initial weight (kg)						
Source of variation	d.f.	s.s.	m.s.	v.r.	Cov.ef.	F pr.
Variety	1	0.213	0.213	0.12	0.99	0.731
Phosphate Level	1	0.466	0.466	0.27	0.89	0.612
Variety X Phosphate Level	1	0.086	0.086	0.05	0.85	0.827
Covariate	1	1.092	1.092	0.63		0.440
Residuals	15	26.016	1.734		0.98	
Total	19	28.122				

Variate: Packed cell volume (%)						
Covariate: Initial weight (kg)						
Source of variation	d.f.	s.s.	m.s.	v.r.	Cov.ef.	F pr.
Variety	1	2.07	2.07	0.13	0.99	0.720
Phosphate Level	1	3.88	3.88	0.25	0.89	0.624
Variety X Phosphate Level	1	0.78	0.78	0.05	0.85	0.826
Covariate	1	9.15	9.15	0.59		0.454
Residuals	15	232.85	15.52		0.97	
Total	19	251.00				

Variate: White blood cells ($\times 10^9$ L)						
Covariate: Initial weight (kg)						
Source of variation	d.f.	s.s.	m.s.	v.r.	Cov.ef.	F pr.
Variety	1	0.442	0.442	0.31	0.99	0.584
Phosphate Level	1	1.016	1.016	0.31	0.89	0.410
Variety X Phosphate Level	1	0.331	0.331	0.72	0.85	0.635
Covariate	1	0.055	0.055	0.23		0.847
Residuals	15	21.171	1.411	0.04	0.94	
Total	19	23.126				

Variate: Albumin (g/l)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	9.557	9.557	3.54	0.079
Phosphate Level	1	2.720	2.720	1.01	0.331
Variety X Phosphate Level	1	1.263	1.263	0.47	0.504
Residuals	15	40.439	2.696		
Total	18	53.632			

Variate: Globulin (g/l)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	63.101	63.101	6.42	0.023
Phosphate Level	1	0.319	0.319	0.03	0.859
Variety X Phosphate Level	1	7.718	7.718	0.79	0.387
Residuals	15	147.331	9.822		
Total	18	216.812			



Variate: Glucose (mmol/l)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.15753	0.15753	2.83	0.113
Phosphate Level	1	0.06903	0.06903	1.24	0.283
Variety X Phosphate Level	1	0.00153	0.00153	0.03	0.871
Residuals	15	0.83550	0.05570		
Total	18	1.03789			

Variate: Total protein (g/l)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	23.545	23.545	2.66	0.124
Phosphate Level	1	4.901	4.901	0.55	0.469
Variety X Phosphate Level	1	15.313	15.313	1.73	0.209
Residuals	15	132.992	8.866		
Total	18	176.226			

Variate: Blood urea nitrogen (mmol/l)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.000	0.000	0.00	0.997
Phosphate Level	1	8.160	8.160	2.66	0.124
Variety X Phosphate Level	1	17.252	17.252	5.62	0.032
Residuals	15	46.047	3.070		
Total	18	68.877			

AP.6 ANOVA Tables for carcass characteristics

Variate: Live weight (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.270	0.270	0.10	0.758
Phosphate Level	1	47.203	47.203	17.85	0.003
Variety X Phosphate Level	1	7.489	7.489	2.83	0.131
Residuals	8	21.161	2.645		
Total	11	76.124			



Variate: Dressed weight (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.0003	0.0003	0.00	0.976
Phosphate Level	1	7.0227	7.0227	22.78	0.001
Variety X Phosphate Level	1	2.4667	1.0920	3.54	0.097
Residuals	8	1.0920	0.3083		
Total	11	10.5817			

Variate: Dressing %					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	1.469	1.469	0.31	0.592
Phosphate Level	1	0.203	0.203	0.04	0.841
Variety X Phosphate Level	1	0.057	0.057	0.01	0.915
Residuals	8	37.800	4.725		
Total	11	39.530			

Variate: Carcass length (m)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.0024083	0.0024083	2.81	0.132
Phosphate Level	1	0.0154083	0.0154083	17.95	0.003
Variety X Phosphate Level	1	0.0052083	0.0052083	6.07	0.039
Residuals	8	0.0068667	0.0008583		
Total	11	0.0298917			

Variate: Back (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.000833	0.000833	0.28	0.612
Phosphate Level	1	0.005633	0.005633	1.88	0.208
Variety X Phosphate Level	1	0.009633	0.009633	3.21	0.111
Residuals	8	0.024000	0.003000		
Total	11	0.040100			



Variate: Chuck (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.003333	0.003333	0.66	0.439
Phosphate Level	1	0.090133	0.090133	17.91	0.003
Variety X Phosphate Level	1	0.000533	0.000533	0.11	0.753
Residuals	8	0.040267	0.005033		
Total	11	0.134267			

Variate: Leg (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.000033	0.000033	0.00	0.955
Phosphate Level	1	0.177633	0.177633	18.38	0.003
Variety X Phosphate Level	1	0.050700	0.050700	5.24	0.051
Residuals	8	0.077333	0.009667		
Total	11	0.305700			

Variate: Loin (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.001633	0.001633	0.71	0.424
Phosphate Level	1	0.020833	0.020833	9.06	0.017
Variety X Phosphate Level	1	0.002700	0.002700	1.17	0.310
Residuals	8	0.018400	0.002300		
Total	11	0.043567			

Variate: Neck (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.04083	0.04083	2.57	0.147
Phosphate Level	1	0.05070	0.05070	3.20	0.112
Variety X Phosphate Level	1	0.05603	0.05603	3.53	0.097
Residuals	8	0.12693	0.01587		
Total	11	0.27450			



Variate: Rib and flank (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.016133	0.016133	3.75	0.089
Phosphate Level	1	0.053333	0.053333	12.40	0.008
Variety X Phosphate Level	1	0.008533	0.008533	1.98	0.197
Residuals	8	0.034400	0.004300		
Total	11	0.112400			

Variate: Shoulder (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.001200	0.001200	0.27	0.617
Phosphate Level	1	0.070533	0.070533	15.91	0.004
Variety X Phosphate Level	1	0.010800	0.010800	2.44	0.157
Residuals	8	0.035467	0.004433		
Total	11	0.118000			

Variate: Liver (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.000033	0.000033	0.01	0.924
Phosphate Level	1	0.024300	0.024300	7.01	0.029
Variety X Phosphate Level	1	0.002700	0.002700	0.78	0.403
Residuals	8	0.027733	0.003467		
Total	11	0.054767			

Variate: Lungs (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.006533	0.006533	5.44	0.048
Phosphate Level	1	0.006533	0.006533	5.44	0.048
Variety X Phosphate Level	1	0.003333	0.003333	2.78	0.134
Residuals	8	0.009600	0.001200		
Total	11	0.026000			



Variate: Empty digestive tract (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.01613	0.01613	1.53	0.252
Phosphate Level	1	0.03000	0.03000	2.84	0.130
Variety X Phosphate Level	1	0.00853	0.00853	0.81	0.395
Residuals	8	0.08453	0.01057		
Total	11	0.13920			

Variate: Heart (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.04320	0.04320	0.99	0.349
Phosphate Level	1	0.04320	0.04813	0.99	0.349
Variety X Phosphate Level	1	0.04813	0.04320	1.10	0.324
Residuals	8	0.34853	0.04357		
Total	11	0.48307			

Variate: Spleen (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.0000083	0.0000083	0.07	0.803
Phosphate Level	1	0.0000083	0.0000083	0.07	0.803
Variety X Phosphate Level	1	0.0000750	0.0000750	0.60	0.461
Residuals	8	0.0010000	0.0001250		
Total	11	0.0010917			

Variate: Kidney (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.0000333	0.0000333	0.33	0.580
Phosphate Level	1	0.0003000	0.0003000	3.00	0.122
Variety X Phosphate Level	1	0.0000333	0.0000333	0.33	0.580
Residuals	8	0.0008000	0.0001000		
Total	11	0.0011667			



Variate: Testis (kg)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.001633	0.001633	0.45	0.523
Phosphate Level	1	0.002700	0.002700	0.74	0.416
Variety X Phosphate Level	1	0.000300	0.000300	0.08	0.782
Residuals	8	0.029333	0.003667		
Total	11	0.033967			



AP. 7 An experimental sheep in a pen



AP. 8 An experimental sheep on a scale ready for weighing



AP. 9 Laboratory technician assisting student to collect blood sample





AP. 10 Experimental carcasses hanged for primal cuts



AP. 11 Experimental samples in water-bathe for *in vitro* gas experiment



AP. 12 Taking gas readings using Manometer

