UNIVERSITY FOR DEVELOPMENT STUDIES

NUTRITIVE VALUE AND GREENHOUSE GAS MITIGATION POTENTIAL OF EIGHT BROWSE PLANTS FROM NORTHERN GHANA

TERRY ANSAH

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 DOCTOR OF PHILOSOPHY DEGREE IN ANIMAL NUTRITION

2015

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BY

TERRY ANSAH (B.Sc. Agriculture Technology, M.Sc. Animal Nutrition and Management)

[DAN/002/11]

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AUGUST 2015

DECLARATION

Student

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature……..Date…….

Name: Terry Ansah

Supervisors

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

Five separate experiments were conducted to assess the nutritive value and greenhouse gas (GHG) mitigation potential of eight browse plants in Northern Ghana. The browse species were *Ceiba pentandra, Khaya senegalensis, Senna siamea, Ficus gnaphalocarpa, Pterocarpus erinaceus, Albizia lebbeck, Azadirachta indica* and *Gmelina arborea*. The nutrient compositions of the browse plants were determined before the various experiments were conducted. The crude protein was highest (229.2 g/kgDM) in *A. lebbeck* and lowest (92.3 g/kgDM) in *K. senegalensis*. The highest NDF was obtained in *P. erinaceus* with the least in *F. gnaphalocarpa*. The ADF ranged between 163.4 and 291.8 g/kgDM in *G.arborea* and *C. pentandra* respectively. The concentration of condensed tannin was highest (114.5 g/kgDM) in *F. gnaphalocarpa* but below measurable limits in *A. lebbeck*. In experiment I, the *in vitro* batch culture was used to evaluate the DM digestibility of the browse plants incubated in nitrogen sufficient and nitrogen deficient media. The experiment was designed as a completely randomised block design in 8×2 factorial arrangement. The interaction between media and browse plants was not significant ($P \geq 0.480$) for all the parameters measured. However, *in vitro* DM digestibility (IVDMD) and ammonia nitrogen (NH_3N) concentration were higher ($P<0.05$) in *Albizia lebbeck* than the other browse plants. The N-sufficient media had higher ($P<0.05$) NH_3N than the N-defficient media, but the IVDMD was similar ($P>0.05$). *In vitro* DM digestibility did not also differ between *A.lebbeck* and the high CT-browse plants (*C. pentandra* and *F. gnaphalocarpa*). In experiment II, the *in sacco* technique was used to evaluate the extent of protein degradation in the rumen using the completely randomised design. The CP degradability parameters all differed ($P<0.05$) among the browse plants. The high CT-browse plants (*C. pentandra* and *F. gnaphalocarpa*) had a lower ($P<0.05$) effective CP degradability compared with the low CT-browse plants. In experiment III, the *in vitro* continuous culture technique was used to investigate the effect of three of the browse plants (*C. pentandra, S. siamea and G. arborea*) on enteric methane emission in a 4×4 Latin square design. Methane

gas was reduced (P>0.05) by 38%, 19% and 19% when wheat straw was supplemented with *C. pentandra, G. arborea and S. siamea,* respectively. In experiment IV, nitrogen metabolism and fiber digestibility of the browse were assessed in a cross-over design. Four browse plants (*C. pentandra, S. siamea, G. arborea* and *A. lebbeck*) were each used as partial replacements for rice straw in a completely randomised block design. Differences (P<0.001) in daily DM intake, daily CP intake, nitrogen balance and CP digestibility were recorded. The effect of supplementing Djallonké sheep grazing on natural pastures with four browse plants on growth and carcass characteristics were assessed in experiment V. A total of 25 intact rams were randomly assigned to four browse plants (*C. pentandra, S. siamea, G. arborea* and *A. lebbeck*) and a control. The highest daily DM intake of the supplement was reported in lambs fed the *G. arborea*. Daily liveweight gain of the supplemented animals improved (P<0.05) when compared to the control animals. Testicular weight increased (P<0.05) in rams that were fed *A. lebbeck* diet. It can be concluded that, the browse plants had potential to reduce enteric methane emission and improve growth performance of Djallonke sheep when used as dietary supplement.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to the almighty God for giving me life and grace to complete this work. My sincere thanks go to Prof. Herbert K. Dei and Prof. Robert G. Wilkinson, my project supervisors for their direction and constructive criticisms in the planning and execution of this study. I also thank, Dr. Jim Huntington of the Harper Adams University for his selfless support during my visit to the Harper Adams University, UK to carry out my *in vitro* and *in Sacco* experiments. My sincere thanks also go to Prof. Hanne Helen Hansen of the Copenhagen University, Dr. Asamoah Larbi of IITA, Africa RISING and Prof. Gabriel A. Teye of the UDS for the mentoring and literature materials they provided. I thank all the staff of the Department of Animal Science for their support especially Mr. Jakper Naandam for proof reading the thesis. I also like to thank Mr. Cudjoe Shadrack, Mr. Daniel Kofi Adgei and Mr. Samoah Njarnab Prince for the help they provided me during the data collection in Ghana. My gratitude also goes to Mr. Samuel Addey, Mr. Mohammed Alhassan and Mr. Bani Bannison Simon for assisting me with the blood collection and analysis.

I will like to express my most profound gratitude to the Common Wealth Scholarship Secretariat for sponsoring my research work in the UK, the University for Development Studies for granting study leave with pay and the Ghana Education Trust Fund for providing partial funding towards my research work in Ghana.

Finally, I will like to thank my parents Rev. and Mrs Ansah, my siblings Stanley Ansah, Stephen Ansah, Gladys Ansah and Naomi Ansah. I like to thank my wife Mrs Janet Ansah and children Michelle A. Oparebia Ansah and Graham Nana Adjei Ansah for their encouragement and prayer support.

DEDICATION

I dedicate this thesis to my parents Rev. Francis Y. Ansah and Mrs Judith Ansah for the trust they had in me and invested their resources in my education.

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LIST OF ABBREVIATIONS

CT: Condensed tannin

DM: Dry matter

NDF: Neutral detergent fiber

ADF: Acid detergent fiber

ADL: Acid detergent lignin

CP: Crude protein

IVGP: *In vitro* gas production

IVDMD: *In vitro* dry matter digestibility

ADWG: Average daily weight gain

ADFom: Acid detergent fibre expressed exclusive of residual ash

aNDFom: Neutral detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash

NEB: Negative energy balance

ED: Effective degradability

RDP: Rumen degradable protein

RUP: Rumen undegradable protein

CHAPTER 1

1.0. INTRODUCTION

The keeping of livestock particularly cattle, sheep and goats are predominant in the three northern regions of Ghana. These regions put together account for more than 30% of the sheep, 35% of the goats and 70% of the cattle produced in Ghana (VSD, 1992-1996). The importance of livestock to Ghanaian households covers a wide range of areas. These include meat, manure for soil fertility improvement and income from sale of animals. It has very important roles in the cultural and religious practices of the people in Ghana. Ansah *et al*. (2006) found that cattle management was used by a church based non-governmental organisation in Northern Ghana as one of the means to broker a peace deal in the Kokomba/Nanumba conflict in 1994. Cattle, sheep and goats are largely owned and managed by household heads who are normally males but sometimes some women, usually widows, also own and manage livestock (Dzoagbe *et al*., 2007). This makes the investment in livestock a worthwhile venture as it has the potential of reducing household poverty and food insecurity.

Despite the numerous benefits derived from livestock, it is faced with challenges which deny livestock owners of maximum gains from this enterprise. The livestock sub-sector accounts for 6.1% of Ghana's Agricultural GDP and this could increase if some of the challenges are addressed (MoFA 2011). One of the major problems faced by livestock farmers in sub-Saharan African has been the problem of feed and feeding (Kassam *et al*, 2009). This problem arises as a result of inaccessibility and poor quality of grasses on natural grazing lands. The northern part of Ghana, which is classified as the savannah zone, has two main seasons. These are the wet (April to September) and dry seasons (October to March). The wet season is usually characterised by wide spread cultivation of food crops on most natural grazing lands thereby reducing availability and accessibility of forage. This practice results in most farmers tethering their animals and feeding them on browse plants and other home-generated agro-by-products.

In the dry season where there is virtually no crop cultivation and animals have access to the grazing lands, the quality of forage is very low with CP levels of some grasses dropping from 12.5% in the wet season to as low as 3% in the dry season (Alhassan *et al*., 1999). Olubajo and Oyenuga (1970) found an 8-10% difference in the digestibility of forages between the rainy and dry seasons in Nigeria, which has similar climatic conditions as Ghana.

The grazing of cereal crop residues on farmlands in the dry season is common (Ansah *et al*., 2006). Crop residues such as rice straw and maize stover together with over grown and dry forages serve as the main energy diet in the dry season. The residues are obtained from farmers' own farms or bought from other farmers (Ansah *et al*., 2014). These crop residues normally complement standing hay and in some cases, take the place of natural grasses in the range in providing the bulk of ruminant feed especially in the dry season. The quality of these crop residues is usually not only but availability is limited due to rampant bush fires within the savannah zone. Rice straw, one of the common crop residues has been reported to contain a CP of about 3.3% (Avornyo *et al*., 2007). Ansah *et al*. (2011) found out that feeding ruminants solely on rice straw resulted in poor growth and high mortality.

Zemmelink *et al*. (1992) suggested that the feeding of low quality forage coupled with rampant bush fires could lead to weight losses ranging from 300-400 g/h/day in ruminants. Large scale feeding of crop residues is also hindered by alternative uses such as fuel source, thatching and the problem of collection in view of the bulkiness of the residue and in some cases distance from settlements (MoFA, 1998). The legume-based crop residues, such as groundnut and cowpea haulms and vines are used as protein sources and are usually harvested and fed to ruminants, but due to problems of harvesting and storage, a lot of it is wasted (Ansah *et al.*, 2014).

The use of pods, fruits and leaves of trees as feed for livestock is gaining significant importance among farmers in the face of these feeding problems in the northern region of Ghana (Ansah and Nagbila, 2011). Leaves of trees or browse plants have been reported to maintain a high amount of CP and low fibre compared with grasses even in the dry season (Le Houérou, 1980; Pellew, 1980). The average CP content for all browse species have been reported to be 125 g/kg DM (Le Houérou, 1980). Several nutritional variations and feeding potentials have been reported in different browse plants using different techniques (Larbi *et al*., 1998). Ouédraogo-Koné *et al*. (2008) also reported seasonal variations in the nutritional content of different browse plants. It is very likely that geographical locations could account for some differences in the nutrient content and feed utilization of these browse plants hence the need to investigate the nutrient content of some of the commonly used browse plants within the savannah regions of Ghana.

The browse plants, despite the appreciable levels of CP and low fiber may contain various levels of plant secondary metabolites (PSM) such as CT and saponnins, which may interfere with the protein degradability in the rumen and in the long run affect carbohydrate fermentation. Tannins are phenolic compounds or water soluble polymers of high molecular weight containing many reactive phenolic hydroxyl (OH) or carboxyl (COOH) groups that enable them to form complex with protein, minerals and other macromolecules (Huang *et al.*, 2010; Jin *et al*., 2012; Patra and Saxena, 2010).

According to Makkar *et al*. (2007), tannins are of two categories comprising the hydrolysable tannin (HT) and CT. The ingestion of plant containing CT decreases nutrient utilization and feed intake whilst the ingestion of HT is potentially toxic to animals (Makkar *et al*., 2007). They appear in many nutritionally important forage trees, shrubs and legumes, fruits, cereals and grains, which often limit their inclusion in the diets of animals (Patra and Saxena, 2010).

Tannins occur in the most vulnerable parts of the plants such as new leaves and flowers and also factors such as light intensity, temperature, water and nutrient stress, soil quality and topography influences the concentration of tannins in plants (Terrill *et al*., 1992; Van Soest 1994; Frutos *et al*., 2004)

Dietary protein utilization in the gut is very important in the growth and reproduction of ruminants. Protein entering the rumen can be broken into the quickly degradable fraction, slowly degradable fraction and rumen undegradable fraction (AFRC, 1992).

McDonalds *et al*. (2011) reported that a number of factors including surface area available for microbial attachment, protective action of other constituents as well as physical, chemical nature of the protein and rate of passage through the rumen affects ruminal protein degradation. Tannins play a significant role in protecting dietary protein from rumen microbial degradation and thus increase total supply of amino acid to the intestine for absorption (Waghorn *et al.*, 1987; McNabb *et al.*, 1996; Jin *et al.*, 2012). Some researchers have reported that when CT increases beyond 9%, the nitrogen concentration in the rumen is reduced (Barry and Forss 1983; Reed *et al.*, 1985).

Tannins bind to different sources of protein included in the diet and within the rumen environment. These sources are proline-rich-protein (PRP), bacterial cell wall (BCW), microbial enzyme and protein present in feed (McSweeny *et al.*, 1999; Waghorn, 2008). According to Waghorn (2008), PRP has a high affinity for CT than proteins present in forage. Min *et al.* (2006) reported a greater affinity between CT and bacterial cells than with plant protein, and according to McSweeney *et al*. (2001), cellulolytic bacteria were affected more than cellulolytic fungi in the CT and bacterial cell affinity. Various authors have reported differences in the effect of CT on dry matter intake and digestibility (Barry and Duncan, 1984; Dawson *et al*., 1999; Animut *et al*., 2008; Dschaak *et al*., 2011). The important factor stressed by most of these authors is the level of concentration in the diet. No effect on intake was found

when Hervás *et al*. (2003) fed low levels of between 0 to 83 g/kg DM of CT but declined sharply when the levels reached 166 g/kg DM. When Hariadi and Santoso (2010) incubated different browse plants with *Pennisetum pupureum* using the *in vitro* gas technique, it was shown that the browse plants with low (19 g/kg DM) level of tannins (*Sesbania grandiflora*) had no effect on *in vitro* gas production whilst the high (54 g/kg DM) CT- browse plants (*Acacia mangium*) decreased gas production significantly. This call for an investigation into the CT levels in local browse plants and how they influence the *in vitro* gas production of these browse plants.

Serum biochemical indices have been used to determine the level of heart attack, liver damage and to evaluate protein quality and amino acid requirements in animals (Harper *et al*., 1979). Levels of urea nitogen, glucose, albumin, cholesterol and other metabolites have been used to measure the functinability of the liver (Kahn, 2010). Very low levels are usually indicative of hepatic diseases whilst high levels indicate a breakdown in liver functioning.

Dietary CT could have some toxic effects in ruminants and may be manifested in irreversible damage to liver cells, hardening of the liver, and loss of liver function, which may lead to jaundice, swelling, and the accumulation of fluids in the stomach and other physiological malfunctions (Wong, 2014). They have also been found to control internal parasites and condition of bloat in ruminants (Waghorn, 1990; Min and Hart, 2003).

Feeding system and nutritional status can affect growth and carcass characteristics (Atti and Abdouli, 2001; Priolo *et al.*, 2001). Fermentation of organic matter in the rumen produces VFA's and also supplies microbial protein. Different diets yield different VFA's and these VFA's act different in on lipogenic nutrients and glucogenic nutrients. Acetic and butyric acids produce more lipogenic nutrients whilst propionic acid supplies more of glucogenic nutrients. Acetic acid has been found to supply 70-80% of acetyl units for lipogensis in subcutaneous adipocytes but only 10-25% in intramuscular adipocytes whilst a greater proportion of

intramuscular adipocytes are derived from glucogenisis from propionic acids (Smith and Crouse, 1984). This indicates that type of diet can significantly influence carcass composition. About 40-70% of NDF digested in the rumen produces 86% lipogenic nutrients and 14% glucogenic nutrients (Tamminga. 2004).

The intake of RUP has been found to favour the production of less fatty carcasses (Fattet *et al.*, 1984; Vipond *et al.*, 1989). The presence of CT in browse plants has been found to increase the supply of RUP due to its ability to form complex with protein.

Rumen fermentation of poor quality fodder such as those with low nitrogen and high fiber (ADF and ADL) has been suggested to produce more methane gas than the fermentation of better quality forages (O'Hara *et al*., 2003). Methane contributes 24% to global warming, and methane emission from rumen fermentation is the largest single source of methane (Hansen *et al*., 2000). The livestock sector is responsible for 18% of total anthropogenic greenhouse gas (GHG) emissions with 30-50% of this being methane (Steinfeld *et al.*, 2006).

Gill *et al.* (2010) suggested that about 80% of the total methane from livestock is of the ruminant origin. This requires urgent steps to find ways to mitigate GHG from ruminant sources without negatively affecting the performance of these animals.

According to IPCC (1996), dairy cattle from Africa and the Middle East produce 36 kg/herd of methane annually. Condensed tannin, a plant secondary metabolite present in most browse plants have received great attention for its ability to reduce methane production (Carulla *et al.*, 2005; Huang *et al.*, 2010). Bhatta *et al*. (2009) reported that Quebracho tannins inhibited methane production linearly (13–45%) with increasing doses (5– 25% of substrates). Min *et al*. (2006) found that Quebracho tannin (75% CT) included at concentrations of 1–2 kg/DMI decreased methane production by 12.3–32.6% in an *in vitro* condition. When rumen liquor from cattle grazing wheat grass at reproductive stage and fed Quebracho tannins 10–20 g/kg DM was tested for methane production, it was found that methane decreased by 25–51% (Min

et al., 2006). Ives *et al*. (2015) observed that CT decreased methane production and inhibited overall *in vitro* fermentation. Beauchemin *et al*. (2007) did not find any effect on methanogenesis when Quebracho tannin extract (10–20 g/kg DM intake) was fed to beef cattle for 28 days.

1.1 RESEARCH QUESTIONS

1. Do common browse plants fed to ruminants in Northern Ghana contain adequate amounts of crude protein?

2. Do browse plants contain condensed tannins and to what extent do these interfere with dietary protein degradation and DM digestibility in the rumen?

3. Can these browse plants be used to reduce enteric methane emission?

4. Can dietary supplementation of these browses plants improve growth performance of ruminants?

1.2 MAIN OBJECTIVE

The main objective of this study was to evaluate the nutritive value and greenhouse gas mitigation potential of eight browse plants from Northern Ghana.

1.3 SPECIFIC OBJECTIVES

The specific objectives were:

1. To determine the chemical composition (CP, Ether extract, NDF, ADF, ADL and CT) of the browse plants and to ascertain the *in vitro* fermentation and gas production of browse plants incubated in nitrogen sufficient or nitrogen deficient media
2. To determine the effect of browse plants on nitrogen degradation using the *in sacco* technique
3. To ascertain the effect of replacing 40% wheat straw with browse plants on *in vitro* enteric methane production
4. To determine the effect of replacing 40% rice straw with browse plants on nutrient digestibility
5. To determine the effect of browse plant supplementation on the growth of semi intensively kept Djallonké sheep

CHAPTER 2

2.0 LITERATURE REVIEW

2.1.0 Feed resource for ruminant production in Ghana

2.1.1 Natural pastures

Natural pastures remain the most common source of forage for large and small ruminants in most communities in Northern Ghana (Ansah *et al*., 2014). According to Oppong-Anane (2006), Ghana has a potential land area of about of 107,000 km^2 for pasture production comprising 360,000 km^2 permanent pasture and 71,000 km^2 unreserved savannah woodland. However little or no effort is made to enhance the yield and quality of these pasture lands for livestock production. Most farmers depend on the natural grasses and legumes that grow on these fields as sources of forage. The growth pattern of the forages follows the rainfall regime within the different agro-ecological zones. The three northern regions also referred to as the interior savannah account for two-thirds of the total grassland in Ghana (Oppong-Anane 2006). The interior savannah has one rainy season which beginsin April through to September and decline in October. The total precipitation is about 1100 mm per annum, with a range from about 800 mm to about 1500 mm (Oppong-Anane 2006). According to Oppong-Anane (2006), the grasses associated with the Interior Savannah are not uniform but differ according to soil type and moisture regime. The grassy background of the zone is invariably dominated by *Andropogon gayanus* with *Hyparrhenia* and *Schizachyrium* as co-dominants in some areas.

The annual total DM is reported to be about 2,170 kg/ha in the Guinea Savannah zones with the highest yield recorded in the growing (rainy) season (Oppong-Anane 2006). The nutritive value of these forages varies with the seasons. Alhassan *et al.* (1999) reported that the CP content in most Ghanaian grasses reduce from 5-15% in the rainy season to 3% in the dry season. Olubajo and Oyenuga (1970) reported an 8-10% difference in the digestibility of forages in the rainy and dry seasons.

2.1.2 Crop residue and agro-industrial by products

According to Ansah *et al*. (2006), about 94% of livestock farmers use crop residue as a source of feed for their animals in the Yendi District of the Guinea Savannah zone in Ghana. These residues are mainly gramineae and leguminosae based, reflecting the kind of crop cultivated in the area. The residues are obtained from farmers own farms or bought from other farmers. Crop residues normally complement standing hay and in some cases take the place of natural grasses in the range in providing the bulk of ruminant feed especially in the dry season. Crop residues, such as groundnut and cowpea haulms, bean vines, maize, sorghum, rice and millet stover, constitute the bulk of ruminant feed during the dry season (Osafo *et al.,* 1993; Owen, 1994; Karbo *et al.,* 1997; Ansah *et al.*, 2014). It is estimated that about 8,000,000 metric tonnes of cereal stalks and 3,500,000 metric tonnes of residues from roots and tubers are potentially available as animal feed in each year (Oppong-Anane, 2006). However, quantities of these that are actually fed to livestock form a very small fraction of the available crop residues (Oppong-Anane, 2006). Large scale feeding of crop residues to livestock is hindered by alternative uses such as fuel and thatch. The problem of collection in view of the bulkiness of the residue and in some cases distance from settlements also serves as a challenge to its use (MoFA, 1998).

The types of agro-industrial by-products used as animal feed varies with the type of crops cultivated and the types of industrial firms that operate in the area. Agro-industrial by-products play a very important role in livestock production especially in periods of forage scarcity. In Northern Ghana, by-products like whole cotton seed, shea nut cake and pito mash are often used in livestock feed (Ansah *et al*., 2006).

Whole cottonseed and shea nut cake contain high amounts of CP and could meet the ammonia nitrogen requirement of rumen microbes to enhance digestibility (Calhoun *et al.,* 1995; Atuahene *et al.,* 1998). It has been estimated that about 22,200 – 24,220 metric tonnes of cotton seed is produced annually in Northern Ghana (Karbo and Bruce, 2000).

One agro by product generated in large quantities in Northern Ghana is the shea nut cake (SNC). Okai and Bonsi (1989) reported that the shea tree from which the shea nuts are harvested is widely distributed in Northern Ghana. The residual cake after removing 45 – 55% of fat from the seeds of the shea fruit is known as SNC. This product has virtually been regarded as waste material in Ghana and disposed via incineration. Shea nut cake contains moderate amounts of CP and fat (Okai *et al*., 1994; Atuahene *et al.*, 1998; Dei *et al*., 2007; Oddoy*e et al*., 2012; Mumeen *et al*., 2013). Ansah *et al*. (2012) and Konlan *et al*. (2012) reported significant improvements in weight gain, feed intake, haematology and serum biochemical properties of sheep fed with SNC based supplement.

2.1.3 Browse plants

According to Sanon (2007), browse refers to the leaves and twigs from shrubs and trees available to ruminants as feed and in a broader sense including also flowers and fruits or pods. In Ghana, it has been estimated that 16% of the land is occupied by different tree crops (MTADP, 1991). Browse plants play very important roles in the sustainable production of livestock and also serve as source of food, medicine and fuel in most communities in Africa. In Benin, it has been reported that cattle farmers depend on browses such as *K. senegalensis*, *Afzelia africana* and *P. erinaceus* as major sources of fodder in the dry season (Brisso *et al*., 2007). The biomass yield from these browse plants was reported to be in the range of 101.9 to 112.5 kgDM/ha with the highest reported for *K. senegalensis*. According to Komwihangilo *et al.* (1995), trees and shrubs are of immense value in agriculture as they have been used to feed as well as to meet the health needs of animals through the ages in the world's arid and semi-arid areas. Supplementing cattle diet with browse plants is practised among some farming communities in Northern Ghana. Ansah and Nagbila (2011) identified a total of thirty-one trees and shrubs used for feeding and medication of livestock in the Talensi-Nabdam District of the Upper East Region of Ghana. Their roles become more important as the dry season becomes

longer with decreasing mean annual rainfall. Most browse species are tolerant to heat, drought, salinity, alkalinity, grazing and repeated cutting. Le Houérou (1980) reported that compared with tropical grasses, browse is richer in protein and some minerals in the dry season. At this time, browse plants are the most valuable feed used by livestock. Browse could therefore supplement the low protein content of grass forage if used effectively during dry periods. The crude fibre content of browse plants also tends to be lower than that of grasses and usually ranges from 200 to 400 g/kg DM and is even lower for shoots and leaves (Pellew, 1980). Given the low content of crude fibre in browse compared with dry grass, the energy content of browse appears to be higher than that of dry grass (Le Houérou, 1978).

2.2.0 Common browse species in northern Ghana

2.2.*1 Ceiba pentandra* (Kapok)

Kapok (*C. pentandra*) (L.) is a large deciduous tree with a height of about 30-40 m (Heuzé and Tran 2015). Kapok has a broad straight trunk and almost horizontally spreading branches. The leaves are compound (5-8 leaflets) (Orwa *et al*., 2009). Kapok produces large quantities of clustered flowers, yellowish white to rose and silky densely hairy on the outer surface (Orwa *et al.*, 2009). Kapok is a fast growing tree and becomes productive within 4-5 years. The leaves and petioles according to Kongmanila and Ledin (2009) contain DM of 300 g/kg, CP of 120 g/kg DM and NDF of 502 g/kg DM. The leaves alone have been reported to contain a CP of 184 g/kg DM and when fed to goats in the dry season, an average daily growth of 103 g/day was obtained (Theng *et al.*, 2006). An average daily intake of 120 g/day was reported when the leaves were harvested and fed to goats (Kongmanila *et al.*, 2008).

Nguyen (1998) reported that the DM digestibility in growing goats fed the foliage as sole diet was 760 g/kg and that growth rates of 74 g/day were similar to what was obtained with foliage of Leucaena spp. *In vitro* organic matter digestibility (IVOMD) of foliage from kapok is reported to be around 440 g/kg (Ogunbosoye and Babayemi, 2010). Reports on the presence

of plant secondary metabolites in kapok leaves are sketchy. Kongmanila and Ledin (2009) reported a CT content of 117 g/kg in the leaves and petioles.

2.2.2 *Khaya senegalensis* (Mahogany)

Khaya senegalensis (Mahogany) occurs in riverine forests and is scattered within the higher-rainfall savannah woodlands (Orwa *et al*., 2009). It is resistant to flooding and can be considered for planting on swampy soils. Except where selectively removed by logging, dry-zone mahogany remains a dominant species in most of its range. Successful plantations of dry-zone mahogany in other parts of the world have generally been in areas with short dry seasons and high rainfall. It is native to countries from West and Central Africa and Uganda in East Africa (Orwa *et al*., 2009).

Foliage biomass yield of 571 kg DM/ha has been reported for *K. senegalensis* (Ouédraogo-Koné *et al.*, 2008). The leaves of *K. senegalensis* is reported to contain a mean DM of 471 g/kg, CP of 106.7 g/kg DM, NDF of 475 g/kg DM, ADF of 375 g/kg DM and ADL of 135 g/kg DM in the early part of the dry season (October - December) (Ouédraogo-Koné *et al.*, 2008). When *K. senegalensis* was fed to sheep as a supplement with *Andropogon gayanus* hay and maize bran, an average daily growth of 48 g/day was recorded (Ouédraogo-Koné *et al.*, 2009). According to these researchers, the average feed intake for *K. senegalensis* was 173g/day with a CP and ME intake of 67 g/day and 4.5 MJ/day respectively.

2.2.3 *Senna siamea* (Cassia)

Senna siamea is a non-nitrogen-fixing leguminous tree attaining a height of 5 m to 20 m (Hassain, 1999). It has a dense, round, evergreen crown and a short bole with smooth, grey bark, slightly fissured longitudinally (Heinsleigh and Holaway, 1988). It has a shallow root system, which can easily be uprooted by strong winds. The leaves are pinnately compound

with an even leaf arrangement of 7-10 pairs of ovate-oblong leaflets of 7-8 cm long and 1-2 cm wide.

Senna siamea foliage is used as green manure for fields with significant contribution of nitrogen to the crop, and reduces weed control problems. In some areas, it is intensively harvested as fodder for cattle, sheep, and goats (Hocking, 1993), but there may be problems with secondary plant compounds (Gutteridge, 1997). An alkaloid in the pods and leaves has been reported to be fatal to pigs (Troup and Joshi, 1983). Fadiyimu *et al.* (2011) reported a DM of 359 g/kg, CP of 197 g/kg DM, Crude fibre of 164 g/kg DM and a tannin concentration of 10.7 g/kg DM in the leaves of *S. siamea*. Little information is available on this tree as feed for sheep in Ghana.

2.2.4 *Ficus gnaphalocarpa* (Large fruited sycanore fig)

Ficus gnaphalocarpa is a large, semi-deciduous spreading savannah tree, up to 21 m in height (Orwa *et al*., 2009). It is a common savannah tree that grows in high water table areas. Often found along watercourses such as streams and rivers, swamps and waterholes. The sycamore fig is sensitive to frost but can withstand some cold. It is native to a number of countries including Ghana (Orwa *et al*., 2009). Its value as forage has not yet been explored in Ghana. Other species of the ficus like the polita in Nigeria is reported to contain a DM of 950 g/kg, CP of 162.10 g/kg DM, Ash of 100 g/kg DM and ether extract of 30 g/kg DM. The fibre composition includes NDF of 373 g/kg DM, 272 g/kg and ADL of 49 g/kg DM. It has total CT of 0.20 mg/g DM (Njidda, 2010).

2.2.5 *Pterocarpus erinaceus* (African teak)

Pterocarpus erinaceus is a nitrogen fixing plant of the Fabaceae family, and is widely distributed in savannah regions of West Africa. The tree can grow to about 11 m in height with a mean diameter at breast height of 36 cm (Petit, 2000). *P. erinacaeus* is resistant to drought and can adapt well to harsh conditions (Bonkoungou, 1999).

Foliage and immature pods are sometimes cut down at the end of the dry season and sold in markets for fattening sheep, goats, cattle and horses. It has a Foliage biomass yield of 1273 kg DM/ha (Ouédraogo-Koné *et al.*, 2008).

Pterocarpus erinaceus in the early part of dry season (October- December) is reported to contain a DM of 523.3 g/kg, CP of 98.3 g/kg DM, NDF of 531 g/kg DM, ADF of 384 g/kg DM and ADL of 173.7 g/kg DM (Ouédraogo-Koné *et al.*, 2008). An average DM feed intake of 238 g/day and CP intake of 86 g/day was reported by Ouédraogo-Koné *et al*. (2009). When *P. erinaceus* was fed as supplement to sheep receiving *Andropogon gayanus* and maize bran as basal diet, an average daily gain of 58.8 g was reported (Ouédraogo-Koné *et al.*, 2009). Olafadehan (2013) reported a linear increase in the intake of *P. erinaceus* when it was supplemented to goats feeding on *Andropogon gayanus*. The author further concluded that maximum nutritional benefits of *P. erinaceus* can be achieved if used as supplement up to 750 g/kg DM rather than as a sole feed, and the sole *P. erinaceus* is better than sole *Andropogon gayanus*.

2.2.6 *Albizia lebbeck* (Lebbek)

Albizia lebbeck is a deciduous, perennial and medium-sized legume tree. It grows as high as 3-15 m in plantations and up to 30 m in the open (Prinsen, 1998). It is found on a wide range of soil types including those that are alkaline and saline but not subject to waterlogging. Reserves

in the root system enable young plants to survive total defoliation from fire or grazing, but with obvious setback to growth. Growth is opportunistic when conditions are suitable but ceases for 2-3 months before leaf drop. Trees are leafless for about 3-4weeks, with new leaf produced at the peak of the dry season, followed by a gregarious flowering in the tropics.

Leaves of *A. lebbeck* are remarkably free of toxins and tannins, and low in soluble phenolic compounds (Romeo 1984; Asif *et al.,* 1986; Sotelo *et al.,* 1986; Ahn *et al.,* 1989). Meanwhile the *A. gummifera* has been reported to contain a CT of 84 g/kg DM (Kebede *et al.,* 2014). Protein and NDF contents are as follows: green leaf, 160-230 and 410-350 g/kg DM; fallen leaf, 100 and 490 g/kg DM; fallen flower, 230 and 510 g/kg DM; pods, 190 and 550 g/kg DM. The fallen leaf has shown surprisingly high voluntary intake by sheep, probably due to its rapid fragmentation. Schlink *et al.* (1991) showed that all fractions fed as supplements produced an increase in digestible DM and intake of low quality basal diet. The effect was greater with the lower quality basal diet of black spear grass. The pods are of particular interest as animal response was poor when fed alone but was positive when fed as a supplement to poor quality grass (Lowry *et al.*, 1998).

2.2.7 *Azadirachta indica* (Neem)

Azadirachta indica is widely known as neem, a tree that has proven value to both city and farm dwellers throughout the dry tropics and subtropics (Stoney, 1997). It is a member of the Meliaceae (mahogany) family. The stem branches at 2-5 m forming a broad, dense, round or oval crown. Total height is 15-25m; occasionally reaching up to 30 m, with a stem diameter ranging from 30 to 90 cm. Neem can grow at altitudes of up to 1500 m, as long as temperatures remain moderate, as it does not withstand cold or frost. Neem tolerates extremely high temperatures, but its normal range is about 9.5°C - 37°C. It is also highly drought tolerant, and

once established, it can survive 7-8 month dry seasons. It requires as little as 150 mm rainfall per year in areas where the root system can access groundwater within 9-12m of the surface. However, it performs best in zones receiving 450-1200 mm/year (Stoney, 1997).

The use of neem in the livestock industry has been focused more on health rather than nutrition. Its use as a feed ingredient is not encouraged much because of the presence of azadirachtin, which makes the carcass of animals bitter. The leaf is reported to contain DM of 948 g/kg, CP of 133 g/kg DM, NDF of 508 g/kg DM, ADF of 339 g/kg DM and CT of 110 g/kg (Kanpukdee and Wanapat, 2008). The mineral composition (ppm) as reported by Bakshi *et al*. (2006) are as follows Ca (29), P (0.6), Mg (67.3), Zn (0.18), Fe (0.059), MN (0.16), Cu (0.068) and Co (0.04). The concentrations of calcium and Magnesium are above the 1mg/l and 5mg/l respectively levels reported to cause a reduction in the growth and cellulose degradation of *Ruminococcus falvefaciens* and *Fibrobacter succinogenes* in the rumen (Van Gylswyk, 1995). A 48 h *in vitro* digestibility of 73.4% DM was reported for neem leaves with predicted DM intake of 8.4 kg/day (Bakshi *et al.*, 2006).

2.2.8 *Gmelina arborea* (Gmelina)

Gmelina arborea is moderately sized to large deciduous tree with a straight trunk. It is wide spreading with numerous branches forming a large shady crown, attains a height of 30 m or more and a diameter of up to 4.5 m. According to Swamy *et al*. (2004), *G. arborea* can survive in areas with harsh conditions (low pH and poor nutrients). It has the ability to appreciably increase soil pH, nitrogen and phosphorus when planted on poor soils. When Onyekwelu *et al.* (2006) has reported a biomass yield of 81.5-392.1 DM t/ha. The leaves of *G. arborea* is reported to contain a moisture content of 747 g/kg CP of 146 g/kg DM, energy of 1368 Kcal/kg DM and a fat content of 127 g/kg DM (Amata and Lebari, 2011). It has a calcium content of

4.5 g/kg and a tannin fraction of 17.6 g/kg DM (Meriales, 1998). The *in vitro* dry matter digestibility (IVDMD) reported for *G. arborea* leaves was 414.5 g/kg DM. A voluntary dry matter intake (DMI) of 481.91 g/day, equivalent to 2.54% of body weight, and CP intake (CPI) of 77.78 g/day was reported when *G. arborea* was fed to sheep. In the same study, an ADG of 36 g/day was reported (Meriales, 1998).

2.3.0 Tannin in browse plants and its effect on use as fodder

2.3.1 Overview of tannins

Tannins are phenolic compounds of high molecular weight containing many reactive phenolic hydroxyl (OH) or carboxyl (COOH) groups that enable them to complex with protein, minerals and other macromolecules (Reed, 1995; Patra and Saxena, 2010). According to Makkar *et al*. (2007), tannins are in two categories comprising the hydrolysable tannin and CT (Figure 2.1). Hydrolysable tannins consist of a central core of carbohydrate such as glucose, glucitol, quinic acids, quercitol and shikimic acid to which phenolic carboxylic acids like gallic acid (3, 4, 5-trihydroxy benzoic acid; gallotannins) or gallic acid dimer hexahydroxydiphenic acid (ellagitannins) are bound by ester linkage (Haslam, 1989; Makkar *et al*., 2007). Condensed tannins or proanthocyanidins, are mainly polymers of the flavan-3-ol (epi) catechin and (epi) gallocatechin units, which are linked by C4–C8 and C4–C6 interflavonoid linkages (Hagerman and Butler, 1989; Ferreira *et al*., 1999).

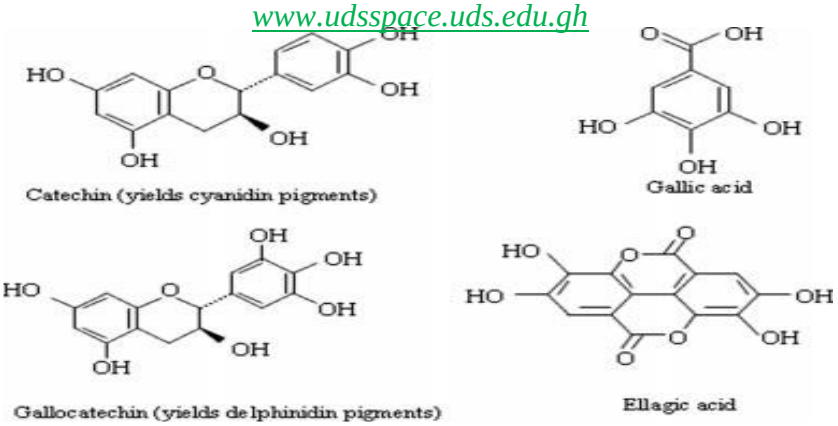

Figure 2. 1 Examples of condensed (catechin and gallocatechin) and hydrolysable (gallic and ellagic acid) tannins. Adapted from Patra and Saxena (2010)

Most plants normally contain both CT and HT but concentration may vary depending on the plant (Haslam, 1989). The ingestion of plant containing CT decreases nutrient utilization and feed intake whilst the ingestion of HT is potentially toxic to animals (Makkar *et al*., 2007). They appear in many nutritionally important forage trees, shrubs and legumes, fruits, cereals and grains, which often limit their inclusion in the diets of animals (Patra and Saxena, 2010). Tannins occur in the most vulnerable parts of the plants such as new leaves and flowers and also factors such as light intensity, temperature, water and nutrient stress, soil quality and topography influence the concentration of tannins in plants (Terrill *et al*. 1992; Van Soest 1994; Frutos *et al*. 2004). Other researchers also reported similar factors affecting the concentration of CT concentrations in plant tissues. These include plant species (Jackson *et al*., 1996), plant part (Foo *et al*., 1982, Barahona *et al*., 1997), plant maturity (Lees *et al*., 1995), growing season (Clarke *et al*., 1939; Donnelly, 1959, Feeny, 1970) and soil fertility (Barry and Forss, 1983; Barry, 1989).

Frutos *et al*. (2004) further explained that during the growth of plants when lots of biomass is produced, fewer resources are available for synthesis of phenolic compounds. However, during

flowering, when growth is minimal, excess carbon may be available for tannin synthesis. This makes the time of harvesting very significant for tannin presence in fodder trees.

Again, different plants may contain different types of CT and this may explain why different CT-plants have different physiological effects on animal performance (Waghorn, 2008). Butler and Rogler (1992) noted that CT with low molecular weight are more reactive and have higher protein-precipitating capacities than the high molecular weight polymeric tannins. However, a recent study by Naumann *et al.* (2014) found no correlation between the molecular weight of CT and the protein p precitable phenolics (R^2 0.11) and amount of protein bound (R^2 0.11). The authors however found a correlation between the concentration of CT and the protein precitable phenolics (R^2 0.81) and amount of protein bound (R^2 0.69). The protein precipitation comes about after a complex has been formed between the CT and protein. Kumar and Singh (1984) suggested that CT and protein complexes could come about through four types of bond: (1) hydrogen bonds between the hydroxyl radicals of the phenolic groups and the oxygen of the amide groups in the peptide bonds of proteins, (2) by hydrophobic interactions between the aromatic ring of the phenolic compounds and the hydrophobic regions of the protein, (3) by ionic bonds between the phenolate ion and the cationic site of the protein (exclusive to HT), and (4) by covalent bonding through the oxidation of polyphenols to quinones and their subsequent condensation with nucleophilic groups of the protein.

Several researchers have found various levels of plant secondary metabolites (PSM) in most browse plants that may limit their utilization as fodder (Fall-Touré and Michalet-Doreau, 1995; Makkar and Becker, 1998; Shayo and Udén, 1999; Aganga and Tshwenyane, 2003). According to Silanikove *et al.* (2001), tannins are found in nearly 80% of all the woody plants. The negative effects of tannins such as low feed intake, low digestibility and toxicity have been reported to occur when ruminants consume forage with a high level of CT, greater than 50-55 g/kg DM (Min *et al.*, 2003).

2.3.2 Effects of CT on feed intake and digestion in the rumen

Reports on the effects of high and low CT-browse plants on voluntary DM intake, organic matter (OM) digestibility and ME intake vary. Barry and Duncan (1984) fed growing sheep a high (105.9 g/kg) or low (45.6 g/kg) CT form *Lotus pedunculatus* (LP) to ascertain the effect on voluntary feed intake. No significance difference was found even though the low tannin had a higher voluntary feed intake and digestibility. When the same feed was fed with one fraction sprayed with polyethleneglycol (PEG), intake for the PEG diet was higher than the non-PEG. Apparent DM digestibility followed in the same pattern with poor performance reported for the animals on the non-PEG. A similar trend was reported by Barry and Manley (1984) when the same plant was offered to sheep in a separate experiment.

Dawson *et al*. (1999) fed weaned lambs 100 g/day of pelleted grass with or without quebracho tannin of 50 g/kg DM. After 6 weeks of feeding, an average daily DM intake of 899.5 g and 893.7 g was recorded for the sole grass and tannin supplemented diet respectively.

Apparent DM and NDF digestibility were 693 g/kg and 684 g/kg DM respectively for sole grass and 625.5 g/kg and 610.8 g/kg respectively for the tannin supplemented diet. Waghorn *et al*. (1998) reported a DM intake of 980 g/day and 990 g/day when *L. corniculatus* (LC) was fed to sheep without PEG and with PEG respectively. When *L.s pedunculatus* (LP) was also fed with or without PEG, the DM intake was 970 g/day and 1080 g/day respectively. A DM digestibility of 690 g/kg and 710 g/kg was reported for non-PEG and PEG LC respectively (Waghorn *et al*., 1998).

Waghorn and Shelton (1997) in another study fed three different groups of pasture (rye grass and clover) fed sheep with 37% fresh LC. The DM intake was not affected. However, when the LC was treated with PEG and fed to another set of animal grazing the same pasture, the DM intake declined marginally. The DM digestibility in the same study was 756 g/kg DM, 743

g/kg DM, and 750 g/kg DM for the sole pasture, pasture + LC and pasture + LC + PEG respectively. It can be concluded from this study that CT from LC included in the diet of lambs at 10 g/kg did not negatively affect dry matter intake and digestibility.

In the study of Carulla *et al*. (2005), feed intake in sheep was enhanced when *A. mearnsii* tannin extract was included in the diet. Dschaak *et al*. (2011) reported a marginal decrease in intakes of DM, CP, NDF and ADF when CT of 30 g/kg DM was added to forage diet and fed to lactating dairy cows. Animut *et al*. (2008) observed no difference in DM intake when browse plants of different CT levels were fed to goats. Hervás *et al*. (2003) noted that addition of Quebracho tannins in the diet of sheep at 0, 0.5 and1.5 g/kg BW did not affect feed intake but when the inclusion rate increased to 3 g/kg BW with a tannin content of 166 g/kg DM, intake was significantly decreased.

Getachew *et al*. (2002) incubated 0.5 g of different browse species from the tropics using the method of Menke and Steingass (1988) in a 40 ml buffered rumen fluid. About 1g of PEG was added to one group of the browse species to incubate. Gas production after 24 h from substrates with or without PEG was 107 ml and 97.6 ml respectively. The total volatile fatty acid (TVFA) was 2.3 mmol/L and 2.2 mmol/L for the non-PEG and PEG substrates respectively.

Hariadi and Santoso (2010) incubated leaves of seven different browse plants containing a total tannin content of between 19 and 54 g/kg DM with *Pennisetum pupureum* (PP) as the main substrate using the method of Menke and Steingass (1998). The samples were incubated as sole PP or 200 g/kg of it replaced with the leaves of browse plants. The browse plants were *Biophytum petersianum*, *Sesbania grandiflora*, *Acacia mangium*, *Jatropa curcas*, *Phaleria papuana*, *Persea americana* or *Psidium guajava*. After 48 h of incubation, the highest volume of gas (64.2 ml/300 mg) was recorded for the *Sesbania grandiflora* substrate with a total tannin content of 19 g/kg DM and the least volume of gas (49.7ml/300mg) recorded for *Acacia*

mangium substrate with a total tannin content of 54 g/kg DM. The trend was the same for the gas production at 16 h and 24 h of incubation. The CP and NDF content of *Sesbania grandiflora* was 390 g/kg DM and 330 g/kg DM respectively and *Acacia mangium* was 165 g/kg DM and 508 g/kg DM respectively.

Hariadi and Santoso (2010) reported the highest TVFA (15.1 mmol/L) for *Sesbania grandiflora* which had the least total tannin and lowest TVFA (12.4 mmol/L) for *Psidium guajava* which had total tannin content of 35 g/kg. Getachew *et al*. (2000) using the *in vitro* method of Makkar *et al*. (1997a) incubated teff straw (400 mg) supplemented with 20% of three different tannin containing browse plants (*Accacia salicina, Claliandra calothyrus* and *Dichrostachys cinerea*). Polyethylene glycol was added to one set of the treatments to measure the effect of CT on digestibility. The substrates were incubated in a media with no nitrogen source added and measurements taken at 8, 12, 16 and 24 h. The results at the end of the study showed a significant increase in potential digestibility for the PEG substrate than the non-PEG. Short chain fatty acid (SCFA) also followed the same pattern for all the browse plants. The low potential digestibility and SCFA production in the non-PEG diet was attributed to the effect of CT binding to protein and also its inhibitory effect on microbial cells or enzymes (Getachew *et al*., 2000).

Four cannulated Polish Holstein-Friesian dairy cows (601 ± 25.3 kg body weight) fed total ixed ration (TMR) supplemented with 140 g of *Vaccinium vitisidaea* corresponding to 2 g of tannins/kg diet DM. The results showed no change in rumen fermentation, pH and apparent digestibility of DM, OM and aNDF (Cieslak *et al*., 2012).

The decrease in the rate of DM feed intake and digestibility of tannin-rich feeds is generally attributed to the astringent taste, which reduces palatability and the lower rate of digestion. The reduction in palatability is caused by the reaction of the salivary muco-protein with tannins or

through a direct reaction with taste receptors, provoking the astringent sensation (McLeod, 1974). Directly infusing CT into the rumen resulted in slow rate of digestion and this was attributed the direct effect of the tannins on rumen microbes (Bae *et al*., 1993). The lower rate of digestion leads to a higher rumen fill providing a feedback to nerve centres involved in intake control. This could slow down voluntary feed intake much more than the astringent taste generated (Waghorn *et al*., 1994). Table 2.1 shows a summarised effect of tannins from different sources at different levels on voluntary feed intake in sheep.

2.3.3 How condensed tannins affect rumen microbial fermentation

Tannins affect rumen microbes through an interaction with the extracellular enzymes secreted and the cell wall of bacteria causing morphological changes of the cell wall and also substrate deprivation (Barry and Manley, 1984; Kumar and Vaithiyanathan, 1990; Leinmüller *et al*., 1991; Scalbert, 1991; Bae *et al*., 1993; Jones *et al*., 1994; McMahon *et al*., 2000; McAllister *et al*., 2005; Smith *et al*., 2005). Tannins exert anti-microbial actions in the rumen (Patra and Saxena, 2009), which may show adverse effects on rumen fermentation and digestion of feeds. Tannins have been reported to alter the permeability of the membranes of rumen microbes (Leinmüller *et al*., 1991; Scalbert, 1991).

Deprivation of microbial substrate according to Frutos *et al*. (2004) is as a result of the tannins interfering or preventing the microbes from attaching to the plant cell walls.

Table 2. 1 Effects of tannins on the voluntary feed intake by sheep

Source	Key comparison	Effect on voluntary feed intake	Reference
Tara tannin	Soybean meal offered as a protein supplement with and without tannins (0.89%)	No difference between treatments.	Driedger and Hatfield (1972)
Lotus pedunculatus	Forage offered had low (4.6%) and high (10.6%) tannin content	No difference in organic matter intake, but lower intake of digestible organic matter with the high tannin forage.	Barry and Duncan (1984)
Lotus pedunculatus	Forage offered with and without PEG (2.4 g/g of tannin)	No difference in organic matter intake, but higher intake of digestible organic matter and metabolizable energy with the PEG-treated forage	Barry and Duncan (1984)
›tus corniculatus	Forage offered contained 0.5% and 3.3% tannins	No difference between treatments.	Waghorn *et al*. (1987)
›tus corniculatus	Forage (2.2% tannins) offered with and without PEG (50 g /day)	No difference between treatments.	Waghorn *et al*. (1987)
ulga (*Acacia ıeura*)	Forage offered with and without PEG (24 g/day)	Supplementation with PEG increased intake of forage by 50%	Pritchard *et al*. (1988)
›ricea lespedeza	Forage offered had high (18.1%) and lower (3.1-8.7%) tannin content	Sheep consumed more of the lower tannin forage	Terrill *et al*. (1989)
ountain mahogany *ercocarpus ontanus*)	Forage (4.1% tannins) offered as a protein supplement with and without PEG (2 g/g of tannin)	No difference between treatments.	Nuñez-Hernandez *et al*. (1991)
ılla (*Hedysarum ›ronarium*)	Forage (4.5-5.0% tannins) offered with and without PEG (100 g/day)	PEG supplementation slightly depressed forage intake	Terrill *et al*. (1992)
›tus pedunculatus	Forage (5.0-5.5% tannins) offered with and without PEG (100 g/day)	No difference between treatments.	McNabb *et al*. (1993)
esmodium ›alifolium	Forage (4.6% tannins) offered with and without PEG (5% of DM)	PEG supplementation increased forage intake by 21%	Carulla (1994)
›tus pedunculatus	Forage (5.5% tannins) offered with and without PEG (100 g/ day)	PEG supplementation increased forage intake by about 9%	Waghorn *et al*. (1994)
Lotus corniculatus	Forage (3.5% tannins) offered with and without PEG (100 g/ day)	No difference between treatments.	Wang *et al*. (1994)
Sesbania sesban and S. *goetzei*	Forage offered as a protein supplement differed greatly in tannin content	Intake of the basal diet decreased as the level of tannins in the supplement increased	Wiegand *et al*. (1995)

Adapted from Rosales (1999).

The CT- protein complex inhibits *Fibrobacter sucinogenes* from attaching to the plant cell wall thereby reducing digestion rate. It has been suggested that the presence of plant protein in the rumen for microbial breakdown aids in microbial attachment to plant cell particularly *Fibrobacter sucinogenes* (Gong and Forsberg, 1989; Mitsumori and Minato, 1993). This was demonstrated by treating *Fibrobacter sucinogenes* S85 cells with trypsin, pronase, or glutaraldehyde, which led to a reduction in the adhesion of cells to cellulose (Gong and Forsberg, 1989). Jones *et al*. (1994) in a study to determine the effect of sainfoin on the growth and proteolytic activities of four rumen microbes found that the growth of proteolytic bacteria (*Butyrivibrio fibrisolvens, Ruminobacter amylophilus* and *Streptococcus bovis*) was inhibited with some morphological changes observed in the microbes. No effect was however found on *Prevotella ruminicola.* Wang *et al.* (2009) in a similar study added phloro tannins at 0.5 g/L to rumen cultures and observed an inhibition of the growth of *F.succinogenes,* with minimal effect on *R. flavefaciens* and *R. albus*. In the same study, the growth of *S. ruminantium, S. bovis, R. amylophilus* and *P. bryantii* were stimulated.

McAllister *et al*. (1994) also showed that *L. corniculatus* CT caused a considerable detachment of *Fibrobacter succinogenes* S85 from colonized filter paper after a 30 min exposure. The effects of tannic acid, ellagic acid, gallic acid and catechin on rumen fungus *Neocallimastix frontalis* strain RE1 was investigated by Muhammed *et al.* (1995). The results showed that all these compounds inhibited the cellulolysis and zoospore attachment to cellulose by this fungus. Gallic acid, ellagic acid and catechin showed more inhibitory effect to cellulolysis than tannic acid. However, ellagic acid was most inhibitory to zoospore attachment.

Tannins also form complexes with protein and carbohydrates and render them unavailable to the microbes (Mangan, 1988; Mueller-Harvey and McAllan, 1992). Tannins are also chelating agents and this could reduce the availability of metallic ions necessary for the metabolism of rumen microorganisms (Scalbert, 1991). Tannins have been found to react with microbial (both

bacterial and fungal) enzymes, inhibiting their activity (Mueller- Harvey and McAllan, 1992; McAllister *et al*., 1994; McSweeney *et al*., 2001). In a study by Bae *et al*. (1993), condensed tannin was found to readily form complexes with microbial enzymes and interfered with the ability of the microbes to get attached to the cell wall of the ingested plant. Hemicellulases have been reported to be affected more compared to cellulase (Waghorn, 1996).

Tannin containing browse plants were incubated in low nitrogen or high nitrogen media in a study by Getachew *et al*. (2000). In each media, there were two treatments (PEG and non-PEG). The results revealed that microbial fermentation of browse plants in high nitrogen media without PEG was lower. The high nitrogen resulted in higher ammonia nitrogen in the rumen but did not correspond to an increase in carbohydrate fermentation. This according to Getachew *et al*. (2000) could be due to the direct effect of the tannins on microbial enzymes. Bae *et al*. (1993) exposed *Fibrobacter sucinogenes* (S85) to various levels of CT and observed a decline in extracellular endoglucanase activity at 0.025 mg/ml. Extracellular and cell-associated endoglucanase activities were almost completely inhibited by 0.4 mg/ml of condensed tannins. In the same study by Bae *et al*. (1993), cellulose digestion was reduced by 8, 10, 45 and 92% when *F. sucinogenes* (S85) were exposed to 0.1, 0.2, 0.3 and 0.4 mg/ml of CT.

2.3.4 Effects of condensed tannin on rumen environment

Condensed tannin could play a very important role in maintaining a stable rumen environment if managed well in the diet of ruminants. Rumen pH and ammonia nitrogen concentration play a very important role in providing stable microbial population to enhance fermentation in the rumen. The fermentation of feedstuff in the rumen by microorganism produces volatile fatty acids (VFA) and lactic acid. Ruminal pH will drop when VFA or lactic acid accumulate in the rumen. To prevent the drop in pH related to production of acids in the rumen and acids ingested with the feed, the acids have to be removed from the rumen or buffered (Dijkstra *et al*., 2012).

The optimal ruminal pH required to maintain normal cellulolysis and microbial protein synthesis is reported to be 6.7 (Van Soest, 1994). McDonald *et al*. (2011) reported a normal rumen pH to be in a range of 5.5-6.5. There is evidence that low pH reduced fiber digestion in both *in vitro* and *in vivo* studies (Mould and Ørskov, 1984; Hoover, 1986; Grant and Weidner, 1992). It has also been reported that a decrease in pH results in a decreased digestion rate and increased lag time of NDF digestion (Grant and Weidner, 1992).

A reduction in rumen pH has been found to cause a marked decrease in CP degradation of soybean meal. In the study of Hariadi and Santoso (2010), different browse plants with total tannins in the range of 19-54 g/kg DM used in an *in vitro* experiment as supplement to *Pennisetum purpureum* Schumach did not significantly affect pH. In that study, the pH reported was all within the range of 6.82-6.95. Williams *et al*. (2011) found significant difference in pH when legumes containing CT of between 4.49-48.5 g/kg DM were incubated in buffered rumen fluid using the continuous culture system. However the pH for all the legumes was in the range 6.19-6.33.

The results was not different from the report of Min *et al*. (2005) when 3 levels of CT (0, 1, 2%) from Quebracho were added to a medium to incubate wheat straw. The pH was in the range of 6.36-6.40. The reports reviewed so far shows no adverse effect of CT on the pH.

The level of ammonia nitrogen in the rumen is a reflection of the extent to which dietary protein is being degraded by rumen microbes.

Satter and Slyter (1974) reported that ruminal ammonia concentrations between 88 and 133 mg NH_3N/L rumen fluid are the optimum to stimulate microbial protein synthesis and a maximum of 50 mg/L is enough to stimulate microbial growth. The growth and protein synthesis of the microbes is very important since almost half of the protein absorbed by the ruminant is in the form of microbial protein. Hariadi and Santoso (2010) found a significant reduction in

ammonia nitrogen when the CT in the diet was 54 g/kgDM. However the ammonia nitrogen recorded was higher (14.8 mg/100ml) than the optimal required for protein synthesis and microbial growth. Waghorn *et al*. (1998) reported a rumen NH_3-N of 379 mg/L and 266 mg/L for *Lotus corniculatus* (LC) with or without PEG respectively and that of LP were 468 mg/L and 175 mg/L with or without PEG. The TVFA was 136 mmol/L and 132 mmol/L for LC with or without PEG respectively and that of *Lotus pedunculatus* (LP) were 96 mmol/L and 65 mmol/L with or without PEG. Supplementation of TMR fed to four Polish Holstein-Friesian dairy cows with *Vaccinium vitisidaea* resulted in 45.9% drop in total ammonia (Cieslak *et al*., 2012).

Getachew *et al*. (2000) incubated different browse plants with CT in the range of 16-262.5 g/kg DM in high or low nitrogen media and with or without PEG. Significant difference was found between the browse plants incubated with or without PEG. The browse plants with PEG had higher ammonia nitrogen than the ones without PEG. It is however worth stating that, the levels of ammonia nitrogen were all within the optimum required for microbial synthesis and growth. These reports seem to suggest that CT in browse plants do not drastically reduce rumen ammonia nitrogen below the optimum.

2.3.5 Effect of tannins on rumen protozoa

Reports of the effect of tannins on rumen protozoa are varied and this variation could be due to the source and concentration of tannins used. Singh *et al.* (2011) observed a significant reduction in rumen protozoa count in experimental animals fed pakar (*Ficus infectoria)* leaves as compared to control animals. Makkar *et al*. (1995) reported lower protozoa numbers when *L. leucocephala* and leucaena hybrid KX2 containing tannins at the levels of 7.3 and 11.6% were fed. Baah *et al.* (2002) reported a decrease in protozoa populations when quebracho

powder was fed to cattle at 0.6% of dietary DM. Monforte *et al*. (2005) reported that protozoa numbers were negatively correlated to some CT rich plants.

According to McSweeney *et al.* (2001) rumen protozoa populations were less affected by tannins from Calliandra. Salem *et al*. (1997) observed a linear increase in protozoa numbers in rumen fluid of sheep fed on Lucerne hay-based diets by addition of increased proportion of *Acacia cyanophylla* foliage, which contained CT of 45 g/kg DM. Similarly, CT present in *L. corniculatus* and *H. coronarium* increased protozoal numbers in the rumen of sheep (Chiquette *et al*., 1989; Terrill *et al*., 1992). Multi-purpose tress such as *Acacia angustissima, Acacia saligna, Chamaecytisus palmensis, Leucaena pallida* and *Sesbania sesban* were fed to Ethiopian sheep as supplement at a level of 30% of the total diet with maize stover as the basal diet. The results of the study showed an increase in protozoa numbers for *Sesbania sesban* and a decrease for *Acacia saligna* (Odenyo *et al.*, 1997). None of the multi-purpose tree supplements eliminated protozoa entirely from the rumen. The mechanism by which tannin affects protozoa population have not yet been confirmed but can be speculated to be through an indirect effect on bacterial cell membrane. The CT- protein (dietary and microbial protein) complex may be reducing bacterial lysis by rumen protozoa. A reduction in bacterial lysis could affect the growth of rumen protozoa in the rumen. Odenyo *et al.* (1997) argued that the effect of multipurpose trees on protozoa numbers might be influenced by the differences in nitrogen content rather than the phenolic present in them.

2.3.6 Effect of tannins on methanogenesis

Various types of forages known to contain CT or tannin extracts have been shown to decrease methane production under both *in vivo* and *in vitro* conditions. Bhatta *et al*. (2009) reported that Quebracho tannins inhibited methane production linearly (13–45%) with increasing doses (5– 25% of substrates). Min *et al*. (2006) found that Quebracho tannin (75% CT) included at

concentrations of 1–2 g/kg DMI decreased *in vitro* methane production by 12.3–32.6%. Similarly, when rumen liquor from cannulated cattle grazing wheat grass in the reproductive stage and ruminally infused with Quebracho tannins at 10-20 g/kg DM intake was incubated, it was observed that the *in vitro* methane gas decreased by 25-51% (Min *et al*., 2006). But cattle grazing wheat grass in vegetative stage did not exhibit anti-methanogenic effect in this same study (Min *et al*., 2006). Beauchemin *et al*. (2007) also did not find any effect on methanogenesis when a Quebracho tannin extract (10–20 g/kg DM intake) was fed to beef cattle for 28 days.

Addition of *Acacia mearnsii* tannin extracts to sheep (Carulla *et al*., 2005) and cattle (Grainger *et al*., 2009) diet resulted in a decrease of methanogenesis. Inclusion of different type of forages containing tannins in diets has also been shown to inhibit methane emissions *in vitro* and *in vivo*. Tannins present in *Calliandra calothyrsus* reduced nutrient degradation and methane released for each gram of organic matter degraded in the Rusitec apparatus (Hess *et al*., 2003). Inhibition of methane production was also observed in Rusitec as the proportion of sainfoin (*Onobrychis viciifolia*) incubated were increased (McMahon *et al*., 1999).

Woodward *et al*. (2002) investigated the feeding of sulla (*Hedysarum coronarium*) on methane emission and milk yield in Friesian and Jersey dairy cows. Cows grazing on sulla had higher daily DM intake (13.1 vs. 10.7 kg DM) and daily milk solid production (1.07 vs. 0.81 kg) than grazing perennial ryegrass pasture. Total daily methane production was similar (253.9 vs. 260 g); however, cows fed sulla produced less methane expressed relative to DM intake (19.5 vs. 24.6 g/kg) and milk solid yield (243.3 vs. 327.8 g/kg). Similar trends in methane emissions and milk production have been observed in dairy cows fed on *L. corniculatus* silage (Woodward *et al*., 2001). There was also a 16% depression in methane production in lambs fed CT *L. pedunculatus* (Waghorn *et al*., 2002).

Tavendale *et al*. (2005) reported that methane production at 12 h was higher for *M. sativa* (25 ml/g DM) than for *L. pedunculatus* (17.6 ml/g DM). Addition of polyethelene glycol increased methane production for *L. pedunculatus* by 17%, but not for *Medicago sativa*.

Animut *et al*. (2008) also observed that feeding of different levels of *L. striata* decreased methane production linearly in goats and attributed the decrease to the presence of CT. Sheep grazed on perennial grass/white clover pasture produced significantly less methane compared with sheep grazed on willow fodder blocks containing 1.2% (Ramirez-Restrepo *et al*., 2010). Methane production was inhibited by inclusion of methanol extract of pericarp of *T. chebula* (a tropical fruit) under *in vitro* conditions up to 90% (Patra *et al*., 2006). Bhatta *et al*. (2009) showed that HT extract from myrabolam (13% DM) had more inhibitory effect on methanogenic archaea (9.4% vs. 11.8% of 16S rRNA) compared with a HT from chestnut (13.2% DM). In an anaerobic sludge, depressing effect on methane production was more pronounced with polymers of gallotannins compared with monomers of HT i.e. gallic acid and pyrogallol (Field and Lettinga, 1987). *Acacia mearnsii* CT decreased methane emissions in cows linearly with increasing doses of tannins (Grainger *et al*., 2009).

While most of the studies on tannins reported either had no effect or decreased methane output, Sliwinski *et al*. (2002) reported an increase in methane production by 32.6% and 21.5% compared with control in sheep fed hay-concentrate-based diet added with chestnut (*Castanea sativa*) wood extract (20% HT) at concentrations of 5 and 10.1 g/kg diet, respectively.

Although the reason for increased methane production is not clear in this study, it suggests that HT at low doses could be ineffective for methane mitigation. Inclusion of tannins in diets might decrease digestibility of fiber, which could result in decreased methane production. It is apparent that the mechanism by which tannin reduce rumen methane production is not

prominent in most of the results presented in the literature, it can however be speculated that since tannins bind to rumen bacterial cell wall and make them unavailable for protozoa lysis, protozoa numbers will be affected and thereby reduce the quantum of hydrogen produced for methanogenesis.

2.3.7 Effect of tannins on post rumen protein production and growth of ruminants

Tannins play a significant role in protecting dietary protein from rumen microbial degradation and thus increase total supply of amino acids to the intestine for absorption (Waghorn *et al.* 1987; McNabb *et al.* 1996). When CT increases beyond 9%, the nitrogen concentration in the rumen is reduced (Reed *et al.,* 1985). Tannins bind to different sources of protein included in the diet or those produced within the animal as a result of ingesting the feed. Waghorn (2008) reported that proline- rich protein (PRP) has a high affinity for CT than proteins present in forage. Min *et al.* (2005) reported a greater affinity between CT and bacterial cells than with plant protein, and according to McSweeney *et al*. (2001), cellulolytic bacteria were affected more than cellulolytic fungi in the CT and bacterial cell affinity.

From both studies by Waghorn (2008) and McSweeney *et al*. (2001), it can be concluded that CT tannins will bind to bacterial cell and PRP first before binding to plant protein. This means that plant or dietary protein will still be available for rumen microbial degradation but perhaps the level of degradation will be minimal to affect post rumen protein supply but enough to enhance rumen ammonia concentration for efficient rumen fermentation. However, this conclusion will also depend on the extent of PRP produced in the saliva during mastication.

The supply of amino acids for absorption in the intestine depends largely on the stability of the tannin-protein complex post ruminally. The tannin-protein complex according to Min *et al*. (2005) is formed at a pH of 4.0-7.0 in the rumen and subsequently dissociate in the presence

of a low pH as is the case in the true stomach and intestine. The protein reaching the abomasum and the intestine for absorption is a mixture of dietary protein and microbial protein and this increased when proteolysis in the rumen is reduced (Patra and Saxena, 2010).

Waghorn *et al.* (1987) observed an increase in both abomasal flow (53%) and net absorption of essential amino acids in the small intestine (59%) when *L. corniculatus* (2.2% CT) was fed to sheep. When CT-rich *L. pedunculatus* (5.5% CT) was fed to sheep, there was a 30% abomasal flow of essential amino acids but absorption in the small intestine declined to about 10% (Waghorn *et al.*, 1994).

Mezzomo *et al*. (2011) found that the amount of rumen degradable protein reaching the abomasum and intestine for absorption was higher when CT was included in the diet of sheep. In the same study, it was also reported that microbial protein that reached the abomasum and intestine did not differ between the CT diet and the control (0% CT). The flux of MP reaching the abomasum increased by 24.2% for the CT diet compared with the control.

In the study of Min *et al*. (2005), different rumen bacterial strains were incubated separately in a media with Rubisco from sunflower as the main source of nitrogen. Exactly 1.5 mg CT was added to each media to provide a CT-nitrogen ratio of 1:1.1(1.5 mg CT and 1.7 mg total N/ml). Another group of the CT- rich media also received PEG in order to bind the CT. The results showed that in the presence of CT, proteolysis was reduced for each of the bacteria strains. Tanner *et al*. (1994) reported a similar result when *L. corniculatus* CT was added to a similar *in vitro* incubation at a CT-protein ratio of 1:5.

The study concluded that the level of CT, bacterial strain and the protein substrate all influenced the extent to which proteolysis occurred and ultimately the amount of dietary and microbial protein that reaches the intestine for absorption. When four browse plants (*Chorisia speciosa*, *Cassia fistula*, *Schinus molle*, *Eucalyptus camaldulensis*) with CT concentrations of 20.8%, 31.6%, 49.2 and 68.1% respectively were incubated in a buffered rumen fluid with or without

PEG, the purine was in the range of 6.9-7.7 for CT without PEG and 7.3-8.2 for CT with PEG. The lowest purine concentration, and highest efficiency of microbial protein synthesis (EMPS), occurred in leaves with higher levels of CT.

Waghorn (2008) reported that CT reduced ruminal digestion of plant protein, rumen ammonia concentrations, protein solubility, rate of amino acid absorption and urinary nitrogen output. In the same study, there was an increase in the proportion of plant protein reaching the intestine and the amount of faecal nitrogen concentration. The reduction in amino acid absorption has been attributed to the effect of CT on endogenous enzyme activity or by association with intestinal mucosa.

Different plant species have been reported to behave differently in their tannin-protein binding ability in the rumen and their subsequent dissociation between the abomasum and small intestine (post ruminally) (McNeill *et al*., 1998).

Since CT have the tendency to reduce feed intake and digestibility in ruminants, it is also possible for it to impact negatively on the growth of ruminants. Barry (1985) observed a significant reduction in the gain of live weight in lambs fed *L. pedunculatus* (which has a high CT content; 76-90 g kg DM). Wang *et al*. (1994) observed that the grazing of L. *corniculatus* (34 g CT /kg DM) reduced feed intake but increased the gain in live weight, carcass weight, and dressing proportion, compared with a group supplemented with PEG, which binds to tannins and inactivates them. Montossi *et al*. (1996) observed a 23% improvement in live weight gain when lambs grazed *Holcus lanatus* (4.2 g CT/kg DM). The flow of CT when plants are ingested from the mouth to the abomasum is shown in Figure 2.2. When the plant cell is disrupted through chewing, the CT and protein are released into solution in the rumen. The CT then binds to the protein and any free CT available goes to bind to beneficial microbes and extra cellular enzymes. When the complex reaches the abomasum where the pH is lower than

what prevails in a normal rumen it dissociates for the protein to be absorbed. The effects of tannins on the degradation, absorption and excretion of nitrogen is summarised in Table 2.2.

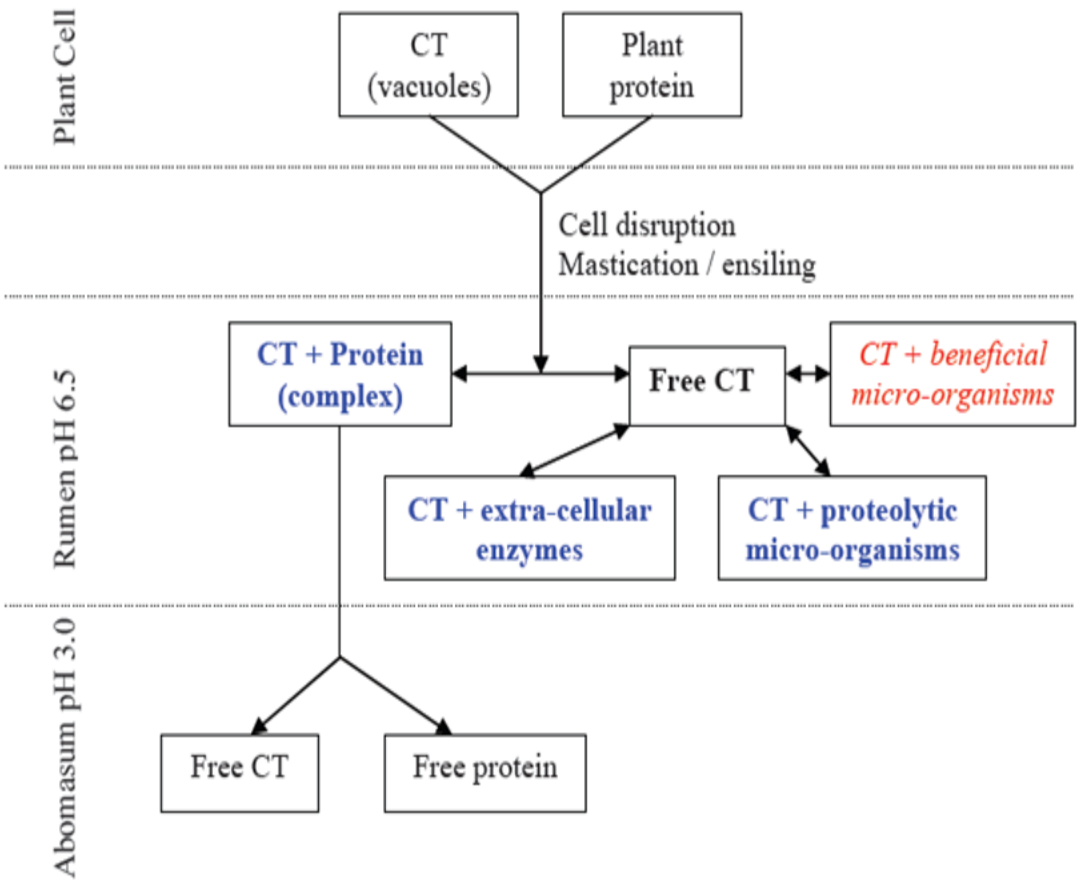

Figure 2. 2 Proposed detrimental (red/italic) and beneficial (blue/bold) interactions of CT with protein and rumen micro-organisms. ↔ indicates a reversible reaction. Compiled by Kenton (2005)

Table 2. 2 Effect of tannins on the degradation, absorption and excretion of nitrogen

Source	Key comparison	Effect	Reference
Tara tannin	*In vitro* ammonia production of soybean meal treated with graded levels of tannins	The presence of 10% tannin resulted in a 90% decrease in deamination	Driedger and Hatfield (1972)
Mung beans (*Vigna radiata*)	*In vitro* protein digestibility of boiled seeds measured with and without tannins	Addition of tannins reduced protein digestibility values by 3-4%	Barroga *et al.* (1985)
72 West African fodder trees	*In vitro* degradability of the protein from 72 fodder trees varying in tannin content	There was an inverse relationship between *in vitro* protein degradability and both soluble phenolics and insoluble tannin concentration	Rittner and Reed (1992)
Lotus corniculatus	*In vitro* degradable protein determined on 12 *L. corniculatus* clones differing in tannin content	As tannin content of the clones increased, *in vitro* protein degradability decreased	Miller and Elke (1994)
Cottonseed hulls	Potential degradability of two cottonseed proteins measured in vitro with and without hulls and PEG	Addition of hulls depressed degradation of the two proteins and the addition of PEG only partially removed that effect	Yu *et al.* (1996)
Faba bean (*Vicia faba*)	*In vitro* degradability of protein in six pairs of near-isogenic lines, in each pair a tannin-free and a tannin-containing line	Seeds from the tannin-free lines had 10% higher in vitro protein digestibility than tannin-containing lines	Helsper *et al.* (1996)
Faba bean (*Vicia faba*)	Rumen degradability of protein of 12 faba bean cultivars determined in vitro	There was a strong correlation between tannin content and in vitro rumen protein degradability	Makkar *et al* (1997b)
12 tropical browse legumes	Nitrogen digestibility from tropical legumes with tannin content ranging from 0 to 2.8% determined *in sacco*	Tannin content (vanillin-HCl and butanol-HCL) was not a reliable indicator of the degradation of nitrogen *in sacco*	Ahn *et al.* (1989)
Temperate fodder legumes	In situ protein degradability determined in cultivars of fodder legumes containing between 0 and 2.8% tannic acid equivalents	Amount of undegraded protein after 12 hours of incubation was positively related to tannin content, with 38% and 86% remaining in non-tannin and tannin legumes, respectively	Messman *et al.* (1996)
Tara tannin	Soybean meal treated with and without 0.89% tannins fed to sheep	There were no differences in nitrogen digestion, but lambs receiving the tannin-treated diet had higher nitrogen retention due to reduced urinary nitrogen excretion	Driedger and Hatfield (1972)
Lotus pedunculatus	Forage with low (4.6%) and high (10.6%) tannin content fed to sheep	More nitrogen reached the duodenum, was gained across the rumen and was excreted in faeces in sheep fed the high tannin forage	Barry and Manley (1984)
Lotus pedunculatus	Forage (9.5% tannins) was fed to sheep treated with zero, low and high rates of PEG	Less nitrogen reached the duodenum, was digested in the post-ruminal region and was excreted in faeces as PEG supplementation increased	Barry *et al.* (1986)

Adapted from Rosales (1999).

2.3.8 Protein and tannin factors that influence CT-protein complex formation

According to Butler (1989), tannin-protein complex can be quite specific for both protein and tannin. Hagerman and Butler (1981) using the competitive binding assay determined the level of affinity of different protein sources (bovine serum, lysosome and albumin) to tannin. The results from the study showed variations in the level affinity and agrees with reports by Mehansho *et al*. (1983) and Asquith and Butler (1985). Specific characteristic features of protein and tannin may be responsible for determining the level of affinity between protein and tannin (Table 2.3).

Table 2. 3 Relative affinity of different protein for sorghum tannin

Protein	Relative affinity
Gelatine	14.0
Proline-rich salivary protein	6.8
Pepsin	1.1
Bovine serum albumin	1.0
Bovine haemoglobin	0.068
Ovalbumin	0.016
β-Lactoglobulin	0.0087
Lysozyme	0.0048
Soybean trypsin inhibitor	<0.0010

(Butler, 1989)

The high molecular weight, open and flexible structures of protein has been reported as the properties influencing the strong affinity of protein for tannin (Asquith and Butler, 1986; Mueller-Harvey and McAllan, 1992). Longer amino acid chains signify a greater number of attachment sites, while greater conformational freedom reduces the limitations caused by steric factors (Rosales, 1999). Apart from the size of the protein, the composition of the amino acids has also been reported to influence the tannin-protein complex (Handley, 1954; Oh *et al.*, 1980). One feature of protein and polypeptide with high affinity for tannins is their richness in imino acid proline (Rosales, 1999; Hagerman and Butler, 1981).

Hagerman and Butler (1981) suggested two major reasons for the high affinity between proline and tannins. The first is that proline-rich polypeptides have much more open and flexible configurations. Second, proline rich proteins have an increased capacity to form strong hydrogen bonds due to increased accessibility of the peptide linkages.

The molecular weight of tannins has been the major factor influencing the protein-tannin complex (Rosales, 1999; Porter and Woddruffe, 1984). The molecular weight of tannin varies with forage species, plant tissue and season (Foo and Porter, 1980; Butler, 1982; Foo *et al*., 1982; Williams *et al*., 1983).

There are varied views as to whether a high molecular weight leads to high protein affinity or vice versa. Bate-Smith (1973) demonstrated that the ability of CT to precipitate the proteins of haemolysed blood increased as the molecular weight increased from 576 to beyond 1134. Porter and Woddruffe (1984) carried out haemanalysis measurements on a number of CT of known molecular weights and reported that CT with a molecular weight of 2400-2700, have a very similar relative astringency to tannic acid. Jones *et al*. (1976) reported that tannins of high molecular weight (17,000-28,000 Dalton) from sainfoin (*Onobrychis vicifolia*) leaves were less effective in precipitating proteins than smaller tannins from other legumes including *Lotus* spp. (6000-7100 Dalton). Kumar and Vaithiyanathan (1990) suggested than when the molecular weight is large (>5000), the CT become rather insoluble in solutions and lose their protein-precipitating capacity. In recent report by Naumann *et al*. (2014), there was no correlation between the molecular weight of CT and the protein precitable phenolics (R^2 0.11) and amount of protein bound (R^2 0.11). The authors however found a correlation between the concentration of CT and the protein precitable phenolics (R^2 0.81) and amount of protein bound (R^2 0.69).

Molecular weight of tannins greatly influences the level of affinity for protein however; it is not clear at what molecular weight the highest affinity will be recorded.

2.3.8 Protein metabolism and microbial protein synthesis in ruminants

Protein used for maintenance, growth and production in ruminants originates from three main sources which include dietary, endogenous and microbial sources (McDonald *et al*., 2011). Fractions of ingested dietary protein is usually degraded in the rumen in order to supply the amino acids, peptides and ammonia required for microbial protein synthesis. Bacteria acting on structural carbohydrate (SC) fraction of the diet rely only on ammonia for cell membrane synthesis and those acting on non-structural carbohydrate (NSC) derive about 65% of their nitrogen from amino acids and peptides and the remainder from ammonia (McDonald *et al*., 2011). The microbial protein synthesised is passed from the rumen and is digested in the small intestine for amino acid supply. The quantum of microbial protein passing to the intestine relies largely on the speed and extent of microbial breakdown of the dietary protein and efficiency of the transformation of the degraded material into microbial protein (McDonald *et al*., 2011).

Bach *et al*. (2005) indicated that the most important factors affecting microbial protein degradation in the rumen included the type of protein, interactions with other nutrients and the predominant microbial population. In a study by Wanapat *et al*. (1982) where four different diets with protein solubility of 23.7%, 50.5%, 18.2% and 24.2% for grass hay plus oat straw (control), control plus urea, control plus soybean oil and control plus sunflower oil respectively were fed to four rumen-cannulated, mature crossbred wethers. The results showed highest digestibility in the control plus urea diet, which also resulted in a high urinary nitrogen excretion. The findings agree with the reports of several other researchers (Whitlow 1978; Beardsley *et al*., 1977; Crooker 1978) who found protein solubility influencing microbial breakdown of protein. The fate of dietary protein for ruminants has been fractionated into various classes based on their level of degradability and digestibility (Table 2.4).

Table 2. 4 Composition, rumen degradation and intestinal digestion of protein fractions

Fraction	Composition	Rumen degradation (%/hour)	Intestinal digestibility (%)
A	Ammonia, nitrate, amino acids, peptides	Instantaneous	None reaches intestine
B1	Globulins, some albumins	200–300	100
B2	Most albumins, glutelins	5–15	100
B3	Prolamins, cell-wall proteins, denatured proteins	0.1–1.5	80
C	Maillard products, nitrogen bound to lignin, tannin-bound protein	0	0

Adapted from Chalupa and Sniffen (1994)

Protein in tropical browse plants may vary in their digestibility due to the presence of tannins in most of them and also due to the varying levels of soluble fractions. Non-ammonia nitrogen is rapidly soluble and its concentration in the plant has been found to increase with increase in nitrogen fertilizer application (Johnson *et al*., 2001). The use of browse plants in Northern Ghana is a common practice especially in the dry season but it is however unclear the extent to which the protein is soluble and also enhance microbial protein synthesis. The fate of dietary protein in the GIT of ruminant is shown in Figure 2.3.

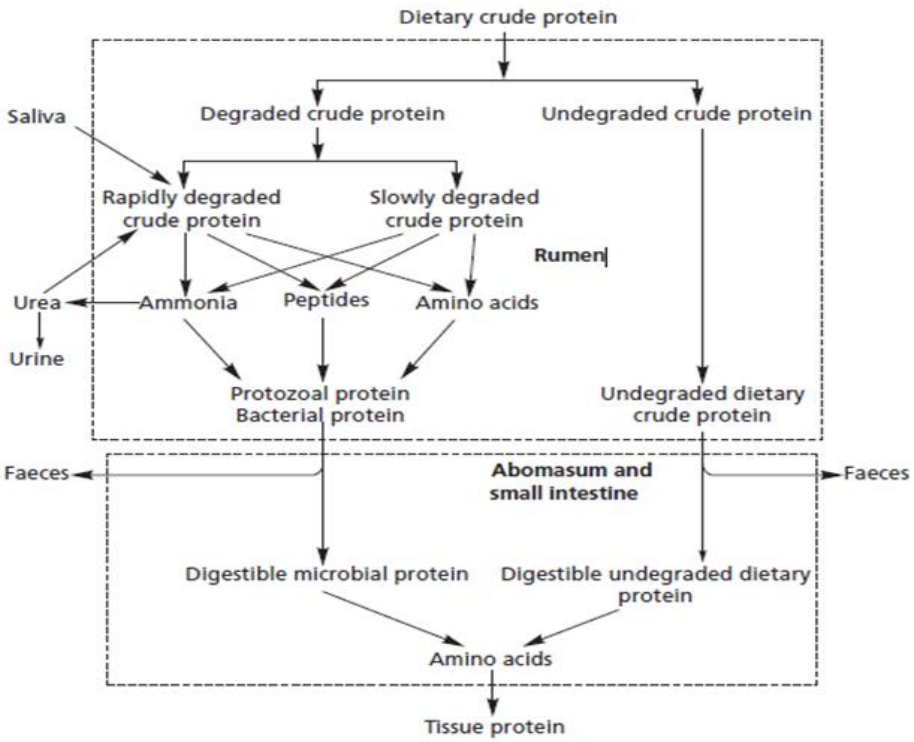

Figure 2. 3 Fate of dietary CP in the ruminant animal (Adapted from McDonald et al., 2011)

2.3.9 INFERENCES FROM LITERATURE REVIEW

- Low quality of crop residue used as basal diets in the dry season may lower animal productivity and increase enteric methane emission
- Browse plants are a major source of supplementary feed for ruminants in the Savanna Regions of Ghana
- The nutrient content and concentration of CT of these browse plants vary according to species, plant part or fraction and season.
- Condensed tannins have been used in diets of ruminants as protein binders to ensure effective degradation and efficient capture of protein by rumen microbes thereby redcuing the amount urinary nitrogen excreted.
- Condensed tannin has been found to significantly influence enteric methane emissions, reduce feed intake and inhibited overall rumen fermentation.

CHAPTER 3

3.0 GENERAL MATERIALS AND METHODS

3.1 Sample collection

Leaves of eight browse plants were collected from a minimum of ten trees for each browse plant within the Northern Region of Ghana (Appendix 2.44). The leaves were collected between the periods of October and November 2011. The eight browse plants were:

Ceiba pentandra (Kapok tree)

Khaya senegalensis (Mahoganny tree)

Senna siamea (Cassia tree)

Ficus gnaphalocarpa (Large fruited sycamore fig)

Pterocarpus erinaceus (African teak)

Albizia lebbeck (Lebbek tree)

Azadirachta indica (Neem tree)

Gmelina arborea (Gmelina)

About 3 kg of each browse plant (unmilled) was packaged and transported to the Harper Adams University, UK for chemical analysis, *in vitro* (batch and continuous culture) and *in sacco* studies.

3.2 Sample milling

Dried samples were milled using a hammer mill (IKA MF 10, UK) fitted with a 1mm sieve screen for chemical analysis and *in vitro* gas study. The samples used for the *in sacco* study were milled to 2 mm.

3.3 Dry matter

Sub-samples of each browse plant were weighed into aluminium bowls and placed in an oven (Binder, Cole-Palmers, UK) at 60^{o}C for 48hours. Dry matter was calculated as:

$$\text{Dry matter (g/kg)} = \left[\frac{Dry\ sample\ weight}{Wet\ sample\ weight}\right] * 1000 \qquad \text{= Equation 3.1}$$

3.4 Ash

Ash was determined according to the procedure of AOAC (2000). Approximately 2 g of dried sample was weighed into a pre-weighed porcelain crucible and heated to 550°C in a muffle furnace (Carbolite, AAF 1100, Hope valley, England) for 4 h. Samples were then cooled in a desiccator and reweighed. The ash content was calculated as:

$$\text{Ash (g/kg DM)} = \left[\frac{Weight\ of\ ash}{Weight\ of\ sample}\right] * 1000 \qquad \text{=Equation 3.2}$$

The organic matter content (g/kg DM) was calculated as 1000 - ash. =Equation 3.3

3.5. Crude protein

Nitrogen was determined according to the method of AOAC (2000) using Leco (Leco FP-528-UK).

Approximately 0.15 g of dry sample was weighed into foil cup and wrapped. The wrapped asmple was transfered to the carousel on top of the Leco instrument in the numbered hole corresponding to the number in the position column. The command was issued from a computer connected to the Leco machine by pressing the analyse button. The carousel rotates and the sample is dropped into the furnace at 900^{o}C. The sample is burnt and the gases produced were analysed for nitrogen. The nitrogen measured was used to calculate the CP using the formulae below.

Crude protein (g/kg DM) = total nitrogen (g/kg DM) x 6.25 =Equation 3.4

The Leco machine was standaized with ethylenediaminetetracetic acid (EDTA) each day before the analysis was done.

3.6 Neutral detergent fibre

Neutral detergent fibre (NDF) was determined according to the procedure of Goering and Van Soest (1970). Approximately 0.5 g of dry milled leaf of each browse plant was weighed into a glass crucible (porosity 1, Soham Scientific, Ely, UK) and were placed into the Fibertec apparatus (1020, FOSS, Warrington, UK). Exactly 25 ml of cold neutral detergent reagent (made by dissolving 93g of disodium ethylene diamine tetra-acetic acid dehydrate (EDTA) and 34 g of sodium borate in 3 L of hot distilled water) was added to each sample. To this, 150 g of sodium lauryl sulphate and 50 ml of tri-ethylene glycol was added. In a separate flask 22.8 g of anhydrous disodium hydrogen phosphate was dissolved in distilled water. The two solutions were then mixed and diluted to 5 L. The pH was adjusted 6.9 - 7.1 (with either 0.1 M NaOH or 0.1 M HCl).

Samples were boiled and refluxed for 30 min, after which an additional 25 ml of cold neutral detergent reagent and 2 ml of α-amylase (2 g of heat stable α-amylase E.C.3.2.1.1. from *Bacillus subtilis* (Sigma, Gillingham, UK) dissolved in 90 ml of distilled water and 10 ml of tri-ethylene glycol was added.

Samples were boiled and refluxed for a further 30 min, drained and then washed three times with 25 ml of hot (80°C) distilled water under vacuum. To each sample, 25 ml of hot (80°C) water and 2 ml of α-amylase solution was added. After 15 min the samples were drained and washed, under vacuum. The crucibles were removed from the Fibretec apparatus and dried at 105°C overnight. After cooling in a desiccator, the crucibles were weighed and then placed in a muffle furnace at 550°C for 4 h. Crucibles were then allowed to cool to room temperature in a desiccator and weighed. Neutral detergent fiber (g) was calculated as:

$$\text{NDF (g)} = (\text{crucible} + \text{dry fibre weight}) - (\text{crucible} + \text{ashed fibre weight}) \quad =$$

Equation 3.5

$$\text{NDF (g/kg DM)} = \frac{\text{NDF weight (g)}}{\text{Sample weight (g)}} * 1000 \quad = \text{Equation 3.6}$$

3.7 Acid detergent fibre

Acid detergent fibre (ADF) was determined according to the procedure of Goering and Van Soest (1970). Approximately 1.0 g of dry milled leaf of each browse plant was weighed into a glass crucible (porosity 2, Soham Scientific, Ely, UK). Crucibles were placed into Fibertec apparatus (1020, FOSS, Warrington, UK). Exactly 100 ml of cold acid detergent reagent was added. Samples were boiled for 60 min to digest, after which they were filtered and washed 3 x 25 ml of hot deionised water. The crucibles were removed from the Fibretec apparatus and dried at 105°C overnight. After cooling in a desiccator, crucibles were weighed and then placed in a muffle furnace at 550°C for 4 h. Crucibles were then allowed to cool to room temperature in a desiccator and weighed. Acid detergent fiber (g) was calculated as:

$$\text{ADF (g)} = (\text{crucible} + \text{dry fibre weight}) - (\text{crucible} + \text{ashed fibre weight}) \quad = \text{Equation 3.7}$$

$$\text{ADF (g/kg DM)} = \frac{\text{ADF weight (g)}}{\text{Sample weight (g)}} * 1000 \quad = \quad \text{Equation 3.8}$$

3.8 Acid detergent lignin

Acid detergent lignin (ADL) was determined according to the procedure of Goering and Van Soest (1970). Acid detergent lignin was determined by following the ADF procedure up to the point of drying overnight in an oven. After drying, 25 ml of 75% sulphuric acid was added to each crucible and placed back in the fibretech apparatus. Each crucible was allowed to stay for 3 h. Within the 3hours, crucible content was mixed by moving the knob on the fibretech to pressure position. This was done 3 times within the 3 h to ensure proper digestion of samples. After the 3 h, the contents of the crucibles were filtered and washed 3 times with 25 ml hot deionised water. The crucibles were removed from the Fibretec apparatus and dried at 105°C overnight. After cooling in a desiccator, crucibles were weighed and then placed in a muffle

furnace at 550°C for 4 h. Crucibles were then allowed to cool to room temperature in a desiccator and weighed. Acid detergent lignin (g) was calculated as:

$$\text{ADL (g)} = (\text{crucible} + \text{dry fibre weight}) - (\text{crucible} + \text{ashed fibre weight}) \quad = \text{Equation 3.9}$$

$$\text{ADL (g/kg DM)} = \frac{\text{ADL weight (g)}}{\text{Sample weight (g)}} * 1000 \quad = \text{Equation 3.10}$$

3.9 Ether extract

Ether extract was determined by the solvent extraction using the Soxtec apparatus (FOSS, Warrington, UK). Approximately 2 g dried sample was weighed into a cellulose extraction thimble (Whatman, Maidstone, UK) and plugged with defatted cotton wool. Total fat was extracted by boiling the samples in 25 ml (30-40°C) petroleum ether (Analar, VWR, Lutterworth, UK) for 30 min. Samples were then rinsed for 30 min, prior to evaporation of the petroleum ether. Ether extract was determined as:

$$\text{Extract (g/kg DM)} = \frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} * 1000 \quad = \quad \text{Equation 3.10}$$

3.10.0 Condensed tannins

3.10.1 Tannins Extract preparation

Tannin extract was prepared by weighing 400 mg of ground sample into 50 ml plastic tubes. Twenty millilitres of 70% aqueous acetone was added and centrifuged (Hettich Zentrifugen D78532, Germany) for 10 min at approximately 3000 *g* at 4°C. The supernatant was collected into clean 50 ml plastic tubes and stored at 4°C for the tannin extraction.

3.10.2 Total Phenol and Tannins analysis

It was estimated according to the procedure of Makkar *et al*. (1993). Standard tannic acid solution of 0, 10, 20, 30,40,50,60,70,80,90 and 100 µl was measured into 5 ml glass test tubes separately. Distilled water was added to each tobe to bring the volume to 500 µl (0.5 ml). To this solution, 250 µl (0.25 ml) of Folin Ciocalteu reagent (1 N) was added and 1.25 ml of sodium carbonate solution also added (to obtain a final volume of 2.0 ml with the tannic acid concentration ranging 0.0 to 10 µg). The content of tube was vortexed (Fisher Scientific, FB15013 top mix-UK) and incubated at room temperature under dark condition. After 40minutes, the absorbance was read at 725 nm using the spectrophotometer. The absorbance was to calibrate a standard tannic acid curve.

Fifty micro litres (50µl) of tannin extract was pipetted into a glass test tube and the volume made up to 1.0 ml with distilled water. Folin Ciocalteu reagent (0.5 ml) was added after which 2.5 ml of 20% sodium carbonate solution was also added. The content was then vortexed (Fisher Scientific, FB15013 top mix-UK) and kept for 40 min at room temperature. Optical density was read at 725 nm on a spectrophotometer and concentration estimated from the standard tannic acid curve. Total phenol was estimated as tannic acid equivalent from the calibration and the results expressed as total phenols grams per 100 g on dry matter basis.

Non-tannins phenol was estimated by precipitating tannins with polyvinyl polypyrrolidone (PVPP) (Fischer Scientific-UK), which binds tannins. Exactly 200 mg PVPP was measured into a test tube and then 2.0 ml distilled water and 2.0 ml tannins extract added. The content of the test tube was vortexed kept in a refrigerator for 15 minutes at 4^{o}C. The mixture was then centrifuged at 3000 *g* for 10 min. After centrifuging, the supernatant which simply contains phenolic other than tannins (PVPP bounds to the tannin) was collected into a cuvette and the absorbance read at 725 nm on a spectrophotometer. From the absorbance, the concentration of

the non-tannin phenolic was estimated from the standard tannic acid curve. The total tannin was the computed using the formulae below.

[Tannin%=Total phenolics (%) -Non-tannic phenolics (%)] = Equation 3.11

3.10.3 Estimation of Condensed Tannin (Proanthocyanidin)

Condensed tannin was estimated according to the method of Porter *et al*. (1986).

Exactly 0.5 ml of tannins extract was pipetted into a test tube in triplicate and 3.0 ml butanol HCl and 0.1 ml of ferric reagent added. The content of the tube was vortexed to ensure proper mixing. The mouth of the tube was covered with glass marble and then boiled for 60 min at a temperature of 97 to 100^{o}C in a water bath.

Similarly, blank was prepared for each sample but without the heating process. The tubes were cooled to room temperature and read at 550 nm using spectrophotometer. Condensed tannins as leucocyanidin equivalent was calculated as below

$$\% \text{ condensed tannins} = \frac{\text{A550nm X 78.26 X dilution factor}}{\% \text{ dry matter}} = \text{Equation 3.12}$$

Dilution factor is 1

3.11. Ammonia nitrogen

Frozen samples from the *in vitro* experiments were analysed for ammonium nitrogen. The method of Watson and Galliher (2001) was adopted and used on the Foss Kjeltac analyser (unit 2300), UK.

Exactly 5 ml of defrosted filtrate from the *in vitro* experiment was measured into digestion tube and 6 ml of magnesium oxide was added (17 g of heavy magnesium oxide dissolved in 100 ml of distilled water).

The sample was steamed via a receiver solution prepared from 50 g boric acid, 50 ml bromocresol green (100 mg bromocresol green in 100 ml methanol) and 35 ml methyl red (100

mg methyl red in 100 ml methanol) in 5 L distilled water. This was back titrated using 5mM sulphuric acid to determine the colour change. Daily calibration of the machine was done using ammonium standard (9 ml ammonium standard diluted in 50 ml deionised water) to obtain a constant recovery of 97.5-102.5%. Ammonium nitrogen was calculated from the titre value obtained for each sample using the following formulae:

$$\text{Ammonia nitrogen (g/L)} = \frac{Sample\ titre\ (ml)}{Weight\ of\ sample\ distilled\ (g)} * 0.01401 * 0.01 * 1000 = \text{Equation 3.13}$$

Where 0.01401 represents the weight of a nitrogen atom and 0.01 is the concentration (normality) of the acid solution.

CHAPTER 4

4.0 EXPERIMENT 1: CHEMICAL COMPOSITION AND *IN VITRO* GAS PRODUCTION OF LEAVES OF EIGHT TROPICAL BROWSE PLANTS

4.1.0 Introduction

This experiment was conducted to investigate the chemical composition and *in vitro* gas production of eight browse plants as protein supplement. A number of authors have reported that the presence of CT in ruminant diet interfere with the degradability of protein in the rumen (Kariuki and Norton, 2008; Huang *et al.*, 2010; Jin *et al.*, 2012; Ives *et al*., 2015). This comes about as a result of the complex formed between the CT and the protein which is reversible when the complex gets into the small intestine. Lack of proteolysis in the rumen can deny the cellulolytic bacteria access to ammonia nitrogen, which is required for microbial cell synthesis (McDonald *et al*., 2011). This phenomenon could lead to poor digestibility of the browse plants.

It has been suggested that when a feed is being tested for nitrogen degradability using the *in vitro* batch culture technique, then two separate media should be used. One media should contain no nitrogen so the source of nitrogen will be from the degradation of the substrate (Rymer *et al*., 2005). The nutrient content analysis on the browse plant in this study showed that except *A. lebbeck*, all the other browse plants contained CT. The other browse plants were therefore compared to the A. *lebbeck*, to confirm whether the presence of the CT could impact negatively on proteolytic activities.

4.1.1 Objectives

- To determine the chemical composition (CP, Ether extract, NDF, ADF, ADL and CT) of browse plants
- To determine the *in vitro* dry matter digestibility, pH, ammonia nitrogen and *in vitro* gas production of browse plants incubated in nitrogen sufficient or nitrogen deficient media

4.1.2 Hypothesis

In vitro dry matter digestibility and ammonia nitrogen production of browse plants will not differ when incubated in nitrogen sufficient and nitrogen deficient media

4.2.0 Materials and methods

4.2.1 *In vitro* gas study

4.2.1.1 Treatments and experimental design

There were eight treatments (browse plants) incubated in two media (ND and NS) with 5 replicates per run. A total of 4 runs of separate incubations were conducted for all the treatments. The samples were incubated in two different media, which were nitrogen, sufficient (NS) and nitrogen deficient (ND). The 8×2 factorial arrangement in randomised complete block design was used for this study. The factors were leaves of browse plants [8] and media type [2]. Three substrate blanks were included in each run.

After each incubation period, the pH of the vessels content was measured. The content was also analysed for dry matter and ammonia nitrogen.

4.2.1.2 Method for in vitro gas studies

The method described by Theodorou *et al*. (1994) was used for the *in vitro* gas studies. By this method, the samples were incubated in a media made up of micro minerals, macro minerals, buffer, reducing solution, indicator (resazurin) and strained rumen fluid. In this study, the nitrogen content of the media was varied in a way that in one medium, Ammonium hydrogen carbonate (NH_4CO_3) was excluded to represent the ND treatment and the other medium with Ammonium hydrogen carbonate (NH_4CO_3) representing the NS treatment. The ND treatment was prepared according to Getachew *et al*. (2000).

Approximately 2.0 g of each treatment was weighed into a 250 ml fischer and duran bottles and placed in an incubator set to a temperature of 39^{o}C overnight.

The stock solution (micro mineral, macro mineral, buffer, reducing solution and indicator) for the various media were prepared a day before the incubation. The macro minerals, buffer, reducing solution and indicator were mixed together in a sterilized 5 L beaker and transferred

into a 10 L Fischer bottle. This was placed in an autoclave for sterilization. The micro mineral was added on the day of incubation.

On the day of incubation the two media were placed in a water bath with the temperature set to 39^{o}C. Carbon dioxide was continuously infused into each media until the rumen fluid was ready.

Rumen fluid was collected from 4 different rams fitted with a fistula. The animals had an average weight of 95 kg and were fed on straw and concentrate (Wynnstay ram master coarse mix, UK) diet at 1.1 x maintenance requirement (AFRC, 1993). The rams were group housed with straw as bedding and under constant light supply. Water was offered *ad libitum*.

Rumen fluid was collected 3-4 h post feeding through a suction process. The fluid was collected into a pre-warmed vacuum flask and quickly sent to the laboratory. The fluid from each of the four animals were pooled and strained using a four layer cheese cloth placed in a funnel over a 5 L conical flask. Carbon dioxide was flushed through the rumen fluid and mixed with each media at 14% inclusion to provide 10 L of media with 1.4 L of strained rumen fluid (Huntington *et al*., 1998).

Approximately 200 ml of the buffered rumen fluid was pumped into the pre-weighed samples in the vessels using a peristaltic pump, gassed for 5 seconds, sealed with rubber corks and placed in the incubator set to 39^{o}C. The rubber corks were fitted with a needle and valve to aid in the measuring of pressure (gas accumulation).

Gas production from each vessel was measured using a pressure transducer (T443; Bailey and MacKay Ltd., Birmingham UK). At each time of reading (0, 3, 6, 12, 18, 24, 36, 48, 60, 72 h), the pressure transducer was connected to one of the openings on the valve and the valve was turned from the closed position to the open position. The pressure in the vessel was then read on the screen of a digital display connected to the transducer in per square inch (psi). The

pressure readings were taken at 0, 3, 6, 12, 18, 24, 36, 48, 60, 72 h and converted to volume (ml/g DM) using Equation 4.2. The pressure reading at time zero was assumed to be zero.

After 72 h, the seals were removed and the bottle contents filtered under vacuum through a dried, pre-weighed sintered glass crucible.

The crucibles were dried overnight at 105°C, cooled in a desiccator and reweighed. The crucibles were then heated (550°C) for 4 h in a muffle furnace, removed and allowed to cool in a desiccator prior to being weighed in order to determine the residual ash. The *in vitro* dry matter (IVDMD) was calculated, after correction for the blank, as follows:

$$\text{IVDMD} = \frac{DM\ in - DMo\ ut}{DM\ in} \quad \text{=Equation 4.1}$$

Gas production (Gp; ml) was calculated from pressure transducer readings (Pt; psi) using the relationship stated in Boyle's Gas Law (Kenton, 2005).

$$\text{Gp} = \frac{Vh}{Pa} * Pt \quad \text{=Equation 4.2}$$

Where Vh represents headspace volume (ml), Pt is pressure reading at time "t" and Pa is an average standard atmospheric pressure (14.7 psi; Metrological Office, Bracknell, UK). A mean Vh of 107.50 ml was determined by filling 100, 250 ml bottles used for the incubation to the brim with water, recording the exact volume and subtracting 200 (i.e. volume of media). From this, a standard predictive equation was derived, where Gp=7.31Pt. After correction for the substrate blanks, cumulative gas production was calculated for 1 g of DM. The gas readings were then fitted to the exponential curve of Ørskov and McDonald (1979) without an intercept using sigma Plot 10th edition (Systat Software Inc).

$$Y = b\ (1 - e^{-ct}) \quad \text{= Equation 4.3}$$

Where Y = gas volume at time t (ml)

b = asymptotic gas production (ml/g DM)

t = time (h)

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

4.2.2 Statistical analysis

Data was analysed using two-way ANOVA with run as block from Genstat version 12.1 (Payne et al., 2009). The difference among treatment means was determined using Fisher's least significant difference test.

4.3 Results

The nutrient compositions of the browse plants are shown in Table 4.1. The DM content of the browse plants was in the range of 345.43 to 448.3 g/kg with the highest and least obtained in *S. siamea* and *F. gnaphalocarpa* respectively. The OM content was also in the range of 129.52 to 383.07 g/kg with *S. siamea* having the highest and *F. gnaphalocarpa* having the least.

The CP content of the browse plants was highest for *A. lebbeck* (229.2 g/kg) and least for *A. senegalensis* (92.3 g/kg). The highest NDF content among the browse plants was recorded in *P. erinaceus* with *F. gnaphalocarpa* having the least (Table 4.1). The ADL for the browse plants ranged from 94.9 g/kg to 282.3 g/kg with highest recorded in *G. arborea*. Condensed tannin (CT) was present in all the plants except *A. lebbeck*. The highest CT was observed in *F. gnaphalocarpa* (114.5 g/kg) and decreased in the order of *F. gnaphalocarpa* > *C. pentandra* > *K. senegalensis* > *A. indica* > *P. erinaceus* > *G. arborea* > *S. siamea*. Based on the CT content, the browse plants can be grouped as higher CT (*F. gnaphalocarpa*, *C. pentandra*, *K. senegalensis*) medium CT (*A. indica*, *P. erinaceus*) and low CT (*G. arborea*, *S. siamea*).

Table 4. 1 Chemical composition of browse plants ± Standard deviation (g/kgDM)

Component	Browse plants							
	A. indica	*A. lebbeck*	*C. pentandra*	*S. siamea*	*F. gnaphalocarpa*	*G. arborea*	*K. senegalensis*	*P. erinaceus*
DM (g/Kg)	399.6±0.6	394.6±0.6	365.3±1.2	448.3±0.3	345.4±0.6	374.3±0.6	434.2±0.2	383.5±0.6
OM	321.9±0.5	304.5±0.9	271.9±1.3	383.1±0.3	129.5±0.7	293.6±0.6	357.6±0.2	294.2±0.3
CP	154.1±1.1	229.2±3.7	126.3±2.8	175.9±2.2	93.4±0.2	151.2±0.6	92.3±1.1	136.7±3.0
Ash	83.5±0.3	85.7±0.8	86.0±0.1	67.2±0.4	83.5±2.7	84.7±0.9	78.3±0.8	85.9±1.8
Ether Extract	21.9±0.3	42.0±0.2	18.1±0.8	46.8±0.8	14.5±2.3	25.0±0.4	46.8±3.2	28.7±1.0
NDF	237.4±3.3	296.1±2.3	271.3±1.3	262.7±2.2	216.2±0.8	248.5±1.0	250.4±1.0	307.3±2.4
ADF	183.7±1.0	193.7±4.6	291.8±0.4	256.9±0.7	274.9±0.7	163.4±3.9	281.9±0.63	205.7±3.3
ADL	134.7±0.2	196.4±0.0	200.4±0.6	111.8±0.5	109.6±0.0	282.3±3.0	94.9±1.0	105.8±0.3
CT	35.2±3.1	0.00	102.8±1.7	1.8±0.19	114.2±1.2	3.7±0.2	66.0±2.9	14.9±0.1

DM=Dry matter; OM=Organic matter; CP=Crude protein; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; ADL=Acid detergent lignin; CT=Condensed tannin

Table 4

Media	Parameters	b	c	pH	NH_3N (mg/l)	IVDMD(g/g)	IVGP (72h)
	Nitrogen sufficient	344.0	0.072	6.72	340.2	0.599	348.0
	Nitrogen deficient	323.0	0.072	6.67	229.6	0.606	325.0
	SED	*20.0*	*0.005*	*0.019*	*14.18*	*0.045*	*21.1*
	P.Value	*0.298*	*0.942*	*0.010*	*0.001*	*0.324*	*0.289*
Plants	*A. lebbeck*	354.4	0.079	6.74	375.6^{a}	0.669^{b}	365.6
	C.pentandra	311.3	0.065	6.72	232.6^{b}	0.563^{ab}	309.0
	K. senegalensis	366.6	0.066	6.68	248.5^{b}	0.380^{a}	358.3
	S. siamea	319.9	0.081	6.66	347.9^{a}	0.671^{b}	325.6
	F. gnaphalocarpa	309.5	0.063	6.69	212.5^{c}	0.615^{b}	313.8
	P. erinaceus	352.5	0.075	6.73	359.8^{a}	0.663^{b}	357.9
	A. indica	279.8	0.076	6.64	218.5^{c}	0.617^{b}	276.3
	G. arborea	383.6	0.071	6.70	283.8^{b}	0.480^{ab}	385.0
	SED	*40.1*	*0.005*	*0.038*	*14.18*	*0.094*	*42.30*
	P.Value	*0.279*	*0.600*	*0.165*	*<0.001*	*0.038*	*0.205*
Media× Plants	*SED*	*56.7*	*0.015*	*0.054*	*40.10*	*0.123*	*59.8*
	P.Value	*0.772*	*0.509*	*0.806*	*0.520*	*0.480*	*0.806*

Means with different superscripts in the same column are significantly different at P<0.05, IVDMD: In vitro dry matter digestibility, IVGP: In vitro gas production, b: asymptotic gas production, c: fractional rate of gas production

Results of the effect of additional nitrogen and browse plants on the *in vitro* gas production (IVGP), *in vitro* dry matter digestibility (IVDMD) and fermentation characteristics are presented in Table 4.2. The browse plants by media interaction did not differ (P>0.05) for all the parameters measured. The main effects of the browse plants and media alone were therefore presented and discussed. There was no difference between the two media on potential

degradability (P=0.298), rate of degradability (P=0.942), IVGP (P=0.289) and IVDMD (P=0.324). However, the nitrogen sufficient (NS) media had a higher (P=0.298) potential degradability (b) than the nitrogen deficient (ND). The ND had a slightly higher IVDMD than the NS even though the difference was not significant (P=0.324). Ammonia nitrogen (P<0.001) and pH (P=0.010) differed significantly between the two media with NS having the highest level of ammonia nitrogen and pH (Table 4.2).

The potential degradability (b) and rate of degradability (c) did not differ (P>0.05) among the browse plants. The highest (P=0.479) potential degradability was recorded in *G. arborea* (383.6 ml/gDM) and the least in *A. indica* (279.8 ml/gDM). The highest rate of degradability (c) was recorded in *S. siamea* ($0.081h^{-1}$) and the least in *F. gnaphalocarpa* ($0.063h^{-1}$).

There was a significant difference (P=0.038) in IVDMD among the browse plants. The highest IVDMD was recorded in *S. siamea* (0.671g/g) and the least in *K. senegalensis* (0.380 g/g). Ammonia nitrogen concentration differed (P<0.001) among the browse plants. *A. lebbeck* had the highest level of ammonia nitrogen (375.6 mg/l) after incubation at 72 h and the least was obtained in *F. gnaphalocarpa* (212.5 mg/l). The pH recorded at 72 h for all the browse plants was above six indicating that the browse plants did not negatively affect the pH of the media.

The cumulative *in vitro* gas production over 72 h for the browse plants is shown in Figure 4.1. After 6 h, there was a rapid increase in IVGP of the browse plants in both media.

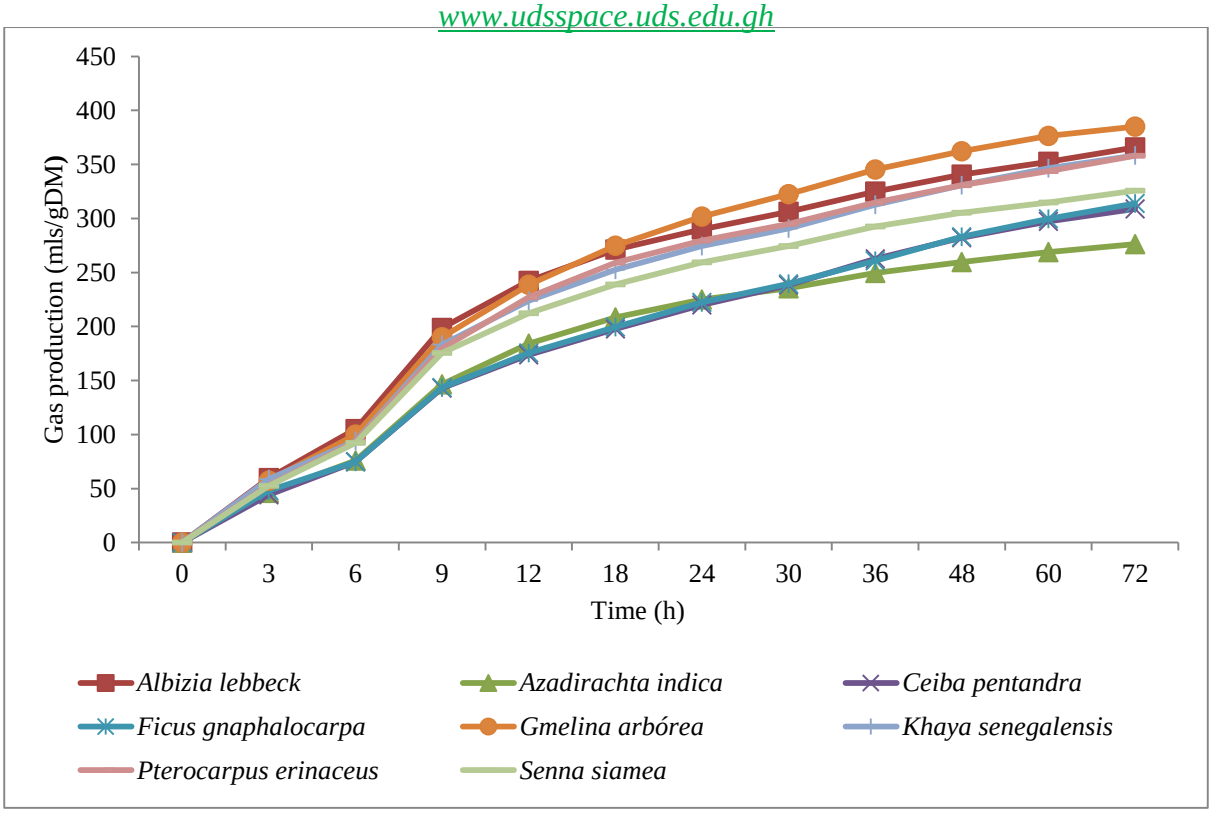

Figure 4. 1 Mean cumulative *in vitro* gas production of browse plants in both media

4.4 Discussion

The low dry matter content of the browse plants compared favourably with what was reported by Ouédraogo-Koné *et al.* (2008) for browse plants harvested during the periods of October to December. This period is often characterised by the sprouting of fresh leaves. The dry matter content values reported could enhance feed intake and digestion rate. The three plants (*F. gnaphalocarpa, C. pentandra* and *K. senegalensis*) with CT above 60 g/kg DM all had higher ADF than NDF. This compared favourably with the results of Getachew *et al.* (2000) who reported a higher ADF than NDF in some CT-plants and attributed this to the CT- protein complex and CT fibre complex formed which is insoluble in the acid detergent solution. The positive relationship found between CT and ADF confirms the fact that as CT in the diet increases, ADF also increases. This could imply that, higher CT in feed has the tendency to increase the ADF fraction of the feed and consequently reduce digestion rate. Acid detergent lignin (ADL) though high, was lower than what was reported by Getachew *et al*. (2000) for other browse plants. The high ADL might be due to the presence of cutin, silica or pectin since these substances according to Van Soest (1994) are insoluble in acid detergent solution. The CT concentration of *C. pentandra*, *F. gnaphalocarpa* and *K. senegalensis* were all above the maximum (30-40 g/kg DM) necessary to improve efficiency of nitrogen degradation and utilization (Barry *et al*., 1986). This was reflected in the extent of ammonia nitrogen concentration of these treatments after incubation. They were among the least in terms of ammonia nitrogen production. However, IVDMD of *C. pentandra* and *F. gnaphalocarpa* compared favourably with the control. The CT according to Bae *et al*. (1993), have a direct inhibiting effect on cellulolytic bacteria. It was therefore expected that this inhibitory effect would have been manifested in the IVDMD of *C. pentandra* and *F. gnaphalocarpa* compared with the control but that did not happen in this case.

The pH recorded for both media fell within the optimal pH range of 6.0-6.7 required to maintain normal microbial function and also microbial protein synthesis (Van Soest, 1994; Russel *et al*., 1992). Ammonia nitrogen concentration in the rumen is a balance between degradation of feed protein and uptake of ammonia for synthesis of microbial protein (Hariadi and Santoso, 2010). Even though the ammonia nitrogen recorded at 72 h was significantly lower in the ND than in the NS, it did not significantly influence the IVDMD of the browse plants in the media. The difference observed in the ammonia nitrogen content of the browse plants agrees with the findings of Hariadi and Santoso (2010), who reported a significant reduction in ammonia nitrogen when the CT in the diet was 54 g/kgDM. The ND media still provided the optimum ammonia nitrogen required for microbial growth, fibre digestion and DM degradation (Slatter and Slyter, 1974; Abdulrazak *et al*., 1997; Boniface *et al*., 1986). This could be an indication of an efficient uptake and utilization of the ammonia nitrogen by the microbes in the ND than in the NS. The finding also suggests that the browse plants provided the needed nitrogen or ammonia for microbial cell synthesis despite the presence of CT in most of the plants. This means the browse plants could be fed to ruminants as protein supplement and will not negatively affect rumen ammonia nitrogen and IVDMD. The pH of the media for each of the browse plants was above 6. This finding is consistent with the report of Hariadi and Santoso (2010) who found no difference in pH when tropical plants containing tannin was supplemented with *Pennisetum purpureum*.

The high ammonia nitrogen recorded in *A. lebbeck, S. siamea* and *P. erinaceus* is an indication of high dietary protein degradation in the media. These browse plants had a relatively lower CT. Lower ammonia nitrogen was obtained in the browse plants with a higher CT content. This findings support other findings that indicate that CT form complex with protein in the rumen and reduce the degradation of the protein (Getachew *et al*., 2000; Hariadi and Santoso, 2010). In the study of Getachew *et al*. (2000), the ammonia nitrogen concentration in the medium

increased when PEG was added to one of the CT treatments. Polyethylene glycol binds CT and inhibits its effect on rumen microbial degradation of dietary protein. The IVDMD for all the browse plant was above 500 g/kg despite the significantly lower ammonia nitrogen concentration of the high CT-plants. This finding suggests that the CT ensured a better synchronous releases and use of nitrogen for carbohydrate fermentation. Even though the CT in two of the browse (*F. gnaphalocarpa* and C. *pentandra*) plants was above the maximum (30-40 g/kg) recommended by Barry *et al*. (1986), it did not negatively affect the IVDMD, pH and ammonia nitrogen supply.

4.5 Conclusion and Recommendation

It can be concluded that excluding nitrogen from the IVGP media did not affect the IVDMD, pH, ammonia nitrogen, IVGP and rate of digestion. Two of the high CT-browse plants (*F. gnaphalocarpa* and *C. pentandra*) accounted for a lower ammonia nitrogen concentration in the study but did not affect IVDMD negatively.

A further study (experiment) is recommended to investigate the extent of rumen protein degradation when all the eight browse plants are incubated in the rumen using the *in sacco* technique. The *in vitro* gas composition of the high CT-browse plants should be investigated further for their methane production.

CHAPTER 5

5.0 EXPERIMENT 2: *IN SACCO* DRY MATTER AND NITROGEN DEGRADABILITY OF LEAVES OF EIGHT BROWSE PLANTS FROM THE SAVANNAH REGION OF GHANA

5.1.0 Introduction

After it was established from Experiment I that the browse plants though contained CT did not negatively affect proteolysis which was measured indirectly as the amount of ammonia nitrogen in the inoculants, experiment 2 was conducted to determine the effect of these browse plants on nitrogen degradability and supply of undegradable dietary protein.

Dietary protein could be broken down into quickly degradable (soluble protein), slowly degradable (insoluble but degradable) and UDP (AFRC, 1993). The quickly degradable and slowly degradable proteins are usually the types of protein, which are broken down by proteolytic microbes to supply the needed ammonia nitrogen to cellulolytic microbes for cell synthesis. The presence of CT could alter the proportions of these fractions hence the need for this study. High CT in the diet is expected to increase the undegradable dietary protein (UDP) because of the complex formed between dietary protein and CT in the rumen (Barry and Duncan, 1984; Messman *et al*., 1996). When proteolysis exceeds the relative rate of carbohydrate digestion, ammonia production can exceed the capacity for it to be assimilated by rumen microbes and the excess is liberated to the environment by the animal as nitrogenous waste (MacRae and Theodorou, 2003). This means the extent of proteolysis must match the amount fermentable carbohydrate present in the rumen. The presence of CT in the rumen as discussed earlier could minimize and efficiently manage the extent of protein degradation and ammonia nitrogen absorption in the rumen. This will minimize the excretion of nitrogenous waste into the environment. On the other hand when the dietary protein is overly protected, the quantum of microbial population is reduced due to low microbial protein synthesis and this could reduce the amount of microbial protein being passed on to the host animal (McDonald *et al*., 2011). Experiment 2 therefore sought to determine the extent of protein degradation in

the rumen and also estimate the amount of UDP that would pass out of the rumen to the abomasum and small intestine for absorption.

5.1.1 Objectives

- To determine the effect of browse plants on degradability parameters of nitrogen using the *in sacco* technique
- To estimate the effect of the browse plants on the supply of undegradable dietary protein

5.1.2 Hypothesis

Dietary protein degradation will not differ among browse plants when incubated using the *in sacco* technique.

5.2.0. Materials and methods

5.2.1 Rumen degradability

The *in sacco* rumen degradability of the leaves of 8 browse plants screened in Chapter 4 was determined according to the method of Sinclair *et al*. (1993) in a completely randomised design. The study was carried out the sheep farm of the Harper Adams University in the UK. Four wether sheep fitted with permanent rumen cannulae were group housed on straw under continuous lighting and with free access to water and a mineral lick. The sheep were fed a basal diet containing straw and concentrate (Ram master coarse mix) (60:40).

Fresh feed was offered twice daily at approximately 09:00 and 16:00 at a rate of 1.1 x maintenance (AFRC, 1993).

Approximately 5.0 g of each dried ground sample was weighed into synthetic fibre bags with a pore size of 42 *um*. Four bags containing the samples were connected to a stainless steel clip, which was attached to the cannula cap by a 30 cm length of nylon cord (Kenton, 2005) and inserted into the rumen of each sheep. The bags with the undigested feed was removed from the rumen at 4, 8, 16, 24, 48 and 72 h and a new set replaced following the complete exchange method of Paine *et al.* (1982).

Each test feed was incubated in each sheep in duplicate at all-time points in order to ensure sufficient residue for analysis. The retrieved bags were placed in a bucket of cold water to remove rumen debris, prior to being washed, on a cold cycle, for 40 min. in a domestic washing machine. In addition, zero h degradability of browse plants were determined by cold washing the nylon bags filled with samples in a domestic washing machine. The bags and contents were dried to a constant weight at 105ºC, and the contents bulked, within each sheep at each time point and analysed for dry matter and nitrogen. The empty bags were re-washed, dried and examined for tears prior to further use.

5.2.2 Calculations and statistical analysis

The DM and CP degradability was determined by curve fitting the data to the exponential equation of McDonald (1981) using sigma plot 10.0 (2006):

$P = A + B (1 – e^{-c(t-L)})$ =Equation 5.1

Where 'P' is the quantity degraded at time t; 'A' is the immediately soluble fraction; 'B' is the insoluble but potentially degradable fraction; and 'c' is the rate of degradation of fraction B at time t. L represents the lag time.

Effective degradability of dry matter and nitrogen were calculated using the equation of Ørskov and McDonald, (1979)

$ED=a+[(b*c)/(c+r)]$ =Equation 5.2

Where 'ED' is effective degradability, 'a' is the immediately soluble fraction, 'b' is the insoluble but degradable fraction, 'c' is the rate of degradability of 'b' and 'r' is the passage rate.

Data was analysed by one-way ANOVA using Genstat version 12.1 (Payne *et al.*, 2009). The variation among the treatment means was determined using Fisher's least significant difference test.

5.3 Results

The results of potential DM digestibility (a+b) and effective degradability (ED) at different outflow rates are shown in Table 5.1. The immediately soluble DM (a) differed (P=0.001) among the browse plants. The highest (a) was in *S. siamea* (416.5 g/kg) with the least in *F. gnaphalocarpa* (241 g/kg). There was however no significant difference among the browse plants for the slowly degradable fraction (b) (P=0.214), rate constant (c) (P=0.682), lag (P=0.687) and effective degradability at 0.08h^{-1}. The degradable fraction (b) was in the range of 302.3 to 570.2 g/kg with the highest recorded in *F. gnaphalocarpa* and the least in *P. erinaceus.*

Table 5. 1 Dry matter degradation parameters and effective degradability (ED) of browse plants (g/kg)

Browse plants	a	b	a + b	c	Lag	ED (0.05h^{-1})	ED (0.08h^{-1})
A. lebbeck	321.8^{c}	400.5	722.4	0.064	-0.71	528.1	491.8
C. pentandra	365.9^{d}	379.7	745.6	0.067	1.61	584.3	531.4
K. senegalensis	311.6^{c}	471.0	782.7	0.096	0.01	620.8	566.3
S. siamea	416.5^{e}	440.5	856.9	0.103	0.48	671.1	619.5
F. gnaphalocarpa	241.1^{a}	572.4	813.5	0.054	1.92	536.2	467.2
P. erinaceus	351.2^{d}	302.3	653.8	0.112	-0.23	556.1	524.1
A. indica	312.4^{c}	490.3	802.7	0.077	-1.71	615.3	563.0
G. arborea	260.5^{b}	570.7	831.2	0.092	0.24	634.2	568.0
SED	*7.82*	*97.4*	*96.6*	*0.03*	*1.81*	*73.5*	*71.1*
P.Value	*0.001*	*0.127*	*0.498*	*0.560*	*0.560*	*0.384*	*0.306*

Means in the same column with different superscripts differed significantly at P<0.05, a:immediately degradable fraction, b: slowly degradable fraction, c: rate of degradation, ED: effective degradability

The results of CP degradability are shown in Tables 5.2 and 5.3. There was significant difference (P<0.001) among the treatments for the immediately soluble protein (a), insoluble

but degradable protein (b), potential degradable (a+b) and effective degradability (ED) at all the outflow rates. The (a) fraction was highest in *A. indica* (504.6 g/kg) with the least obtained in *F. gnaphalocarpa* (360.1 g/kg). With the exception of *A. lebbeck* and *S. siamea*, all the other browse plants had a (b) fraction of below 200 g/kg. The highest potential degradability was observed in *A. lebbeck* (787.8 g/kg) with the least in *C. pentandra* (393.4 g/kg). The ED was highest in *A. lebbeck* at all outflow rates and the UDP recorded for the *A. lebbeck* also lowest at all outflow rates.

Table 5. 2 Crude protein degradation characteristics of browse plants (g/kg DM)

Browse plants	a	b	a + b	c	Lag	ED $0.05h^{-1}$	ED $0.08h^{-1}$
A. lebbeck	470.3^{d}	317.5^{d}	787.8^{d}	0.059	4.6	659.7^{c}	600.8^{c}
C. pentandra	377.5^{b}	15.9^{a}	393.4^{a}	0.079	17.9	400.9^{a}	389.1^{a}
K. senegalensis	469.3^{d}	129.0^{c}	598.2^{c}	0.066	9.7	568.3^{b}	531.8^{b}
S. siamea	418.3^{c}	253.0^{d}	671.2^{c}	0.054	6.0	560.9^{b}	515.4^{b}
F. gnaphalocarpa	360.1^{a}	65.4^{abc}	425.5^{a}	0.061	20.5	404.5^{a}	384.7^{a}
P. erinaceus	390.8^{b}	29.2^{ab}	420.0^{a}	0.077	14.8	425.3^{a}	410.1^{a}
A. indica	504.6^{e}	91.0^{bc}	595.6^{b}	0.064	16.3	591.7^{b}	547.8^{b}
G. arborea	457.7^{d}	86.6^{bc}	544.3^{b}	0.073	13.0	548.3^{b}	507.2^{b}
SED	*7.38*	*31.49*	*33.16*	*0.008*	*5.26*	*25.7*	*22.5*
P.Value	*0.001*	*0.001*	*0.001*	*0.074*	*0.059*	*<0.001*	*<0.001*

Means in the same column with different superscripts differed significantly at P<0.05, a: immediately degradable fraction, b: slowly degradable fraction, c: rate of degradation, ED: effective degradability

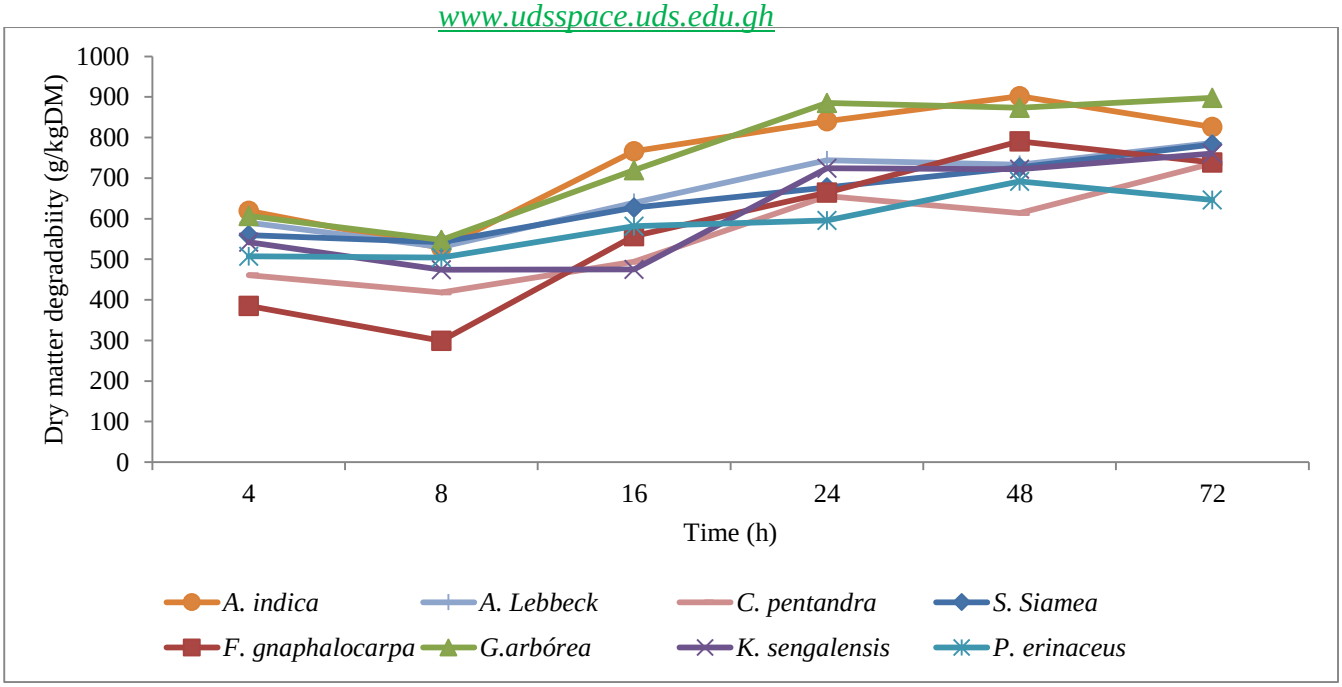

Figure 5. 1 *In sacco* DM degradation of browse plants

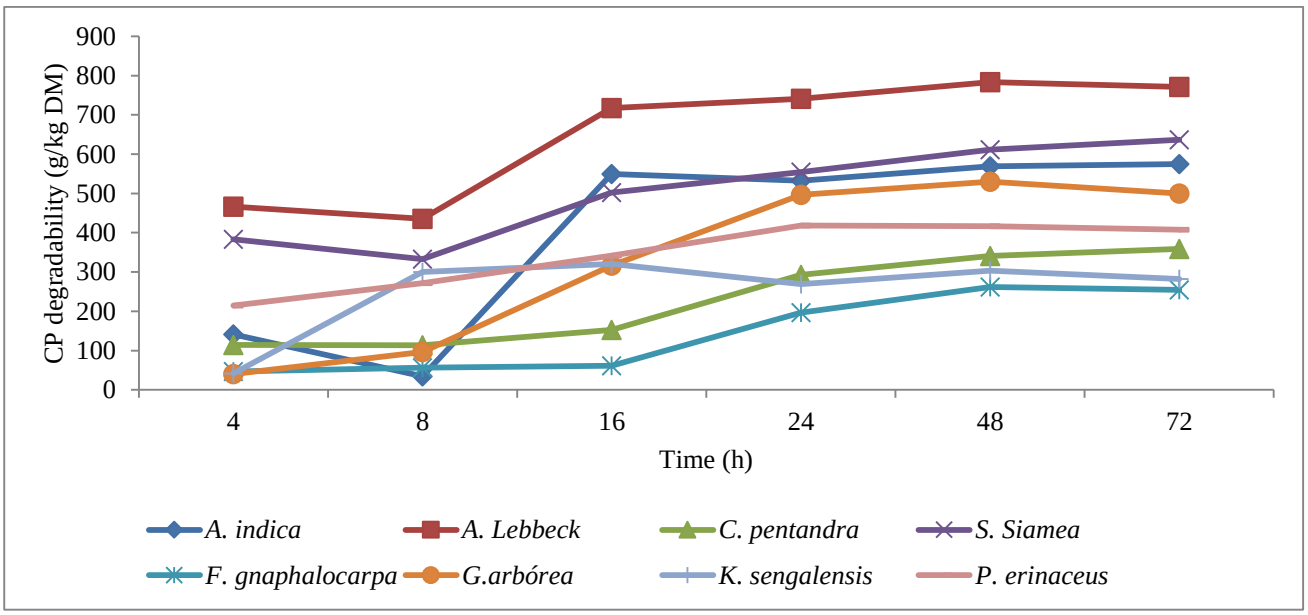

Figure 5. 2 *In sacco* CP degradation of browse plants

Figure 5.1 and 5.2 show the trend of degradability for DM and CP over time. In terms of DM degradability, there was a decline between 4 and 8 h after which they all began to rise (Figure 5.2). A similar trend was observed for the CP of *A. Lebbeck*, *S. siamea* and *A. Indica*. The other five browse plants did not follow that trend.

5.4 Discussion

Effective dry matter degradability for all the browse plants decreased with increase in outflow rates and agrees with the findings of Mupangwa *et al*. (1997) and Ikhimioya *et al*. (2005). The ED of all the browse plants was above 500 g/kg at flow rates of $0.05h^{-1}$ and decreased (<500 g/g) at flow rates of $0.08h^{-1}$. The relatively high loss of the soluble DM (>200 g/kg) observed in this study was higher than what was reported by Ngodigha and Oji (2009). These authors reported a soluble DM loss of less than 190 g/kg for some other tropical browse plants. It has been suggested that soluble carbohydrate in the diet has the potential of increasing microbial protein outflow rate and also increase the supply of amino acids to the small intestine (Obara *et al*., 1991; Lee *et al*., 2002).

The soluble DM loss reported for *A. lebbeck* and *S. siamea* in this study was similar to what was reported by Larbi *et al*. (1996) for the same browse plants harvested in the wet season but lower than what was reported for dry season harvest by the same authors. The difference might be due to the difference in the amount of soluble carbohydrate present in the plants used which could have been influenced by time of harvesting. In this study, the leaves were harvested between the periods of October and November during which matured leaves would have been shed leaving the immatured on the trees. Immatured leaves are high in soluble carbohydrates than matured ones and could result in higher losses of soluble DM in the rumen. The variety or location of the browse plants could also influence the extent of digestibility. The relatively higher ADF and ADL fraction reported for *A. lebbeck* and *S. siamea* in this study compared

with that of Larbi *et al*. (1996) could have also accounted for the lower slowly degradable fraction.

The lower lag time observed for the browse plants may have been due to the particle size of the incubated samples. Lopez (2005) suggested that milling size influences the surface area available for microbial attachment and degradation. The ED reported in this study at the different outflow rates suggest these plants have higher potential when used as feed for small ruminants.

The immediately soluble protein (a) was above 200 g/kg DM for all the browse plants. The (a) fraction reported in this study was higher than what was reported by Larbi *et al*. (1996) for *A. lebbeck* and *S. siamea*. *Azadirachta indica* had the highest loss of soluble protein even though it contained some amount of condensed tannin and had a lower CP compared with *A. lebbeck* and *S. siamea*. Perhaps this might be due to difference in plant species and could mean that higher protein may not necessarily result in higher solubility. The composition or molecular structure of the protein could have also influenced the high loss of soluble protein in *A. indica.* This suggests that, browse plants harvested in the early dry season (October to November) had appreciable levels of soluble protein (ammonia, nitrate, amino acids and peptides) in them and could therefore be used as protein supplement for ruminants.

The insoluble but degradable protein of the browse plants was below 150 g/kg DM for all the browse plants except for *A. lebbeck* and *S. siamea*. The low degradation of this fraction could be attributed to the binding effect of the CT present in the browse plants. This low degradation of protein (b) did not however, affect the potential DM digestibility negatively.

A positive correlation has been reported between DM degradability and protein solubility (Peter *et al*., 1971; Nishimuta *et al.*, 1973). Condensed tannin in these browse plants did not negatively affect the solubility of the protein of browse plants within the rumen. The lowest (b)

was reported in *C. pentandra* which is reported to have a CT fraction of (102.8g/kg). The high CT in *C. pentandra* affected the proportion of slowly degradable protein but not the immediately soluble protein. This means that, CT in the browse plants used did not affect protein solubility but significantly redcued protein degradation in the rumen. This explains why the potential DM digestibility was still high for the high CT-browse plants despite the low protein degradation. The CT concentrations were able to ensure an efficient degradation and utilization of protein in the rumen.

5.5 Conclusion and Recommendation

From the study, it can be concluded that *F. gnaphalocarpa* and *C. pentandra* reduced effective protein degradation. The CT levels did not negatively affect protein solubility in the rumen.

CHAPTER 6

6.0 EXPERIMENT 3: *IN VITRO* METHANE GAS PRODUCTION OF WHEAT STRAW SUPPLEMENTED WITH THREE DIFFERENT TROPICAL BROWSE PLANTS (*C. pentandra, S. siamea and G. arborea*)

6.1.0 Introduction

The initial screening of the browse plants in experiment 1 showed varying levels of CT concentration. Despite the high level of CT in *C. pentandra*, it did not have a negative effect on *in vitro* and *in sacco* protein degradation as found in Experiments 1 and 2. Could the high level of CT in *C. pentandra* be exploited as an avenue to reduce enteric methane gas production?

The level of CT was used as a basis to select three browse plants for Experiment 3.The *C. pentandra* was incubated alongside low CT-*S. siamea* and *G. arborea*.

The feeding of cereal based crop residue like rice straw is a very common practice in Ghana especially in the dry season (Osafo *et al*., 1993; Karbo *et al*., 1997; Ansah *et al*., 2014). It serves as a readily available source of energy for ruminants during this period. Cereal based straws are often characterised by high fibre and low nitrogen content which has a negative impact on intake and digestibility (Nurfeta, 2010; Ansah *et al*., 2011). Methane production is a natural and inevitable outcome of rumen fermentation of feedstuff. Many factors influence ruminant methane production, including level of intake, type and quality of feeds, time of forage harvesting, energy consumption, animal size, growth rate, level of production, genetics, and environmental temperature (Johnson and Johnson, 1995; Hegarty, 2004; Shibata and Terada, 2010).

Brask *et al*. (2013) demonstrated that the choice of forage species and most critically the time of harvesting influences the amount of methane produced during rumen fermentation. Forage maturity has the tendency to increase the level of recalcitrant fiber such as lignin. The feeding

and fermentation of highly lignified forage leads to a longer mean rumen retention time, which could lead to a higher methane and carbon dioxide emission. This has been demonstrated by Hammond *et al*. (2014) who found that shorter mean retention time of feed resulted in less methane production in sheep. O'Hara *et al*. (2003) has also indicated that rumen fermentation of poor quality forage produces more methane gas than fermentation of better quality forages. Mills *et al.* (2003) suggested that increased feed intake results in lower methane gas production. The contribution of methane to global warming has been estimated to be about 24% and emissions from rumen fermentation is the largest single source of methane (Hansen *et al*., 2000; Goodland and Anhang, 2009). According to IPCC (1996), Dairy cattle from Africa and the Middle East produce 36 kg/head of methane annually.

Condensed tannins (CT) present in most browse plants have received great attention for their ability to reduce methane production (Carulla *et al.,* 2005). Ives *et al*. (2015) found that CT form *Acacia molissima* is able to mitigate *in vitro* methane production. Lower methane gas production was rcorded when some browse plants from Ghana were incubated for 24 h and this was attributed to the presence of plant secondary metabolites such as CT and saponnins in the browse plants (Meale *et al*., 2012).

The present study sought to investigate the extent to which methane gas is reduced when wheat straw is replaced with different CT-browse plants at 40% inclusion.

6.1.1 Objective

To determine the methane gas production from wheat straw when 40% of it is replaced with varying levels of CT-browse plants

6.1.2 Hypothesis

Overall methane gas production of wheat straw will not differ when supplemented with CT-browse plants.

6.2.0 Materials and methods

6.2.1 Experimental feed formulation and design

Leaves of three browse plants (*C. pentandra*, *S. siamea* and *G. arborea*) were used as partial replacement for low nitrogen containing wheat straw in a proportion of 40% browse plant to 60% straw. The treatments were *C. pentandra*+straw (CPen+S), *S. siamea*+Straw (SS+S) and *G. arborea*+Straw (GA+S). Sole straw (S) without any supplementation was used as a control. The DM and chemical composition of the experimental diet was determined following the methods described in sections 3.2, 3.4, 3.5, 3.6 and 3.7. The CT content of the diet was not determined in this experiment but rather adopted from Table 4.1 for the various browse plants used.

The 4×4 Latin square design was used for this study with each experiment (run) lasting a total of fourteen days. The first eight days were used as adjustment period with actual data collection commencing on the 9th to the 14th day.

6.2.2 Method for continuous culture

A total of four double layered fermenter vessels measuring 1.18 L were used. The top cover of each vessel had 25 mm (internal diameter) port which will allow for feeding and two 12 mm (internal diameter), which was used for continuous infusion of artificial saliva from a peristaltic pump and total gas collection. The effluent was collected through an outlet on the vessels with an internal diameter of 20 mm. A 1m rubber tube was connected to the outlet into a container positioned in ice to maintain a temperature of not more than 3^{o}C below the vessels. Each vessel was connected to a water heater jacket (Lauda ecoline RE 16, Germany) that supplied warm water within the layer of the vessel to ensure the temperature in the vessel is kept at 39^{o}C.

Artificial saliva was prepared according to the method of McDougall (1984) and modified to contain 13.82 mg/day (ammonium sulphate) $(NH_4)_2SO_4$ to supply 2.93 mg/day of N from days

1-8. The buffer consisted of 9.8 g/l $NaHCO_3$, 2.77 g/l Na_2HPO_4 0.47 g/l NaCl, 0.57 g/l KCl, 0.04 g/l $CaCl_2$ and 0.06 g/l $MgCl_2$.

Rumen fluid was collected from four different cannulated intact rams. The animals had an average weight of 95 kg and were fed on straw and concentrate (Wynnstay ram master coarse mix, UK) at 1.1x maintenance requirement (AFRC, 1993). The rams were group housed with straw as bedding and under constant light supply. Water was offered *ad libitum*.

Rumen fluid was collected 3-4 h post feeding through a suction process. The fluid was collected into a pre-warmed vacuum flask and quickly sent to the laboratory. The fluid from each of the four animals were pooled and strained using a four layer cheese cloth placed in a funnel over a 5 L conical flask. Carbon dioxide was flushed through the rumen fluid prior to addition to the vessels.

On the day of inoculation, each vessel was filled with approximately 510 ml buffer. The saliva was diluted with tap water in the proportion of 60:40 respectively (Rufener *et al.,* 1963) to get the buffer. The water heater jacket was filled with water with the temperature set to 39^{o}C to keep the vessel warm. Carbon dioxide was flushed through the vessel to ensure it is anaerobic. When the rumen fluid was ready, 670 ml was poured into each vessel and 15 g (dry) of each sample was added immediately.

The same amount of feed (treatments) was fed into the vessels after every 12h (09.00h and 21.00h). The buffer was fed continually at an infusion rate of 59 ml/h throughout the experiment.

The content of each vessel was stirred continuously with the help of a rotor inserted through the head plate to the bottom of the vessel at approximately 20 turns per min.

6.2.3 Sampling procedure

1. The pH of the vessel content was taken each day at 12h. On day 8,9,10 and 11, the pH was taken at 9.00, 11.00, 13.00, 15.00, 17.00 and 19.00h.

2. On days 9 and 10, 10 ml of effluent was taken at the same time as in 1 above and stored at -20^{0}C for ammonia nitrogen analysis.
3. Total gas was collected into gas-tight bags from the 9th day for methane, hydrogen and carbon dioxide analysis. After 24 h, the total volume of gas in each bag was measured using a gas meter (Dry test gas meter, DC-1, Japan). After the volume has been recorded, the gas bags were connected to a biogas analyser (PGD3 Status Scientific Controls LTD, UK) to determine the composition of methane, hydrogen and carbon dioxide gas produced from each treatment.

6.2.4 Statistical analysis

The data was analysed using ANOVA in Latin square design from Genstat 12.1 and the variation among the treatment means was determined using Fisher's least significant difference test. The vessels and period were used as rows and columns, respectively.

6.3 Results

The results of the chemical composition of the four diets are shown in Table 6.1. The CP determined was relatively lower for sole straw than the other 3 diets which were all higher than 90 g/kg DM. The NDF was relatively higher for SS (759.9 g/kg DM) compared to the other 3 diets and the same trend was observed for ADF. The CT was analysed for all the treatments except the sole straw and the results show that *C. pentandra* had the highest CT (102.8g/kg DM).

Table 6. 1 Chemical composition of experimental diets (g/kg DM

	DM	OM	CP	Ash	NDF	ADF	CT
straw	924.5±1.6	914.9±1.9	35.9±1.1	78.7±1.6	759.9±3.9	576.2±10.7	ND
straw	922.2±0.7	922.8.0±0.1	95.4±0.9	71.2±0.1	385.8±20.5	385.3±16.2	1.8±0.19
straw	920.7±2.2	914.2±1.2	96.9±1.0	79.0±1.3	324.6±9.8	389.1±4.4	3.7±0.21
+straw	913.8±12.3	909.7±5.2	92.3±0.3	77.8±5.9	357.3±14.6	416.3±4.8	102.8±1.70

CT-condensed tannin, ND: Not determined, The CT reported in this table represents CT in only the browse plants and not browse plants and straw, SS+straw: Senna siamea, GA+staw:Gmelina arborea, CPen+straw: Ceiba pentandra.

Table 6. 2 *In vitro* gas production, gas composition and ammonia nitrogen of the treatments

Diet	Total gas (ml/g DM)	Methane (ml/g DM)	Co_2 (ml/g DM)	H_2 (ml/g DM)	NH_3N (mg/L)
Sole straw	37.33	5.38 (0)	16.19 (0)	0.06	75.00
SS+straw	30.33	4.34 (19)	15.48 (4.4)	0.08	77.85
GA+straw	33.17	4.37 (19)	15.86 (2.0)	0.06	74.72
CPen+straw	28.83	3.34 (38)	11.74 (27.5)	0.05	75.47
SED	*6.86*	*1.46*	*4.59*	*0.02*	*1.91*
P.Value	*0.64*	*0.61*	*0.75*	*0.59*	*0.41*

Figures in parenthesis are a percentage of reduction of methane and carbon dioxide between the treatments and the control (Sole straw).

The results of the IVGP and gas composition are presented in Table 6.2. The gas composition did not differ for methane (P>0.05) and carbon dioxide (P>0.05). Also there was no significant difference in total gas and H_2. Total gas production was in the order of Sole straw>GA+straw>SS+straw>CPen+straw. Methane production followed a similar order as the total gas production. This indicates that though a higher gas production was obtained in sole straw, the methane contribution was quite high. The percentage carbon dioxide decreased for all treatments.

Ammonia nitrogen produced over 10 h did not differ significantly (P=0.952) among the treatments. The results of the trend of ammonia nitrogen production and pH over 10 h periods are shown in Figure 6.1 and 6. 2. The trend of ammonia nitrogen production varied among the treatments with a rise in ammonia nitrogen level after 2 h for all treatments except sole straw which showed a marginal decline from the initial level. The maximum level of ammonia nitrogen production for the treatments was attained at different times. *Senna siamen* attained maximum ammonia nitrogen after 4 to 5 h of feeding, sole straw was after 2 to 3 h and *G. arborea* was after 2 h and there after it declined. *Ceiba pentandra* which had the lowest ammonia nitrogen attained its peak after 6 h of feeding.

A varied trend was also observed for pH among the treatments. However, at all the time points within the 10 h period, the pH was above 6.

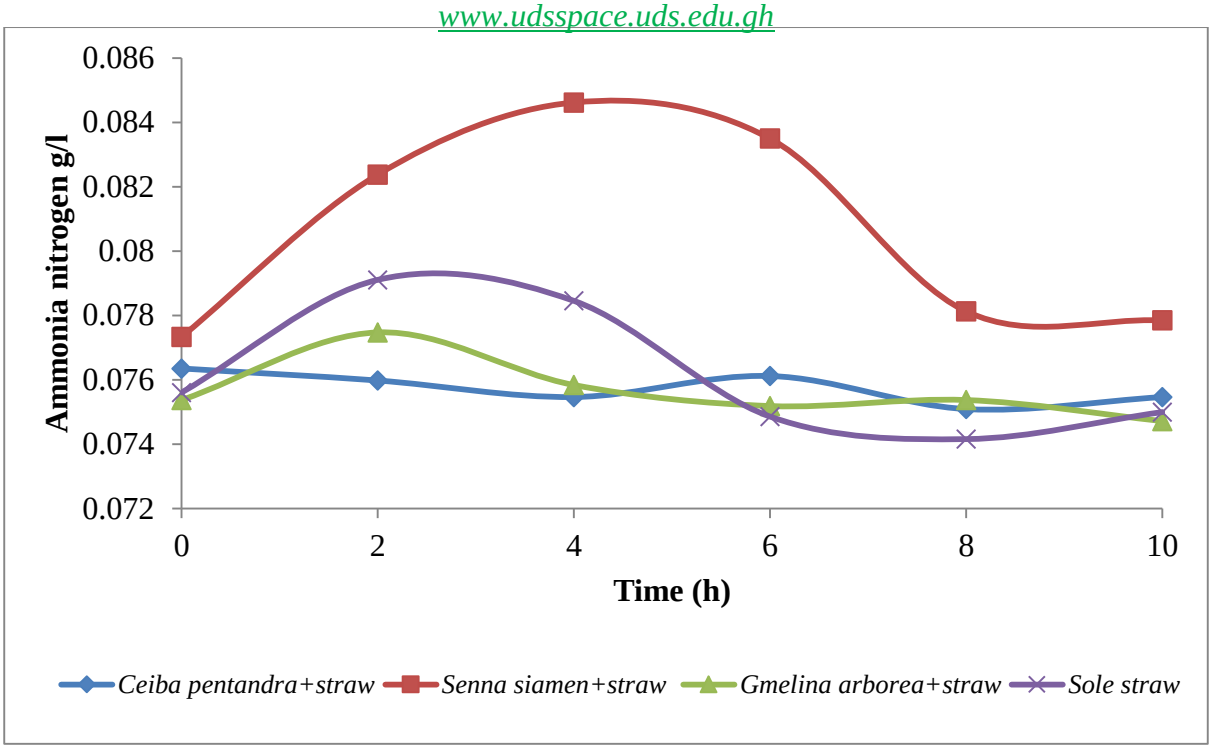

Figure 6. 1 Effect of browse plants on ammonia nitrogen concentrations

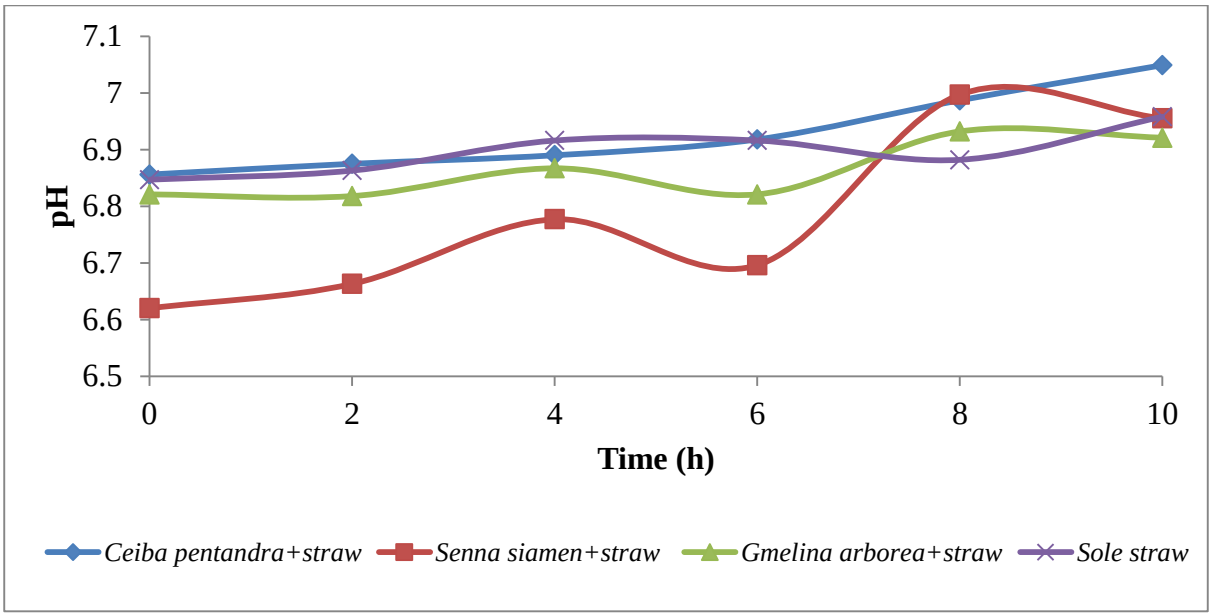

Figure 6. 2 Effects of browse plants on pH

6.4 Discussion

The CP content of the sole straw was lower than the minimum 60-80 g/kg DM required for sustenance of microbial growth (Van Soest, 1982). The replacement of 40% wheat straw with browse plant decreased the NDF fraction for all the supplemented diet and increased the ADF fraction for GA+straw and CP+straw. The higher ADF than NDF fraction for the GA+straw and CPen+straw is in line with the report of Getachew *et al.* (2000) who reported a higher ADF than NDF in some CT plants and attributed this to the CT-protein complex and CT-fibre complex formed which was insoluble in the acid detergent solution.

In vitro gas production (IVGP) gives an indication of the extent to which the carbohydrate of the substrate is being fermented by anaerobic microbes from the rumen (Lopez *et al*., 1998, France *et al*., 2000). The IVGP from CPen+straw was about 23% lower than the gas produced from sole straw and the methane gas was also 38% lower. The result shows that the combination of *C. pentandra* and straw reduced both digestibility and methane production.

Ceiba pentandra contains a CT of 102.8 g/kg (Table 6.1) and this relatively high CT may have accounted for the reduction in IVGP and methane gas production (Ives *et al*., 2015). The reduction in methane might be attributed to the inhibition of the two main bacteria, *R. flavefasciencs* and *R. albus* in the rumen responsible for production of hydrogen, which serves as a primary raw material for methanogenic bacteria to produce methane (Van Gylswyk, 1995). The results also suggest that the CT did not only inhibit the hydrogen producing bacteria but also the non-hydrogen bacteria (*Fibrobacter succinogenes*) hence the decline in the IVGP.

This finding is in line with the reports of Bae *et al*. (1993) who observed a decline in cellulose digestion when *Fibrobacter succinogenes* digesting cellulose was exposed to CT of 0.4 mg/ml. The replacement of wheat straw with 40% *C. Pentandra*, *G. arborea* and *S. siamea* resulted in 38%, 19% and 19% reduction of methane production respectively. This result agrees with that

of Hariadi and Santoso (2010) who observed a decrease in IVGP and methane production when *Pennisetum purpureum* was supplemented with *Acacia mangium*, which contained total tannin of 54 g/kg.

Condensed tannin has been reported to directly inhibit microbial attachment to the cell wall of plants in the rumen there by reducing the digestibility of the browse plants (Bae *et al*., 1993). Condensed tannins also reduced methane production through a reduction in fibre digestion, which decreases the amount of H_2 production and also by directly inhibiting the growth of methanogens (Tavendale *et al*., 2005). The results suggest that more energy was lost to methane production in sole straw compared to *CPen* + straw.

Condensed tannin form complexes with protein at higher pH (6-7) and reduces protein degradation in the rumen. Lower protein degradation is reflected in the concentration of ammonium nitrogen within the rumen or *in vitro* cultures. In this study, the level of ammonia nitrogen did not differ significantly among the treatments and was above 70 mg/l after 10 h. However, higher ammonia nitrogen concentration was recorded at 4 h when 40% straw was replaced by sole straw (figure 6.1). There was a direct inhibition of the microbes and microbial enzymes hence the reduction in overall gas production. The ammonia nitrogen for the *CPen* +straw did not change much over the 10 h period whilst that of GA+staw and sole straw all increased after 2 h and gradually declined afterwards.

6.5 Conclusion and Recommendation

It can be concluded that replacing straw with 40% browse plants reduced methane production by 19-39% with *C. pentandra* resulting in the highest reduction. Ammonia nitrogen and pH were not negatively affected with the inclusion of the browse plants.

CHAPTER 7

7.0 EXPERIMENT 4: EFFECTS OF PARTIALLY REPLACING RICE STRAW WITH TANNIFEROUS BROWSE PLANTS ON THE NITROGEN METABOLISM AND FIBRE DIGESTIBILITY IN DJALLONKÉ SHEEP

7.1.0 Introduction

Experiment 4 was a follow-up to the previous experiments and it sought to measure the extent of CP, NDF and ADF metabolism when these browse plants are fed to Djallonké sheep. Nutrient metabolism gives an indication of how much of the nutrient is being retained in the host animal. It was therefore imperative that this study was conducted to provide further information on the metabolism of nutrients in these browse plants.

Evaluation of trees to ascertain their feeding value has been on going for some time now (Kang *et al*., 1990; Duguma *et al*., 1994; Larbi *et al*., 1998; Ouédraogo-Koné *et al*., 2008). The leaves of trees have been shown to contain high amounts of CP and low fiber particularly in the dry season when the quality of most forages have dwindled (Le Houérou, 1980; Pellew, 1980). Leaves of tropical browse plants may also contain varying levels of CT, which could have an impact on rumen function, and the degradability of dietary protein (Makkar and Becker, 1998; Shayo and Udén, 1999; Aganga and Tshwenyane, 2003). Condensed tannins could suppress feed intake, rumen protein degradability, DM, CP and NDF digestibility (Waghorn *et al*., 1998; Getachew *et al*., 2000; Waghorn, 2008; Hariadi and Santoso, 2010). The negative effects of tannins (low feed intake, low digestibility, toxicity) have been reported to occur when ruminants consume forage with a high level of condensed tannins concentration (>50-55g/kg DM) (Min *et al*., 2003). The use of CT-browse plants could play an important role in reducing the amount ammonia excreted via urine into the environment through the reduction of rumen protein degradation. This study was a follow up to the *in sacco* experiment (Experiment 3) to ascertain the digestibility of DM, N and NDF of CT-browse plants when used as a partial replacement for rice straw.

7.1.1 Objective

Determine the DM, N, NDF and ADF digestibility of tanniferous browse plants

7.1.2 Hypothesis

Nitrogen, NDF and ADF digestibility of tanniferous browse plants will not differ when fed to Djallonké sheep as a replacement for rice straw

7.2.0 Material and methods

7.2.1 Study area

The study was carried out at the Nyankpala Campus of the University for Development Studies, Tamale, Ghana. It is located on latitude 9° 25′ 41″ N and longitude 0° 58′ 42″ W at an altitude of 183 m above sea level. The area is in the Guinea Savannah Zone and characterized by a unimodal rainfall pattern. Rains begin in April, rising to a peak in August –September and ending in October or November. Rainfall averages 1060 mm per annum. Temperatures range from as low as 15˚C in January when the weather is under the influence of the North Easterly (harmattan) winds and as high as 42˚C around the end of the dry season in March.

7.2.2 Experimental animal and housing

Eight intact rams which were eight months old with an average initial weight of 13.88 ±1.56 kg (mean±SD) were randomly assigned to a total mixed ration made of browse plants (*A. lebbeck, S. siamea, C. pentandra and G. arborea* leaves) and rice straw. The animals were housed in a metabolism cages (46×91×40 cm) made of metal.

7.2.3 Source and processing of browse plants

Albizia lebbeck, S. siamea, C. pentandra and *G. arborea* leaves were harvested from trees in and around the Nyankpala campus in January to February 2014 and shade-dried for about three days. The leaves were then separated from the branches and stored in sacks.

7.2.4 Experimental design

Eight animals were randomly assigned to four treatments comprising T0 (58% straw+40% *A. lebbeck*), T1 (58% straw+40% *C. pentandra*), T2 (58% straw+40% *G. arborea*), and T3 (58% straw+40% *S. siamea*). *Albizia lebbeck* was used as the control diet since it had no CT present in it (Table 4.1). The cross over design was used in two different periods. The animals were allowed ten days adjustment to the feed and three days to the cages.

Data collection lasted for 5 days. After the first period, the animals were rested for three days and another three days for adjustment to the new treatment diet. The animals were fed on the treatment diet during each adjustment phase.

The Experiment was conducted within the months of January and February, 2014.

7.2.5 Feed formulation, feeding and Watering

The feed was prepared by chopping the rice straw into pieces (approx. 8-10 cm long). The dried leaves of each browse plant were weighed and mixed with rice straw. About 2% (1.5% premix and 0.5% salt) of the total weight of each diet was computed and used to measure the quantity of salt and vitamin to be included in the diet. The salt and vitamin were dissolved in 1L tap water and sprinkled onto the diet. The feed was thoroughly mixed by hand and packed into sacks and their weights taken.

Feed was offered *ad-libitum* in a plastic container. Before feeding each day, a sample of the feed was collected into plastic bags and stored. Water was served at 10:00 am in the morning and 16:00 pm in the afternoon in a plastic container.

Table 7. 1 Composition of ingredients (g) in experimental diet, chemical composition of experimental diet and chemical composition of browse plants

Item	T0 *A. lebbeck*	T1 *C. pentandra*	T2 *G. arborea*	T3 *S, siamea*
Browse	400	400	400	400
Untreated rice straw	580	580	580	580
Premix	150	150	150	150
Salt	050	050	050	050
Chemical composition of experimental diet (g/kg DM)				
DM	914.2±2.4	953.0±0.1	914.4±31	934.2±0.3
CP	111.4±1.3	70.3±0.3	80.5±0.8	90.1±1.2
NDF	575.7±1.2	610.3±14.6	536.1±5.8	538.6±16.9
ADF	393.6±2.3	473.2±16.6	402.1±2.1	370.2±4.8
Chemical composition of the browse plants (g/kg DM)				
CP	229.2±3.6	126.2±2.7	151.2±0.5	175.8±2.0
NDF	296.1±2.2	271.3±1.2	248.5±1.0	262.7±2.2
ADF	193.7±4.5	291.7±0.4	163.4±3.9	256.9±0.6
CT	0.00	102.8±1.7	3.7±0.2	1.8±0.2

T0AL: A. lebbeck; T1CP: C. pentandra; T2 GA: G. arborea; T3 SS: S. siamea, Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg

7.2.6 Sample collection and calculation

Daily feed samples were taken and stored in plastics bags for DM, N, and NDF analysis.

About 100 g of the stored feed was sampled and oven dried at 105^{o}C for 4 h. The dry weight of the feed was recorded and used for calculating the total DM intake and subsequently average daily DM intake. Dry matter feed intake was computed by subtracting the sum of the feed refused and left over feed for each treatment from the total feed formulated.

Nutrient digestibility (N, NDF and ADF) was calculated after converting to DM basis using the following formulae

$$\text{Nutrient digested}=\frac{Nutrient\ in\ feed-nutrient\ in\ faeces}{Nutrient\ in\ feed} \quad \text{=Equation 7.2}$$

In the case of nitrogen and crude protein metabolism, the formulae was modified to

$$\text{Nutrient metabolised}=\frac{CP\ in\ feed-(CP\ in\ faeces+CP\ in\ urine)}{CP\ in\ feed} \quad \text{=Equation 7.3}$$

The animals were fitted with faecal collection bags to collect the total faeces voided. The bags were removed at 07:00 daily and the faecal matter weighed. After weighing, 10% subsample of each faecal matter was frozen for DM, N, and NDF analysis.

Urine was collected daily at 07:00. The urine collected each day was weighed and 10% subsample stored in plastic containers for N analysis. Approximately 30 ml of 6N HCl was added daily to the urine collection containers to prevent volatilization of ammonia (Dabiri and Thonney, 2004).

At the end of the experiment, a subsample of the pooled daily faecal samples for each sheep at each period was taken for determination of N, NDF and ADF.

Pooled urine samples from each sheep was also subsampled at each period and analysed for N using the micro Kjeldahl following the AOAC (2000) procedure described below.

Approximately 1 g of each subsample (Dry Faecal matter or feed) was weighed into glass cylinder (Pyrex glass measuring cylinder). In the case of the urine, 2 ml was weighed. About10 ml of sulphuric acid and 10 g of catalyst ($CuSO_4$-Na_2SO_4 mixture) was added to each sample. The samples were then digested in a Kjeldahl Electrothermal Apparatus for 45 min and 15-20 min for dry feed or faecal matter and urine respectively.

The samples were allowed to cool after which 50 ml of distilled water was added and mixed to form a light green solution. Approximately 20 ml of 40% NaOH was added to 10 ml of digested sample for neutralization and distilllation against 10 ml of 4% Boric acid using Kjeldahl Nitrogen Distillation Apparatus (Kjeltech, Tecator model, Denmark). The distillate was titrated against 0.1N Hydrochloric acid and the titre value recorded. The total nitrogen of the sample was computed using the formula below:

For feed or faecal samples

$$\text{Total Nitrogen } (N_T)\ (g/kg) = \frac{[(\text{Titre value (ml)}]\text{x } 14.01 \text{ x } 0.1\text{N HCl}]}{0.2\text{w/v of sample x } 10} = \text{Equation 7.1}$$

For urine samples

$$\text{Total Nitrogen } (N_T)\ (ml/L) = \frac{[(\text{Titre value (ml)}]\text{x } 14.01 \text{ x } 0.1\text{N HCl}]}{0.4\text{w/v of sample x } 10} = \text{Equation 7.2}$$

Nitrogen balance was calculated as the difference between N consumed and the sum of faecal N plus urinary N.

$$\text{Nitrogen balance} = Nitrogen\ intake - (Nitrogen\ faeces + Nitrogen\ in\ urine) \quad = \text{Equation 7.3}$$

7.2.7 Blood urea nitrogen analysis (BUN)

Blood samples (10 ml) were taken at end of each period from the jugular vein into a set of clean test tubes. This was centrifuged (Selecta-centro 8, Spain) at a speed of 5000 rpm to extract the serum. The serum was then transferred to another set of clean test tubes for BUN analysis. The blood urea nitrogen was analysed following the method of Amoako *et al*. (2014) using the BT 3000 Random Access Chemistry analyzer.

3.2.8 Neutral detergent fiber and Acid detergent fiber analysis

The aNDFom (NDF assayed with a heat stable amylase and expressed exclusive of residual ash) and ADFom (ADF assayed and expressed exclusive of residual ash) in the experimental diet and the faecal matter were analysed using Ankom^{200} fiber analyser (Ankom Technology, Macedon, NewYork) following the method of Goering and Van Soest (1970). The analysis was carried out at the Forage Evaluation Unit of the Agricultural Sub-sector Improvement Project (AgSSIP) Laboratory at the Nyankpala campus of UDS.

For the aNDFom analysis, approximately 0.45-0.55 g of milled (1mm) and dry sample was weighed into filter bags (F57) and placed on a bag suspender. About 2 L of NDF solution was prepared and poured into the bucket of the analyser. Approximately 4 ml of heat stable α-amylase E.C.3.2.1.1 from *Bacillus subtilis* (Sigma, Gillingham, UK) and 20 g sodium sulphite were added to the NDF solution. The bag suspender together with the samples was lowered into the solution and a weight placed on the suspender to keep the samples stable during agitation. The bucket was then sealed with metal lid and fastened tightly.

The heat and agitator buttons were activated for digestion at a temperature of 100^{o}C. After 75 min, the heat was turned off and the solution drained through an exhaust into a waste collection gallon. About 2 L of hot (70-80^{o}C) water with 4 ml of heat stable α-amylase was poured into the digestion bucket to wash the samples. The washing process was repeated for 3 times after which the samples were removed and placed in a beaker filled with acetone. After 5 min, the samples were removed from the acetone and allowed to dry at room temperature.

After about 20 min, the samples were placed in an oven set to a temperature of 105^{o}C for about 4 h.

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

aNDFom (g/kg) = $\frac{(W3-(W1*C1))}{W2} * 1000$ =Equation 7.4

Where: W1= Bag tare weight

W2= Sample weight

W3= Dried weight of bag with fiber after extraction

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

The ADFom analysis was done by weighing approximately 0.45-0.55 g of dry milled sample into fiber bags and placed in a bag suspender. About 2 L of ADF solution (60 g of Cetyl trimethylammonium bromide (CTAB) dissolved in 2 L of 1N sulperhic acid solution) was poured into the digestion bucket and the samples together with suspender lowered into the solution. A weight was placed on the suspender to keep the samples stable during agitation. The bucket was then sealed with metal lid and fastened tightly. The heat and agitator buttons were activated for digestion at a temperature of 100^{o}C. After 60 min, the heat was turned off and the solution drained through an exhaust into a waste collection gallon. About 2 L of hot (70-80^{o}C) water was poured into the digestion bucket to wash the samples. The washing process was repeated for 3 times after which the samples were removed and placed in a beaker filled with acetone. After 5 min, the samples were removed from the acetone and allowed to dry at room temperature. After about 20 min, the samples were placed in an oven set to a temperature of 105^{o}C for about 4 h.

The weight after drying was recorded and used to compute the ADFom content using the following equation:

ADFom (g/kg) = $\frac{(W3-(W1*C1))}{W2} * 1000$ =Equation 7.5

Where: W1= Bag tare weight

W2= Sample weight

W3= Dried weight of bag with fiber after extraction

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

7.2.8 Data analysis

The effect of the treatment was analysed using the analysis of variance as randomised block. Period was used as a block and analysed in Genstat 12.1 (Payne *et al.*, 2009). The means were separated using Fisher's unprotected least significant difference test.

7.3 Results

The results of the chemical composition of the four browse plants used and experimental diets are shown in Tables 7.1. The CP was in the range of 151.2 to 229.2 g/kg DM for *G. arborea* and *A. lebbeck* respectively. The aNDFom was in the range of 248.5 to 296.1 g/kg DM with the highest recorded in *A. lebbeck*. The ADFom was lowest in *G. arborea* whilst the highest CT was obtained in *C. pentandra*. The DM of the experimental diets ranged between 914 and 953 g/kg DM for *A. lebbeck* and *C. pentandra* respectively. Replacing 40% of the rice straw resulted in the CP ranging between 70.3 and 111.4 g/kg DM.

The effects of partially replacing rice straw with browse plants on DM intake and nutrient digestibility are shown in Table 7.2. Dry matter intake (DMI) differed significantly (P=0.001) among the experimental diets with *S. siamea* having the least (255.1g/h/d). The difference between the control (*A. lebbeck*) and *C. pentandra* and *G. arborea* did not differ (P>0.05). The trend was similar for crude protein intake (CPI) and nitrogen intake. The DM digestibility coefficient did not differ (P>0.05) between the control and the other treatments. The DM digestibility was in the range of 0.57 to 0.69 for *S. siamea* and *A. lebbeck* respectively. The CP digestibility differed (P<0.001) between the control and the other treatments. The lowest CP digestibility was obtained in *S. siamea*. The aNDFom digestibility and ADFom digestibility differed (P<0.05) among the treatments with the lowest digestibility reported *in G. arborea*. There was no difference between the *A. lebbeck* (control) and the high CT-supplement (*C. pentandra*) for both aNDFom and ADFom digestibility. The faecal nitrogen (Faecal N g/h/d) differed (P=0.041) among the treatments with the least recorded in *S. siamea*. Faecal N was highest in *C. pentandra* and *G. arborea* but was not different from the control. The lowest (P>0.05) Urine nitrogen (Urine N) was obtained in animals on *C. pentandra* diet. The nitrogen balance (N balance) differed significantly (P<0.001) and was in the range of 3.16 to 7.84 g/h/d.

Blood urea nitrogen (BUN) did not differ (P>0.05) among the experimental diets and was highest in *A. lebbeck* (12.45 mmol/L) but least in *G. arborea* (7.11 mmol/L).

Table 7. 2 Effect of browse plants on intake and nutrient digestibility inyoung Djallonké rams

Parameter	*A. lebbeck*	*C. pentandra*	*G. arborea*	*S. siamea*	*SED*	*P.Value*
DMI (g/h/d)	468.3^{b}	456.1^{b}	509.1^{b}	255.1^{a}	*24.91*	*0.001*
CPI (g/h/d)	52.39^{d}	32.17^{b}	40.99^{c}	23.12^{a}	*3.35*	*<0.001*
N intake (g/h/d)	8.38^{d} d	5.15^{b} b	6.56^{c}	3.70^{a}	*0.537*	*<0.001*
DM digested	0.69	0.64	0.59	0.57	*0.06*	*0.141*
aNDFom digested	0.68^{b}	0.65^{ab}	0.55^{a}	0.73^{b}	*0.05*	*0.02*
ADFom digested	0.62^{b}	0.56^{ab}	0.46^{a}	0.67^{b}	*0.06*	*0.03*
CP digested	0.94^{c}	0.91^{b}	0.91^{b}	0.84^{a}	*0.01*	*<0.001*
Faecal N (g/h/d)	0.25^{ab}	0.30^{b}	0.37^{b}	0.17^{a}	*0.06*	*0.041*
Urine N (g/h/d)	0.29	0.15	0.22	0.36	*0.08*	*0.099*
N balance	7.84^{d}	4.69^{b}	5.97^{c}	3.16^{a}	*0.53*	*<0.001*
Blood Urea N (mmol/L)	12.45	8.26	7.11	8.85	*2.09*	*0.100*

DMI: Dry matter intake (gram per herd per day); CPI: Crude protein intake; N: Nitrogen; aNDF: Neutral detergent fiber not corrected for ash; ADFom: Acid detergent fiber not corrected for ash.

7.4 Discussion

The CP for *C. pentandra* and *G. arborea* were within the minimum range of 60-80 g/kg DM required to sustain microbial growth whiles that of *A. lebbeck* and *S. siamea* were above the minimum (Van Soest, 1982). The replacement of rice straw with 40% browse plants increased the CP content of the diet beyond what was reported previously (33 g/kg DM) by Avornyo *et al.* (2007) to over 70 g/kg DM in this present study. This is due to the superiority of the browse plants over rice straw in terms of CP.

The DM intake did not reflect the CP content of the experimental diets as was suggested by Konlan *et al*. (2012). The difference might be due to the presence of CT and also the type of supplement used. *Senna siamea* was second in terms of CP among the experimental diets but recorded the least DM intake and agrees with the findings of Dabiri and Thornney (2004) who reported that the level of protein did not affect DM intake. The DM intake for the experimental diet was higher than the 215 g/d reported for sole rice straw fed to Djallonké sheep under the intensive system, indicating that the 40% replacement of rice straw with browse plants increased DM intake (Ansah *et al*., 2012). The high CT content of *C. pentandra* did not significantly affect DMI. This agrees with findings of other researchers who found no difference in DMI when varying levels of tannin containing browse plants were fed to growing sheep (Barry and Duncan 1984; Hervás *et al*., 2003; Animut *et al*., 2008).

The decrease in DM intake of tannin-rich feeds is generally attributed to the astringent taste which reduces palatability. The reduction in palatability is caused by the reaction of the salivary muco-protein with tannins or through a direct reaction with taste receptors, provoking the astringent sensation (McLeod, 1974).

The CP and N intake (g/h/d) were least in *S. siamea* and could be attributed to the low DM intake reported for that diet (Table 7.4). The lack of significant difference in DM digestibility

between *A. lebbeck* and the CT-browse plants could be due to the 40% inclusion levels used in this study. Several other researchers did not find significant difference in DM digestibility when CT-browse plants were fed to ruminants (Barry and Duncan 1984; Hervás *et al*., 2003; Animut *et al*., 2008).

The significantly higher CP digestibility of *A. lebbeck* relative to the other treatment confirms the reports by other researchers who found a decline in protein digestibility when varying levels of tannin containing diets were fed to ruminants (Rittner and Reed, 1992; Yu *et al*., 1996; Makkar *et al*., 1997). However it is interesting to note that the treatment with the highest concentration of CT had a higher CP digestibility than those with low levels. This might be due to differences in the composition of the protein. The molecular weight of CT has been suggested to be the reason for variation in the effect of CT on nutrient digestibility (Butler and Rogler, 1992). This is a reflection of a high intestinal protein digestion.

This difference could also mean that the source of the CT affects CP digestibility more than the concentration.

The trend in CP digestibility in this present study might be due to differences in molecular weight of the CT and also differences in intestinal digestion of CP in the diet. The lack of difference in aNDFom and ADFom digestibility between the *A. lebbeck* and *C. pentandra* suggest that there was no effect of the CT on the digestibility of NDF and ADF. The finding agrees with several other authors who found no difference in NDF and ADF digestibility when different levels of CT were fed to sheep or incubated using *in vitro* techniques (Waghorn *et al*., 1987; Nuñez-Hernandez *et al*., 1991; Carrula, 1994; Mohammad *et al.*, 2015).

The DM, CP and NDF digestibility reported in this study was higher than the 38.4%, 46.9% and 38.4%, respectively, reported by Jeon *et al*. (2013) for rice straw in an *in situ* study. This indicates that the replacement of rice straw with browse plants enhanced the digestibility of the

rice straw. The improved digestibility of DM and NDF might be attributed to the high percentage of CP degradability reported in experiment 2.

The effect of CT in the *C. pentandra* was manifested in the low urine nitrogen reported. The complex between CT-protein reduces the extent of protein degradability thereby reducing the amount of nitrogen excreted in the urine. The BUN was again highest in the control indicating a high level of proteolysis occurring when *A. lebbeck* was fed. Despite the high CP degradation in the control diet, the DM digestibility did not differ, suggesting a possible asynchronous use of nitrogen and carbohydrate fermentation in the *A. lebbeck* diet in contrast to the CT-browse plants. It also suggests that cellulolytic bacteria were not affected by the presence of CT in the treatment diets.

It has been suggested that the CT-protein complex interferes with the ability of cellulolytic bacteria especially *Fibrobacter succinogenes* to form an attachment with plant cell wall thereby reducing digestion rate since the presence of plant protein in the rumen for microbial breakdown aids in microbial attachment to plant cell in particular (Gong and Forsberg, 1989; Mitsumori and Minato, 1993).

7.5 Conclusion and Recommendation

Replacing 40% of rice straw with browse plants enhanced the CP content. The CT-browse plants as supplements did not negatively affect the DM, NDF and ADF digestibility. It is recommended that varying inclusion levels of the browse plants be fed to ascertain their effects on nutrient digestion. The high N metabolism recorded in this study for all the treatments requires further investigation.

CHAPTER 8

8.0 EXPERIMENT 5: EFFECT OF BROWSE PLANTS ON GROWTH, BLOOD METABOLITES AND CARCASS CHARACTERISTICS OF DJALLONKÉ LAMBS

8.1.0 Introduction

Experiments 1, 2 and 4 have shown great potentials for these browse plants in terms of nutrient content and digestibility. Relatively high levels of CT were also reported in some of the browse plants in the previous experiments. *Ceiba pentandra* despite its high level of CT compared favourably with the low CT-browse plants in terms of IVDMD, ammonia nitrogen, and effective degradability of CP in Experiments 1 and 2. It also showed great potential in reducing enteric methane emissions from ruminants in Experiment 3.

In vitro and *in sacco* techniques of feed evaluation are unable to provide information related to growth, blood metabolites and carcass characteristics. It was therefore important, to further conduct an *in vivo* experiment to measure the effect of the browse plants on the growth characteristics of Djallonké sheep.

The low quality of forages and non-leguminous based crop residues like rice straw especially in the dry season calls for appropriate feed supplement. Cost and availability of conventional feed supplement can be a major setback to its use among most smallholder livestock producers in developing nations. Tree planting is being promoted in most African countries as a means of restoring degraded lands and also for timber. The integration of plantation with livestock production could maximise returns to the farmers. Over 5000 trees and shrubs have been listed as being suitable for feeding livestock (Le Houérou, 1980; Brewbaker, 1986; Okoli *et al.*, 2003).

Serum biochemical indices have been used to determine the level of heart attack, liver damage and to evaluate protein quality and amino acid requirements in animals (Harper *et al*., 1979). Levels of urea nitogen, glucose, albumin, cholesterol and other metabolites have been used to

measure the functinability of the liver (Kahn, 2010). Very low levels are usually indicative of hepatic diseases whilst high levels indicate a breakdown in liver functioning.

Dietary CT could have some toxic effects in ruminants and may be manifested in irreversible damage to liver cells, hardening of the liver, and loss of liver function, which may lead to jaundice, swelling, and the accumulation of fluids in the stomach and other physiological malfunctions (Wong, 2014). They have also been found to control internal parasites and condition of bloat in ruminants (Waghorn, 1990; Min and Hart, 2003).

Feeding system and nutritional status can affect growth and carcass characteristics (Atti and Abdouli, 2001; Priolo *et al.*, 2001). Fermentation of organic matter in the rumen produces VFA's and also supplies microbial protein. Different diets yield different VFA's and these VFA's act different in on lipogenic nutrients and glucogenic nutrients. Acetic and butyric acids produce more lipogenic nutrients whilst propionic acid supplies more of glucogenic nutrients. Acetic acid has been found to supply 70-80% of acetyl units for lipogensis in subcutaneous adipocytes but only 10-25% in intramuscular adipocytes whilst a greater proportion of intramuscular adipocytes are derived from glucogenisis from propionic acids (Smith and Crouse, 1984). This indicates that type of diet can significantly influence carcass composition. About 40-70% of NDF digested in the rumen produces 86% lipogenic nutrients and 14% glucogenic nutrients (Tamminga. 2004).

The intake of RUP has been found to favour the production of less fatty carcasses (Fattet *et al.*, 1984; Vipond *et al.*, 1989). The presence of CT in browse plants has been found to increase the supply of RUP due to its ability to form complex with protein.

There is very little information on the role of these browse plants in enhancing growth, blood metabolites and carcass characteristics of sheep.

The purpose of this experiment was therefore to determine the effect of CT-browse plants on the growth, blood metabolites and carcass characteristics.

8.1.1 Objective

To determine the effect of feeding browse plants as supplement to semi intensively kept Djallonké sheep grazing on native pastures

8.1.2 Hypothesis

Liveweight gain of Djallonké lambs grazed on natural pasture will not differ with browse plant supplementation

8.2.0 Materials and methods

8.2.1 Study area

The study area is the same as the description provided in section 7.1.1 above.

8.2.2 Source of experimental animals and quarantine measures

A total of 20 male Djallonké lambs (8-12 months old) were purchased from the livestock market at Katingdaa in the Tolon District. The animals were fitted with ear tags for easy identification.

8.2.3 Experimental design

The animals (12.8±1.7kg) were assigned to 5 treatments with 4 replicates each in completely randomised design. The treatments were shade dried *A. lebbeck, G. arborea, S. siamea* and *C. pentandra*. Four (4) animals were used as control and so did not receive any browse plant supplement. The duration of the experiment was 56 days and was between the months of June and July, 2014.

8.2.4 Housing and feeding during experiment

The animals were housed individually in wooden cages with concrete floors. The cages were fitted with wooden feeding troughs and plastic watering bowls. The animals were fed the supplementary diet *ad libitum* at 07:00 h and were released for grazing on natural pastures at 10:00 h. Water was also supplied *ad libitum* and was replaced at the same time of feeding. A 500 g of iodated salt was dissolved in 10 L of tap water and sprinkled on the each browse plant before feeding to improve taste. The weight of the feed was recorded before feeding commenced.

8.2.5 Data collection

8.2.5.1 Supplementary feed intake

The orts from each treatment were put together and weighed daily. At the end of the experiment (56 days), the total weight of the ort for each treatment was added to the weight of what was left in each feed bag. This was subtracted from the total amount of feed prepared for each treatment group to get the supplementary feed intake. Daily samples of the supplementary feed were taken and stored in a refrigerator until the experiment was over. After the experiment, the sampled feed was bulked for each treatment and sub-sample taken for drying in the oven. Duplicates of each subsampled treatment were weighed and oven dried at 60^{o}C for 48 h. The DM percentage was computed and used to estimate the total DM intake of the supplement for each treatment group.

8.2.5.2 Live weight gain

The weekly weight of the animals were taken using a hanging scale (Camry hanging scale, ISO9001:2008, China). The initial liveweight of each animal was subtracted from the final weight to get the livewitgh gain. This was then didved by the duration of the experiment (56 days) to get the daily gain.

8.2.5.3 Blood collection and processing

Blood was taken at about 07:00 h in the morning before feeding. Approximately 10 ml of blood was taken with the help of a syringe from the jugular vein and transferred into clean test tubes without an anti-coagulant for the blood metabolite analysis.

The blood was centrifuged at a speed of 5000 rpm and the serum separated. The serum was then transferred into another set of clean test tubes and stored at 4^{o}C until analysis was conducted.

8.2.5.4 Serum biochemistry

The Random Access, Fully-Automated, "Walk Away" Clinical Chemistry Analyzer (Flexor XL, Vital Scientific, Netherlands) was used for the serum biochemistry analysis. Before the start of each test, the machine was calibrated using a multi-calibrator (ELICAL 2 Multiparametric Calibrator, CALI-0550). Two separate controls were also run on the machine and these were the ELITROL I Normal Multiparametric Control (CONT-0060) and ELITROL II Abnormal Multiparametric Control (CONT-016).

8.2.5.5 Albumin (ALB)

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.

8.2.5.6 Total protein (TP)

Estimation of total protein in this study was 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 at 540 nm is directly proportional to the TP concentration.

8.2.5.7 Globulins (GLO)

Globulin was a calculated parameter estimated by subtracting albumin from total protein.

8.2.5.8 Glucose (GLU)

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

8.2.5.9 Carcass characteristics

Three animals were randomly selected from each treatment group for carcass analysis at the Meat unit of the University for Development Studies, Tamale. Feed was withheld from the animals 24 h before slaughter and the slaughter weights were taken on the day of slaughter. All animals were slaughtered and cut into parts on the same day. Immediately after slaughter, the bodies were skinned; head and testis (external organs) were removed and weighed. The carcasses were eviscerated and the internal organs were removed and separately weighed. The internal organs measured included, full and empty digestive tracts, liver, kidney, lungs and spleen. Hot carcass weight was taken after all the internal organs, head, skin and testis have been removed. The carcass was then cut into neck, shoulder, hind thigh, chuck, loin and back.

8.2.6 Data analysis

The effects of the treatments on all parameters were analysed using one way ANOVA from Genstat 12.1 (Payne *et al*., 2009). The initial weight of the animals was used as a covariate. The means were separated using Fisher's least significant difference.

8.3 Results

The results presented are based on the supplements only since data could not be taken on the forage intake while the animals were grazing on native pastures. The results of the effects of browse plants on the growth and serum metabolites are shown in Table 8.1.

Table 8. 1 Effects of browse plants on DM intake, growth and serum profile

Variables	Control	*G. arborea*	*C. pentandra*	*S. siamea*	*A. lebbeck*	*SED*	*P.Value*
DM intake (g/h/d)	-	87.70	35.28	4.15	48.71	-	-
Initial live weight (kg)	12.00	12.75	13.63	12.62	13.12	*1.35*	*0.807*
Final live weight (kg)	14.07^{a}	15.34^{ab}	15.04^{ab}	15.24^{ab}	16.14^{b}	*0.59*	*0.048*
Daily live weight gain (g)	12.71^{a}	33.11^{b}	41.95^{bc}	38.43^{b}	55.59^{c}	*6.47*	*<.001*
Serum parameters							
Alb (g/l)	20.43	20.15	21.77	22.05	21.38	*1.25*	*0.49*
TP (g/l)	63.80	64.60	63.60	66.30	65.80	*3.81*	*0.93*
Glb (g/l)	43.4	44.4	41.8	44.2	44.4	*3.70*	*0.94*
Glucose (mmol/l)	0.23	0.34	0.36	0.38	0.35	*0.11*	*0.67*
BUN (mmol/l)	8.55	8.43	7.02	6.80	7.52	*1.27*	*0.55*

Alb=Albumin, TP=Total protein, Glb=Globulin, BUN=Serum urea nitrogen

Supplementary feed intake per animal per day was in the range of 4.15 g to 87.70 g for *S. siamea* and *G. arborea* respectively. The gain/DM intake between the browse plants was highest (P<0.001) in *S. siamea* (9.21) with the control having the least. Three of the browse plant supplemented animals showed no difference in final liveweight when compared to the control and also *A. lebbeck*. The daily liveweight gain differed significantly between the browse plant supplemented animals and the control. Animal supplemented with *A. lebbeck*

differed (P<0.001) from *S. siamea* and *G. arborea* but did not differ from C. *pentandra.* There was no difference between C. *pentandra*, *S. siamea* and *G. arborea* supplemented animals in terms of daily liveweight gain.

The serum metabolites did not differ significantly among the treatments. The total protein (TP) was in the range of 63.60-65.80 g/l for the control and *A. lebbeck*. The blood urea nitrogen (BUN) was in the range of 6.80-8.55 mmol/l with the highest reported in the control and the least in *S. siamea*.

The results of the carcass and non-carcass components for all the treatments are presented in Table 8.2 and 8.3 respectively. There was no difference (P>0.05) among the treatments for all carcass components.

Table 8. 2 Effects of browse plants on carcass components

Carcass characteristics	Control	*G. Arborea*	*C. Pentandra*	*S. siamea*	*A. Lebbeck*	*SED*	*P.Value*
Slaughter weight (kg)	12.92	13.45	13.12	14.17	14.34	*0.774*	*0.372*
Carcass weight (kg)	5.23	5.63	5.37	5.41	6.72	*1.080*	*0.656*
Dressing %	40.21	42.27	41.41	39.61	43.93	*2.249*	*0.384*
Neck (kg)	0.55	0.50	0.47	0.48	0.51	*0.087*	*0.917*
Hind Thigh (kg)	0.72	0.85	0.76	0.75	0.91	*0.1395*	*0.631*
Chuck (kg)	0.25	0.29	0.28	0.24	0.28	*0.042*	*0.724*
Back (kg)	0.30	0.28	0.29	0.32	0.34	*0.052*	*0.795*
Shoulder (kg)	0.24	0.29	0.27	0.28	0.31	*0.058*	*0.794*

Means with different superscript show significant difference (P<0.05)

The non-carcass components differed for testis and spleen. In contrast to the other treatments, *A. lebbeck* had the highest (P<0.05) testicular weight (0.47 kg). The spleen was highest

(P<0.05) in *C. pentandra* and least in *A. Lebbeck*. The slaughter weight followed the increasing order of (Control<*C. pentandra*<*G. arborea*<*S. siamea*<*A. lebbeck*). The lowest (P>0.05) dressing percentage was obtained in *S. siamea* (39.93%) with the highest obtained in *A. lebbeck* (43.47%).

Table 8. 3 Effects of browse plants on non-carcass components

Carcass characteristics	Control	*G. Arborea*	*C. Pentandra*	*S. siamea*	*A. Lebbeck*	*SED*	*P.Value*
Skin (kg)	1.15	0.97	1.05	1.15	1.40	*0.188*	*0.282*
Head (kg)	1.11	1.09	1.09	1.12	1.29	*0.095*	*0.267*
Testis (kg)	0.36^{b}	0.33^{b}	0.35^{b}	0.36^{b}	0.47^{a}	*0.039*	*0.043*
Liver (g)	257.0	290.1	256.0	283.1	289.8	*27.88*	*0.568*
Lung (g)	164.8	184.1	188.4	158.8	186.3	*21.25*	*0.538*
Spleen (g)	35.0^{a}	30.9^{b}	48.9^{a}	20.9^{c}	21.5	*3.639*	*0.007*
Heart (g)	74.1	99.5	80.3	74.6	79.0	*14.46*	*0.434*
Kidney (g)	53.0	55.1	56.3	52.1	54.7	*6.29*	*0.962*
Full Digestive tract (kg)	3.44	3.35	3.73	3.60	4.09	*0.737*	*0.865*
Empty Digestive tract (kg)	0.99	1.32	1.29	1.21	1.61	*0.201*	*0.116*

Means with different superscript in the same row show significant difference (P<0.05)

8.4 Discussion

Supplementing Djallonké sheep grazing native pasture with browse plants showed varied responses. The four browse plants had a CP level above the minimum 60-80 g/kg DM below which appetite and voluntary feed intake are depressed (Minson 1990). The lowest feed intake reported in *S. siamea* despite the appreciable level of CP (175.87 g/kg DM) may have been due to the bitter taste of the leaves of *S. siamea*.

The finding in this present study does not agree with Konlan *et al*. (2012) who attributed the high DM intake in Djallonké sheep to the high CP in the concentrate. Paterson and Clinch (1993) attributed the low acceptability of *S. siamea* as fodder to the problem of toxicity.

The CT content in *S. siamea* was lower than what was reported in *C. pentandra* but the DM intake did not reflect this. It has been reported elsewhere that high tannin levels have a negative effect on feed intake by causing astringency (Chang *et al*., 1998; Al-Mamary *et al*., 2001). The daily maximum DM intake reported in Djallonké sheep fed the browse plants and grazing native pastures was 4.15 g/h, 35.28 g/h, 48.71 g/h and 87.70 g/h for *S. siamea, C. pentandra, A. lebbeck* and *G. arborea* respectively.

All the animals gained weight during the period of study. However, the control animals consistently had the lowest final liveweight and final liveweight gain compared with the animals on supplementary diet. This is an indicication that the liveweight of Djallonké sheep grazing native pastures could be significantly improved with the supplementation of browse plants. The improved liveweight may be due to the high CP levels of browse plants. In terms of the final weight, there was no difference between the control and the other supplements except *A. lebbeck*. The superiority of *A. lebbeck* and *C. pentandra* in terms of liveweight gain over the control suggests a better utilization of the nutrients from these supplements. In the case of *C. pentandra*, the high CT did not negatively affect liveweight gain.

The total protein (TP) reported in the present study (Table 8.1) was slightly higher than the normal TP (63 g/l) reported for West African Dwarf sheep by Konlan *et al*. (2012) and this could be due to a slightly higher digestibility and absorption of the dietary protein by the animals on supplementation. The blood urea nitrogen (BUN) has been described as a reflection of the extent of ammonia nitrogen production in the rumen (Lewis, 1957). The lack of significant difference among the treatments and the control suggest that the *C. pentandra* supplement did not affect proteolysis in the rumen despite the relatively high CT. The high BUN level reported in the control as against the low weight gain could suggest that there was some level of body protein catabolism in the control group (Leibholz, 1970).

From Table 8.2, the supplemented animals had higher (P>0.05) slaughter and carcass weight (P>0.05) than the control. The carcass weight and slaughter weights were lower than what was reported by Fasae *et al.* (2014) who fed crop residues as supplement to semi intensively managed Djallonke sheep. The difference might be due to the supplements used and the forage grazed. The dressing percentage recorded in this study was lower than the 46.38% reported by Fasae *et al*. (2014) for the same breed of sheep. The lowest dressing percentage was reported in *S. siamea* even though it was the second highest in terms of slaughter weight.

Undegradable rumen protein has been found to influence testicular weight of rams (Fernández *et al*., 2004). This does not support the results showing *A. lebbeck* having a heavier testicular weight than the CT-browse plants. When RDP does not match the amount fermentable carbohydrate, extra energy is needed to excrete the excess ammonia nitrogen from the rumen. This situation puts the animal in a state of NEB and could results in less energy being available for tissue development. This means that the RDP in *A.lebbeck* matched well with the amount of fermentable carbohydrate hence the heavier testicular weight and feet. The testis and spleen

were however; lower than the 0.89 kg and 0.33 kg respectively reported by Fasae *et al*., (2014) for semi intensively kept Djallonké sheep.

8.5 Conclusion and recommendation

Supplementing Djallonké sheep grazing on native pasture with browse plants had a positive effect on final liveweight and daily liveweight gain with *A. lebbeck* having the highest. *S. siamea* had the highest gain per DM supplement intake. There was no negative effect of supplement on the blood metabolites, carcass and non-carcass characteristics. Any of these browse plants could be used, as supplement, however, the use of *S. siamea* should be investigated further for better treatments methods to address the problem of DM intake.

CHAPTER 9

9.0.0 General discussion

The nutrient composition and condensed tannin levels were similar to what has been reported by previous researchers in other West African countries (Ahn *et al.,* 1989; Larbi *et al.,* 1998; Ouédraogo-Koné *et al.,* 2008). The slight variations in CP, NDF and ADF levels may be attributed to the season of harvesting, geographical location and agronomic conditions. The high CP reported for *A. lebbeck* and *S. Siamea* confirms the superiotiy of leguminous plants over non-leguminous in terms of nitrogen content. The CP content of the entire browse plants studied was however, above the 60-80 g/kg DM required to support the growth of rumen microbes (Van Soest, 1982). The NDF and ADF reported for the browse plants were all below what has been reported for most rice straw (NDF; 774 g/kg and ADF; 546 g/kg) (Gunun *et al*., 2013). The presence of CT in the browse plants is expected to impact positively on CP degradability and enteric methane emmision in the rumen.

In the *in vitro* gas, study showed a much higher ammonia nitrogen concentration in the NS media than in the ND media eventhough the IVDMD did not differ. This is an indication that the browse plants could supply adequate amounts of degradable protein despite the high CT presence in some of them. There were significant variations in the ammonia nitrogen concentration of the browse plants with non-CT and low CT-plants having the highest. This however did not reflect in the IVDMD. This meant that using the low CT-brwose plants as a sole diet could lead to excesive protein degradation in the rumen and could possibly result in the animals getting into a condition of negative energy balance (NEB). When ammonia nitrogen in the rumen is not balanced with fermentable CHO, animasl tend to use energy that would have been used for growth in removing excess ammonia from the rumen (Tamminga, 2006). There could aslo be the problem of water pollution because of high excretion of urea via the urine of animasl consuming the browse plants as sole diet. The pH reported in the *in*

vitro gas study was within the normal rumen pH suggesting that the CT in the browse plants did not negatively affect the rumen pH.

In the *in sacco* study, the quickly degradable protein fractions were higher than the slowly degradable fraction in the high CT-plants. The quickly degradable fraction was not affected by the presence of CT unlike the slowly degradable fractions. This confirms the effect of CT on CP degradation in the rumen. It is possible that the ammonia nitrogen reported in the *in vitro* gas study was largely as a result of the presence of an appreciable levels of non protein nitrogen in the high CT-plants which may not have been affected by the CT. The effective protein degradability was higher in the non-CT and low CT-plants compared to that of the high CT-plants indicating a possible high supply of UDP from the high CT-plants to the small intestine for digestion and absrobtion.

The browse plants showed some potentials to reduce GHG emission and this can be attributed to the presence of CT in the plants. The high CT-browse plant redcued methane production by 38% compared to the sole wheat straw. The other low CT-browse plants also accounted for some 19% reduction in methane production. While methane production was decreasing for the CT-browse plants, IVGP also reduced suggesting a decline in DM digestibility. Several other researchers who reported decrease in methane production also reported a decline in overall DM digestibility (Hariadi and Santoso, 2010; Ives *et al*., 2015.). The reduction in both methane and DM digestibility may suggest that the CT in the browse plants did not only inhibit methanegenes but also cellulolytic bacteria. Hydrogen gas producing cellulolytic bacteria have been reported to be negatively affected by CT in the rumen (Bae *et al.*, 1993).

Ammonia nitrogen was not negatively affected when 40% of wheat straw was replaced with browse plants. Boniface *et al*., (1986) found that about 45 mg/l of ammonia nitrogen in the rumen was enough to ensure maximum CHO fermentation. The ammonia nitrogen reported in

this study was above the 45 mg/l suggesting that CP degradation was not affected by the presence of CT. The decline in potential DM digestibility in this study could therefore not be attributed to a lack of ammonia nitrogen fro microbial cell synthesis but a rather an inhabition of cellulolytic microbes in the rumen.The pH of the media and substrate was not affected by the presence of CT. The reduction in methane could improve energy retention in animals when used as supplement since methane production represents a loss of gross energy.

There was an improvement in dietary CP when the browse plants were used as a partial replacement for rice straw in the nutrient digestibility study and this is due to the superiority of the browse plants over rice straw interms of CP content. The DMI was not affected by the differences in CP concentration of the diets since *S. siamea*, which had a higher CP compared with *C. pentandra* and *G. arborea* had the least DMI. The low intake might be due to the taste of *S. siamea* which is known to be bitter when consumed. The high DMI reported in animals on *G. arborea* may have been caused by differences in the physical appearance of the leaves of the browse plants. Physical appearance such as structure of the leaves, thickness, texture, smell and size has been suggested as possible sources of variation in DM intake (Van, 2006). *Gmelina arborea* leaf is bigger in size than the other browse plants and this could influence bite size. The N intake was again lower in *S. siamea* and this can be attributed to the low DMI reported. The CP digestibility was above 80 % in all the browse plants fed. The low faecal N and urine N in the animals fed on the browse plant diets may be due to the low CP intake. The high CP digestibility in the high CT-browse plants may suggest that the CT-protein complex, which may have been formed in the rumen, was reversed in the small intenstine due to pH differences to supply UDP. In the *in sacco* study, protein degradability and effective degradability were very low in the high CT-plants

The lack of significant difference in DM digestibility in the metabolism study between the *A. lebbeck* and the other browse plants despite the presence of CT could be due to the 40% inclusion levels used in this study. Several other researchers did not find significant difference in DM digestibility when CT-browse plants were fed to ruminants (Barry and Duncan 1984; Hervás *et al*., 2003; Animut *et al*., 2008).

The significantly higher CP digestibility of *A. lebbeck* relative to the other treatments confirms the reports by other researchers who found a decline in protein digestibility when varying levels of tannin diets were fed to ruminants (Yu *et al*., 1996; Makkar *et al*., 1997; Theodoridou *et al*., 2012). However, it is interesting to note that the treatment with the highest concentration of CT had a higher CP digestibility than those with low levels. The trend in CP digestibility in this present study might be due to difference in molecular weight of the CT and perhaps differences in browse plants. This could also suggest that the intestinal digestion of the by-pass protein was effective in the high CT-plant.

The low urine and faecal N in this experiment could be attributed to the low intake of nitrogen. It has been reported that reduction N intake could possibly result in lower urine N excretion (Tamminga 1992; Jonker *et al*., 2002). Lower rumen CP degradation or efficient use of degraded CP for microbial cell synthesis and growth could have also accounted for low urine N reported.in this study.

The lower N excreted when the animals were fed browse plants and rice straw based diet could help minimise the extent of water pollution from ruminant sources.

The animals fed the treatment diets in the metabolism study all maintained a positive nitrogen balance, which could impact positively on meat and milk production.

The lack of difference in aNDFom and ADFom digestibility between the control and *C. pentandra* suggest there was no effect of the CT on the digestibility of NDF and ADF. The finding agrees with several other authors who found no difference in NDF and ADF digestibility when different levels of CT were fed to sheep or incubated using *in vitro* techniques (Waghorn *et al*., 1987; Nuñez-Hernandez *et al*., 1991; Carrula, 1994). The significantly higher CP digestibility in the control diet did not correspond to a higher DM, NDF and ADF digestibility suggesting an inefficient microbial utilization of the degraded CP. The replacement of rice straw with 40% browse enhanced the DM, CP and NDF digestibility of sole rice straw reported by Jeon *et al*. (2013) and this can be attributed to the improved CP content of the diet.

The lowest feed intake in the lamb growth study was reported in *S. siamea* despite the appreciable level of CP (175.87 g/kg). This indicates that the CP in *S. siamea* did not influence feed intake. Lower palatability of *S. siamea* in ruminants has been reported by Paterson and Clinch (1993) who attributed this to the problem of toxicity. Condensed tannin is noted to cause an astringent taste in animals when they consume CT-diet but this cannot be the cause of the low intake of *S. siamea* since the CT content was relatively lower (Chang *et al*., 1998; Al-Mamary *et al*., 2001).

The animals on the control consistently had the lowest final live weight gain and daily live weight gain compared with the supplemented animals. It is interesting to note that *G. arborea* which had the highest DM intake did not correspond to a higher live weight gain. This could be explained by the low NDF and ADF digestibility reported for *G. arborea* (Table 8.4) and low IVDMD reported in table 4.2. The superiority of *A. lebbeck* and *S. siamea* in terms of live weight gain over the other treatments suggests a better utilization of the nutrients (protein and energy) from these supplements. The highest daily DM intake per head for the browse plants

were 4.15 g, 35.28 g, 48.71 g and 87.70 g for *S. siamea*, *C. pentandra*, *A. lebbeck* and *G. arborea* respectively.

The total protein (TP) reported in the present study (Table 8.2) was slightly higher than the normal TP (63 g/l) reported for West African Dwarf sheep by Konlan *et al.* (2012) and this could be due to a better digestibility and absorption of the dietary protein by the supplemented animals. This is reflected in the high CP digestibility and nitrogen balance recorded.

The blood urea nitrogen (BUN) has been described as a reflection of the extent of ammonia nitrogen production in the rumen and subsequent absorption into the blood (Lewis, 1957; Jolazedah *et al.*, 2015). The lack of significant difference among the treatments suggests that the *C. pentandra* supplement did not negatively affect proteolysis in the rumen despite the relatively high CT. The high BUN level reported in the control as against the low weight gain could suggest that there was some level of body protein catabolism in the control group as has been suggested by Leibholz (1970).

Except for testicular weight, carcass and non-carcass characteristics were not affected the treatments. Testicular weight has been found to increase when UDP is supplied in the diet of rams (Fernandez *et al.*, 2004). However, this was not the case in the present study as the *A. lebbeck* which had a high RDP rather had a heavier testicular weight. The cause of low testicular weight and other fertility indicators have been attributed to a negative energy balance (NEB) (Tamminga, 2006). According to the same author, negative ENB arises when there is an asynchronous supply of RDP and fermentable carbohydrate in the rumen. The ENB is as a result of the extra energy cost of detoxifying excess ammonia escaping from the rumen (Staples and Thatcher, 2001). When this happens, energy that should have been directed to tissue growth is used for detoxification. This therefore suggests that, though *A. lebbeck* had no CT, the level of protein degradation was well balanced with the amount of available fermentable carbohydrate hence more energy was available for tissue deposition.

In contrast to *C. pentandra*, which had lower dietary protein degradation in both *in vitro* batch and *in sacco* incubation, *A. lebbeck,* had a higher dietary protein degradability when they were both used as sole diets. However, the IVDMD and DM degradability did not differ between the two samples. In the nitrogen metabolism and growth studies where *A. lebbeck* and *C. pentandra* were used as supplements, the overall CP digestibility was higher in *A. lebbeck* and it also resulted in higher liveweight gain and testicular weight.

This finding suggests that the use of *A. lebbeck* (no CT) as a sole diet could provide enough degradable protein but not enough fermentable carbohydrate However, when it is fed as a supplement to grazing animals, the use of the degraded protein becomes more efficient possibly due to the supply of more fermentable carbohydrate from the forage being grazed.

In the case of the high CT-browse plant, there seems to be an efficient degradation and utilization of the nitrogen when used as a sole diet hence, the similar IVDMD and DM digestibility as well as the improved liveweight gain reported.

It therefore implies that *A. lebbeck* (no CT) is more beneficial to animal performance when it used as a supplement whereas the high CT-browse plants could be used as a sole diet without negatively affecting digestibility and growth.

9.1.0 General conclusion and recommendation

9.1 Conclusion

- All browse plants tested in this study had adequate amount of CP to serve as supplements for ruminants
- The presence of CT in the browse plants did not affect the fraction of immediately soluble protein but reduced the amount of slowly degradable protein and effective protein degradability. The potential DM degradability of the browse plants were not affected by the presence of the CT.
- Replacing 40% of wheat straw with CT-browse plants resulted in 38% reduction in methane for *C. pentandra*, 19% reduction for *G. arborea* and *S. siamea*. Overall *in vitro* fermentation was found to reduce with the presence of CT.
- Liveweight gain of supplemented animals improved without any negative effects on serum metabolites.

9.2 Recommendation

- *A. lebbeck* or low tannin browse plants should be used as supplements instead of a sole diet whereas the high CT-browse plants could be used as a sole diet.
- Different inclusion levels of the CT-browse plants should be tested with rice straw to establish the optimum levels to achieve the least enteric methane production.
- The effect of these browse plants on reproductive hormones in males should be investigated further since the browse plants affected testicular weight.
- The browse plants should be investigated further for their effects on CP digestibility at increasing CP intake.

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APPENDIX

AP.1 ANOVA Tables for *in vitro* gas production

Variate: b						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Run stratum	3		175390.	58463.	9.09	
Run.*Units* stratum						
Plant	7		58001.	8286.	1.29	0.279
Media	1		7125.	7125.	1.11	0.298
Plant.Media	7		25875.	3696.	0.57	0.772
Residual	43	(2)	276440.	6429.		
Total	61	(2)	536843.			

Variate: c						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Run stratum	3		0.0039701	0.0013234	3.01	
Run.*Units* stratum						
Plant	7		0.0024308	0.0003473	0.79	0.600
Media	1		0.0000023	0.0000023	0.01	0.942
Plant.Media	7		0.0027994	0.0003999	0.91	0.509
Residual	43	(2)	0.0189257	0.0004401		
Total	61	(2)	0.0279153			

Variate: Ammonia nitrogen mg_l					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Run stratum	3	9844.	3281.	1.02	
Run.*Units* stratum					
Sample	7	252135.	36019.	11.20	<.001
Media	1	195482.	195482.	60.77	<.001
Sample.Media	7	20108.	2873.	0.89	0.520
Residual	45	144753.	3217.		
Total	63	622323.			

Variate: pH					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Run stratum	3	2.352043	0.784014	134.04	
Run.*Units* stratum					
Sample	7	0.064844	0.009263	1.58	0.165
Media	1	0.042766	0.042766	7.31	0.010
Sample.Media	7	0.021775	0.003111	0.53	0.806
Residual	45	0.263210	0.005849		
Total	63	2.744638			

Variate: DM_loss_g_g					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Run stratum	2	0.02151	0.01076	0.41	
Run.*Units* stratum					
Sample_ID	7	0.45706	0.06529	2.49	0.038
Media	1	0.02633	0.02633	1.00	0.324
Sample_ID.Media	7	0.17428	0.02490	0.95	0.485
Residual	30	0.78694	0.02623		
Total	47	1.46613			

Variate: in vitro gas at 72hours					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Run stratum	3	171541.	57180.	8.00	
Run.*Units* stratum					
Sample_ID	7	73198.	10457.	1.46	0.205
Media	1	8228.	8228.	1.15	0.289
Sample_ID.Media	7	26556.	3794.	0.53	0.806
Residual	45	321462.	7144.		
Total	63	600985.			

AP. 2 ANOVA tables for *in sacco* CP degradability

Variate: a					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	75222.7	10746.1	98.68	<.001
Residual	24	2613.7	108.9		
Total	31	77836.4			

Variate: b					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	322774.	46111.	23.25	<.001
Residual	24	47602.	1983.		
Total	31	370375.			

Variate: c					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	0.0022195	0.0003171	2.18	0.074
Residual	24	0.0034985	0.0001458		
Total	31	0.0057180			

Variate: a_b					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	529854.	75693.	34.41	<.001
Residual	24	52791.	2200.		
Total	31	582645			

Variate: ED_0_05					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	263725.	37675.	28.36	<.001
Residual	24	31882.	1328.		
Total	31	295607.			

Variate: ED_0_08					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	183243.	26178.	25.76	<.001
Residual	24	24393.	1016.		
Total	31	207636.			

AP. 3 ANOVA table for *in sacco* DM digestibility

Variate: a					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	88947.7	12706.8	103.84	<.001
Residual	24	2936.9	122.4		
Total	31	91884.7			

Variate: b					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	243295.	34756.	1.83	0.127
Residual	24	455267.	18969.		
Total	31	698562.			

Variate: a_b					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	121980.	17426.	0.93	0.499
Residual	24	447543.	18648.		
Total	31	569524.			

Variate: c					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	0.012133	0.001733	0.82	0.578
Residual	24	0.050551	0.002106		
Total	31	0.062684			

Variate: ED_0_05					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	71743.	10249.	1.12	0.384
Residual	24	219808.	9159.		
Total	31	291551.			

Variate: ED_0_08					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	7	64993.	9285.	1.27	0.306
Residual	24	175508.	7313.		
Total	31	240501.			

AP. 4 ANOVA tables for Methane studies

Variate: Total_gas_mls_gDM					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Vessels stratum	3	629.89	209.96	2.23	
Run stratum	3	1706.11	568.70	6.04	
Vessels.Run stratum					
Plant	3	167.67	55.89	0.59	0.642
Residual	6	564.67	94.11		
Total	15	3068.33			

Variate: Methane_mls_gDM					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Vessels stratum	3	20.722	6.907	1.62	
Run stratum	3	16.277	5.426	1.27	
Vessels.Run stratum					
Plant	3	8.313	2.771	0.65	0.611
Residual	6	25.534	4.256		
Total	15	70.847			

Variate: Co2_mls_gDM					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Vessels stratum	3	370.36	123.45	2.94	
Run stratum	3	367.10	122.37	2.91	
Vessels.Run stratum					
Plant	3	51.48	17.16	0.41	0.753
Residual	6	252.35	42.06		
Total	15	1041.29			

Variate: H2_mls_gDM					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Vessels stratum	3	0.0054009	0.0018003	1.81	
Run stratum	3	0.0115558	0.0038519	3.88	
Vessels.Run stratum					
Plant	3	0.0020366	0.0006789	0.68	0.594
Residual	6	0.0059547	0.0009924		
Total	15	0.0249480			

Variate: After_12hrs					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Vessels stratum					
Plants	3	54.366	18.122		
Run stratum	2	8.715	4.357	0.45	
Vessels.Run stratum					
Plants	3	39.534	13.178	1.35	0.405
Residual	3	29.216	9.739	8.63	
Vessels.Run.*Units* stratum	12	13.543	1.129		
Total	23	145.375			

Variate: t10					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Vessels stratum	3	0.09138	0.03046	2.81	
run stratum	3	0.85882	0.28627	26.38	
Vessels.run stratum					
Plant	3	0.03627	0.01209	1.11	0.414
Residual	6	0.06512	0.01085		
Total	15	1.05158			

AP 5 ANOVA tables for nutrient digestibility

Variate: Daily_DM_FI_g					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period stratum	2	116.	58.	0.03	
Period.*Units* stratum					
Trmt	3	232517.	77506.	41.62	<.001
Residual	18	33517.	1862.		
Total	23	266150.			

Variate: Digst_Coeff					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period stratum	2	0.040225	0.020113	2.10	
Period.*Units* stratum					
Trmt	3	0.059251	0.019750	2.07	0.141
Residual	18	0.172030	0.009557		
Total	23	0.271506			

Variate: Daily_CP_intake_g					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period stratum	1	0.46	0.46	0.02	
Period.*Units* stratum					
Plants	3	1874.19	624.73	27.78	<.001
Residual	11	247.38	22.49		
Total	15	2122.03			

Variate: Daily_N_intake_g					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period stratum	1	0.0119	0.0119	0.02	
Period.*Units* stratum					
Plants	3	47.9792	15.9931	27.78	<.001
Residual	11	6.3328	0.5757		
Total	15	54.3240			

Variate: Daily_N_F_g					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period stratum	1	0.006852	0.006852	1.00	
Period.*Units* stratum					
Plants	3	0.079586	0.026529	3.89	0.041
Residual	11	0.075074	0.006825		
Total	15	0.161512			

Variate: Daily_N_U_g					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period stratum	1	0.02642	0.02642	2.12	
Period.*Units* stratum					
Plants	3	0.10011	0.03337	2.67	0.099
Residual	11	0.13740	0.01249		
Total	15	0.26394			

Variate: Digst_Coeff_CP					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period stratum	1	0.0010317	0.0010317	5.06	
Period.*Units* stratum					
Plants	3	0.0209685	0.0069895	34.31	<.001
Residual	11	0.0022411	0.0002037		
Total	15	0.0242412			

Variate: N_Balance					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period stratum	1	0.1256	0.1256	0.22	
Period.*Units* stratum					
Plants	3	47.1602	15.7201	27.61	<.001
Residual	11	6.2625	0.5693		
Total	15	53.5484			

AP. 6 ANOVA tables for growth, metabolites and carcass

Variate: Gain_per_day_g						
Covariate: Initial_weight_kg						
Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
Treatment	4	3669.12	917.28	11.25	0.97	<.001
Covariate	1	320.72	320.72	3.93		0.067
Residual	14	1141.68	81.55		1.20	
Total	19	5968.83				

Final liveweight						
Covariate: Initial_weight_kg						
Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
Treatment	4	8.7343	2.1836	3.30	0.98	0.048
Covariate	1	66.2200	66.2200	100.00		<.001
Residual	12	7.9467	0.6622		8.62	
Total	17	87.0000				

Variate: Back					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.006667	0.001667	0.41	0.795
Residual	10	0.040267	0.004027		
Total	14	0.046933			

Variate: Carcass_weight					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	4.363	1.091	0.62	0.656
Residual	10	17.481	1.748		
Total	14	21.845			

Variate: Chuck					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.005600	0.001400	0.52	0.724
Residual	10	0.026933	0.002693		
Total	14	0.032533			

Variate: Dressing_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	35.235	8.809	1.16	0.384
Residual	10	75.853	7.585		
Total	14	111.088			

Variate: Empty_Dig_tract					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.58763	0.14691	2.43	0.116
Residual	10	0.60507	0.06051		
Total	14	1.19269			

Variate: Full_digestive_tract					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	1.0120	0.2530	0.31	0.865
Residual	10	8.1547	0.8155		
Total	14	9.1667			

Variate: Heart_g					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	1302.1	325.5	1.04	0.434
Residual	10	3135.1	313.5		
Total	14	4437.2			

Variate: Kidney_g					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	33.78	8.44	0.14	0.962
Residual	10	593.12	59.31		
Total	14	626.89			

Variate: Live_weight					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	8.267	2.067	0.39	0.813
Residual	10	53.333	5.333		
Total	14	61.600			

Variate: Liver_g					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	3598.	900.	0.77	0.568
Residual	10	11662.	1166.		
Total	14	15261.			

Variate: Alb_gl					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plants	4	11.063	2.766	0.89	0.495
Residual	15	46.702	3.113		
Total	19	57.765			

Variate: Cholesterol_mmol					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plants	4	0.06655	0.01664	0.51	0.729
Residual	15	0.48865	0.03258		
Total	19	0.55520			

Variate: Globulin_gl					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plants	4	20.49	5.12	0.19	0.941
Residual	15	409.60	27.31		
Total	19	430.09			

Variate: Gluc_mmol					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plants	4	0.05635	0.01409	0.58	0.679
Residual	15	0.36202	0.02414		
Total	19	0.41837			

Variate: TP_gl					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plants	4	22.98	5.75	0.20	0.936
Residual	15	435.52	29.03		
Total	19	458.51			

Variate: Triglyceride_mmol					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plants	4	0.06675	0.01669	0.19	0.938
Residual	15	1.29677	0.08645		
Total	19	1.36352			

Variate: Urea_mmol					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plants	4	10.235	2.559	0.79	0.547
Residual	15	48.333	3.222		
Total	19	58.568			

AP. 7 Experiment 4 Carcass hanged for cuts

AP. 8 Experiment 4 Sheep grazing after release from pen

AP.9 Pictures of browse plants used in this study

Gmelina arborea

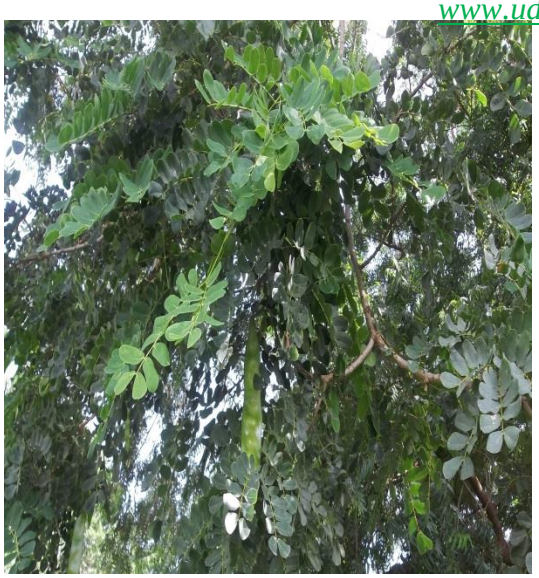
Azadirachta indica

Senna siamea

Pterocarpus erinaceous

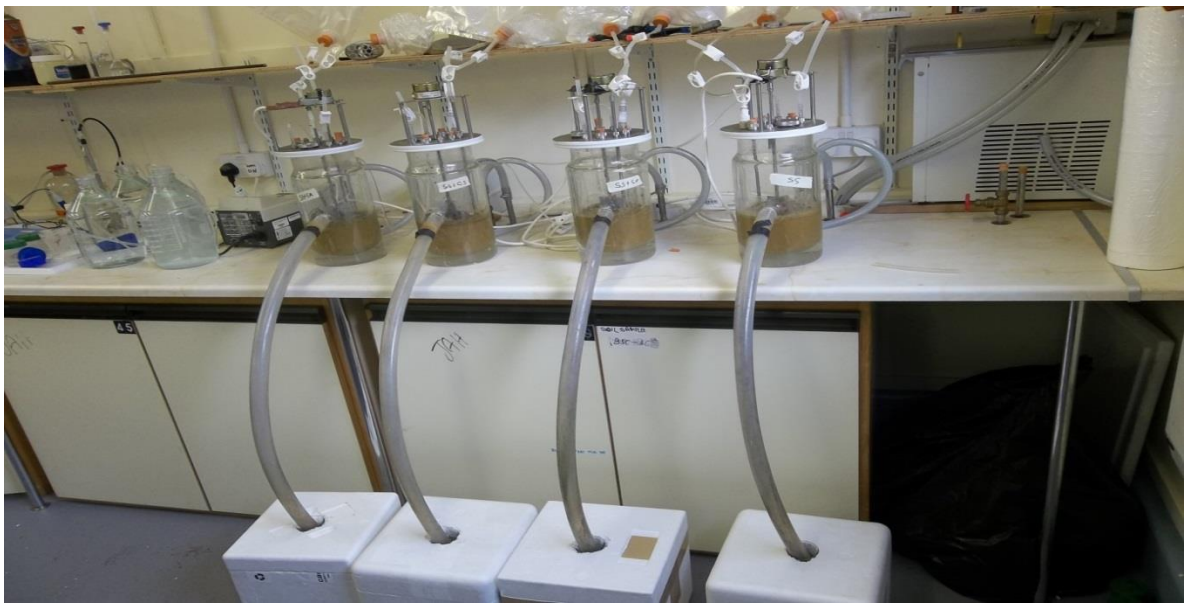
Ficus gnaphalocarpa

Ceiba pentandra



Albizia lebbeck

Khaya senegalensis



AP. 10 Vessels containing samples, gas collection bags, and other apparatus for the continuous culture experiment