**Chemical composition and *in vitro* ruminal fermentation characteristics of cowpea varieties in the Limpopo Province, South Africa**

by

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A thesis submitted in the partial fulfilment of the requirements for the degree of

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## Declaration

I **Mbuso Jethro Mbukwane** declare that this thesis, which I hereby submit for the degree of Masters in Animal Nutrition at the University of Pretoria, is my own work, except where reference has been made, and it has not previously been submitted by me for the degree at this university or any other university or tertiary institution.

Date-------------------------------------------------------------

Signature--------------------------------------------------------

# Dedication

*This work is wholeheartedly dedicated to my lovely wife, Ntombikayise Blessing Mbukwane*.

## Acknowledgements

This has been a journey of highs and lows. However, I enjoyed it and I am looking forward to apply the skills I acquired during this study in future challenges.

First and for most I would like to thank God almighty who gave me the gift of life and supported me till this far, His promises are true and genuine "**I will go before you and will level the mountains; I will break down gates of bronze and cut through bars of iron**" Isaiah 45:2.

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## List of abbreviations

ADF: acid detergent fibre

ADFd: acid detergent fibre digestibility

ADG: average daily gain

ADL: acid detergent lignin

ANF: anti-nutritional factors

ANOVA: analysis of variance

AOAC: association of official analytical chemists

a.s.l: above sea level

b: Insoluble but slowly fermentable fraction

c: rate of fermentation

C: carbon

Ca: calcium

$CH_4$: Methane

CP: crude protein

CPd: crude protein digestibility

$CO_2$: carbon dioxide

CV: coefficient of variation

$^oC$: degrees in celsius

DM: dry matter

DMd: dry matter digestibility

DMI: dry matter intake

DoA: Department of Agriculture

ED: effective degradability

EZ: ecological zone

FAO: Food and Agriculture Organisation

FSS: fine stem stylo

GDP: gross domestic product

GHG: greenhouse gases

GLM: general linear model

GV: gas volume

$H_2SO_4$: sulphuric acid

ILCA: International Livestock Centre for Africa

IVOMD: *in vitro* organic matter digestibility

LSD: least significant difference

ME: metabolisable energy

mg: milli-gram

µmol: micro-mole

$MgSO_4.7H_2O$: magnesium sulphate anhydrous

$MgCl_2.6H_2O$: magnesium chloride anhydrous

MJ: mega-Joule

mL: milli-Liter

mm: milli-meter

MPTS: multi-purpose trees

N: nitrogen

NDF: neutral detergent fibre

NDFd: neutral detergent fibre digestibility

NEPAD: New Partnership for Africa's Development

NRC: National Research Council

NTP: non-tannin phenols

OM: organic matter

OMd: organic matter digestibility

P: phosphorus

PA: proanthocyanidin

PGP: potential gas production

pH: potency of Hydrogen

RCD: randomised complete design

RCBD: randomised complete block design

RDP: rumen degradable protein

SAS: statistical analysis system

SD: standard deviation

SEM: standard error of the mean

SSA: sub-Saharan Africa

t $ha^{-1}$: ton per hectare

TCT: total condensed tannin

TP: total phenols

TT: total tannin

VFA: volatile fatty acids

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**Abstract**

Shortage of quality feed is the major constraint in livestock production particularly under smallholder subsistence farmer's conditions in sub-Saharan Africa, including South Africa. This is due to the fact that livestock production under smallholder subsistence farmers predominantly depends on communal grazed natural pastures which are often inadequate in both quality and quantity particularly during the dry season. In order to improve livestock production in these areas, there is a need to address shortage of feed both in terms of quantity and quality. Consequently cowpea (*Vigna unguiculata* (L.) Walp.) varieties adaptable in Limpopo province of South Africa were evaluated for their nutritive value and *in vitro* gas production (fermentation) attributes in order to identify superior varieties as potential forage source for ruminants animals.

Several laboratory experiments (including proximate analysis, *in vitro* digestibility estimate and gas production) were conducted at the University of Pretoria, South Africa. In this study, 12 varieties of cowpea were evaluated for their chemical composition, *in vitro* organic matter digestibility and gas production (fermentation) attributes. Generally the adaptable varieties had more than 15% crude protein (CP) content with relatively low neutral detergent fibre (NDF), thus they were highly digestible. This indicates that these varieties could be potentially utilised as supplementary forage and nitrogen source to complement poor quality forage. However, of the 12 varieties, three, Bechuana white, IT 97K-499-35 and TX 08-30-1 were relatively superior as they had greater CP, ME and IVOMD with low fibre values. Hence, these three varieties are chosen for further evaluation in terms of their potential in improving ruminal fermentation and digestibility of poor quality grass hay when used as

supplement at three different levels of inclusion. This was done by conducting an *in vitro* gas production experiment and measuring parameters such as, ruminal fermentation, *in vitro* organic matter digestibility and NDF degradability of forage. Generally supplementing poor quality grass hay with cowpea varieties improved grass hay fermentation. Among the cowpea varieties, high levels of inclusion resulted in greater gas production for Bechuana White and IT 97K-499-35, while for TX 08-30-1 variety, there was no difference between 15 vs 30 % or 50% inclusion level. Thus Bechuana White and IT 97K-499-35 can be used as a forage source while TX 08-30-1 varieties can be recommended as a protein source to supplement poor quality forage. However, there is a need to determine their dry matter intake, digestibility and animal performance response in order to utilise them in the feeding system of ruminants. *In vitro* supplementary results to poor quality forage suggests that thirty percent level of inclusion of the two cowpea varieties (Bechuana White and IT 97K-499-35) has the maximum benefit.

# GENERAL INTRODUCTION

**Background**

Natural pastures play a critical role in livestock production in sub-Sahara Africa as it is the major feed resource that is cheaply available. However, insufficient quantity and quality of forages, which is often associated with overgrazing, climate change and poor management of natural pastures negatively affects livestock production, especially under subsistence farmers' situation. Moreover, the challenge of increasing population in sub-Sahara Africa leads to greater food demand; hence there is a need to intensify agricultural production (Singh *et al*., 2005). Many tropical and subtropical countries are characterised by low productivity of ruminants due to inadequate nutrition associated with low nitrogen concentration and reduced digestibility of available feed materials (Singh *et al*., 2005).

On the other hand, livestock play a significant role in the livelihoods of smallholder subsistence farmers and their economies in tropical and subtropical regions of Africa. This is because livestock is normally kept for food (meat and milk), as a source of cash income, draught power, fibre and hides (Thomas and Sumberg, 1995; Barrett *et al*., 2005; Kiptarus, 2005; Behnke and Centre, 2012). In addition to that, livestock also provides energy for tillage, transportation of goods and people, and weeding. Livestock also provides manure for improving soil fertility hence saving on fertilizer costs (Sumberg, 1998).

South Africa has about 80% agricultural land that is suitable for production of livestock and communal farmers own approximately 65% of the livestock population ((Department of Agriculture (DoA), 2003)). There is an estimate of 240 000 smallholder subsistence farmers in South Africa, (Olivier, 2004); however, livestock production under these farmers is very poor, hence they contribute less to the gross domestic product (GDP). This could be due to a number of factors, which includes poor management, prevalence of diseases, inadequate nutrition, inadequate feed supply and shortage of the grazing land (Kunene *et al*., 2003).

Ruminant animals in most rural areas of Africa survive under very poor nutritional conditions, utilizing feed resources from crop residues and available natural pastures, which are mostly of poor-quality (Osuji and Odenyo, 1997; Ravhuhali, 2010). Approximately 240 000 smallholder subsistence farmers in South Africa provide local, regional or informal markets with beef (Olivier, 2004). These farmers mainly depend on a whole range of natural pastures including grass, tree legumes, trees or shrub leaves, legumes and pods with little or no supplementation for their livestock feeds. Generally, in winter or dry-season, forages lose their quality and during such periods, animals lose a great deal of weight (Nsahlai *et al*., 1998). Therefore fluctuation of forage quality and quantity coupled with high stocking rate poses a major challenge to livestock production (Scogings *et al*., 2004; Dziba *et al*., 2007). Consequently, this challenge leads to over grazing of communal areas due to lack of appropriate grazing management and over utilization of natural vegetation (Paterson *et al*., 1998; Fuhlendorf and Engle, 2001; Masafu, 2006; Sultan *et al*., 2008). In addition, low productivity of livestock in sub-Sahara Africa is usually caused by a combination of poor livestock husbandry practices and diseases (Anele *et al*., 2011). According to Anele *et al*. (2011), sound nutrition strategies and programmes aimed at improving the nutrition of livestock are critically important for improved production.

Supplementation with various grains and or by-products is necessary to achieve desired market weights at weaning. Most of the grains are imported into South Africa at greater costs than by-products, which are likely to increase due to the escalating price of fuel and demand caused by an increase in the prevalence of droughts. In addition, seasonality of production and variability in quality of by-products limits their use in cattle rations. Using concentrates, by-products and nitrogen (N) fertilizers usually results in the net importation of nutrients into farms, which is undesirable from an environmental and cost stand-point. In contrast leguminous plants are more digestible than warm-season grasses because they contain greater crude protein (CP) and non-structural carbohydrate (NSC) concentrations (Ball *et al*., 2002). They can be used in place of concentrate supplements to optimize the growth of animals.

Different strategies of improving the quality of natural pastures and crop residues have been exploited in the past including the use of straws and forage legumes to feed livestock (Gebreyowhans and Gebremeskel, 2014). Grain legumes such as field peas, lupin seeds and faba beans can be used as dietary protein supplementation to feeds of lower quality (Batterham and Egan, 1986; Dixon and Hosking, 1992). It is, therefore, very important to evaluate the utilisation of legumes in order for ruminant livestock producers to design strategies aimed at improving the poor-quality roughages fed on their livestock (Ravhuhali, 2010).

Legumes generally have the ability to fix atmospheric nitrogen to the soil for its use and subsequent crop use thereby improving soil fertility (Etana *et al*., 2013). They are palatable and play an important role as a source of proteins for humans and livestock, hence warm-season legumes, such as cowpeas, also known as *Vigna unguiculata*, is the crop of choice in the tropics (Muli and Saha 2000; Food and Agricultural Organisation (FAO), 2009; Etana *et al*., 2013). The performance of cowpeas in terms of biomass production and persistence and their feeding value in east and west Africa is well documented (Anele *et al*., 2011). However, from our knowledge very little has been done in the sub-tropical climate of South Africa.

The current study did not focus on the feeding value of cowpea variety; however, it was a baseline study in South Africa to determine the chemical composition and *in vitro* gas production. Although a preliminary study was conducted in Limpopo Province on the comparison of the feeding value of four cowpea hay-cultivars by Ravhuhali (2011) in semi-arid tropical climate of Limpopo Province, the study by Ravhahali (2011) did not focus on this newly developed varieties used in the present study.

The varieties included in the current study have been undergoing the agronomical evaluation in Limpopo, South Africa. However, its nutritive value and feeding potential as a forage source for ruminant animals is still not known. Therefore, to address the knowledge gap that exists in terms of their potential nutritive value, chemical composition and *in vitro* ruminal fermentation characteristics of *Vigna unguiculata* varieties were studied for those varieties found to be drought tolerant, adaptable and productive under the sub-tropical environment in Limpopo province.

## Objectives

### General objective

The overall objectives of this study was to identify superior varieties that can be used to improve the utilisation of poor-quality roughages during the dry-season by small ruminants kept under smallholder subsistence farmer's condition in South Africa.

### Specific objectives

The specific research objectives of the study were:

- To characterize twelve cowpea varieties that have been agronomically evaluated in the Limpopo Province of South Africa in terms of chemical composition and *in vitro* gas production characteristics.
- To relate the chemical composition of cowpea varieties to their digestibility and *in vitro* gas production attributes.
- To evaluate the effect of using cowpea as supplement to poor quality forage on *in vitro* ruminal fermentation and *in vitro* digestibility.

**Hypothesis**

In this study the following hypotheses were formulated and different laboratory experiments were undertaken to test them.

- Ho: There is no variation among the varieties of cowpeas in terms of their chemical composition and potential nutritive value and *in vitro* gas production characteristics.
- Ho: There is no relationship between the chemical composition, digestibility and *in vitro* gas production attributes of the cowpea varieties grown in the Limpopo Province of South Africa.
- Ho: Supplementing poor quality grass hay with selected cowpea varieties forage does not affect *in vitro* ruminal fermentation and digestibility of forage based diets.

# CHAPTER 1

## Literature review

### 1.1. Livestock production and its role in food production in sub-Sahara Africa

Livestock keeping serves as the anchor for the pastoral community and also provides social and cultural value (Onono *et al*., 2013). In sub- Saharan Africa (SSA), livestock accounts for 25 to 30 % of the agricultural domestic products (Winrock International, 1992). Cattle, goats, camels and sheep as well as mixed herds and flocks are domesticated in SSA. These classes are commonly kept by smallholder subsistence farmers in mixed farming systems that combine livestock and crop production (McIntire *et al*., 1992; Onwuka *et al*., 1997; Valbuena *et al*., 2012). According to Brumby (1986), mixed farming by small-scale subsistence farmers in Africa is 74% by estimation of total cattle.

Livestock production ranges from smallholder to intensive and sophisticated commercial systems that contributes a majority of animal and feed needs for most poor people (Tothill *et al*., 1989; Herrero *et al*., 2010). The advantage of crop-livestock mixed farming is that animals get their feed from the crops while the livestock provides manure to the crops and traction in planting and harvesting (McIntire and Gryseels, 1987). Farmers are also able to buy food, inputs and other goods from cost savings and cash income generated from the system (Christiaensen *et al*., 1995; Fafchamps *et al*., 1998; Moll, 2005; Hoddinott, 2006). In sub-Sahara Africa, livestock are kept for food (milk and meat), cash income, draught power, manure, hides and fibre (Thomas and Sumberg, 1995; Sumberg, 1998; Swanepoel *et al*., 2000; Stroebel, 2004; Barrett *et al*., 2005, Kiptarus, 2005; Behnke and Centre, 2012).

There is an estimate of 240 000 small scale farmers in South Africa whose livestock are normally fed a range of natural pastures including grass, tree legumes, trees or shrub leaves, legumes and pods with little or no supplementation (Olivier, 2004). In SSA, livestock account for about 53% of the agricultural capital stock and about 30% of agricultural gross domestic product (GDP) ( New Partnership for Africa's Development (NEPAD), 2006). Lenne and Thomas (2006), pointed out that approximately 20% of the animal protein in African diets are contributed by eggs, meat and milk and these feed approximately 70% of people dependent mainly on livestock animals.

Some of the challenges faced by farmers in the region are frequent disease outbreaks, drought, poor livestock infrastructure, water shortages and shortage of extension services to advise farmers on best livestock production practices (Gitau *et al*., 2001; Mati *et al*., 2006;

Pavanello, 2010; Wesonga *et al*., 2010; Opiyo *et al*., 2011). Feed shortages, poverty and inequity also presents some challenges to the development of smallholder farmers in these sites (Ralevic *et al*., 2010; Sharma *et al*., 2010; Moebius-Clune *et al*., 2011). Resource-poor smallholder subsistence farmers whom depend on small ruminants are constantly under pressure to purchase animal feeds due to rangelands being severely degraded (Ben Salem and Smith, 2008; Valbuena *et al*., 2012).

## 1.2. Predominant feed resources available for ruminant animal

Forages are the primary feed component for livestock globally. These include legumes and grasses, which can be grazed and/or conserved as silage or hay to cater for periods of feed shortages (Smith *et al*., 2008; Havilah, 2011). Annual forages may be classed as cool-season grasses, warm-season grasses, cool-season legumes, warm-season legumes or Brassicas (Havilah, 2011). These species may be sown in mixtures or alone. In southern Africa, the savanna biome, defined as overcrowded seasonal bushes that form a continuous and an overgrown herbaceous layer and an open, scattered layer of trees and shrubs is the most prevalent form (Frost *et al*., 1985; Smith and Goodman, 1986; Tefera *et al*., 2008). It constitutes extensive, dynamic and diverse ecosystems supporting great numbers of wildlife and livestock (Tefera *et al*., 2008).

The best varieties of feed resources are well adaptable to local climatic environment as well as disease and insects tolerant. Best varieties can be often associated with high digestibility, early maturity and improved feed quality (high energy and protein content) (Franzel *et al*., 2014). Energy and protein sources are of significance importance in ruminant's nutrition due to their ability to stimulate ruminal microbes and enhance the production function of the animals (Wanapat, 2008). However, the use of such feed resources, especially by resource poor farmers, is limited because of seasonal variation and affordability. Ruminant animals are subjected to roughages of lower quality which are normally of poor nutritional value (lower energy, minerals, and vitamin and protein contents) (Kumar *et al*., 2015). To partially overcome such problem, farmers keep crop residues for later use in stall feeding during dry seasons (Wapanat, 2008). However, during prolonged dry-seasons, this runs out and livestock is left without adequate feed.

Countries in SSA are usually affected by forage shortage due to inadequate rainfall (Ben Salem and Smith, 2008). Consequently, livestock thrive under serious nutrient shortages and as a result, animals often depend on crop residues of low quality (e.g. stubbles, straws) (Figure 1.1), agricultural by-products, other non-conventional feed sources and expensive feed supplements (Ben Salem and Smith, 2008; Akinfemi *et al*., 2012; Kumar *et al*., 2015). The nutritive value of some typical feeds that are normally utilized by livestock animals for dry-season feeding are shown in Table 1.1. Livestock in this region has reduced weights and production due to inadequate feed resources (Ajayi *et al*., 2005; Anele *et al*., 2011). The strategic use of shrubs, cacti and fodder trees has been shown to improve livestock production in the savanna regions due to their ability to remain green and maintain their protein content (Olafadehan and Adewuni, 2009; Olafadehan and Okunade, 2016)

Ruminants may also make use of cultivated fodders such as pearl millet (*Pennisetum glaucum*), oat (*Avena sativa*), maize (*Zea mays)* and sorghum (*Sorghum bicolor*); leguminous fodders such as cowpea; by-products from the agro-industrial sector such as wheat straw, wheat bran, and concentrates such as soybean meal, mustard oil cake, groundnut cake, cotton seed cake, and grains like maize, oat, barley and wheat (Kumar *et al*., 2015). The use of legume residues is often managed differently from cereal residues due to their greater feed value (Valbuena *et al*., 2012). The same authors in their findings concluded that in Africa, the use of crop residues for stall feeding and stubble grazing has generally increased. Some of these feed resources may not be viable for adoption by smallholder subsistence farmers due to availability and affordability. Farmers can also exploit food-feed systems which offer some synergistic benefits in that the crops grown on the farm are mainly grown for human food whereas the crop residues are used for livestock feed (Wanapat, 2008). Cassava (*Manihot esculenta*), *Phaseolus calcaratus*, sweet potato (*Ipomoea batatas*), *Trichanthera gigantean* and *Flemingia macrophylla* have been extensively studied Nigeria and Kenya as potential feed resources for ruminants in sub-Sahara Africa (Banful *et al*., 2000; Wapanat *et al*., 2006; An *et al*., 2003; Hong *et al*., 2003; Chanthakoun *et al*., 2008).

**Figure 1.1** Cattle grazing straw on farm where grazing land has been severely affected by drought (Photo taken in Lichtenburg, North-West province of South Africa, in 2015 by Waldo Swiegers)

**Table 1.1** Protein and crude fibre content and dry matter digestibility, of some typical feeds available for dry season feeding

| Type | Protein content | Crude fibre | Digestibility |
|---|---|---|---|
| Maize stover ( straw) | Low | High | Low |
| Rice straw | Low | High | Low |
| Sugarcane tops | Low | High | Moderate |
| Bagasse | Low | High | Low |
| Dry season grazing | Low | High | Low |
| Legume tree leaves | Moderate to high | Moderate to high | High |
| Haulms and tops | Moderate to high | High | Moderate to high |
| Cassava leaf meal | High | High | |
| Brewers grains | High | High | Moderate to high |
| Tree fruits (e.g. Acacia spps) | Moderate | Moderate to high | Low to moderate |
| Molasses | Low | Low | |
| Citrus pulp | Low | High | Moderate |
| Oilseed cakes | High | Low to High | High |
| Cereal bran | Moderate to high | Moderate to high | High |
| Cage layer manure | High | High | |
| Cereal grains | Moderate | Moderate | High |

Source: Jayasuriya, 2002; Smith, 2005; protein content (g $kg^{-1}$ DM): low = < 60, moderate = 6 to 110, high > 11; crude fibre (g $kg^{-1}$ DM): low = < 60, moderate = 6 to120, high = > 12; digestibility (g $kg^{-1}$ DM): low= < 40, moderate = 40 to 600, high = > 600

### 1.3. Warm season legumes as livestock feeds

According to De Faria *et al*. (1989), legumes are broadly defined as podded fruit that are defined by a structure of unusual flower and have a potential of nodulating with *rhizobia*. Legumes (*Fabaceae Lindl., syn. Leguminosae Juss.*) have many important economic benefits. Amongst the plant families, they are considered as one of the largest herbaceous plants in size. Perennials, annuals, trees and shrubs from species of tropical and temperate origins are types of legumes (Mikic *et al*., 2011). Annual legume species such as bitter vetch (*Vicia ervilia (L.) Wild),* pea (*Pisum stadium L.*), grass peas (*Lathyrus sativus L.*), faba bean (*Vicia faba L.*), chickpea (*Cicer arietinum L.*) and lentil (*Lens culinaris Me*dik.) are considered some of the first domesticated crops where crop rotation with cereals was practiced (Mikic *et al*., 2011a).

High-quality feed is produced by summer annual legumes even during autumn and late summer when there is a tremendous decline in forage quality in sub-tropical and tropical environments (Mikic *et al*., 2011a). During this time, leaf digestibility is 60 to 75% while stem is 50 to 55%, whereas the corresponding crude protein concentrations for the stem and leaf are 10 and 20%, respectively. It is very important to control the stocking rate at this time to allow for re-growth of the leaves since overgrazing may restrict re-growth (Havilah, 2011). Summer annual legumes can be grown with sorghums and millets in mixtures as a source of nitrogen (Havilah, 2011). In Africa, grain legumes reportedly fixed about 15-210 kg N $ha^{-1}$ in a season, whereas 43-581 kg N ha-$^{1}$ $y^{-1}$ was reportedly fixed by tree legumes (Dakora and Keya, 1997).

Fertilizer is often unavailable to smallholder subsistence farmers due to its high cost and poor market infrastructure. This limits the use of fertilizers, hence a bulk of the nitrogen needed to produce crops has to come from biological fixation by legumes to benefit itself, companion crops and subsequent crops (Dakora and Keya, 1997). Therefore, legumes are a valuable addition in farming systems of resource poor farmers. Nitrogen fixing legumes have striking characteristics of growing in drought-stricken areas where no other crop can survive and thrive in poor soils. Additionally, these species are able to form nodules with several *bradyrhizobia* and *rhizobia*. Dakora and Keya (1997) point out *Vigna unguiculata* (cowpea), *Macrotyloma geocarpum* (Kersting's bean) and *Vigna subterranean* (Bambara groundnut) to be the main legumes that are usually cultivated throughout sub-Saharan Africa. Similar authors also emphasize the use of creeping legumes, like forage species and cover crops to improve soil fertility through biological N fixation, ground cover, organic matter retention, as well as in preserving soil moisture.

Examples of commonly grown warm season forage legumes are cowpea (*Vigna unguiculata*), soybeans (*Glycine max*) and lab-lab (*Lablab purpureus*), while lucerne (*Medicago sativa L.*), white (*Trifolium repens L.*) and red (*Trifolium pratense L.*) clovers, and sainfoin (*Onobrychis viciifolia Scop.*) constitutes of those grown in temperate climates (Havilah, 2011). The chemical composition of legumes commonly used as forage for ruminant livestock are shown in Table 1.2. Cultivated legume species are a cheaper source of high quality protein for both animals and humans. Legumes maintain and restore soil fertility because of its symbiotic relationship with nitrogen fixing bacteria (Singh *et al*., 2006) and are inevitable in the remediation of wastelands, heavily degraded agroecosystems, and to reduce cropping systems dependency toward nitrogen mineral fertilizers. Similarly, Graham and Vance (2003) state that legumes play a significant role in agriculture, natural ecosystems and agroforestry by fixing nitrogen in environments that are low in nitrogen, thereby improving crop production. The contribution of legumes in intercropping systems is widely acknowledged because of greater yields of the non-fixing intercrop components in comparison to sole crops (Corre-Hellou *et al*., 2006). Due to their ability of biological nitrogen fixation to the soil, legumes are important in both the facilitation and dynamics of nitrogen in various plant communities (Hauggaard-Nielsen and Jensen, 2005; Fustec *et al*., 2010).

**Table 1.2** Chemical composition of legumes commonly used as forage for ruminant livestock

| Species | ...... | …… | g kg$^{-1}$ | DM | …… | MJ kg$^{-1}$ DM |
|---|---|---|---|---|---|---|
| | NDF | ADF | CP | NPN | EE | ME |
| Lucerne (*Medicago sativa*) | 358 | 283 | 206 | 8.2 | 13 | 9 |
| Red clover (*Trifolium pratense*) | 395 | 262 | 242 | 22.2 | 18 | 9.2 |
| Sulla (*Hedysarum coronarium*) | 378 | 296 | 218 | 21.1 | 25 | 9.3 |
| White clover (*Trifolium repens*) | 276 | 210 | 242 | 16.1 | 18 | 10 |
| Lab lab (*Lablab purpureus*) | 453 | 404 | 178 | - | - | 8.4 |
| Cowpea (*Vigna unguiculata*) | 353 | 311 | 175 | 3.4 | 13 | 8.5 |

Source: Fulkerson *et al*. (2007); NDF: neutral detergent fibre; ADF: acid detergent fibre; CP: crude protein; NPN: non-protein nitrogen; EE: ether extract; ME: metabolisable energy

## 1.4. Cowpea production in South Africa

In South Africa, cowpea is locally known as *dinawa a*nd it is mostly grown by smallholder farmers for its leaves as *morogo/imifino* (Department of Agriculture, Forestry and Fisheries (DAFF), 2011). According to the National Department of Agriculture (NDA) (2009) cowpea production in South Africa is mostly small scale by smallholder farmers under dry conditions. Its production is low compared to other staple crops, such as maize, due to lack of funding for research purposes (Asiwe, 2009). There are, however, few cases of commercial production used for animal fodder, and recently, an increase in grain production due to food insecurity challenges amongst most rural house-holds (DAFF, 2011). There are no recorded figures for the quantities of cowpea produced or the size of area under production. According to the DAFF (2011), cowpea producing provinces in South Africa are Limpopo, North-West, Kwazulu Natal and Mpumalanga. Moswatsi (2014) argues that cowpea underutilization deprives the resource poor farmers of its numerous advantages and/ or nutritional benefits for both human and livestock consumption. It is therefore necessary to develop better yielding varieties that are well adapted for local conditions for production to be increased.

### 1.5. Brief history and production of cowpea

As a fodder crop with wide adaptation characteristics, a vegetable and a grain legume, cowpea is also tolerant to stress and is cultivated in about 7 million hectares worldwide, in hot and warm regions (FAO, 2009). Africa is the main cowpea producing continent with countries like Niger, Nigeria, Sudan, Kenya, Senegal, Angola, Botswana, and Mozambique producing the most of cowpea (Ehlers and Hall, 1997). It is also produced in countries like South America (Brazil), Asia (India, Myanmar), the south and western regions of North America (USA, West Indies) and Europe (Italy) (Mortimore *et al*., 1997; FAO, 2009; Etana *et al*., 2013). According to the FAO (2000), Nigeria and Niger represents 49% of the global cowpea production at 850 000 and 271 000 tons annually.

#### 1.5.1. Cowpea agronomic characteristics

Cowpea (*Vigna unguiculata* (L). Walp), is a legume of the Phaseolea tribe (Polhill and Vander der Maesen, 1985). It is a legume plant that belongs to the Fabaceae family (Mortimore *et al*., 1997). Cowpea offers a new promise in crop-livestock systems and pasture production systems (Anele *et al*., 2011), and is often referred to as a “hungry season crop” because it is usually harvested first during the cropping season before other cereal crops are ready for harvest (Etana *et al*., 2013). Under conventional farming systems of the drier regions of tropical Asia, Central America and Africa, *Vigna unguiculata* is considered as a major component of such systems (Mortimore *et al*., 1997). According to Anele *et al*. (2011), dual purpose cowpea is of significance importance to resource-poor farmers because provides biomass for livestock supplementation during the dry season, it provides extra food and income for the household and also requires very little inputs for its production. Several authors point out that it is adapted to high temperatures and drought, tolerant to low fertility, has symbiosis with mycorrhizae, provides nitrogen fixation and thrives in several soil pH conditions (Kwapata and Hall, 1985; Fery, 1990; FAO, 2009). Etana *et al*. (2013) argues that cowpea can fix up to 240 kg N $ha^{-1}$ and leaves up to 60 to 70 kg N $ha^{-1}$ ha nitrogen to subsequent crops.

Cowpea is a very important legume in semi-arid regions of the tropics due to green leaves or pods, a valuable forage source for livestock animals and grain as leafy vegetables for human consumption (Ali *et al*., 2004; Adeyanju *et al*., 2007). In west Africa, the crop can be used to feed livestock during dry season (Tarawali *et al*., 2002). In addition, the crop can be used as livestock feed (vines and leaves) and it can be grazed fresh, dried as hay or preserved as silage (Anele *et al*., 2010). Cowpeas husk which are obtained after threshing are as valuable as the leaves for livestock feed (Oluokun, 2005). Cowpea leaves are an important source of protein for human beings and are often consumed similarly to spinach (FAO, 2009).

Ali *et al*. (2004), reported high cowpea forage yields of 729 to 880 kg$^{-1}$ ha$^{-1}$ after cowpea was planted in sandy loam soil that was irrigated. According to Dawit *et al*. (2009), cowpea has the ability to suppress weeds, particularly the *Striga species*. Cowpea can be conserved as nutritious hay that can support livestock animal production even during dry seasons in west Africa (Tarawali *et al*., 2002; Singh *et al*., 2003). Several authors reported cowpea intercropping with traditional cereals like maize and sorghum with higher quality and yields of these cereals than when grown alone (Ahmad *et al*., 2007; Dahmardeh *et al*., 2009; Etana *et al*., 2013). In crop rotation systems, resistant cowpea varieties have potential to suppress root-knot nematode (*Meloidogyne spp.*) reproduction, hence it can be a valuable crop (Ehlers and Hall, 1997).

### 1.5.2. Description of cowpeas

Cowpea is an herbaceous fast growing annual food legume with leaves and crop residues used as forage (Ehlers and Hall, 1997). Cowpeas have large leaves and is vining. Cowpea is fairly tolerant to moderate soil acidity, drought, low fertility and heat (Smith *et al*., 2008). Generally, cowpea has high nutritive value and does not cause bloat in cattle. Cowpeas have a DM yield potential of 2000 to 3000 kg DM ha$^{-1}$ from dry-land and 8000 kg DM ha$^{-1}$ from irrigation. Dry matter yield between 5 to 7 tons is possible in the first, out of three possible cuts, which declines to 4 tons in the second and third cutting (USAID, 2008). In terms of performance, they range about 3-10 t ha$^{-1}$ DM in 12 to 16 weeks as crop residue at grain harvest. Crude protein in crop residue is 100 to 250 g kg$^{-1}$ DM and up to 300 g kg$^{-1}$ DM at early flowering (Smith *et al*., 2008).

To obtain proper re-growth of indeterminant cowpea varieties, grazing must be delayed to flowering and with this practice, 2 to 3 grazing events are possible. Cowpeas are harvested when pods begin to turn yellow (NDA, 1997). According to Le Houerozi (2006), cowpeas are cut to a height of 10 cm. Their limitation to use is attributable to heavy grazing and such practice should be avoided. When cowpeas are harvested at mid flowering, they make silage of greater quality (Havilah, 2011). They are very palatable and have high nutritive value that can be conserved as hay or silage or be used as fresh cut and carry forage (FAO, 2009). Ehlers and Hall (1997), states that cowpeas are sensitive to cold, heavy rain and frost and may be susceptible to pest and diseases. Table 1.3 shows the chemical composition of cowpea forages harvested from different ecological zones of Africa.

**Table 1.3** Chemical composition of cowpea forages harvested from different ecological zones in Africa

| Country | Cowpea varieties | Chemical composition (g kg$^{-1}$ DM) | | | | | | References |
|---|---|---|---|---|---|---|---|---|
| | | CP | DM | OM | NDF | ADF | IVOMD | |
| South Africa | -Pan 311 | 229 | 933 | 867 | 453 | 303 | - | Ravhuhali, (2010) |
| | -Red Colona | 195 | 867 | 880 | 449 | 289 | - | |
| | -Agripes | 245 | 880 | 873 | 472 | 333 | - | |
| | -Black eye | 260 | 859 | 813 | 426 | 236 | - | |
| South Africa | -Not Specified | 220 | 900 | 830 | 380 | 320 | - | Gwanzura *et al.* (2012) |
| Nigeria | -IITA 97K-1069-6 | 181 | 948 | - | 569 | 399 | 635 | Anele *et al*. (2010) |
| | -Peu | 147 | 942 | - | 612 | 419 | 585 | |
| Ethiopia | -12668 | 186 | 892 | 863 | 607 | 533 | 551 | Gebreyowhans & Gebremeskel (2014) |
| | -White wonder | 177 | 899 | 865 | 580 | 523 | 574 | |
| | -9333 | 180 | 895 | 861 | 570 | 572 | 573 | |
| | -Small seed | 177 | 896 | 855 | 583 | 552 | 562 | |
| | -Black eyed | 186 | 894 | 849 | 563 | 471 | 602 | |
| Ethiopia | IT82D 899 | 198 | 874 | 769 | - | - | - | Etana *et al*. (2013) |
| | TUX1948-01F | 192 | 873 | 754 | - | - | - | |
| | TVU11424 | 194 | 875 | 764 | - | - | - | |
| | IT85F2687 | 201 | 887 | 782 | - | - | - | |
| | 82D 504 -4 | 209 | 886 | 773 | - | - | - | |
| | IT84D-448 | 193 | 872 | 779 | - | - | - | |
| | IT93K 2046 -2 | 223 | 879 | 760 | - | - | - | |
| | IT 87D 551 -1 | 202 | 883 | 793 | - | - | - | |
| | IITAUK 91-12 | 222 | 889 | 772 | - | - | - | |
| | 87D - 1802 | 211 | 877 | 778 | - | - | - | |
| Ethiopia | WWT | 164 | 917 | - | 427 | 311 | 697 | Geleti *et al*. (2014) |
| | ILRI 9325 | 182 | 909 | - | 429 | 341 | 687 | |
| | ILRI 11976 | 180 | 910 | - | 434 | 317 | 687 | |
| | ILRI 6782 | 165 | 919 | - | 437 | 378 | 656 | |
| | ILRI 6783 | 178 | 915 | - | 438 | 365 | 636 | |
| Ethopia | - | 185 | 945 | - | 401 | 354 | - | Koralagama *et al*. (2008) |
| Zimbabwe | - | 154 | 905 | - | 372 | 212 | - | Baloyi *et al*. (2008) |
| Zimbabwe | - | 226 | 909 | 919 | 507 | 388 | - | Chekeredza *et al*. (2002) |
| Zimbabwe | - | 180 | 855 | - | - | - | - | Simbarashe *et al*. (2015) |

### 1.5.3. Anti-nutritional factors found in cowpeas and other forage legumes

Most forage legumes contain some anti-nutritional factors (ANF), such as tannins, with the effect of feeding value reduction to ruminant animals (Reed, 1995; Gwanzura *et al*., 2012). Woodward and Reed, 1989 argue that these compounds also interfere with the concept of low fibre and high protein contents, usually used to indicate a high feeding value of a forage. Other secondary compounds or anti-nutritional factors found in legumes are glucosinolates, saponins, alkaloids or gossypol, lectins, non-protein amino acids, and these compounds may result in ruminant production being limited (Table 1.4). Reduction in nutritive value for ruminants have been associated with high contents of condensed tannins in forage legumes (Ahn *et al*., 1989; Palmer and Schlink, 1992). Legumes nutritive value is altered by polyphenolics by altering the composition of ruminal microbial species or reducing availability or making unavailable nutrients or the rumen or postruminally as a result of compounds complexing with carbohydrates, minerals and proteins (Baloyi *et al*., 2001). In their study, Baloyi *et al*. (2001), found less tannin concentrations, which may not adversely affect livestock productivity as compared to tannin concentration in Silverleaf Desmodium (Table 1.4). Saponins have been found to inhibit microbial synthesis and fermentation in the rumen and altering the site of nutrient digestion in sheep (Lu and Jorgensen, 1987). Toxicosis at tissue level may result from polyphenols complexing with and inhibiting extracellular microbial cellulolytic enzymes (Butler *et al*., 1982).

**Table 1.4** Chemical composition (g kg$^{-1}$ DM) and anti-nutritional factors of legumes commonly used as livestock forages

| | Cowpea | Silverleaf | FSS | Fitzroy | Veld |
|---|---|---|---|---|---|
| Chemical composition | | | | | |
| Crude protein | 154 | 98.3 | 107 | 134 | 14.1 |
| Neutral detergent fibre | 372 | 551 | 497 | 454 | 752 |
| Acid detergent fibre | 212 | 366 | 334 | 280 | 434 |
| Acid detergent lignin | 71.9 | 93.0 | 70.6 | 146 | 61.1 |
| PA (g kg$^{-}$1 Mimosa tannin equivalent) | | | | | |
| Soluble | 1.45 | 84.2 | 2.70 | 55.6 | 2.63 |
| Protein-bound | 13.8 | 71.7 | 17.7 | 19.7 | 13.5 |
| NDF-bound | 2.75 | 7.20 | 1.69 | 5.63 | 5.21 |
| Total | 18.0 | 163 | 22.1 | 80.9 | 21.4 |
| % Protein bound | 76.6 | 43.9 | 76.3 | 23.3 | 66.9 |
| % NDF bound | 15.3 | 4.48 | 6.04 | 7.06 | 19.0 |

Source: Baloyi *et al*. (2001); FSS: fine stem stylo; PA: proanthocyanidin

### 1.5.4. Feeding value of cowpea

Singh and Tarawali (1997) points out that cowpea is an equally important fodder for livestock. It has a high nutritive value, with the grain, leaves and haulms having a crude protein content ranging from 22 to 30% (Bressani, 1988; Nielsen *et al*., 1997), and from 13 to 17% in the haulms with low fibre concentration and a high digestibility (Tarawali *et al*., 1997; Singh *et al*., 2003). Koralagama *et al*. (2008) concluded that the utilization of cowpea haulms was a cost effective strategy of increasing livestock animal production in rural areas compared to the use of concentrate feeding. Sollenberger, *et al*. (2004) reported that cowpea had greater CP (160 g kg$^{-1}$ DM) and *in vitro* organic matter digestibility (IVOMD) (600 g kg$^{-1}$ DM) than bahia grass (*Paspalum notatum*) (110 g kg$^{-1}$ DM CP and 490 g kg$^{-1}$ DM).

Vendramini *et al*. (2012) highlighted the benefits of warm-season legume intercropped in pasture grazing systems for beef cattle producers. Foster *et al*. (2009c), showed the importance of cowpea to cattle producers in mid-to late-summer in contributing nitrogen when cowpea was used as a supplement in the south-eastern USA. In addition, Foster *et al*. (2009a; 2009b; 2009c) demonstrated that cowpea supplementation was similar to soybean

(*Glycine max* (L.) Merr.) meal supplementation at maintaining nitrogen retention in lambs fed basal bahia-grass haylage or hay at modest dietary CP levels. Cowpea has been used successfully as forage for wildlife in Florida (Vendramini *et al*., 2012). The same author observed increased interests of intercropping cowpea on existent bahia-grass pastures for beef cattle due to their fast regrowth attributes after defoliation and superior nutritive value. Several authors reported an improvement in nutrient supply and growth of livestock on a cowpea haulm diet over the use of low quality forages alone but degree of weight change varied relative to total nutrient supply (Schlecht *et al*., 1995; Ngwa and Tawah, 2002; Baloyi *et al*., 2006; 2008). Anele *et al*. (2010) argues that there are limited numbers of studies that report the specific cowpea variety and the corresponding animal response and in the few cases reported, there were variations in different cowpea lines used, as well as its associated forage quality. Singh *et al*. (2003) reported greater weight gain in rams that were supplemented with the cowpea haulms of variety IT90K-277-2 compared to Dan Ila; and Akinlade *et al*. (2005) reported that cowpea haulms of variety IT96D-716 increased milk yield when used to supplement cows compared to cowpea haulms of variety 994-DP.

According to Grings and Tarawali, 2010, the importance of using legume fodder as supplement is that it boosts the nitrogen content of the feed, which in turn allow microbes to utilize the feed. Supplementation also increases the digestibility of poor quality forage due to the addition of more readily digestible fibre of legumes versus grass. Chekeredza *et al*. (2002) found a 22.7% increase in microbial protein supply when cowpea haulms were supplemented to maize stover. The minimum requirement of CP for maintenance is between 70 to 80 g $kg^{-1}$ DM for ruminants, whereas high producing animals like dairy cows requires between 130 to 140 g CP $kg^{-1}$ DM (Meissner, 1997).

### 1.5.5. Supplementation of poor quality roughages with forage legumes

Ruminant animals in Africa thrive under poor nutritional conditions and systems, utilize feedstuff from crop residues and poor native pastures (Osuji and Odenyo, 1997). Available forage and pasture tends to decline in quality and quantity during the dry seasons (Savadogo *et al*., 2000). According to McDonald *et al*. (2002), forages with a high content of slowly or indigestible cell wall structures are poorly digested and thus the intake drops. Consequently, livestock lose body weight due to deficiency of nitrogen, digestible energy and elements such as sulphur, and sometimes phosphorus, sodium, calcium, zinc, iodine, cobalt or copper in the feed (Chekeredza *et al*., 2002; McDonald *et al*., 2002). Since the available feed resources are very poor in terms of nutrient availability; their nutritional value needs to be improved to achieve high production performance in livestock. Ratio of Ca: P should be within the range of 1:1 or 2:1, although there is evidence that ruminants tolerate rather higher ratios, provided phosphorus requirements are met (McDonald *et al*., 2002). Simbrarashe *et al*. (2015) reported P and Ca concentrations of 3.2 g $kg^{-1}$ DM and 3.8 g $kg^{-1}$ DM, respectively. Anele *et al*. (2011) recorded Ca and P concentrations of 13.34 and 2.79 g $kg^{-1}$ DM in the wet season for commercial cowpea and further states that, Ca and P make up to 70% of total mineral elements in the animal's body. Minerals also play important roles in the optimum function of the rumen microorganisms responsible for the digestion of plant cellulose in the rumen. Furthermore, they also assist in the utilization of feed energy and in protein metabolism (McDowell *et al*., 1993). McDowell *et al*. (1993), recommends 3g $kg^{-1}$ DM Ca for ruminants animals in warm, wet climates.

Several authors concluded that forage legumes supplemented to low quality roughages, such as maize stover and hay, improves feed quality (Koralagama *et al*., 2008; Ravhuhali, 2010; Vendramini *et al*., 2012). This is because forage legumes contain proteins, vitamins and minerals that are essential for rumen micro-organism growth which degrade feedstuffs prior to gastric and intestinal digestion by the host animal (Ravhuhali, 2010).

### 1.5.6. Effect of supplementation on intake and digestibility

The feeding value of legumes, including cowpea hay has long been recognized, as it has been used extensively for all kinds of livestock in Africa (Thompson *et al.*, 1988). Most legumes may be fed to livestock animals as fresh herbage or conserved as hay. The principal value of legume hay lies in its greater percentage of digestible protein than grass hay or low quality feedstuff. Leng *et al.* (1992) suggested that the role of cowpeas in ruminant diets can be seen as three fold, firstly, as a nitrogen and mineral supplement to enhance fermentation and microbial growth efficiency. It can be a source of post-ruminal protein for digestion. Mupangwa *et al*. (2000) indicated that dry matter digestibility was greater for legume hays. The organic matter digestibility ranged from 58% for cassia hay to 62% for stylo hay and there were no differences among the legume hays.

Cowpeas are also total feeds because they supply essential nutrients needed to meet both the maintenance and production needs for the animal, as well as meeting the quantity needs. Tarawali *et al.* (1997) found the cowpea species valuable after studying the use of haulms as fodder in various places globally. Several authors (N'Jai, 1998; Singh *et al.,* 2003; Singh *et al.,* 2006) have also described the use of cowpea residues as a supplement to low quality roughages in animal production. The level of supplement required varies on the quality of the basal diet used (Norton *et al.,* 1992). Singh *et al.* (2003) also found that incremental levels of cowpeas as a supplement to poor quality roughages indicate that they are valuable to animals. When cowpeas were fed to lambs under dry lot conditions the animals gained 80 g per animal per day with 200 – 400 g per day of cowpea haulms as a supplement to a basal diet of sorghum stover (Singh *et al.,* 2003). Animals receiving an oat hay-corn- soybean diet also reportedly had increased live weight gain (Thompson *et al.,* 1988).

On the value of legume hays for dairy heifers, Dvorachek (1929) found that cowpea hay was equal to other leguminous hays (Lucerne hay) for producing body weight gains on dairy heifers. Cowpea hay was not as palatable, nor was it consumed with as little waste as alfalfa hay. Cowpea provides adequate mineral source when fed to animals in high amounts. However, animals may require supplementation in dry feeds deficient in minerals with 20 to 30% of the total dry matter intake of cowpea (Goodchild and McMeniman, 1994). Table 1.5 shows dry matter intake and digestibility of legumes used to supplement hay whereas the dietary intake digestibility, live weight changes of Pedi goats supplemented with different levels of cowpea cultivars are shown in Table 1.6.

**Table 1.5** Average dry matter intake and apparent digestibility of legumes used to supplement hay

| | Treatment diet | | | | |
|---|---|---|---|---|---|
| Trait | V | VU | VC | VS | VF |
| N (%) | 4 | 5 | 6 | 6 | 6 |
| Veld hay (g $day^{-1}$) | 327 | 422 | 452 | 446 | 453 |
| Legume hay (g $day^{-1}$) | - | - | 141 | 154 | 196 |
| Total DM intake (g $day^{-1}$) | 327 | 422 | 593 | 601 | 649 |
| Crude Protein intake (g $day^{-1}$) | 4.58 | 19.4 | 28.0 | 21.3 | 27.3 |
| Total DM intake ($kg^{-1}W^{0.75}day^{-1}$) | 32.8 | 44.2 | 55.9 | 53.3 | 55.6 |
| DMD | 0.541 | 0.514 | 0.557 | 0.497 | 0.507 |
| OMD | 0.631 | 0.490 | 0.583 | 0.564 | 0.519 |

Source: Baloyi *et al*. (2008); V: veld hay; VU: veld hay sprayed with 10g kg-1 urea; VC: veld cowpea; VS: Silverleaf desmodium; VF: fine stem stylo; DMD: dry matter digestibility: OMD: organic matter digestibility

**Table 1.6** Dietary intake, digestibility and live weight changes of Pedi goats supplemented with different levels of four cowpea cultivars fed on *ad libitum* buffalo grass hay

| | Treatments | | | |
|---|---|---|---|---|
| | Pan 311 | Red colona | Agripes | Black eye |
| Variable | 161g goat$^{-1}$day$^{-1}$ | 159g goat$^{-1}$day$^{-1}$ | 148g goat$^{-1}$day$^{-1}$ | 119g goat$^{-1}$day$^{-1}$ |
| Intake(g goat$^{-1}$day$^{-1}$) | | | | |
| DM | 501 | 510 | 823 | 821 |
| OM | 449 | 459 | 288 | 280 |
| CP | 48 | 42 | 48 | 88 |
| NDF | 271 | 284 | 180 | 186 |
| ADF | 171 | 178 | 113 | 102 |
| Intake (g kg$^{-1}$ W$^{-0.75}$) | | | | |
| DM | 53.9 | 55.4 | 35.2 | 37.3 |
| OM | 48.2 | 51.9 | 31.2 | 32.6 |
| CP | 5.17 | 4.57 | 4.69 | 4.42 |
| NDF | 29.2 | 30.9 | 19.6 | 21.6 |
| ADF | 18.4 | 18.8 | 12.3 | 11.9 |
| Digestibility coefficient | | | | |
| DM | 0.67 | 0.70 | 0.66 | 0.65 |
| OM | 0.78 | 0.78 | 0.65 | 0.64 |
| CP | 0.74 | 0.71 | 0.70 | 0.66 |
| NDF | 0.51 | 0.45 | 0.42 | 0.40 |
| ADF | 0.36 | 0.84 | 0.32 | 0.30 |
| Live weight changes | | | | |
| Initial (kg) | 18.50 | 18.37 | 18.50 | 17.07 |
| Final (kg) | 19.58 | 19.27 | 19.20 | 17.63 |
| Weight gain (g goat$^{-1}$day$^{-1}$) | 206 | 180 | 140 | 112 |

Source: Ravhuhali, (2010); DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre

# CHAPTER 2

## Chemical composition and *in vitro* gas production of cowpea varieties in the Limpopo Province, South Africa

**Abstract**

Twelve varieties of cowpea grown under rain fed conditions in the Limpopo province, South Africa were evaluated for their chemical composition, *in vitro* gas production and *in vitro* organic matter digestibility (IVOMD) attributes. Forage material of each variety was harvested from a 4 x 4 m plot at 100% flowering stage and sub-samples were taken in duplicate for determination of chemical composition, *in vitro* gas production and IVOMD. The crude protein (CP) content, neutral detergent fibre (NDF), acid detergent lignin (ADL) and non-fibrous carbohydrates were significantly ($P < 0.05$) different among the varieties, whereas organic matter content was not significantly ($P > 0.05$) different. There was a significant ($P < 0.05$) variation among varieties in terms of IVOMD, metabolisable energy (ME) and mineral concentrations (calcium and phosphorus). Generally, CP and ME contents of the cowpea varieties were above the minimum concentration level required to meet the requirement of ruminant animal for maintenance purpose, while fibre content (NDF) was below the upper limit of 60% which reduces intake. Greater CP content (126 to 216 g $kg^{-1}$ DM), IVOMD (475 to 653 g $kg^{-1}$ DM) and *in vitro* gas production (78.3 to 88.2 mL 400 $mg^{-1}$ DM) indicates the potentials of these varieties to provide good quality forage material during the time of utilization, as well as for strategic supplementation of nitrogen to improve poor quality forage utilization during the dry-season. Thus, of the twelve varieties studied, the following three varieties: Bechuana White, IT 97K-499-35 and TX 08-30-1 were ranked at the top as they have shown the greatest IVOMD, gas production and CP concentration respectively. Hence, these varieties have good potential as source of good quality forage and nitrogen supplement. However, these varieties need to be tested further in terms of their feeding value using *in vitro* and *in vivo* studies.

**Keywords:** *in vitro* digestibility; ruminal fermentation; *Vigna unguiculata*

## 2.1. Introduction

Livestock farming is the main activity for the pastoral community while also serving the purpose of providing social and cultural values (Onono *et al*., 2013). Livestock production under these systems is normally sustained by natural pastures while in the mixed crop-livestock system, crop residues are also major sources of feed (Rebero and Mupenzi, 2012). These feed resources are, however, prone to seasonal variation and during the dry-season the roughages are poor in quality, often inadequate to meet animal nutritional requirements (Mendieta-Araica *et al*., 2009; Kumar *et al*., 2015). According to Thomas and Sumberg (1995), poor quality and feed scarcity are major factors responsible for poor livestock performance during dry-season period. Due to these, livestock lose body weight with increasing rate of mortality (Ajayi *et al*., 2005).

Feed shortages has been characterized by several authors as inadequate forage supply and/ or low forage quality (high fibre and/ or low protein). This, as well as poverty and inequity, presents some challenges to the development of smallholder farms in South Africa (Ralevic *et al*., 2010; Sharma *et al*., 2010; Moebius-Clune *et al*., 2011). In addition to that, the necessity to buy feeds and the degradation of rangelands have persistently put pressure on resource-poor small-holder subsistence farmers to shift their focus to small ruminants (Ben Salem and Smith 2008; Valbuena *et al*., 2012). Therefore, it is very important when evaluating forage legumes to select for those species and varieties that are adaptable, high in forage yield, better in feed quality ( high protein content, energy and digestibility) and also acceptable in terms of time taken to reach maturity when selecting alternative forage as animal feed for specific areas (Franzel *et al*., 2014). During the dry-season, inclusions of energy and protein sources in the diet are of significance importance in ruminant nutrition due to their ability to stimulate rumen micro-organisms and to enhance production of the animals (Wanapat, 2008). However, forage legumes from shrubs, trees and leguminous fodders such as cowpea have been used to improve livestock production in the savanna regions due to their ability to remain green and maintain their protein content during the dry season (Olafadehan and Adewuni, 2009; Olafadehan and Okunade, 2016).

Cowpea, known for its stress tolerance and wide adaptation traits, is a grain legume, fodder crop and vegetable that is grown in regions ranging from warm to hot in about an area of 7 million ha (Ehlers and Hall, 1997). Sixteen African countries are known for their extensive cowpea production and of this, Niger and Nigeria account for two-thirds of the world cowpea production at 271 000 and 850 000 tons per year respectively, which in proportion represents 49% of the global crop (FAO, 2000). Cowpea is drought tolerant, thrives well under low fertility soils and fixes atmospheric nitrogen in the soil (Ehlers and Hall, 1997; Etana *et al*., 2013). Cowpeas have a potential to produce dry matter of 2000 and 3000 kg DM $ha^{-1}$ in dryland areas and up to 8000 kg DM $ha^{-1}$ under irrigation (FAO, 2009). Crude protein in the crop residue and forage ranges from 10 to 25% and it reaches up to 30% when the forage is harvested at early flowering stage (Smith *et al*., 2008). The objective of the present study was to evaluate the potential nutritive value of twelve cowpea varieties grown in the Limpopo province of South Africa. This was done by determining their chemical composition and *in vitro* organic matter digestibility of the cowpea forage.

## 2.2. Materials and methods

### 2.2.1. Location

The varieties of cowpeas that were used in the study were obtained from an anonymous farm in the Limpopo Province of South Africa with latitudes of 24°32'58.1"S 28°06'21.1"E and elevation of 1237 metres above seal level (a.s.l) near Modimolle in the Waterberg district of Limpopo Province. These were the new cowpea varieties developed by Texas A&M University and by The Institute of Tropical Agriculture (ITTA), as well as local cultivars; and were agronomically tested at this farm. The farm is geographically located at 24°32'58.1"S 28°06'21.1"E at an elevation of 1237 metres above seal level (a.s.l) near Modimolle in the Waterberg district of Limpopo Province.

The area is characterised as a mineral-rich region known as Waterberg Massif, containing one of the world's largest platinum deposits. Soil in this farm is classified as sandy clay loam, derived from sandstone, quartzite and shale (Fenta, 2012). The area has mild temperatures ranging from 14 to 30$^{o}$C and an annual rainfall of 623 mm (Fenta, 2012).

### 2.2.2. Forage establishment and management

Twelve different cowpea varieties were evaluated as experimental treatments in this study. The varieties were as follows TX 08-30-1, GEC, IT 98K-598-2, TX 08-49-1, IT 86D1010, PAN 311, IT 97K-499-35, IT98K-205-8, IAR 48, IT 98K-491-4,IT 98K-391-2 and Bechuana White. The 12 varieties were replicated 4 times in a completely randomized block design

(CRBD) in a plot size of 4m x 4m with inter-row spacing of 0.75m. Dual (Metachlor) was applied at the rate of 0.5 litres per hectare as a pre-emergent herbicide during planting. The cowpea fodders were harvested approximately 4 months after planting at maturity (pod yellowing) and after pod harvest. An area of 0.75m by 0.75m was harvested per plot and plants were harvested to ground level. After the harvesting, the above ground forage material was shade dried in order to achieve partial drying before transporting it to the University of Pretoria.

### 2.2.3. Chemical composition

Upon on their arrival at the Nutrition Laboratory, University of Pretoria, the forages were further oven-dried at 55 $^{o}$C for 48 hours. Forage samples were grounded thereafter to pass through a 1 mm sieve in a Wiley mill (Arthur H. Thomas, Philadephia, PA, USA). Dry matter content (DM) was determined by oven drying samples at 105 $^{o}$C for 16 hours, and thereafter were ignited in muffle furnace at 550 $^{o}$C for 4 hours to determine ash (AOAC, 2000) method 942.05.

Determination of acid detergent fibre (ADF) and neutral detergent fibre **(**NDF) were done by following the procedure described by Van Soest *et al*., (1991). An assay on the NDF was performed without sodium sulphite and α-amylase and both NDF and ADF were expressed without residual ash. Acid detergent lignin (ADL) determination was done by solubilisation of cellulose with sulphuric acid in the ADF residue (Van Soest *et al*. 1991). The non-fibre carbohydrate (NFC) was calculated as follows: NFC = 100 – CP – ash – EE – NDF and the variables were expressed as g kg$^{-1}$ DM (Sniffen *et al*. 1992). The difference between NDF and ADF was used to estimate hemicellulose since both NDF and ADF were assayed serially on the same sample, while for cellulose estimation, the difference between ADF and ADL was used.

### *In vitro* organic matter digestibility determination

The Tilley and Terry (1963) method, modified by Engels and Van der Merwe (1967) was used in determination of the IVOMD. Rumen fluid collection was done before the morning feeding from two Merino wethers (70 kg body weight) with cannulas. They were fed lucerne (*Medicago sativa*) hay *ad libitum* and the quantity collected was approximately 500 mL. Two digestion phases were involved in this method and 200 mg samples of feed, replicated in triplicate with standards and blanks in every batch were incubated with rumen liquor under anaerobic conditions for 48h at 39 $^{o}$C in the first phase of digestion. The next phase involved acid pepsin digestion for 48h at 39 $^{o}$C, under anaerobic incubation conditions and terminated after 48h. Thereafter, the collected residual plant materials were oven-dried for 12h at 105 $^{o}$C. Combustion at 550 $^{o}$C for 2h in a muffle furnace was used to determine the ash contents (Engels and Van der Merwe 1967).

Nitrogen content of the forage was measured by Dumas technique from which crude protein (CP) was calculated as N x 6.25 (AOAC, 2000) method 994.12. Analysis of phosphorus was done using an ultraviolet spectrophotometer (Chemical lab instruments method no. 075-01, Bavaria, Germany), and Calcium by atomic absorption spectrometer (Perkin Elmer, 1982).

### 2.2.4. *In vitro* ruminal gas production procedure

#### 2.2.4.1. Buffer media preparation

Preparations of buffer solution, micro-mineral and macro-mineral solution followed the procedure of Menke and Steingass (1988). The micro-mineral was slightly modified by replacing magnesium sulphate ($MgSO_4.7H_2O$) with magnesium chloride ($MgCl_2.6H_2O$) to reduce the amount of sulphate ($SO_4$) in the media as suggested by Mould *et al*. (2005). Before the beginning of the experiment, the appropriate amount of distilled water, macro- and micro-mineral solutions and buffer solution were mixed with prepared 0.1% (wt. /vol.) resazurin and tryptose. After all chemicals had dissolved, L-cysteine hydrochloride was added directly to the solution and the buffer solution was put in a 39 $^{o}$C water bath and bubbled continuously with $CO_2$ to remove oxygen which was indicated by the clearing of the buffer solution. Rubber stoppers were used to seal the serum bottles and were left at 39 $^{o}$C.

#### 2.2.4.2. Rumen fluid collection

Rumen fluid collection was done before the morning feeding from two Merino wethers (70 kg body weight) with cannulas. They were fed lucerne (*Medicago sativa*) hay *ad libitum* and the quantity collected was approximately 500 mL. Rumen fluid collected from each donor animal was thoroughly mixed and strained through four layers of cheesecloth, and immediately transferred to a pre-warm thermos flask to preserve microbes. In the laboratory, the rumen fluid was emptied into an industrial blender and purged with $CO_2$ (Grant and

Mertens, 1992). The blended rumen fluid was transferred to a large glass beaker and purged with $CO_2$ inside a 39 $^oC$ water bath and continuously stirred as recommended by Goering and Van Soest (1970). After that, 25 mL buffer solution was mixed with 15 mL rumen fluid and added to each incubation bottle.

#### 2.2.4.3. Gas production measurement

Gas production by incubation of bottles containing cowpea forage at 39 $^oC$ was measured by using a semi-automated gas production system following the procedure of Theodorou *et al.* (1994). This system consists of a digital data tracker (Traker series 220 indicators; Omega Engineering Inc., Laval, QC, Canada) connected to a pressure transducer (PX4200-015GI; Omega Engineering Inc.) with a needle on the tip. Approximately 400 mg of each cowpea forage sample was weighed into 120 mL serum bottles, and then 40 mL rumen fluid + medium was added under a stream of $CO_2$ to each of the serum bottles closed with rubber stoppers and crimp seal caps. A needle was inserted through the rubber stopper of each serum bottle for about 5 seconds to release small amounts of gas that might have built up since the incubation started. The incubator and the rotary shaker were turned on at 120 rpm after the placement of all serum bottles to mark the onset of incubation. Gas pressure measurements were taken at 2,4,8,12,24 and 48 hours of incubation. Three blanks were included in each analysis in order to quantify the gas production derived from each culture medium and the rumen inoculums. Three replicates were used in each run and four runs conducted. Transducer readings were correct for blanks and thereafter gas pressure was converted in gas volume (mL) by using Boyle's law relationship as described by Mauricio *et al*. (1999),

$$\text{Gas volume (mL)} = \frac{Vh}{Pa} \times Pt$$

Where Pa is the atmospheric pressure (psi); Vh is the volume of head space in the serum bottle (mL); Pt is the reading from pressure transducer attached to a data pressure (psi).

Fermentation was terminated after 48 hours by removing serum bottles from the incubator and placing them on ice.

### 2.2.5. Calculations and statistical analysis

Data for the chemical composition and *in vitro* fermentation of the cowpea varieties were statistically analysed using the general linear models (GLM) procedure of SAS (2002). The main effect was cowpea variety. Where the F-test has shown significant difference, the means were separated by using Tukey test. To fit non-linear regression models using the NLIN procedure, *in vitro* incubation times were used (SAS 2002)
Calculation of Non-fibrous carbohydrate was as follows:

NFC = 100 – (CP + fat + ash + (NDF – NDIN))
Where NFC = Non-fibrous carbohydrates, CP = Crude protein, NDF = Neutral detergent fibre, NDIN = Neutral detergent insoluble nitrogen

Metabolisable energy (ME, MJ $kg^{-1}$ DM) was estimated according to Menke and Steingass (1988) as:
ME (MJ$kg^{-1}$ DM) = 2.20 + 0.136 IVGP24 (mL per 0.4 g DM) + 0.057 CP (%DM)
Where ME = Metabolisable energy, IVGP24 = *in vitro* gas production over 24 h, CP = Crude protein.

The rate and extent of gas production was determined for each of the forage legume species by fitting gas production data to the non-linear equation (Ørskov and Mcdonald 1979):
$Y = b\ (1-e^{-ct})$
Where Y is gas production at time t; b is gas production (volume) associated with the insoluble but slowly fermentable fraction (g $kg^{-1}$ DM); and c is the rate (% $h^{-1}$) of fermentation of fraction b. Effective gas production (EGP) was estimated using the Ørskov and McDonald (1979) equation, assuming the flow rate constant (k) of 0.05 $h^{-1}$. EGP = b*c / (k + c).

## 2.3. Results

### 2.3.1. Plant chemical composition and *in vitro* organic matter digestibility

Chemical composition and *in vitro* organic matter digestibility of cowpea varieties grown in the Limpopo province of South Africa are presented in Table 2.1. The cowpea varieties did not show significant differences ($P > 0.05$) in terms of OM concentrations. However, the cowpea varieties were significantly different ($P < 0.05$) in terms of all other studied parameters. The CP concentrations in this study ranged between 126 g $kg^{-1}$ DM and 216 g $kg^{-1}$ DM. The greatest CP concentration was for TX -08-30-1, while both PAN 311 and IT 86D 1010 had the least CP of 126 and 132 g $kg^{-1}$ DM, respectively, the remaining varieties were in between.

Among cowpea varieties, PAN 311 had the greatest NFC (475 g $kg^{-1}$ DM), whereas, varieties TX -08-30 and IT 98K-205-8 had the least NFC values of 374 g $kg^{-1}$ DM and 369 g $kg^{-1}$ DM, respectively. Variety IT 86D 1010 recorded the greatest NDF (522 g $kg^{-1}$ DM), while Bechuana White recorded the least NDF (414 g $kg^{-1}$ DM). Among the studied cowpea varieties, ADF ranged between 367 g $kg^{-1}$ DM and 455 g $kg^{-1}$ DM, with Bechuana White recording the least ADF value (367 g $kg^{-1}$ DM) and IT 98K-205-8, the greatest (455 g $kg^{-1}$ DM). However, varieties IT 98K-598-2, IT 86D 1010, IT 97K-499-35 and IT 98K-391-2 did not significantly ($P < 0.05$) differ from IT 98K-205-8 in terms of ADF concentrations. The greatest ADL value was recorded for IT 98K-205-8 (119 g $kg^{-1}$ DM) and TX 08-30-1 (120 g $kg^{-1}$ DM), whereas the lowest ADL value was recorded for Bechuana White. The IVOMD ranged between 475 g $kg^{-1}$ DM and 653 g $kg^{-1}$ DM. The greatest value was observed for Bechuana White, while IT 98K-205-8 had the least IVOMD value.

**Table 2.1** Mean chemical composition and IVOMD (g kg$^{-1}$ DM) of cowpea varieties grown in Limpopo province.

| | **Chemical components (g kg$^{-1}$ DM)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Variety** | OM | CP | NFC | NDF | ADF | ADL | IVOMD | Hemice | Cellulose |
| TX 08-30-1 | 828 | $216^{a}$ | $374^{ef}$ | $454^{d}$ | $390^{bc}$ | $120^{a}$ | $520^{bcde}$ | $64.1^{abc}$ | $270^{c}$ |
| GEC | 840 | $199^{ab}$ | $384^{def}$ | $466^{cd}$ | $401^{b}$ | $110^{b}$ | $531^{bcd}$ | $65.3^{abc}$ | $291^{bc}$ |
| IT 98K-598-2 | 847 | $138^{ef}$ | $399^{cd}$ | $514^{ab}$ | $450^{a}$ | $89^{f}$ | $564^{b}$ | $63.5^{abc}$ | $361^{a}$ |
| TX 08-49-1 | 830 | $186^{b}$ | $408^{c}$ | $448^{d}$ | $378^{bc}$ | $110^{b}$ | $552^{bc}$ | $69.8^{abc}$ | $268^{c}$ |
| IT 86D 1010 | 841 | $132^{f}$ | $403^{cd}$ | $522^{a}$ | $447^{a}$ | $110^{b}$ | $488^{eb}$ | $74.2^{ab}$ | $338^{a}$ |
| PAN 311 | 837 | $126^{f}$ | $475^{a}$ | $465^{cd}$ | $385^{bc}$ | $103^{d}$ | $554^{bc}$ | $80.4^{a}$ | $282^{bc}$ |
| IT 97K-499-35 | 835 | $163^{cd}$ | $407^{cd}$ | $490^{bc}$ | $410^{a}$ | $107^{c}$ | $567^{b}$ | $79.7^{a}$ | $303^{b}$ |
| IT 98K-205-8 | 841 | $162^{cd}$ | $369^{f}$ | $513^{ab}$ | $455^{a}$ | $119^{a}$ | $475^{e}$ | $58.0^{bcd}$ | $336^{a}$ |
| IAR 48 | 836 | $194^{b}$ | $395^{cde}$ | $456^{d}$ | $386^{bc}$ | $92^{e}$ | $507^{cde}$ | $70.4^{abc}$ | $294^{bc}$ |
| IT 98K-491-4 | 836 | $180^{bc}$ | $404^{cd}$ | $446^{d}$ | $387^{bc}$ | $91^{ef}$ | $555^{bc}$ | $58.7^{bcd}$ | $296^{bc}$ |
| IT 98K-391-2 | 842 | $145^{efd}$ | $395^{cde}$ | $495^{b}$ | $441^{a}$ | $90^{ef}$ | $522^{bcde}$ | $53.1^{cd}$ | $351^{a}$ |
| Bechuana White | 832 | $156^{de}$ | $452^{b}$ | $414^{e}$ | $367^{c}$ | $85^{g}$ | $653^{a}$ | $46.7^{d}$ | $282^{bc}$ |
| ***SEM*** | 5.6 | 6.7 | 7.0 | 8.4 | 9.8 | 0.9 | 0.99 | 5.20 | 9.9 |
| ***P- value*** | *>0.000* | *<0.000* | *<0.000* | *<0.000* | *<0.000* | *<0.000* | *<0.000* | *<0.000* | *<0.000* |

Means with different letters (superscripts) within columns differ significantly at $P < 0.05$; ADF: acid detergent fibre; ADL: acid detergent lignin; CP: crude protein; Hemice: hemicellulose; IVOMD: *in vitro* organic matter digestibility; NDF: neutral detergent fibre; NFC: non-fibre carbohydrates

### 2.3.2. Metabolisable energy, calcium, phosphorus and calcium to phosphorus ratio

Table 2.2 shows the metabolisable energy, calcium, phosphorus and calcium to phosphorus ratio values of 12 cowpea varieties evaluated in this study. Generally, the cowpea varieties differed in terms of their ME values. Variety TX -08-30-1 had the greatest ME value of 8.83 MJ $kg^{-1}$ DM, while IT 86D 1010 had the least ME value of 7.6 MJ $kg^{-1}$ DM.

Calcium and phosphorus values of the studied cowpea varieties differed ($P < 0.05$) significantly. Calcium concentrations ranged from 2.55 g $kg^{-1}$ DM to 4.85 g $kg^{-1}$ DM with IT 86D 1010 variety having the least and TX -08-30-1, the greatest value. The greatest P value was recorded for Bechuana White (15.7 g $kg^{-1}$ DM), while the least phosphorus value was recorded for IT 98K-205-8 at 11.2 g $kg^{-1}$ DM.

In terms of Ca: P ratio, the cowpea varieties differed significantly ($P < 0.05$), with both IT 86D 1010 and PAN 311 having a ratio of 1: 5.3 Ca: P, whereas TX -08-30-1 a Ca: P ratio of 1: 2.5.

**Table 2.2** Calculated metabolisable energy, calcium, phosphorus and calcium: phosphorus ratio of selected cowpea varieties used in this study

| | | Main mineral (g kg$^{-1}$ DM) | | |
|---|---|---|---|---|
| **Variety** | ME (MJ kg$^{-1}$ DM) | Ca | P | (Ca : P) |
| TX-08-30-1 | 8.83$^{a}$ | 4.85$^{a}$ | 12.2$^{bc}$ | 1 : 2.5$^{de}$ |
| GEC | 8.57$^{ab}$ | 3.82$^{bcd}$ | 11.6$^{bc}$ | 1 : 30$^{cd}$ |
| IT98K-598-2 | 7.88$^{ab}$ | 3.25$^{cde}$ | 11.3$^{bc}$ | 1 : 3.5$^{c}$ |
| TX08 -491-1 | 8.81$^{a}$ | 4.14$^{abc}$ | 12.8$^{abc}$ | 1 : 3.1$^{cd}$ |
| IT 86D1010 | 7.60$^{b}$ | 2.55$^{e}$ | 13.5$^{abc}$ | 1 : 5.3$^{a}$ |
| PAN311 | 7.76$^{ab}$ | 2.57$^{e}$ | 13.7$^{ab}$ | 1 : 5.3$^{a}$ |
| IT97K -499-35 | 8.17$^{ab}$ | 4.33$^{ab}$ | 13.9$^{ab}$ | 1 : 3.2$^{c}$ |
| IT98K -205-8 | 8.24$^{ab}$ | 4.31$^{ab}$ | 11.2$^{c}$ | 1 : 2.4$^{e}$ |
| IAR 48 | 8.57$^{ab}$ | 4.02$^{abcd}$ | 13.6$^{abc}$ | 1 : 3.4$^{c}$ |
| IT98K -491-4 | 8.62$^{ab}$ | 3.91$^{bcd}$ | 12.9$^{abc}$ | 1 : 3.3$^{c}$ |
| IT98K -391-2 | 8.06$^{ab}$ | 3.91$^{de}$ | 14.1$^{ab}$ | 1 : 4.4$^{b}$ |
| Bechuana White | 8.44$^{ab}$ | 3.45$^{bcde}$ | 15.7$^{a}$ | 1 : 4.6$^{b}$ |
| *SEM* | 7.498 | 0.156 | 0.65 | 0.18 |
| *P- value* | <0.000 | <0.000 | <0.000 | <0.000 |

Means with different letters (superscripts) within columns differ significantly at indicated *P* value, $P < 0.05$; ME: metabolisable energy; Ca: P: calcium to phosphorus ratio; Ca: calcium; P: phosphorus; SEM: standard error of the mean

### 2.3.3. *In vitro* gas production characteristics

Figure 2.1 shows the total gas production of cowpea varieties over 48hrs of incubation, which represents the extent of *in vitro* ruminal fermentation of different varieties of cowpea forages over 48 hr time. Bechuana White and IT 97K-499-35 had greater *in vitro* gas production at 48 hour incubation times while TX -08-30-1 and GEC were relatively the least fermentable varieties amongst the studied cowpea varieties.

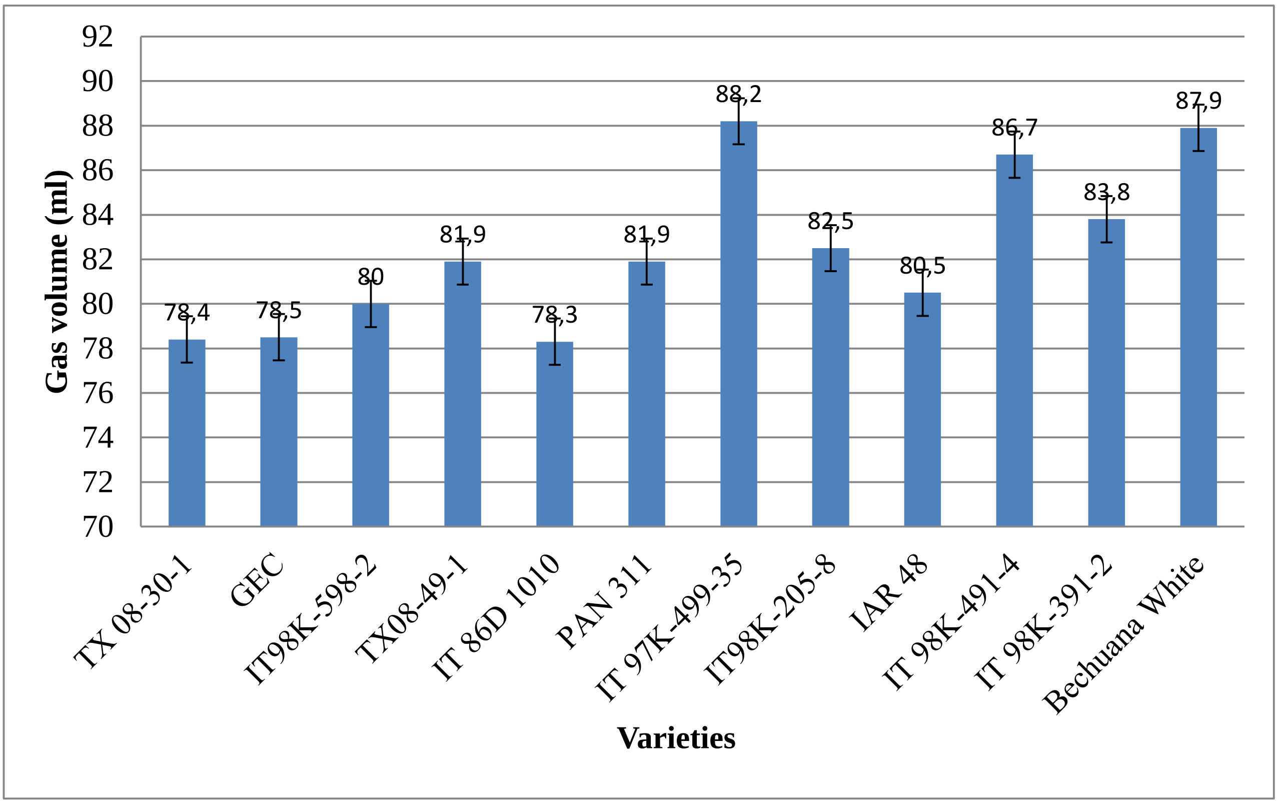


**Figure 2.1** Cumulative gas volume produced from cowpea varieties incubated after 48hrs

Figure 2.2 shows gas production pattern of cowpea varieties over time of incubation. The cowpea varieties significantly differed in their potential fermentation attributes. At early incubation periods (2-12 hr.), TX 08 -491-1 had the greatest volume of gas, while at late incubation periods (12 – 24hr.), Bechuana White consistently produced the greatest volume of gas. However, there was no difference between gas volume recorded for Bechuana White and IT 98K-491-4 and IT 97K-499-35 at 48 hr incubation. TX -08-30-1, IT 86D1010 and GEC were the least fermentable of the cowpea varieties at all incubation periods.

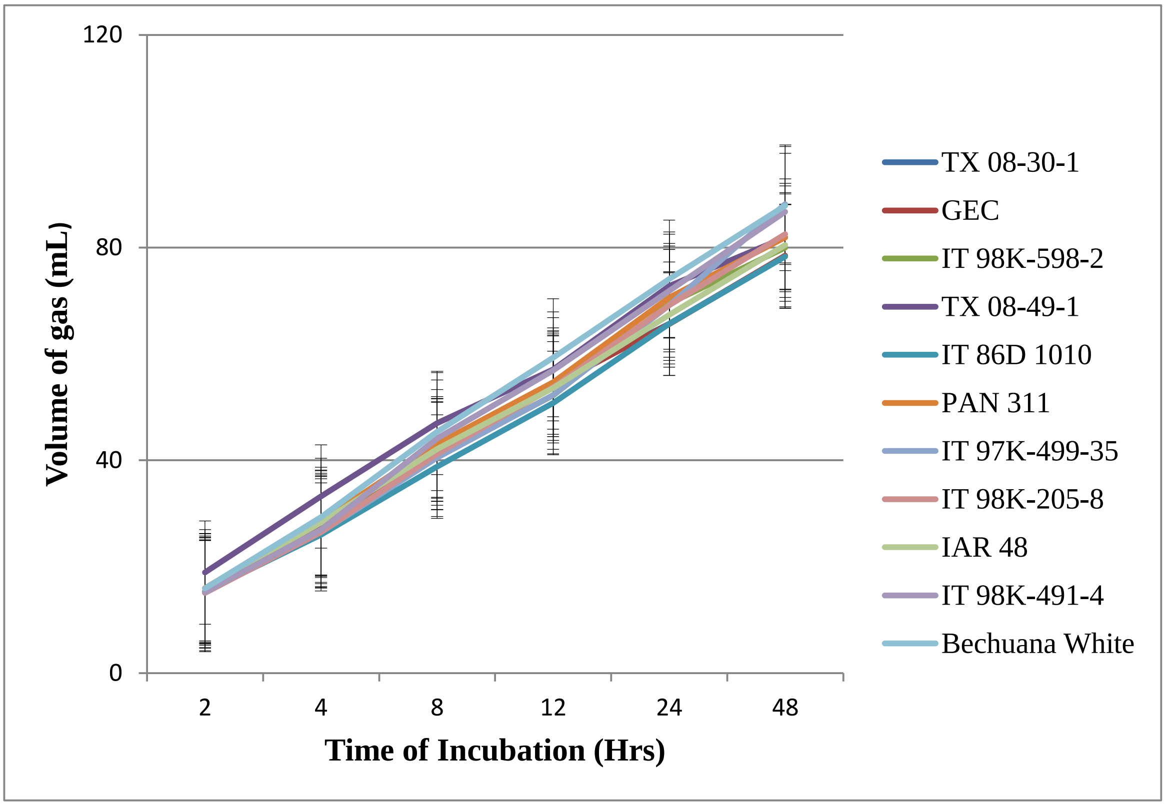


**Figure 2.2** Gas production patterns of cowpea varieties used in this study

### 2.3.4. *In vitro* gas production kinetics

*In vitro* gas production kinetics of cowpea varieties grown in the Limpopo province of South Africa are shown in Table 2.4 Cowpea varieties generally had lesser insoluble but slowly fermentable fraction and greater rate of gas production compared to grass hay. However, among the cowpea varieties, IT 97K-499- 35, IT 98K-491-4 and Bechuana White had greater insoluble but slowly fermentable fraction compared to IAR 48.

Studied cowpea varieties differed significantly ($P < 0.05$) to grass hay in terms of the effective gas production (EGP). There was also a significant ($P < 0.05$) difference of EGP between the cowpea varieties. Bechuana White had the greatest EGP whereas IT 97K-499-35 had the least EGP.

**Table 2.3** *In vitro* gas production kinetics of cowpea varieties used in the study

| Treatment | b (ml) | c (ml) | EGP (ml 400 $mg^{-1}$) |
|---|---|---|---|
| Grass hay | $170.5^{a}$ | $0.01^{f}$ | $33.0^{g}$ |
| TX 08-30-1 | $73.6^{bc}$ | $0.09^{abc}$ | $25.3^{bc}$ |
| GEC | $73.0^{bc}$ | $0.09^{abc}$ | $46.6^{de}$ |
| IT 98K-598-2 | $76.5^{bc}$ | $0.08^{abcd}$ | $47.1^{cd}$ |
| TX 08-49-1 | $73.9^{bc}$ | $0.09^{a}$ | $48.0^{cd}$ |
| IT 86D 1010 | $73.6^{bc}$ | $0.07^{d}$ | $44.1^{f}$ |
| PAN 311 | $77.2^{bc}$ | $0.08^{abcd}$ | $48.4^{c}$ |
| IT 97K-499-35 | $84.6^{b}$ | $0.06^{e}$ | $18.1^{d}$ |
| IT 98K-205-8 | $78.8^{bc}$ | $0.08^{cd}$ | $48.0^{cd}$ |
| IAR 48 | $70.8^{c}$ | $0.09^{ab}$ | $45.1^{ef}$ |
| IT 98K-491-4 | $83.3^{b}$ | $0.08^{bcd}$ | $51.5^{ab}$ |
| IT 98K 391-2 | $80.5^{bc}$ | $0.08^{abcd}$ | $50.5^{b}$ |
| Bechuana White | $84.0^{b}$ | $0.09^{abcd}$ | $52.9^{a}$ |
| *SEM* | 3.44 | 0.003 | 0.49 |
| *P- value* | *<0.000* | *<0.000* | *<0.000* |

Means within a column with different letters in superscript differ significantly (*P* <0.05); *b*: insoluble but slowly fermentable fraction; c: rate of fermentation of fraction *b*; EGP: effective gas production; SEM: standard error of mean

### 2.3.5. Correlation coefficient between *in vitro* gas production and chemical composition of incubated substrates

The correlation coefficient between *in vitro* gas production and chemical composition of incubated substrates is shown in Table 2.5. *In vitro* organic matter digestibility of incubated substrates showed a significant (*P* < 0.05) positive correlation with non-fibre carbohydrates, whereas, it showed a strong negative correlation with cell wall contents (NDF, ADF and ADL). Crude protein content of cowpea varieties showed a significant (*P* < 0.05) negative correlation with organic matter, cellulose and non-fibrous carbohydrate components. However, gas volume of substrate at mid incubation hours (8 and 12hours), showed a significantly (*P* < 0.05) strong negative correlation with cell wall contents (NDF and ADF).

**Table 2.4** Correlation coefficients between *in vitro* gas production and chemical composition of incubated substrates

| Parameter | OM | CP | NFC | NDF | ADF | ADL | IVOMD | HEMIC | CELU |
|---|---|---|---|---|---|---|---|---|---|
| CP | -0.600** | _ | -0.548** | -0.491 | -0.473 | 0.331 | 0.124 | -0.117 | -0.592** |
| NFC | -0.141 | -0.548** | _ | -0.397 | -0.470 | -0.426 | 0.647** | 0.175 | -0.300 |
| NDF | 0.752** | -0.491 | -0.397 | _ | 0.951** | 0.267 | -0.631** | 0.280 | 0.834** |
| ADF | 0.793** | -0.473 | 0.470 | 0.951** | _ | 0.152 | -0.564** | -0.032 | 0.926** |
| ADL | -0.284 | 0.331 | -0.426 | 0.267 | 0.152 | _ | -0.566** | 0.388 | -0.231 |
| IVOMD | -0.292 | -0.124 | 0.647** | -0.631** | -0.564** | -0.566** | _ | -0.287 | -0.339 |
| HEMIC | -0.030 | -0.117 | 0.175 | 0.280 | -0.032 | 0.388 | -0.287 | _ | -0.180 |
| CELU | 0.889** | -0.592** | -0.300 | -0.834** | 0.926** | -0.231 | -0.339 | -0.180 | _ |
| GV2 | -0.490 | 0.152 | 0.268 | -0.399 | -0.474 | 0.097 | 0.350 | 0.181 | -0.504 |
| GV4 | -0.506 | 0.193 | 0.335 | -0.568 | -0.585* | -0.079 | 0.518 | -0.018 | -0.546 |
| GV8 | -0.470 | 0.178 | 0.369 | -0.718** | -0.618* | -0.342 | -0.483 | -0.402 | -0.478 |
| GV12 | -0.567 | 0.250 | 0.380 | -0.849** | -0.695** | -0.334 | 0.544 | -0.587* | -0.556 |
| GV24 | -0.175 | -0.305 | -0.550 | -0.427 | -0.314 | -0.537 | 0.386 | -0.406 | -0.104 |
| GV48 | -0.208 | -0.184 | -0.385 | -0.344 | -0.269 | -0.437 | -0.053 | -0.276 | -0.098 |

*P < 0.05, ***P* < .001

ADF: acid detergent fibre; ADL: acid detergent lignin; Celu: cellulose; CP: crude protein; GV2: gas volume after 2 hours incubation ...., GV48: gas volume after 48 hours of incubation; Hemic: hemicellulose; IVOMD: *in vitro* organic matter digestibility; NDF: neutral detergent fibre; NFC: non-fibrous carbohydrates; OM: organic matter

## 2.4. Discussion

Lack of reliable feed supply throughout the year (especially during the dry season) in the tropical and subtropical regions of sub-Sahara Africa (SSA) is a major cause of concern for small holder subsistence farmers (Thomas and Sumberg, 1995). The available feed resources are usually high in fibre content and lower in protein content, resulting in poor fermentation and digestibility as a result of low rumen degradable nitrogen supply to the rumen microbes (Mendieta-Araica *et al*., 2009). Inclusion of forage legumes provides a protein source that improves utilization of these poor quality forages due to their ability to supply rumen degradable nitrogen to stimulate ruminal micro-organisms and subsequently to enhance animal production (Wanapat, 2008). The cowpea varieties tested in this study were found to be of good quality forage with high crude protein concentration ranging from 126 to 216 g $kg^{-1}$ DM (Table 2.1). All studied cowpea varieties had CP contents above the critical threshold level (7%) which is necessary for normal intake of dry matter and rumen functioning of the animal (Meissner, 1997; Ikhimioya, 2008). Furthermore, most of the cowpea varieties used in this study had CP values above the range of 110 – 130 g $kg^{-1}$ DM, which is a concentration adequate to support the maintenance and growth of beef cattle (Meissner, 1997; NRC, 1998; Valarini and Possenti, 2006). Some of the studied cowpea varieties were within the range of 16 – 20% CP that is considered adequate for lactating dairy animals (Poppi and McLennab, 1995; Schingoethe 1998). Cowpea varieties TX 08-30-1, IAR 48 and TX 08-49-1 had a CP concentration in the same range as those reported by Chekeredza *et al*. (2002), Baloyi *et al*. (2008), Ravhuhali (2010) and Simbarashe *et al*. (2015) who recorded CP values of 195 – 260 g $kg^{-1}$ DM, 154 g $kg^{-1}$ DM, 180 g $kg^{-1}$ DM and 226 g kg-1 DM respectively in studies conducted in South Africa and Zimbabwe. The CP concentration of some of the cowpea varieties found in the study was above the CP concentration of cowpea varieties reported in Nigeria (14.7 and 18.1 g $kg^{-1}$ DM) by Anele *et al*. (2011). Such variation in quality of forage or crop residues may be due to variation in genetic characteristics (Singh and Schiere, 1995), environment (soil characteristics, rainfall) and crop management practices (level of fertilization, plant density, stage of maturity at harvest, drying methods, and storage) between the studies (Walli *et al*., 1994). The results of this study are in agreement with those found by others elsewhere (Etana *et al*., 2013 and Geleti *et al*., 2014).

Generally, the fibre content (ADF and NDF) of the varieties tested were above the values recorded in other studies in South Africa and Zimbabwe (Chekeredza *et al*., 2002; Baloyi *et al*., 2008; Ravhuhali, 2010 and Gwanzura *et al*., 2012. However, the NDF content of the varieties tested was below the upper limit of 60%, above which dry matter intake is depressed (Meissner *et al*., 1991; Van Soest *et al*., 1991). The relatively low content of fibre among varieties may facilitate the colonization of the feed by the microbial rumen population, which in turn might induce greater fermentation rate, therefore improving digestibility and intake (Van Soest, 1994). The range of NFC content among the studied varieties showed that the varieties can be fermented or be degraded easily because NFC are easily broken down by rumen microbes and used as energy source for both the host animal and the micro-organisms to increase feedstuff digestibility (Arigbede *et al*., 2011). According to Tylutki *et al*. (2008), there is a positive relationship between NFC and ruminal ammonia ($NH_3$-N) utilization. The NFC has a significant role to play in forage digestion since N utilization by rumen microflora is related to amount of fermentable energy. We can postulate that the adequate NFC contents, especially from PAN 311 and Bechuana White could promote efficient microbial protein synthesis in the rumen by enabling better ruminal ammonia utilization that is released from feeds with greater rumen degradable protein (RDP) content, which in turn may be absorbed by the animal to enhance its production (Cabrita *et al*., 2006). According to Njidda and Nasiru (2010), high level of rumen degradable protein in feeds enhances microbial multiplication, which in turn determines the extent of fermentation. It achieves this by the enhancement of activity of cellulytic and proteolyic microbes in microbial protein synthesis, which is provided in adequate amount by legumes (Abule *et al*., 1995; Baloyi *et al*., 2008).

Calcium and phosphorus play an important role in the proper functioning of the rumen microorganisms especially those which digest cellulose, there-by influencing utilization of energy from feeds and protein metabolism amongst other functions (McDowell *et al*., 1993). The range of values recorded for Ca (2.55 to 4.85 g $kg^{-1}$ DM) in this study are above the critical level of 3 g $kg^{-1}$ DM recommended to satisfy ruminant needs in warm wet climates, except for varieties PAN 311 and IT 86 D1010 (McDowell *et al*.,1993). The calcium and phosphorus results of this study are within those found by Anele *et al*. (2011) and Simbarashe *et al*. (2015) in studies done in Zimbabwe and Nigeria. Calcium values of 3.8 and 13.34 g $kg^{-1}$ DM and phosphorus values of 3.2 and 2.79 g $kg^{-1}$ DM were found in the two countries. This represent Ca : P ratio of 1.2 : 1 and 4 : 4.78.

*In vitro* gas production provides a basis from which energy value (e.g. metabolisable energy), short-chain fatty acids and organic matter digestibility can be predicted (Gatechew *et al*., 2004). The amount of organic matter substrate fermented is influenced by protein and fibre content of the forage material (Babayemi *et al*., 2009). Cowpea varieties had IVOMD values ranging from 475 to 65.3 g $kg^{-1}$ DM. These values are within the range reported by Gebreyowhans and Gebremeskel (2014). Anele *et al*. (2010) also found an IVOMD value of 63.5%, which is similar to the one obtained for Bechuana White in this study. Metabolisable energy values calculated in this study were between 7.60 and 8.83 MJ $kg^{-1}$ DM. The ME values reported in this study were above 2.32 MJ $kg^{-1}$ DM concentration level recommended for confined goats with body weight of 10 kg (Steele 1996) and half of the studied cowpea varieties were above the 8.37 ME $kg^{-1}$ DM recommended for dry ewes (NRC 1985).

Several factors such as fibre content, presence of plant secondary metabolites and potency of rumen fluid used influences the fermentation and gas production (Babayemi *et al*., 2004) of substrates *in vitro*. The trend of gas production illustrated in figure 2.2 shows that forage materials of cowpea varieties were highly fermentable in early hours (2 to 12 hrs.) of incubation; however, as incubation times advanced further, the rate of fermentation slowed down. Amongst studied cowpea varieties, Bechuana White and IT 98K-491-4 and IT 97K-499-35 recorded the highest total gas volume of 87.8, 86.7 and 88.2 ml $400mg^{-1}$ DM at 48 hr incubation, respectively.

This study showed that cell wall contents (NDF, ADF and ADL) negatively affected the *in vitro* organic matter digestibility of incubated substrates. In contrast, *in vitro* organic matter digestibility of incubated substrates had significant ($P < 0.05$) positive correlation with non-fibre carbohydrates. Gas volume produced by cowpea varieties differed mainly due to differences in fermentable organic matter and fibre concentrations. These in turn may affect the extent and rate of the fermentation of available substrate to short-chain fatty acids, methane ($CH_4$) and carbon dioxide (Blümmel and Becker, 1997).

## 2.5. Conclusion

This study showed that forage from the studied cowpea varieties had relatively high protein and adequate concentrations of calcium and phosphorus. Further, the cowpea varieties had low fibre (NDF) content and good fermentation attributes. The positive correlation between IVOMD and NFC indicate that selecting cowpea varieties for greater NFC may result in improved digestibility of the organic matter. Results from this study suggest that forage materials from cowpea varieties grown in the Limpopo province are suitable as roughage source, protein and mineral supplements to complement low quality forages. Cowpea varieties Bechuana White, IT 97K-499-35 and TX 08-30-1 showed a good potential as a source of high quality roughage and/or nitrogen supplement to animals fed on poor quality grass. However, there is a need to determine the actual benefit using *in vivo* and *in vitro* supplementation studies.

# CHAPTER 3

## *In vitro* ruminal fermentation and digestibility *of Eragrostis curvula* hay supplemented with selected varieties of cowpea forages

**Abstract**

This study evaluated the effect of supplementing poor quality *Eragrostis curvula* grass with forage of three cowpea varieties on *in vitro* ruminal fermentation and digestibility. Grass hay was obtained from the University of Pretoria's Hatfield experimental farm and cowpea varieties were harvested at 100% flowering stage from the field trial at an anonymous farm in the Limpopo province of South Africa. Grass hay and forage from the three cowpea varieties were oven dried, milled and analysed for chemical analysis. *In vitro* gas production of grass hay, cowpea varieties and grass hay supplemented with cowpea forage at three different levels (15, 30 and 50%) of inclusion were recorded using a semi-automated system. Generally supplementing poor quality grass hay with cowpea varieties significantly ($P < 0.05$) improved gas production. Among the cowpea varieties, high levels of inclusion resulted in greater gas production for Bechuana White and IT 97K-499-35, while for TX 08-30-1 variety, there was no difference between 15 vs 30 % or 50% inclusion level. Cowpea supplemented grass hay at 30 and 50% level of supplementation resulted in a significantly ($P < 0.05$) greater rate of gas production (c-value) when compared to the control. Of the three varieties, Bechuana White and TX 08-30-1 are recommended as forage supplement to improve poor quality basal diet fermentation and subsequently utilisation of the poor quality diet. However, there is a need for *in vivo* evaluation to determine the actual feeding value of this variety through measurement of dry matter intake, digestibility and animal performance.

**Keywords:** digestibility, fermentation, poor quality forage, *Vigna unguiculata*, supplementation

## 3.1. Introduction

Livestock production in many tropical regions is constrained by insufficient quality and quantity of available forage materials. Environmental fluctuations, acidic and low fertile soils and long annual dry seasons contribute immensely to the reduction of livestock production (Anele *et al*., 2011). The available feed resources are often not adequate to meet the animal nutritional requirements for maintenance and production purposes (Mendieta-Araica *et al*., 2009). Consequently, livestock lose body weight due to deficiency of nitrogen, digestible energy and elements such as sulphur, and sometimes phosphorus, sodium, calcium, zinc, iodine, cobalt or copper in the feed (Chekeredza *et al*. 2002).

To improve production performance of livestock, there is a need to improve quality and quantity of feed. Supplementation of forage legumes to low quality roughages such as maize stover or hay improves their feeding quality (Koralagama *et al*., 2008; Ravhuhali, 2010; Vendramini *et al*., 2012). This is because forage legumes contain minerals, proteins and vitamins essential for growth and maturity of rumen micro-organisms that degrade feedstuffs before undergoing intestinal digestion by the animal (Ravhuhali, 2010).

In regions characterized by farming systems with very limited use of purchased farming inputs, cowpea can be regarded as a fulcrum to sustain such farming systems (Anele *et al*., 2010). Leng *et al.* (1992) suggested that the role of cowpeas in ruminant diets can be seen as three fold, as a nitrogen source and mineral supplement for digestion improvement and ruminal microbial growth efficiency of ruminants fed on poor quality forage. Cowpea residues have been used successfully as a supplement to low quality diet (N'Jai, 1998; Singh *et al.*, 2003; Singh *et al.*, 2006). The objective of the study was to determine the effect of supplementing forage from cowpea varieties from *in vitro* ruminal fermentation and NDF degradation of poor quality *Eragrostis curvula* grass hay.

## 3.2. Material and methods

### 3.2.1. Selection of cowpea varieties

Three promising cowpea varieties identified previously based on their chemical composition, ruminal fermentation and digestibility, were used for further study to test their effects as supplemental feed on poor quality basal diet. The selected cowpea varieties were Bechuana White, IT 97K-499-35 and TX 08-30-1.

### 3.2.2. Treatment setup

*Eragrostis curvula (EC)* was obtained from the Hatfield Experimental Farm of the University of Pretoria, dried and milled to pass through a 1mm sieve in a Wiley mill for chemical analysis. Thereafter, it was stored in airtight plastic container for subsequent analysis of chemical composition and *in vitro* gas production studies. Cowpeas were mixed with grass hay in the ratios, 15: 85, 30: 70 and 50: 50 and 400g of these were added in serum bottles for incubation. A control treatment which was constituted of *Eragrostis curvula* hay was also prepared. The mixed cowpea – grass hay sample were incubated in a serum bottle to measure *in vitro* gas production of the different mixed diets.

### 3.2.3. Buffer preparation and rumen fluid collection

The buffer, micro and macro mineral solutions were prepared as described by Goering and van Soest (1970). However, the macro-mineral solution was modified slightly by replacing Magnesium Sulphate ($MgSO_4.7H_2O$) with Magnesium Chlorite ($MgCl_2.6H_2O$) in order to reduce Sulphate in the media as recommended by Mould *et al*. (2005). These solutions were prepared a day before and kept in separate containers in a 4 °C fridge. Resazurin solution was prepared by dissolving 100 mg of resazurin into 100 mL distilled water and it was kept in dark container in a fridge due to light sensitivity. Before the start of the experiment, an appropriate amount of distilled water was measured and used to dissolve tryptone. All solutions were measured as described by Goering and van Soest (1970) and mixed together with distilled water in conical flask and blended with a magnetic stirrer for 2 minutes, placed in water bath (39 °C) and purged with $CO_2$ till light pinkish in colour. Appropriate amount of L-Cysteine hydrochloride and

Sodium Sulphite were weighed and added directly to the rest of the solution and flushed with $CO_2$ till pinkish colour turned colourless which indicates a complete removal of oxygen.

Two South African Merino sheep (males) fitted with permanent rumen cannula were used as the rumen fluid donors. Donor sheep were daily fed alfalfa hay *ad libitum* as a basal roughage with free access to water. Rumen fluid of approximately 500 mL per animal was collected before the morning feeding. The fluids were mixed and strained through four layers of cheese-cloth into pre-warmed thermos flask and immediately transported to the laboratory within 20 minutes. Upon arrival the rumen fluid was purged with carbon dioxide ($CO_2$) to maintain the anaerobic condition.

### 3.2.4. Incubation of test feed and gas measurement

Each pure substrate of grass hay and forage from three varieties of cowpea and nine combinations of grass hay supplemented with three cowpea variety (≈0.4 g) at three different levels (15, 30 and 50%) of inclusion as described under section 3.2.2. The various treatments were weighed into 120 mL serum bottles and each treatment was prepared in four replicates per run and studied in 2 separate runs. Aliquots (40 mL) consisting of 15 mL rumen fluid and 25 mL buffer solution, were dispensed into the serum bottles previously warmed at 39 °C and flushed with $CO_2$. The serum bottles were sealed with a butyl rubber stopper and aluminium crimp seals. To release gas that might have built up at the start, a small needle was inserted into the rubber stopper for 5 seconds and the serum bottles were incubated at 39 $^{o}$C in a continuous rotary shaking incubator at 120 rotations per minute (rpm). The gas volumes (GV) were recorded at 2, 4, 8, 12, 24 and 48 hours post incubation. Gas volume was measured by inserting a 23 gauge (0.6 mm) needle attached to a pressure transducer connected to data tracker with a visual display. Two runs were conducted in different weeks with four replicates per run and for each run four blanks containing the buffered rumen fluid without substrate were included. The average volume of gas produced from blanks was subtracted from the volume of gas produced from the incubated sample. The gas volume produced was plotted against incubation time, and Ørskov and McDonald (1979) equation was used to estimate gas production characteristics.

$GV = b\,(1 - e^{-ct})$

Where *GV* is the total gas volume (ml 0.4 $g^{-1}$ DM) at time t, *b* is the insoluble but slowly degradable/ fermentable fraction (mL), *c* is the rate constant of gas production from insoluble fraction per hour.

### 3.2.5. 30-h neutral detergent fibre degradability and *in vitro* organic matter digestibility

To determine the 30h *in vitro* NDF degradability the method proposed by Goering and Van Soest (1970) was used. Rumen fluid was collected from two donor South African Merino sheep as described in section 3.2.3 above. Approximately 0.5 g of each test feed sample was weighed in duplicate per 2 runs and transferred into 120 mL serum bottles. Two blank samples which contained only buffered rumen fluid without substrate were used as control for each run. Serum bottles were filled with 15 mL rumen fluid and 25 mL medium, after which bottles were incubated in the *in vitro* water bath set at a temperature of 39 °C for 30 hours under constant $CO_2$ positive pressure. After 30 hours of incubation each sample was used to determine NDF following the method of Mertens (2002).

The Gebreyowhans and Terry (1963) method, modified by Engels and Van der Merwe (1967) was used in determination of the IVOMD. Two digestion phases were involved in this method and 200 mg samples of feed, replicated in triplicate with standards and blanks in every batch were incubated with rumen liquor under anaerobic conditions for 48h at 39 $^{o}$C in the first phase of digestion. The next phase involved acid pepsin digestion for 48h at 39 $^{o}$C, under anaerobic incubation conditions that was terminated after 48h. Therafter, the collected residual plant materials were oven-dried for 12h at 105 $^{o}$C. Combustion at 550 $^{o}$C for 2h in a muffle furnace was used to determine the ash contents (Engels and Van der Merwe 1967).

### 3.2.6. Calculations and statistical analysis

Effective gas production (EGP) was estimated using the Ørskov and McDonald (1979) equation, assuming the flow rate constant (k) of 0.05 $h^{-1}$.

EGP = b*c / (k + c),

*b* is the insoluble but slowly degradable/ fermentable fraction (mL), *c* is the rate constant of gas production from insoluble fraction per hour.

To determine the fermentation and digestion kinetics of gas production, NLIN procedure of SAS (2002) was used to fit non-linear regression model of *in vitro* incubation data. To determine the NDF degradability, NDF after incubation was subtracted from the NDF of samples without incubation. Data was subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS 2002).

## 3.3. Results

### 3.3.1. *In vitro* gas production of grass hay and cowpea varieties

Table 3.1 shows the effect of supplementing poor quality grass hay with different cowpea varieties on *in vitro* gas production. Generally cowpea supplementation improved gas production at 48hrs of incubation compared to the grass hay alone substrate. Among the cowpea varieties, high levels of inclusion (50% level of inclusion) resulted in greater gas production for Bechuana White, IT 97K-499-35 and TX 08-30-1 varieties. There was a significant difference ($P < 0.05$) between varieties in terms of gas volume when supplemented to grass hay, and in particular grass hay supplement by TX 08-30-1 variety produced less volume of gas at 15 and 50% level of inclusion. At 15% level of supplementation, TX 08-30-1 had lesser gas volume than Bechuana White or IT 97K-499-35. In contrasts, at 30% level of inclusion, IT 97K-499-35 produced the least gas volume compared to the other two varieties after 48hrs of incubation. At 50% level of inclusion the gas production after 48hr of incubation ranked greatest for Bechuana White, followed by IT 97K-499-35 and then TX 08-30-1.

**Table 3.1** Effect of supplementing poor quality grass hay with different cowpea varieties on *in vitro* gas production

| Treatment | Incubation period | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3h | 6h | 9h | 12h | 24h | 30h | 48h |
| GRASS | $6.5^{f}$ | $12.6^{h}$ | $19.7^{g}$ | $28.7^{g}$ | $47.3^{g}$ | $60.3^{f}$ | $80.2^{f}$ |
| GRASS +15% BW | $9.1^{cd}$ | $17.0^{de}$ | $24.9^{e}$ | $34.1^{e}$ | $52.2^{e}$ | $64.9^{d}$ | $84.8^{d}$ |
| GRASS + 30% BW | $9.4^{cd}$ | $17.9^{d}$ | $26.4^{d}$ | $35.6^{d}$ | $53.6^{d}$ | $65.0^{d}$ | $82.6^{e}$ |
| GRASS+ 50% BW | $14.0^{a}$ | $23.0^{a}$ | $32.3^{a}$ | $42.4^{a}$ | $61.1^{a}$ | $73.4^{a}$ | $92.0^{a}$ |
| GRASS+15%IT 97K-499-35 | $8.7^{d}$ | $16.5^{ef}$ | $24.5^{e}$ | $33.7^{e}$ | $51.8^{e}$ | $64.4^{d}$ | $84.1^{d}$ |
| GRASS+30%IT 97K-499-35 | $7.2^{ef}$ | $15.7^{fg}$ | $24.2^{ef}$ | $33.4^{ef}$ | $51.4^{ef}$ | $62.7^{e}$ | $80.0^{f}$ |
| GRASS+50%IT 97K-499-35 | $11.4^{b}$ | $20.4^{b}$ | $29.8^{b}$ | $39.8^{b}$ | $58.5^{b}$ | $70.7^{b}$ | $89.4^{b}$ |
| GRASS+15%TX 08-30-1 | $7.4^{e}$ | $15.4^{g}$ | $23.3^{f}$ | $32.6^{f}$ | $50.7^{f}$ | $63.3^{e}$ | $83.8^{e}$ |
| GRASS+30%TX 08-30-1 | $8.9^{d}$ | $17.6^{d}$ | $26.1^{d}$ | $35.3^{d}$ | $53.3^{d}$ | $64.6^{d}$ | $82.2^{e}$ |
| GRASS+50% TX 08-30-1 | $10.0^{c}$ | $19.0^{c}$ | $28.4^{c}$ | $38.4^{c}$ | $57.2^{c}$ | $69.4^{c}$ | $88.0^{c}$ |
| *SEM* | 0.32 | 0.30 | 0.30 | 0.30 | 0.30 | 0.31 | 0.28 |
| *P- value* | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 |

Within columns, means followed by the same letter are not significantly different at $P < 0.05$; BW: BechuanaWhite; SEM: standard error of the mean

### 3.3.2. *In vitro* gas kinetics

*In vitro* gas production kinetics of grass hay, and cowpea supplemented grass hay is shown in table 3.2. The grass hay significantly ($P < 0.05$) differed in terms of insoluble but slowly fermentable fraction when compared to grass hay supplemented with the cowpea varieties, however, among grass hay supplemented with cowpea varieties at different levels, there was no significant ($P > 0.05$) difference in terms of the slowly fermentable fraction. Furthermore, grass hay had a significantly ($P < 0.05$) slower rate of fermentation compared to grass hay supplemented with the cowpea varieties. Cowpea supplemented grass hay at 30 and 50% level of supplementation resulted in a significantly ($P < 0.05$) faster rate of gas production (c-value) when compared to the control. For all varieties at 15% level of supplementation, the rate of gas production did not differ compared to the control. However, supplementing TX 08-30-1 at 30% level of supplementation, significantly ($P < 0.05$) improved the fermentation rate. There was a significant ($P < 0.05$) difference between grass hay and cowpea varieties as well as grass hay supplemented with varieties in terms of effective gas production. Generally, a 50% level of supplementation resulted in a higher effective gas production compared to the control.

**Table 3.2** *In vitro* gas production kinetics of grass hay, and cowpea supplemented grass hay

| Treatment | b (ml) | c (ml) | EGP (ml 400 $mg^{-1}$) |
|---|---|---|---|
| Grass hay | $102.4^{a}$ | $0.01^{d}$ | $20.7^{cd}$ |
| Grass hay + 15% BW | $57.8^{b}$ | $0.04^{bcd}$ | $25.3^{bc}$ |
| Grass hay + 30% BW | $52.9^{b}$ | $0.05^{bc}$ | $26.6^{bc}$ |
| Grass hay + 50% BW | $59.9^{b}$ | $0.05^{bc}$ | $27.5^{b}$ |
| Grass hay + 15%IT 97K-499-35 | $60.2^{b}$ | $0.03^{cd}$ | $21.7^{bcd}$ |
| Grass hay + 30%IT 97K-499-35 | $55.0^{b}$ | $0.05^{bc}$ | $26.3^{bc}$ |
| Grass hay + 50%IT 97K-499-35 | $50.6^{b}$ | $0.06^{b}$ | $28.2^{b}$ |
| Grass hay + 15%TX 08-30-1 | $55.7^{b}$ | $0.02^{cd}$ | $18.1^{d}$ |
| Grass hay + 30%TX 08-30-1 | $53.3^{b}$ | $0.10^{a}$ | $35.9^{a}$ |
| Grass hay + 50%TX 08-30-1 | $56.3^{b}$ | $0.05^{bc}$ | $26.3^{a}$ |
| *SEM* | 5.42 | 0.09 | 1.95 |
| *P- value* | <0.0001 | <0.0001 | <0.0001 |

Means within a column with different letters in superscript differ significantly ($P < 0.05$); *b*: slowly fermentable fraction; *c*: rate of fermentation of fraction *b*; EGP: effective gas production; BW: Bechuana White; SEM: standard error of the mean

### 3.3.3. 30 h *in vitro* NDF degradation and *in vitro* organic matter digestibility

Table 3.3 shows the 30 hour (hr) *in vitro* neutral detergent fibre digestibility (*iv*NDFd) and IVOMD of grass hay, and cowpea supplemented grass hay. The 30 hour *iv*NDF digestibility of grass hay supplemented with 30% BW and 50% IT 97K-499-35 cowpea varieties were significantly ($P < 0.05$) greater than the poor quality grass hay. At 30 and 50% inclusion, there was no difference between cowpea varieties in terms of *iv*NDF digestibility than IT 97K-499-35 supplemented grass hay. However, at 15% inclusion level, TX 08-30-1 resulted in a greater NDF digestibility of the poor quality grass hay. The cowpea varieties used in the study did not differ significantly ($P < 0.05$) in terms of their IVOMD. All cowpea varieties, with the exception of TX 08-30-1 at 30% level of supplementation, improved the IVOMD when compared to poor quality grass hay.

**Table 3.3** 30 h *in vitro* NDF degradation and *in vitro* OM digestibility of cowpea varieties, grass hay and mixtures

| Treatments | *iv*NDFd (%DM) | IVOMD (% DM) |
|---|---|---|
| Grass hay | 18.8[ef] | 35.0[g] |
| BW | 48.2[a] | 69.3[ab] |
| TX49 | 47.0[a] | 66.1[bc] |
| TX08 | 37.8[b] | 66.6[bc] |
| Grass hay + 15% BW | 20.5[def] | 43.9[f] |
| Grass hay + 30% BW | 24.3[cd] | 48.4[ef] |
| Grass hay + 50% BW | 23.5[cde] | 60.0[cd] |
| Grass hay + 15%IT 97-499-35 | 18.3[f] | 43.9[f] |
| Grass hay + 30%IT 97-499-35 | 22.8[def] | 44.3[f] |
| Grass hay + 50%IT 97-499-35 | 27.7[c] | 74.0[a] |
| Grass hay + 15%TX 08-30-1 | 22.9[de] | 42.0[fg] |
| Grass hay + 30%TX08-30-1 | 20.6[def] | 56.1[d] |
| Grass hay + 50%TX08-30-1 | 23.2[cde] | 53.8[de] |
| *SEM* | 1.36 | 2.35 |
| *P- value* | <0.000 | <0.000 |

Means within a column with different letters in superscript differ significantly ($P < 0.05$); *iv*NDFd: 30 hour *in vitro* detergent fibre; IVOMD: *in vitro* organic matter digestibility; BW: Bechuana White; SEM: standard error of the mean

### 3.4. Discussion

Gas produced by *in vitro* fermentation reflects the extent of feed fermentation, digestibility and microbial synthesis of the tested feed (Getachew *et al*., 1998; Sommart *et al*., 2000 and Sallam 2005). Generally, low gas produced would indicate low degradability, but feedstuffs high in CP normally produce less gas during fermentation because protein fermentation produces ammonia, which influences the carbonate buffer equilibrium by neutralizing $H^+$ ions from VFA without release of carbon dioxide (Cone and van Gelder 1999). Cowpea varieties supplemented to grass hay were highly fermentable compared to grass hay alone. This could be attributable to greater crude protein content coupled with lesser fibre concentrations in cowpea varieties as compared to grass hay alone. The cowpea varieties provided a source of nitrogen to the microbes, which in return resulted in greater fibre degradation and hence producing more gas. High fermentation of cowpea varieties in the first 24 h of incubation indicates the quick digestibility of cowpea forage compared to grass hay. According to Akinfemi *et al*. (2012) gas production is regarded as an indicator of carbohydrate degradation. Generally, supplementation of cowpea varieties to poor quality grass hay provided readily accessible and rapidly fermentable carbohydrate and rumen degradable nitrogen, thus the overall fermentation was improved. Consequently, increasing supplementation led to a significant ($P < 0.05$) improvement of total substrate throughout the incubation period (Table 3.1). This led to a drop of insoluble but slowly fermentable fraction with improved rate of fermentation (Table 3.2).

According to Abegunde *et al*. (2011) the rate at which feed substrate or constituents are degraded in the rumen is as important as the extent of digestion. This study showed that supplementing cowpea varieties to poor quality grass hay improved IVOMD of mixed substrate. This could be associated with an improved rate of gas production observed in table 3.2. All cowpea varieties, with the exception of TX 08-30-1 at 30% level of supplementation, improved IVOMD when compared to poor quality grass hay. According to Kafilzadeh and Heidary (2013), degradability of NDF is imperative component for forage quality. In this study, supplementing poor quality grass hay with cowpea varieties significantly improved 30hr *iv*NDF digestibility when 30% BW and 50% IT 97K-499-35 were used as supplement. At 30 and 50% inclusion, there was no difference between cowpea varieties in terms of 30hr *iv*NDF digestibility due to supplementation.

### 3.5. Conclusion

This study showed that supplementing poor quality grass hay with Bechuana White and IT 97K-499-35 at 50% inclusion level improved fermentation. When TX 08-30-1 is used as a supplement, 30 % level of inclusion is recommended over 50% inclusion because it resulted in greater IVOMD, and rate of gas production and similar effective gas production as 50% supplementation level. Among the three varieties, TX 08-30-1 variety is recommended, as a supplementary source of forage and nitrogen to improve poor quality hay fermentation and hence utilization by ruminant animals due to its superiority in terms fermentation attributes when used as supplement to poor quality forage.

# CHAPTER 4

## General conclusions and recommendations

## 4.1. General conclusions

The purpose of this study was to characterize twelve cowpea varieties that have been agronomically evaluated in the Limpopo Province, South Africa in terms of their chemical composition and *in vitro* gas production characteristics. Cowpea varieties were evaluated in order to identify variety(s) that could be utilised as potential alternative forage source for supplementation of poor quality feed. Consequently two laboratory based experiments were conducted at University of Pretoria in order to 1) determine nutritive value through proximate lab analysis and 2) *in vitro* gas production and an *in vitro* cowpea supplementation to poor quality grass hay. The overall results from this study showed that Bechuana White, IT 97K-499-35 and TX 08-30-1 are suitable varieties as supplementary source of protein and roughage sources to animals fed poor quality forage.

The varieties have a satisfactory nutritive value (high protein concentration, adequate metabolisable energy and produced a reasonably high amount of gas) and good *in vitro* organic matter digestibility. This suggests that the promising varieties could be strategically used as supplemental feed source to improve poor quality forage utilisation by small ruminants. Generally the digestible organic matter of the varieties was on the range of 52 to 65% and the crude protein content for the varieties ranged between 15 to 21%. This indicates the potential of promising varieties to meet the maintenance and production requirement of ruminants.

The effect of supplementing poor quality grass hay with varying levels of selected cowpea varieties indicated that it improved ruminal fermentation for the first 30 h when supplemented at both 30 and 50% level. The 50% level of supplementation will result in substitution effect. Thus, supplementing poor quality grass hay with cowpea varieties increased the protein content of the diet to the level in which microbes are able to ferment the feed efficiently. At 15% inclusion level, TX 08-30-1 resulted in a greater NDF digestibility of the poor quality grass hay. The 15% will result in supplementation effect. Additionally, NDF degradability of grass hay was improved when supplemented by this variety at 50% level. This means that supplementing poor quality grass hay with variety IT 97K-499-35 at 50% level may reduce the retention period of poor quality feed in the rumen and thus may improve feed intake of the poor quality forage.  In this study, supplementing poor quality grass hay with cowpea varieties significantly improved 30hr *iv*NDF digestibility when 30% BW and 50% IT 97K-499-35 were used as supplement. At 30 and 50% inclusion, there was no difference between cowpea varieties in terms of 30hr *iv*NDF digestibility due to supplementation.

### 4.2. Recommendations

This study showed that cowpeas can be produced with relatively good nutritive value (high in crude protein, highly digestible, adequate metabolisable energy and fibres). These attributes indicate the suitability of using forage from cowpeas after harvesting of the grain as a supplementary feed source to improve poor quality forage utilisation by animals. This may be particularly important in the situation of resource poor farmers or small-holder subsistence farmers who cannot afford to supplement their animals in times of feed shortage. However, there is a need for a further research for strategic use of the forage material in the diet of animals under communal and smallholder production systems in South Africa. Such research will help to establish the level in which production of livestock will be improved particularly under smallholder subsistence farmers, who at the moment could not able to afford commercial feed supplements. Further research is required to determine how the benefit of using promising varieties (Bechuana White, IT 97K-499-35 and TX 08-30-1) identified in this study could be converted into animal product, e.g. how it can affect milk and meat production and also quality. Hence, the relative preference for, palatability and intake of cowpea varieties adapted in Limpopo need to be determined since they can give complementary information to the data derived from their nutritive value and further consolidates their importance as source of feed for livestock.

The *in vitro* trial conducted in this study showed that 30 and 50% cowpea supplementation to poor quality grass hay improved fermentation. Additionally these varieties at both 30 and 50% supplementation level also improved NDF degradability of grass hay. On the other hand supplementing, IT 97K-499-35 at 50% level increased the *in vitro* organic matter digestibility of the poor quality grass hay, suggesting that supplementing this variety at 50% level may reduce the retention period of poor quality feed in rumen of the animal due to substitution effect and perhaps increase dry matter intake. A 15 and 30% inclusion with maximum benefit denotes supplementation effect, whereas a 50% inclusion level denotes substitution effect. Therefore, both Bechuana White and IT 97K-499-35 are recommended as a roughage source whereas, TX 08-30-1 may be recommended as a source of protein supplement. Furthermore, it is recommended that an *in vivo* trial should be conducted which will help in assessing the effect of these varieties (Bechuana White, IT 97K-499-35 and TX 08-30-1) on poor quality forage digestibility and retention period.

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