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Forage quality of some Kalahari browse species and its ability to reduce methane emission

by

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Table of contents

List of figures

List of tables

List of abbreviations

ADF	acid detergent fiber
ADL	acid detergent lignin
CH_4	methane
CO_2	carbon dioxide
CP	crud protein
CT	condensed tannins
EE	ether extract
GE	gross energy
Gg	Giga gram
GP	gas production
IVDMD	*in vitro* dry matter digestibility
IVOMD	*in vitro* organic matter digestibility
Kg	kilograms
ME	metabolizable energy
mg	milligrams
MJ	mega joules
mL	milliliters
MSE	mean standard error
MW	molecular weights
NDF	neutral detergent fiber
NDFN	neutral detergent fiber nitrogen
NFC	non fiber carbohydrates
NTP	non-tannin phenols

OM	organic matter
PEG	polyethylene glycol
PVPP	polyvinyl-polypyrrolidone
SCVFA	short chain volatile fatty acid
TP	total phenols
TT	total tannins
VFA	volatile fatty acid

Declaration

I Jacobus Johannes Francois Theart declare that this dissertation, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution.

Signature:……

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Summary

Forage quality of some Kalahari browse species and its ability to reduce methane emission

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The nutritional value of browse foliage from shrub and tree species in the Kalahari region of the Northern Cape, South Africa is not well quantified and analysed. In this study, nineteen browse (shrub and tree) species were selected and their foliage harvested during April 2012, when the plants are at mid vegetative stage of growth in order to determine its chemical composition, nutritional values and its potential to reduce methane production. The foliage materials were analysed for crude protein (CP), ash content, dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), total phenols and condensed tannins. The *in vitro* gas production technique was used to determine the volume of gas and methane (CH_4) produced. *IIn vitro* organic matter digestibility (IVOMD), volatile fatty acids (VFA) and rumen ammonia (NH_3-N) concentration were also determined. In the gas production study the forage samples were studied either in the absence or presence of polyethylene glycol 8000 (PEG) to determine the effects of tannin on various parameters of interest. Crude protein ranged from 70-320 g/kg DM, ash 40-210 g/kg DM, NDF 350-508 g/kg DM, ADF 270-530 g/kg DM, ADL 85-320 g/kg DM and neural detergent fibre nitrogen (NDFN) 47-93 g/kg DM. Total tannins ranged from 9-320 g/kg DM, condensed tannins 2-125 g/kg DM and hydrolysable tannins which ranged from 3-195 g/kg DM. The CP concentration of all 19 browse

species included in the current study, except *Olea europaea*, *Terminalia sericea* and *Monechma genistifoluim* will meet the maintenance requirements of ruminants based on CP concentrations as indicated in the NRC (2007) guidelines. Among the shrubs and trees, *Acacia luederitzii* and *Monechma incanum* showed the best potential to decrease methane production by up to 90 % after 48 h of incubation. The secondary compounds (mainly tannins) of the browse species had no significant ($p<0.001$) effect on IVOMD and rumen ammonia concentration but the VFA, methane and gas production was decreased significantly ($p<0.001$). In the last experiment, an *in vitro* gas production experiment was conducted to evaluate the potential of 6 selected browse species (with high, medium and low condensed tannin concentrations) when supplemented to *Eragrostis trichopophora.* This was done in order to determine their potential as anti-methanogenic additives in the diet of ruminant animals. These browse species were supplemented with *Eragrostis trichopophora* at a ratio of 30:70. The effects of addition of these browse as a supplement, on rumen fermentation and methane production were studied. Of the six browse included in the current study, *A. luederitzii* and *M. incanum* decreased methane production by more than 50 %, but digestibility and VFA production was decreased. From these 3 studies, it seems that *Boscia albitrunca* and *Rhus lancea* has the best potential to be used as a feed supplement during times of drought, depending on the availability of these browse foliage, while *A. luederitzii* and *M. incanum* seems to have the best potential to consistently decrease methane production, but at the expense of digestibility. An *in vivo* feeding trail inside a methane chamber should be considered in the future in order to complement this study to determine the nutrient availability and degradability of these browse species and to estimate the actual potential reduction in enteric methane production.

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General Introduction

The production of greenhouse gas (GHG) by livestock and its impact on global warming is a worldwide problem. Methane is one of the three main greenhouse gases, together with carbon dioxide and nitrous oxide that contributes to global warming (Steinfeld *et al*., 2006). According to Stats South Africa, (2010) there are 13.7; 28.8 and 2 million herd of cattle, sheep and goats in South Africa, respectively.

In 2004, commercial beef cattle contributed 45% while emerging/communal cattle 33% of the total enteric fermentation of 1225 Giga gram (Gg) CH_4 in South Africa with mature cows and bulls having the highest CH_4 emission factors for enteric fermentation (Otter, 2010). Most of these animals are kept on big extensive farms or on communal lands where the nutritional value of forage is generally low. Browse tree legumes and shrub foliage (*viz.*; leaves, twigs, pods, fruits and bark) are important components in the diets of cattle, sheep, goats and wild ungulates in arid and semi-arid regions of sub-tropical South Africa, which may serve as a supplement during times of prolonged drought (Forbes & Clement, 1998).

Methane production from ruminants is affected by the type of feed consumed. Several authors (Johnson & Johnson, 1995; Moss *et al*., 1995) reported reductions in methane output by increasing the proportion of concentrates to forage in ruminant diets. However, in grassland-based systems which predominate South Africa, ruminants usually receive relatively small amounts of concentrates during their production cycle. In these situations, offering foliage from browse of high nutritional value is essential to enhance animal production. Supplementing with browse foliage is capable of simultaneously reducing methane emissions per unit of daily weight gain (Hart *et al*., 2009; Moss *et al*., 2000). Enteric methane from ruminants account for about 11-17% of methane generated globally (Beauchemin *et al*., 2009). Methane arises from the activity of methanogens in the rumen that use hydrogen and carbon dioxide as precursor, thereby preventing the accumulation of reducing equivalents, which would otherwise impede rumen fermentation (Beauchemin *et al*., 2009). Although the production of methane is desirable from a fermentative perspective, it is energetically costly, as cattle emit 2-12% of their gross energy intake in this potent GHG (Beauchemin *et al*., 2009). Thus, decreasing the production of enteric methane from ruminants without altering animal productivity is desirable both as a strategy to reduce global GHG emissions as well as a means of improving feed conversion efficiency (Beauchemin *et al*., 2009). Most of the enteric CH_4 produced by ruminants has its origin in the rumen and thus dietary and/or rumen manipulation can be one possible target to mitigate CH_4 emissions. Fermentation of feed components in the rumen by the micro-flora, under anaerobic conditions, result in the production of volatile fatty acids (VFA'S), that are used by the

animal as a source of energy and fermentation gasses (CO_2 and CH_4) that will be eliminated through eructation.

Browse tree legumes and shrub foliage (*viz.*; leaves, twigs, pods and fruits and bark) with its high crude protein (CP) content of 100–250 g/kg dry matter (DM) (Le Houérou, 1980) have the potential as CP supplements in ruminant diets. However, its utilisation could be limited by the high content of polyphenolic compounds (*viz.*; phenolics and tannins), especially when fed at high levels, due to the adverse effects on feed digestibility and nutrient availability (Le Houérou, 1980).

Most tropical browse plants contain secondary compounds. Condensed tannins (CT) are secondary phenolic compounds in plants that may play a role in reducing nitrogen availability for fermentation but animals avoid plants with these high secondary phenolic compounds. Condensed tannins vary considerably in chemical structure but share a common property by having a high affinity for protein and have been identified to be effective in reducing methane emissions in ruminant animals (Waghorn, 2008). Tannins bind with proteins forming tannin-protein-complexes (McAllister & Newbold, 2008) which reduce degradation of plant protein to ammonia in the rumen, thereby enhancing the flow of feed protein to the small intestine. For diets containing high nitrogen content, the binding effect of CT could be beneficial for the environment, as less nitrogen would be expected to be changed to rumen ammonia and thereby reducing excretion of highly volatile ammonia via urine (Beauchemin *et al*., 2009). Tannins could be beneficial or detrimental to ruminant animals depending on the type of tannin, the amount consumed and the physiological status of the animal (Hagerman & Butler, 1991). It has been reported that consumption of low to moderate concentration of tannin does not affect voluntary feed intake, whereas high tannin concentration was reported to result in reduced feed intake (Barry & Duncan, 1984; Waghorn *et al*., 1994).

Among ruminants, browsers are less affected by the effect of tannins compared to grazers. The efficient utilization of tanniferous plants by browsers compared to grazers might be related to the high concentration of proline in their saliva (Kumar & Singh, 1984; Hagerman & Butler, 1991). Proline has a high affinity to bind with tannins to form tannin proline protein complexes which are stable across the entire pH range of the digestive tract (Hagerman & Butler, 1991). The association of tannins with proline may cancel the negative effects of tannins on palatability and therefore improves feed intake and digestion of tannin- rich feeds (McArthur *et al*., 1995; Narjisse *et al*., 1995). Furthermore, Field *et al.* (1989) and Tavendale *et al.* (2005) reported that tannins with lower molecular weights could be more effective against methanogens than tannins with higher molecular weights. This is the consequence of the latter having a lower H-bonding strength and the ability to penetrate bacteria and bind to microbial enzymes. Several studies have indicated that tannins have anti-methanogenic activity, either by direct inhibition of methanogens or indirectly through inhibition of protozoa (Moss *et al.*, 2000, Kamra *et al.*, 2006; Animut *et al.*, 2008; Bhatta *et al.*, 2009; Jayanegara *et al.*, 2009). This means that tannins from different browse species may vary vastly in type and concentration, which suggests that there is a need to generate more information in relation to

key browse species used by livestock to exploit their potential to reduce enteric methane. The general objective of this study is to select out of 19 browse species two to three tannin rich browse species that can be used as a feed supplement or additive in order to reduce enteric methane production in ruminant animals without significantly reducing feed digestibility.

CHAPTER 1

Literature review

1.1.1 Plant bioactive compounds

Generally, trees occupy a significant niche in the farming systems and overall way of life in the Kalahari-bush-dune-veldt. Fodder shrubs and trees (browse) in this region play a significant role both in farming systems and in livestock production. Livestock in this zone largely depends on browse for dietary protein. Compared with tropical grass, browse is generally richer in protein and minerals (Le Houérou, 1980). The importance of browse increases with increasing aridity and is generally most essential in the dry seasons, when most other feed resources depreciate in forage quality and quantity. Browse intake increases total dry matter intake, crude protein intake, and improves the digestibility of low-quality forages. The effect of browse on livestock feeding is shown in increased survivability (*viz*.; lower mortalities, especially over the dry season) and increased productivity (Le Houérou, 1980).

The most commonly occurring browse foliage species in the Kalahari-bush-dune-veldt are *Acacia erioloba, Acacia haematoxylon, Dichrostachys cinerea, Grewia flava, Hermannia tomentosa, Acacia mellifera* and *Terminalia sericea.* Browses varied in their seasonal availability but *A. haematoxylon, G. flava* and *T. sericea* are present on the farm lands all year round. Browse leaves and pods form a major component of the diet of goats, which meets over 60% of dietary intake. There are several types of leguminous and non-leguminous trees utilized as forage by goats but the predominant species in the Kalahari are *Acacia* species and *G. flava*. The nutritional importance of browse is especially significant for free ranging goats and cattle (Devendra 1993). Goats have a great tendency to change their diet according to seasonal feed availability and growth rate of plants. Some parts of browse species can be found during the dry season (winter) and these include pods, fruits and leaves of evergreens. Most trees/shrubs produce its leaves during the wet season (summer), thus browse is more available during the summer (November to March) (Palgrave, 1983). Cattle tend to consume browse during early spring when new leaves develop and during times of drought. Devendra (1993) reviewed the significance of shrub and tree foliage as sustainable feed resources. The extent of this contribution to meet dietary requirements is dependent on the type and quantity of browse available, preference of animals, accessibility, palatability and presence of toxic ingredients (Devendra, 1995).

In most situations, the practical use of browse as a supplement is to enhance the intake and utilization of other fibrous grasses, and thus meet the maintenance and variable levels of production requirements. Goats browse extensively and a lot of the browse plants contain plant secondary metabolites such as tannins (Topps, 1992). The researcher also stated that these phenolics appear to be

the major constraint in the use of legume shrubs and trees as animal fodder because of its effect on intake, digestibility and the animal's metabolism. Hill & Tamminga (1998) stated that there are two major nutritional advantages of the consumption of feeds high in tannins for ruminants. The first relates to the prevention of bloat when animals eat pastures that are rich in soluble proteins (Griffiths, 1991). The second advantage is the ability of tannins to form complexes with free protein in the rumen and thus protect the protein from degradation in the rumen. Therefore, bloat is not a problem for grazing ruminants in the tropics (Cheeke & Shull, 1985).

Fermentation is an oxidative process, during which cofactors such as NADH, FADH, NADPH have to be reduced to NAD^+, $NADP^+$, FAD^+ through dehydrogenation reactions by releasing hydrogen in the rumen (Martin *et al*., 2009). As soon as reduced cofactors are produced, hydrogen is used by methanogenic Archaea, a microbial group distinct from Eubacteria, to reduce CO_2 by forming CH_4 according to the following equation: $CO_2+4H_2= CH_4 + 2H_2O$ (Martin *et al*., 2009). Methane in the rumen is predominantly produced via this pathway. Methanogenesis is essential for an optimal performance of the rumen because it avoids hydrogen accumulation, which would lead to inhibition of dehydrogenesis activity involved in the oxidation of reduced cofactors (Martin *et al*., 2009).

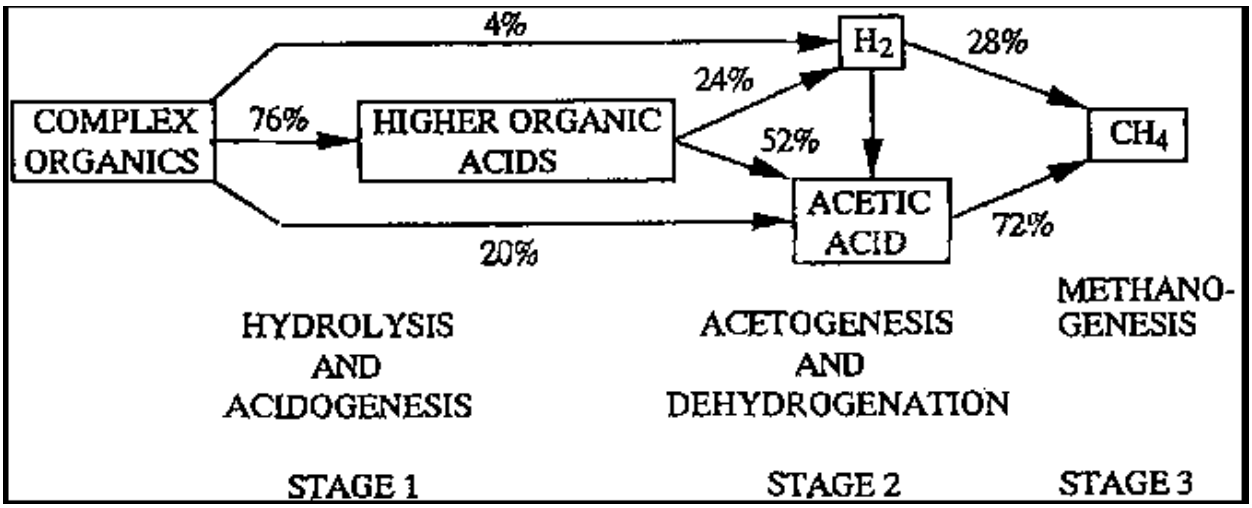

Figure 1: Methane formation during fermentation (McCarty *et al*., 1982)

The metabolic pathways involved in the hydrogen production and utilization, as well as methanogenic community are important factors that should be targeted when strategies are developed to control CH_4 emissions by ruminants (Martin *et al.* 2009). The potential use of plant extracts to reduce CH_4, is receiving renewed interest as it's seen as a natural alternative to chemical additives and is well perceived by consumers. Tannins and saponins have been extensively studied and have showed promise for its mitigating potential within this category (Beauchemin *et al*. 2007). Tannins as feed supplements or as tanniferous plants have often shown a potential for reducing enteric CH_4 emission by up to 20 % (Beauchemin *et al*., 2007).

1.1.2 Tannins (condensed and hydrolysed)

Tannins will inevitably be anti-nutritional when dietary CP concentrations are limiting because it reduces the absorption of amino acids (AA) (Waghorn, 2008). Structure, molecular weight and concentration of condensed tannins affect the nutritive value of the diet. It is important that the benefits of reduced CH_4 counter weigh any detrimental effects of tannins on digestion and production. According to Grainger *et al*. (2009), CH_4 emission was reduced by up to 30 % and at the same time milk production of the cows were also reduced by about 10 %. However, nutritional and animal health (anthelmintic, bloat safe) properties of tannin ingestion coinciding with reductions in methanogenesis and especially N_2O emissions and the absence of N requirements for plant growth (most are legumes) makes these plants attractive for environmentally sustainable ruminant production. Thus, any viable strategy has to achieve in one or more of the following goals: either a reduction of hydrogen production that should be achieved without impairing feed digestion or a stimulation of hydrogen utilization towards pathways producing alternative end products beneficial for the animal such as propionate production or an inhibition of the methanogenic Archaea bacterial activity and numbers in the rumen. This should ideally be done with a concomitant stimulation of pathways that consume hydrogen in order to avoid a build-up of high hydrogen concentrations in the rumen and its negative effect on fermentation as described by (Martin *et al*. 2009).

Some mitigation options have been proposed (*viz*.; vaccines, chemical additives) but diet manipulation is the most direct, and arguably the most effective means of lowering methane emissions from ruminants in most animal production systems (Hassanat & Benchaar 2013). Dietary inclusion of biologically active plant compounds such as condensed tannins and saponins have been reviewed (Jouany & Morgari, 2007). Wide varieties of biological compounds in plants have been explored for their ability to mitigate ruminal methane production (Martin *et al*., 2009).

A report by Verdier *et al*. (2012) indicated successful expression of a key regulator of the production of proanthocyanidins (*viz*.; one form of condensed tannin), MtPAR (*Medicago truncatula* proanthocyanidin regulator) in alfalfa resulting in detectable levels of proanthocyanidin in shoots. This suggests a possible pathway to the production of condensed tannins in this popular forage legume. A meta-analysis of *in vivo* experiments with tannins by Jayanegara *et al*. (2012) reported a direct relationship between dietary tannin concentration and CH_4 production per unit of digestible OM. Jayanegara *et al*. (2012) and Verdier *et al*. (2012), however, reported a trend ($p = 0.08$) for decreased feed intake and a statistically significant decrease in digestibility, particularly CP, with increasing dietary tannin concentrations (a 0.16 % decrease per g/kg DM extra tannin in the diet) but also for NDF with a 0.11 % decrease per g/kg DM extra tannin in the diet. Results from a meta-analysis of C3 *versus* C4 grasses and warm and cold climate legumes by Archimède *et al*. (2011) concluded that CH_4 production was lower for animals fed high tannin legumes compared with animals fed low tannin legumes (37.2 *versus* 52.2 L CH_4/kg digestible OM intake). In studies with diets of good nutritional quality containing condensed tannin (Waghorn, 2008), feed intake and

animal performance have not been negatively affected. Reduced digestibility in diets containing condensed tannin is almost universal and unavoidable (Waghorn, 2008; Patra, 2010). This is an important factor that must be considered when feeding supplemental tannins or tanniferous plants but relationships with digestion are affected by the type of tannin and the composition of the diet. In addition, the carbon fraction of condensed tannin is excreted in the faeces (Terrill *et al*., 1994), so high concentrations of indigestible tannins in diets further limit digestible OM available to the animal.

Crude protein digestibility was also reduced when diets containing a lower tannin concentration (2.8 g/kg DM) were fed (Waghorn, 2008). The effect of tannins is conditional on their composition (Waghorn, 2008; Goel & Makkar, 2012). As reported by Pellikaan *et al*. (2011), *in vitro* gas and CH_4 production depended on tannin composition, such as type (condensed *versus* ellagitannins *versus* gallotannins), solubility, cis-trans configuration and browning rate. In the study of Pellikaan *et al*. (2011), valonea and myrobalan tannins were most effective at reducing CH_4 production, with only a minor impact on total gas production. Waghorn, (2008); Pellikaan *et al*. (2011) and Goel & Makkar, (2012) also pointed out that more research in ruminants is needed with these compounds to establish its antimethanogenic effect.

Tannins are high molecular weight (>3000 Da) phenolic compounds that bind to proteins to form complexes and precipitates dietary feed nutrients such as carbohydrates, proteins and minerals (Mangan, 1988). The effects of polyphenolics on the nutritive value of browse in ruminants may vary a lot depending on the following: the species composition of the micro flora in the rumen, its ability to bind with proteins, carbohydrates and minerals and thus reducing or completely preventing: its availability, its effects on inhibiting extracellular microbial cellulolytic enzymes and its extent of absorption from the rumen and which may result in toxicities at tissue level (Butler *et al*., 1986; Mueller-Harvey & McAllan, 1992).

In contrast, polyethylene glycol (PEG), a non-nutritive synthetic polymer, has a high affinity for phenolic compounds, especially tannins, and thereby deactivates it by forming tannin-PEG complexes (Makkar *et al*., 1995). PEG can prevent the formation or liberate protein from tannin-protein complexes (Barry & Manley, 1984) and it has been used to mitigate adverse effects of secondary compounds on rumen fermentation as well as improving the performance (*viz*., growth and milk yield) of ruminants fed diets high in secondary compounds. On the other hand, tannins at low to moderate concentrations may be beneficial in the treatment of bloat (Waghorn *et al*., 1994) and may increase protein utilization in ruminants (Reed, 1995).

Methods for quantification of hydrolysable tannins have become available (Makkar, 2003). It should be noted that hydrolysable tannins are hydrolysed in the rumen and some hydrolysable tannins could be toxic (Lowry *et al*., 1996; McSweeney *et al*., 2003). As with other CH_4 mitigating agents, the long-term effects of tannins have not been established. In addition, as indicated by Goel & Makkar (2012), a substantial reduction in CH_4 emission with these compounds, particularly tannins, would be difficult without compromising animal production.

1.1.3 Other secondary plant components

1.1.3.1 Saponins

Saponins are of interest to CH_4 mitigation because it inhibits rumen ciliate protozoa (Lila *et al*., 2003; Makker *et al*., 1998; Wang *et al*., 1998) by altering the cell membrane permeability (Klita *et al*., 1996), but it appears that saponins do not directly inhibit methanogens. Saponins show great promise as rumen fermentation modifiers, by using the free hydrogen ions that are floating in the rumen or by using it in other metabolic processes such as propionic acid formation or by exhibiting bactericidal action on methane producing bacteria (Goel & Makker, 2012). Saponins are glycosides found in most plants. These effects have been explained partly by the action of saponins on ruminal microbes, resulting in a decrease in rumen degradation of feed proteins and an increase in microbial protein synthesis in the rumen, which both increase the intestinal flow of amino acids (Makker & Becker, 1996).

Administration of saponins have been found to improve the assimilation of feed nitrogen by animals because less NH_3^+ -N is produced in the rumen and subsequently less urea is excreted in the urine (Santoso *et al*., 2004). Two mechanisms have been considered to explain the effect of saponins on N metabolism in the rumen: saponin extracted from leaves of *Sesbania sesban* and *Medicago sativa* roots have significantly reduced the number of protozoa (Klita *et al*., 1996; Newbold *et al*., 1997) which play a major role in ruminal feed protein degradation (Jouany, 1996). Ammonia nitrogen resulting from microbial protein degradation can be bound by saponins in a balanced chemical reaction regulated by NH_3^+-N concentration (Newbold *et al*., 1997).

An adequate amount of NH_3^+-N is continuously available for microbial protein synthesis in the rumen (Hussain & Cheeke, 1995). Hess *et al*. (2003a) have found no effect of saponins on protozoa numbers. This could be due to the capacity of some rumen bacteria to hydrolyse saponins into free glycosyl and saponin fractions (Newbold *et al*., 1997), therefore removing its toxicity against protozoa. The foaming property of saponins increases the surface tension of the bulk solution and accelerated lyses of microbial cells with weakened membranes (Newbold *et al*. 1997). In addition, bacterial growth inhibition may be caused by complexion of essential minerals and steroids with saponins, thus limiting their bioavailability for bacterial metabolism (Simons *et al*., 2006).

Of the nine studies with saponins summarized by Goel & Makkar (2012), six reported a decreased in CH_4, from about 6 to 27 % (absolute production, or per unit of BW or DMI). In three of these studies, however, OM digestibility was decreased and in another three, digestibility was not reported (Goel & Makkar 2012). From the analysis, they noted that there was no difference in the CH_4-mitigation effect between steroidal saponins (*Yucca schidigera*) and triterpenoid saponins (*Quillaja saponaria*); *Y. schidigera* and *Q. saponaria* have been studied the most as sources of

saponins because of its commercial availability. Hu *et al*. (2006) fed goats 0, 3 and 6 g/day tea saponins and observed an increase in feed intake and a consequent increase in average daily gain (ADG) with the 3 g/day dose.

Wang *et al*. (2009) reported an approximately 15 % decrease in CH_4 production by sheep fed 170 mg/day of *Y. schidigera* extract. Mao *et al*. (2010) reported no effect of tea saponins (3 g/day) on ADG of lambs but a 28 % decrease in CH_4 production. In another study from the same group, Zhou *et al*. (2011) reported a 6 to 10 % mitigating effect of tea saponins on CH_4 production in restricted-fed sheep. *Yucca schidigera* or *Q. saponaria* fed to dairy cows at 10 g/day had also no effect on milk production, total-tract nutrient digestibility, rumen fermentation or CH_4 production in the study of Holtshausen *et al*. (2009). Similarly, 3 g yucca powder per kg diet DM fed to dairy cattle did not affect feed intake, milk production and composition, digestibility, energy balance or CH_4 production in the study of Van Zijderveld *et al*. (2011). Overall, with perhaps the exception of some data on tea saponins that requires further validation, there is not enough evidence of consistent (or long-term) effect of saponins on enteric CH_4 production or animal performance.

1.1.3.2 Essential oils

A large number of *in vitro* experiments investigated the CH_4 mitigating potential of essential oils and its active ingredients (Calsamiglia *et al*., 2008; Bodas *et al*., 2008; Benchaar *et al*., 2009). Unfortunately, few have followed up the *in vitro* work with *in vivo* experiments. In most cases, these plant bioactive compounds **(**PBAC) have not been successful as CH_4 mitigating agents (Beauchemin and McGinn, 2006; Benchaar *et al*., 2007; Van Zijderveld *et al*., 2011). In a review on the topic, Benchaar & Greathead (2011) concluded that some essential oils (*viz*.; garlic and its derivatives, and cinnamon) reduced CH_4 production *in vitro*. These compounds, however, have not been studied extensively *in vivo*, and there is no evidence that it can be used successfully to inhibit rumen methanogenesis. In some cases, as with *Origanum vulgare* leaves, the effect on CH_4 mitigation was significant, and there was also a trend for increased milk production and feed efficiency in dairy cows (Tekippe *et al*., 2011; Hristov *et al*., 2013) but these results remain to be confirmed in long-term experiments.

1.1.4 Plant species with the potential to be used as a feed supplement during times of drought

The use of browse species as fodder for ruminant is increasingly becoming important in many parts of the tropics. Generally, tree fodder is richer in crude protein (CP), minerals and digestible nutrients than grasses (Devendra, 1990; Topps, 1992). In Southern Africa, protein supplements can be obtained from indigenous legume trees, such as *Colophospermum mopane*, *Brachystegia spiciformis*,

D. cinerea and the *Acacia genus* (Dube, 2000; Mlambo *et al*., 2004; Tefera *et al*., 2008). The use of tree legume fodder as supplement has been found to improved intake, digestibility and animal performance (Norton, 1994; Abdulrazak *et al*., 1996). *Acacia* trees are ubiquitous in many parts of the arid and semi-arid areas of Southern Africa and have multiple uses, by providing food, medicine and fodder, besides from being resistant to diseases and the harsh climatic conditions (Le Houérou, 1980).

The genus of *Acacia*, *Acacia karroo* in particular, is the most widespread and is often leafy at the end of the dry season (Barnes *et al*., 1996). *Acacia karroo* epitomises the concept of a multi-purpose tree until it was a serious encroacher, threatening rangelands and animal productivity (Nyamukanza & Scogings, 2008). Focus has now shifted from its eradication as a weed to its utilisation as a protein supplement for livestock, particularly goats and cattle. *Acacia. karroo* contains thorns and tannins, which deter herbivory and reduce browse utilisation by herbivores (Teague, 1989; Dube *et al*., 2001). The effects of these anti-quality factors can be easily moderated by harvesting leaves and air drying it in the sun prior to feeding (Mapiye *et al*., 2009). The leaf meal of *A. karroo* contains between 100 and 160 g crude protein (CP) per kg of dietary dry matter (Aganga *et al*., 2000; Halimani *et al*., 2005). To meet a steer's daily CP requirement of between 110 and 160 g/kg of dietary DM (NRC, 2000) a steer weighing between 150 and 300 kg should be fed an additional 1000–1500 g of *A. karroo* leaf meal per day to gain between 200 and 350 g/day (Mapiye, 2009). One 2.0 m tall tree can produce up to 1 kg of leaf meal per annum. The recommended optimum plant density for *A. karroo* production ranges between 500 and 1000 plants/ha (O'Connor, 1995; Barnes *et al*., 1996) which translates to a leaf meal biomass yield of 1000 kg/ha/annum. Such an amount of leaf meal can meet CP requirements of up to 16 steers for 60 days. Since *A. karroo* is adapted and widely distributed in the dry-areas, easily propagated from seed (Scogings & Mopipi, 2008) has high growth rates and copping ability (Barnes *et al*., 1996), it is possible to get sufficient and continuous supply of leaf meal for sustainable beef production in semiarid areas. Considering that herd sizes per household in communal areas of Southern Africa vary between 5 and 10 cattle (Mapiye *et al*., 2009) and replacement rates are less than 10% of the total herd per annum (Musemwa *et al*., 2008) use of *A. karroo* leaves could be a feasible and viable resource for sustainable beef producers.

According to Aganga *et al*, (2000) *A. haematoxylon* has the potential to be used as a supplement during times of drought. In comparison to other *Acacia* species, *Acacia gerrardii* had relatively low ADL, CT and TP, and higher CP and IVDMD (Ndagurwa *et al*., 2013). Previous studies have also reported high digestibility in browse with a high CP (Kaitho *et al*., 1998) and low fibre (Van Soest, 1994) concentration. Lignin cannot be degraded anaerobically, and has been found to depress cell wall digestion (Van Soest, 1994). The lower IVDMD recorded for *A. Karroo* than other *Acacia* species can be attributed to a relatively higher concentration of ADL and ADF (Mapiye *et al*., 2011). *Acacia Karroo* has high amounts of non- extractable and fibre bound proanthocyanidins, which may interfere with digestibility (Dube *et al*., 2001). Previous studies also reported the negative effects of CT on digestibility (Ammar *et al*., 2005; Mokoboki *et al*., 2005). *Acacia nilotica* contained more total

phenols (TP) than the other *Acacia* species which is consistent with previous studies (Dube *et al*., 2001). Most of the phenolics in *A. nilotica* are catechingallates, which may be toxic to ruminant animals (Mueller-Harvey *et al*., 1987). Although *A. nilotica* phenolic concentration may be toxic, its digestibility remains high (Dube & Ndlovu, 1995).

The presence of phenolic compounds in A*cacia* species has a negative effect on its nutritional value and on its intake by ruminant animals (Degen *et al*., 1998). Tannins are attributed to be one of the major causes of the limited use as a livestock fodder (Makkar *et al*., 1993). Studies on some *Acacias* have shown tannins have either a positive (Salem *et al*., 1999) or a negative effect (Degen *et al*., 1998) on animal production. This variable effect could be attributed to the type of species, season and nutritive value.

1.1.5 Plant species with potential to decrease methane production

One characteristic of research involving effects of tannins on animal digestion and productivity is the variation in responses among studies. Some of the variation may be explained by the type, concentration and protein binding capacity of the tannins and concentration of dietary CP (Jayanegara *et al*., 2009). Other causes of inconsistencies include variable techniques to measure the tannin concentration (Makkar, 2003) and failure to distinguish between condensed and hydrolysable tannins (Mueller-Harvey, 2006) as well as the level of intake expected or required for optimal production. Condensed tannins can reduce the rate of digestion (Makkar *et al*., 1995) but this will have little effect on animals fed at the maintenance level of intake, because the rumen can accommodate more feed. However, in a lactating animal, production can be reduced because of bulk fill limitations on feed intake (Grainger *et al*., 2009). Other author's findings on the effect of tannins (condensed or hydrolysed) are summarized in Table 1.1.

Table 1.1 Effect of tannins on fermentation

Plant specie	**Effect of tannin on protein availability**	**Effect of tannin on OM digestibility**	**Effect of tannin on methane production**	**Reference**
Acacia mearnsii	Decreased	NA	Decreased	Grainger *et al*. (2009)
Lespedeza cuneata	Decreased	No effect	Decreased	Amimut *et al*. (2008)
Prosopis cineraria	Decreased	Increased	NA	Bhatta *et al*. (2005)
Lespedeza striata	Decreased	No effect	Decreased	Amimut *et al*. (2008)
Prosopis juliflora	No effect	No effect	Decreased	Soltan *et al*. (2012)
Acacia saligna	Decreased	No effect	Decreased	Soltan *et al*. (2012)
Atriplex halimus	No effect	No effect	Decreased	Soltan *et al*. (2012)
Leucaena leucocephala	Decreased	No effect	Decreased	Soltan *et al*. (2012)

NA = not available

The study by Grainger *et al*. (2009) is a good example of how digestibility, feed intake and ultimately production (and milk fat and protein yields) may be negatively affected if tannins (condensed in this case) are overdosed.

1.1.6 Conclusion

In conclusion, hydrolysable and condensed tannins are plant bioactive components that may offer an opportunity to reduce enteric CH_4 production, although intake and animal production may be compromised. Tea saponins seem to have the potential, but more and long-term studies are required before it could be possibly recommended for use. Where CH_4 production was reduced *in vivo*; the long-term effects on animal performance and product quality were not established. Browse offers opportunities for utilization in semi-arid regions of South Africa. The tannin content helps reduce methane but the effect is not analyzed. Therefore, research is required from browse in the Kalahari bush-dune-veld to determine the effects of tannin rich plants on methane reduction, either directly or indirectly, to get more accurate, consistent results by making use of *in vitro* studies that allows screening specific plants with methane reducing properties.

1.1.7 Study objectives

- To characterise a range of tropical and sub-tropical forage species collected from the Kalahari-bush-dune-veldt in terms of chemical composition, plant secondary compounds and potential nutritive value.
- To determine the effect of tannins on methane emission from a range of tropical and sub-tropical browse species (using *in vitro* techniques), with or without the use of PEG (poly-ethylene glycol) to bind with tannins.
- To investigate the possible beneficiary effect of supplementation of tannin rich plant materials on fibre degradation and reduction of methane from *Eragrostis trichopophora* forage using *in vitro* techniques.

1.1.8 Hypotheses

- H_0: There are no species differences in the chemical composition, potential nutritional value and *in vitro* methane production of the 19 different tropical and/or subtropical browse species collected from the Kalahari-bush-dune-veldt in South Africa.
- $H_{1:}$ There are species differences in the chemical composition, potential nutritional value and *in vitro* methane production of the 19 different tropical and/or subtropical browse species collected from the Kalahari-bush-dune-veldt in South Africa.
- H_0: Inclusion of tannin rich plant materials at different levels will not affect the *in vitro* gas production, digestibility and methane production when incubated in a flask with *Eragrostis trichopophora* hay as the major substrate.
- H_1: Inclusion of tannin rich plant materials at different levels will affect the *in vitro* gas production, digestibility and methane production when incubated in a flask with *Eragrostis trichopophora* hay as the major substrate.

CHAPTER 2

Chemical compositions of some Kalahari browse forage species

2.1 Abstract

The study aimed to quantify and analyse the nutritional value of selected shrub and tree species in the Kalahari region located in the Northern Cape of South Africa. The following nineteen browse (shrub and tree) species namely *Acacia erioloba, Boscia albitrunca, Acacia haematoxylon, Olea europaea, Ziziphus mucronata, Terminalia sericea, Rhus lancea, Acacia karroo, Prosopis glandulosa, Acacia luederitzii, Acacia mellifera, Acacia hebeclada, Grewia flava, Dichrostachys cinerea, Hermannia burchelli, Lycuim cinereum, Monechma genistifolium, Hermannia tomentosa* and *Monechma incanum* were selected for this study and its forage harvested during the medium maturity vegetative stage The chemical composition and nutritional value of the edible forage biomass (leaves and <2 mm stem) was determined by analysing the crude protein (CP), ash, dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), total phenols and condensed tannin in the foliage. Crude protein ranged from 70-320 g/kg DM, ash 40-210 g/kg DM, dry matter 908-942 g/kg, NDF 350-508 g/kg DM, ADF 270-530 g/kg DM, ADL 85-320 g/kg DM and neural detergent fibre nitrogen (NDFN) 47-93 g/kg DM. Total tannins ranged from 9-320 g/kg DM, condensed tannins 2-125 g/kg DM and hydrolysable tannins ranged from 3-195 g/kg DM. All 19 browse species used in this study, except *O. europaea*, *T. sericea* and *M. genistifoluim* will meet animal maintenance requirements based on CP concentrations according to the NRC (2000) guidelines for sheep. *Boscia albitrunca*, *R. lancea*, *L. cinereum* and *H. burchelli* seems promising as a supplement during times of drought, having moderate CP values with low NDF, ADF and ADL values. The availability of these browse species is however limited, and there is no cultivation of these browse species due to the Kalahari being in a low rainfall area. This study classified browse species according to its chemical composition and nutritional value.

Key words: browse, chemical composition, digestibility, tannin

2.2 Introduction

Although the nutritive value of conventional feeds on animals have been studied extensively, little information is available about the nutritive value of alternative feeds such as browse. However, this may become an important source of nutrients for grazing animals under harsh conditions such as in the semi-arid Kalahari-bush-dune-veldt, especially during the dry season when the nutritional value and quantity of green herbages are limited (Rooyen, 2001). Browse also have the potential to alleviate some feed shortages and nutritional deficiencies experienced during the dry season by grazing animals in these areas where few alternative feedstuffs are available (Salem *et al*., 2006). Browse foliage are important components in the diets of cattle, sheep, goats and wild ungulates in arid and semi-arid regions of tropical Africa, including the Kalahari (Rooyen, 2001). However, the rational use of these species as fodder for ruminants requires the knowledge of its nutritive value. The nutritive value of browse species is highly variable (Papachristou *et al*., 2007) being affected by plant species, plant part, plant age and environmental factors such as seasonality, sunlight intensity, temperature and water availability (Wilson, 1977).

Seasonal variations are mainly attributed to the physiological changes which occur in plants during the growing season. Moreover, species vary in its response to climatic and physiological changes (Dann & Low, 1988). These differences in seasonal variations determine the practical value as a forage source for ruminants. The recognition of the potential of tree foliage to produce considerable amounts of high protein biomass and energy, especially in harsh and arid conditions has led to the development of animal farming systems that integrate the use of tree foliage with bulky feed sources (Devendra, 1990). However, the nutritional value of shrubs and tree species are not quantified and the optimal utilization by herbivores may be restricted by high levels of secondary compounds in their leaves (Salem, 2005; Salem *et al*., 2006). Some browse species may contain anti-nutritive factors that reduce intake as well as protein and dry matter digestibility (Reed, 1986). An important group of these allelochemicals found in tropical browse species is polyphenolics, among which the major one is tannins (proanthocyanidins and hydrolysable tannins) (Reed *et al*., 1990). The main objective of the present study was to determine the chemical composition of indigenous browse species found in the Kalahari bush veldt.

2.3. Materials and methods

2.3.1 Sample collection and preparation

Samples of edible forage from 19 browse species were collected in the Kalahari (S 26° 46.610' E 22° 34.557') area located in the Northern Cape Province of South Africa between March and April 2012. Emphasis was given to browse species commonly utilized by livestock in this area. The list of browse samples used for this study is indicated in Table 2.1. The plant samples were collected when the browse plants were at a medium maturity vegetative stage. During the sampling period, approximately 5 kg of fresh plant leaves and twigs (fine stems) were hand plucked from the browse species to be used in an *in vitro* study. This plant material were dried in a cool dark place, but was not washed. The forage samples were sub-sampled and dried at 55 °C for 48 h in a forced air oven. Subsequently the samples were ground to pass through a 2 mm and 1 mm sieves in a Willey mill and was stored until used for analysis. These browse species were systematically divided into two categories as trees (with a height of >2 m) and shrubs (with a height <2 m). The browse species were analysed, compared and discussed between and within the two broader groupings.

2.3.2 Determination of chemical composition of forage sample

Samples of the forage materials were analysed for DM and total ash (method 934.01 AOAC, 2000). Nitrogen was determined with the Leco Dumas method 968.08 (AOAC 2000). The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to the method of Van Soest *et al*. (1991). Fat concentration was determined using ether extraction (EE) in the Tecator Soxtec (HT6) system (AOAC, 1999). The non-fibre carbohydrate (NFC) concentration of the feeds was calculated by subtraction of CP, NDF, fat, and ash from total DM (Sniffen *et al*., 1992).

The concentration of macro and micro minerals such as calcium, iron, zinc and manganese were analysed using atomic absorption spectrophotometer following method 935.13 (AOAC, 2000). Phosphorous concentration was determined using an auto analyser (AOAC, 2000). All browse samples were replicated twice during laboratory analysis, to enhance accuracy and to determine the mean error during the measurements. Where the difference between the averages of duplicated samples was greater than 5%, the analysis was repeated.

Determinations of total phenols (TP), total tannins (TT) and condensed tannins (CT) were done based on the method describe by Makker (2003). The TP and TT were determined by a modified folin-ciocalteu method using polyvinyl-polypyrrolidone (PVPP) to separate tannin phenols from non-tannins phenols (NTP). Both TP and TT were calibrated against tannic acid solution as a standard (Sigma–Aldrich) and values were expressed as tannic acid equivalents. Condensed tannins were

determined by the butanol–HCl–iron method (Singh *et al*., 2005). The CT values obtained in the plant samples were expressed as leucocyanidin equivalents. The HT was estimated as the difference between TT and CT (Singh *et al*., 2005).

2.3.3 Statistical analysis

Tree and shrub foliage nutrient and secondary plant compound concentrations were statistically analysed using the 'GLM' option of SAS (9.1) with methods of Steel & Torrie (1980). Differences among foliage species were determined using Duncan's multiple-range test (Duncan & Brant 1983).

2.4. Results and discussion

2.4.1 Chemical composition of the browse leaves

The chemical composition of the forage from the browse species collected in the Southern Kalahari region of South Africa is summarized in Table 2.2. There is a significant ($p<0.05$) variation in chemical compositions of the studied browse species.

There were some plant species (*M. genistifolium* and *L. cinereum*) that had an ash concentration of >100 g/kg DM. The CP concentration also varied considerably between the shrub and tree species. Among the tree forage species (>2m), *B. albitrunca* had the highest CP value and *O. europaea* had the lowest CP concentration. Within the shrub forage sample (<2m), *L. cinereum* had the highest and *M. genistifolium* the lowest CP concentration. In this study it appears that forage from shrub species had higher crude protein concentrations (173.3 g/kg DM) than tree species (142.3 g/kg DM). In South Africa, *Acacia* species such as *A. karroo* have been reported to be a valuable source of forage for herbivores, particularly during dry periods (Aganga *et al*., 2000; Dube, 2000; Tefera *et al*., 2008). Although *A. karroo* leaves contain high levels of CP and essential amino acids (Ngwa *et al*., 2000). In the current study the CP levels of *A. karroo* leaves (125 g/kg) are on the lower side compared to other indigenous *Acacia* species as reported by many authors (Abdulrazak *et al*., 2000; Aganga *et al*., 2000; Ngwa *et al*., 2002; Mokoloki *et al*., 2005; Ngwa *et al*., 2000; Rubanza *et al*., 2005), but it is within the optimal range of 110–160 g CP/kg DM recommended by the NRC (2000) for beef production. This makes *A. karroo* a potentially important source of CP supplement for cattle, goat and sheep grazing low quality forages in the specific study area. Hassen *et al*. (2008) studied the chemical composition of *Z. mucronata* foliage and the reported CP concentration (179 g/kg DM) of that study was relatively higher than the CP concentration recorded in the present study (157.3 g/kg DM). The CP concentrations of the tree and shrub species may differ from one geographical location to the next as can be seen in this study and those of Abdulrazak *et al.* (2000), Aganga *et al*. (2000), Mokoboki *et al*. (2005), Ngwa *et al*. (2000), Rubanza *et al*. (2005) and Hassen *et al*., (2008). Out of the 19 browse species in the present study *O. europaea*, *T. sericea* and *M. genistifolium* were the only species that

cannot be used as a potential source of protein supplement during times of drought, due to its low CP concentrations, according to the recommendations for sheep production by the NRC (2001).

The fibre concentrations of the tree and shrub foliage was analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) and the results are shown in Table 2.2. It was recorded that *A. haematoxylon* and *T. sericea* had the highest NDF and ADF concentrations respectively while *A. erioloba* had the highest ADL concentrations among the tree species analysed. The highest concentration found among the shrub species were *H. tomentosa*, *D. cinerea* and *H. tomentosa* was recorded respectively for NDF, ADF and ADL while the lowest concentration for NDF, ADF and ADL was recorded for the trees *A. karroo*, *Z. mucronata* and *A. luederitzii* and shrubs *M. incanum*, *L. cinereum* and *H. burchelli*, respectively. The ADF and NDF concentrations recorded in the current study for *Z. mucronata* foliage were lower than those results recorded by Hassen *et al.* (2008) and Rothauge *et al.* (2003) for the same species. The NDF and ADF concentration recorded in the current study for *D. cinerea* were lower and higher, respectively, than the NDF and ADF value reported for the same species by Tefera *et al*. (2008). After analysing all the browse species in the present study, according to its CP, NDF, ADF and ADL concentrations, it seems that some of the trees (*B. albitrunca* and *R. lancea*) and shrubs (*L. cinereum* and *H. burchelli*) species can be used as a potential supplement during times of drought. These species will meet the daily requirements for animal production according to the NRC (2000) having high CP concentrations and low fibre values. The other aspect that needs to be considered is the availability of these browses. Relative amounts of *B. albitrunca* and *R. lancea* can be harvested during times of drought, but the availability of DM from the shrub species *L. cinereum* and *H. burchelli* was low. Further research will be required to determine the digestibility and availability of nutrients of *B. albitrunca*, *R. lancea*, *L. cinereum* and *H. burchelli* before it can be recommended as a source of protein supplement for beef cattle.

2.4.2 Plant secondary compound composition of browse species

There was a significant ($p<0.05$) variation of phenolic composition of forage from the studied browse species (Table 2.3). *A. luederitzii* and *D. cinerea* had the highest concentration of total phenols, total tannins, condensed and hydrolysed tannins among the tree and shrub forage species, respectively. The tree forage species *B. albitrunca* had the lowest phenolic concentrations, while *L. cinereum* had the lowest concentration of total phenols, total tannins and hydrolysed tannin. *A. hebeclada* had the lowest condensed tannin concentration among all the shrubs test in the current investigation.

In a review, Min *et al*. (2003) noted that at equivalent concentrations different CT sources had variable effects on degradation of CP due to differences in molecular weight and chemical structure affecting the biological activity of CT. Bhatta *et al*. (2005) noted a marked increase in CP digestibility

when goat kids were fed diets containing CT-rich *Prosopis cineraria* leaves and supplemented with polyethylene glycol. In the study of Getachew *et al*. (2002), NH_3-N and short chain volatile fatty acid (SCVFA) concentrations were lower ($p<0.05$) when CT was included. It seems that high levels of CT have a negative effect on digestibility. The phenolic components of the browse species studied are similar to the results obtained from these authors (Getachew *et al*. 2002; Animut *et al*. 2008 and Jayanegara *et al*. 2011). Optimal utilisation of CP from *Acacia* browse leaves could be limited by high (viz.>80 mg/g DM) concentration of phenolics and tannins and even higher (*viz*., >50 mg/g DM) concentrations of polymerised condensed tannins Rubanza *et al*. (2008). Such high levels of tannins could impair utilisation of CP from browse supplements to ruminants (Aerts *et al*., 1999) due to tannin interactions through chemical formations with dietary nutrients. This causes depressed feed digestibility and affects overall nutrient availability to the body (Mangan, 1988). Reed (1986), Abdulrazak *et al*. (2000) and Rubanza *et al*. (2003) also reported high concentrations of phenolics and tannins in *Acacia* species leaves. Slight variations between observed and literature values in phenolic and tannin concentrations could be due to the method used to extract tannins, nature of tannins in different fodder species, molecular weight of tannins, stage of growth and the proportion of leaf sample harvested (Waterman & Mole, 1994). The high levels of phenolics in *A. luederitzii* and *A. karroo* observed in the present study were much higher compared to other *Acacia* species reported by Rubanza *et al*. (2003).

Differences in levels of phenolics and tannins among species of *Acacia* leaves could also be explained by differences in genotypic factors that control biosynthesis and accumulation of polyphenolic compounds in *Acacia* (Wong, 1973; Haslam, 1998). Relatively high CT fractions in *A. luederitzii* (125.4 g/kg DM) and *D. cinerea* (124.4 g/kg DM) recorded in this study could result in depressed palatability as well as intake of these species as CT had been associated with reduced palatability and feed intake in ruminants (Muhammed *et al*., 1994). Adverse effects due to high proportions of condensed tannins bound to protein would be through a reduced CP digestibility, mainly by formation of protein–tannin complexes (Aerts *et al*., 1999). Negative effects of the fibre bound CT fraction on feed digestibility would be mainly through formation of complexes with dietary carbohydrates (Muhammed *et al*., 1994). Variable CT fractions among the *Acacia* spp. could be related to variable tannin activity, the close relationship between proanthocyanidin composition and distribution of CT fractions, tannin stereochemistry (Waterman, 2000) and the effect of tannin structure on tannin biological activity (Haslam, 1998). In the study of Charels *et al*. (2008) tannins in forage reduced the incidence and severity of bloat. As discussed in section 2.1, *B. albitrunca* and *R. lancea* are among the browse species that seems to have the best potential to be used as a supplement during times of drought. From the analysis of the phenolic composition of *B. albitrunca*, bloat might occur if it is fed alone due to its very low phenolic concentrations. Forages containing tannins are non-bloating because tannins bind excess plant proteins, precipitating them out of rumen fluid, and in the process, preventing the creation of the stable foam that's characteristic of pasture bloat (MacAdam

et al., 2011). Further research needs to be done on the phenolic components of the browse species selected in this study, to analyse its potential effects on digestibility and animal production.

2.4.3 Mineral composition of browse species

It is well known that the soil of the Kalahari-bush-dune-veldt contains low concentrations of phosphorus while it is rich in Fe and Mn. The mineral compositions of the browse species were evaluated to determine the nutritional composition of the selected browse spesies. Focus was on Ca, P, Fe, Mn and Zn dew to limited resources available.

There was a significant ($p<0.05$) difference between species in terms of the mineral composition of forage from the studied browse species (Table 2.4). For the tree forage species, *Z. mucronata*, *R. lancea*, *A. luederitzii*, *A. hebeclada* and *T. sericea* had the highest Ca, P, Mn, Fe and Zn concentrations, respectively. In contrast, *B. albitrunca* and *A. erioloba* had the lowest Ca and P concentrations, while *R. lancea* had the lowest Mn, Fe and Zn concentrations. Among the shrub forage sample, *A. melliferia* and *M. incanum* had respectively the highest Ca and Zn concentrations, while *L. cinereum* had the highest P, Mn and Fe concentrations. In contrast the lowest concentrations of Ca and Zn were observed in *D. cinerea*. Whereas *A. melliferia* had the lowest P and Fe concentrations and *M. incanum* had the lowest Mn concentration. In this study the P and Ca values recorded for tree species such as *R. lancea* and *Z. mucronata* were lower than those reported by Aganga *et al*. (1998) for the same species, while the Fe and Mn concentrations were much higher in the present study compared to that of Aganga *et al*. (1998). The P and Ca concentrations of *A. karroo* from the Kalahari dune bush veldt were lower than *A. melliferia* which in turn had a higher Fe and Zn profile than that reported by Abdulrazak *et al*. (2000), but showed a lower Mn concentration. The Ca and Zn values recorded in this study for *D. cinerea* was lower than those reported by Tefera *et al*. (2008), while the P concentration was slightly higher in this study. Overall the values reported by Aganga *et al*. (1998), Abdulrazak *et al*. (2000), Ngwa *et al*. (2000) and Tefera *et al*. (2008) for Ca and P are relatively higher than those recorded in this study while Fe and Mn values in this study are much higher than those reported by Aganga *et al*. (1998), Abdulrazak *et al*. (2000), Ngwa *et al*. (2000) and Tefera *et al*. (2008). The higher Fe and Mn concentrations in this study could be related to the soils being rich in these two minerals. The Ca, P and Mn concentrations of *Z. mucronata* reported by Hassen *et al.* (2008), tended to be higher, the same and lower respectively compared to the present study. The P concentrations of the browse species in this study had similar concentrations to those reported by Hassen *et al.* (2008) for all browse species (except *L. cinereum*) where the P concentration levels was below the critical level of 3 g/kg DM suggested by McDowell (2003) for maintenance requirements of ruminants. It seems that browse species from the Kalahari-bush-dune-veldt have a high Fe and Mn concentration, while the P concentration of browse species are deficient.

2.5 Conclusion

This study concludes that the chemical composition and nutritional value of browse species differ largely between the tree and shrub species. There is not enough relevant data available from other articles regarding the nutritional value and chemical composition of different browse species of the Kalahari-bush-dune-veldt. It can be concluded that the required data gathered in this study falls within the range of other researched data analyses and gives a good representative value of the chemical composition of browse species from this region of the Kalahari. The nutritional value of browse species *B. albitrunca*, *R. lancea*, *L. cinereum* and *H. burchelli* could possibly meet ruminant maintenance requirements. These browse species could be used as a feed supplement, depending on DM availability during times of drought. Taking its availability into account, *B. albitrunca* is likely the superior option to be used as a supplement, but bloat might be a problem, because *B. albitrunca* has low concentrations of condensed tannins. Further research will be required to determine the digestibility and availability of nutrients in *B. albitrunca*, *R. lancea*, *L. cinereum* and *H. burchelli* to determine if they can be used as a supplement for ruminants.

Table 2.1 List of browse species included in the study

Scientific name	Common Name	Plant type	Palatability	Forage value reference
Acacia erioloba	Camel thorn	Tree	Highly palatable	Poynton R. J. 1984
Boscia albitrunca	Shepherd's tree	Tree	Highly palatable	Poynton R. J. 1984
Acacia haematoxylon	Grey camel thorn	Tree	Highly palatable	Rooyen *et al*., 2001
Olea europaea	Wild olive	Tree	Moderately palatable	Poynton R. J. 1984
Ziziphus mucronata	Buffalo thorn	Tree	Highly palatable	Poynton R. J. 1984
Terminalia sericea	Silver cluster-leaf	Tree	Moderately palatable	Poynton R. J. 1984
Rhus lancea	Karee tree	Tree	Highly palatable	Poynton R. J. 1984
Acacia karroo	Sweet thorn	Tree	Highly palatable	Poynton R. J. 1984
Prosopis glandulosa	Mesquite	Tree	Highly palatable	Rooyen *et al*., 2001
Acacia luederitzii	False umbrella thorn	Tree	Highly palatable	Rooyen *et al*., 2001
Acacia mellifera	Black thorn	Shrub	Highly palatable	Poynton R. J. 1984
Acacia hebeclada	Candle thorn	Shrub	Highly palatable	Rooyen *et al*., 2001
Grewia flava	Velvet raisin	Shrub	Highly palatable	Poynton R. J. 1984
Dichrostachys cinerea	Sickle bush	Shrub	Highly palatable	Poynton R. J. 1984
Hermannia burchelli	Tea bush	Shrub	Highly palatable	Rooyen *et al*., 2001
Lycuim cinereum	Kriedoring	Shrub	Highly palatable	Rooyen *et al*., 2001
Monechma genistifolium	Perdebos	Shrub	Highly palatable	Rooyen *et al*., 2001
Hermannia tomentosa	Lusernbos	Shrub	Highly palatable	Rooyen *et al*., 2001
Monechma incanum	Blouganna	Shrub	Highly palatable	Rooyen *et al*., 2001

Table 2.2 Chemical composition and gross energy content of browse species used in the study

Species name/ Plant type	DM (g/kg DM)	Composition (g/kg DM)								GE (MJ/kg DM)
		ASH	CP	EE	NDF	ADF	ADL	NFC	NDFN	
Trees										
Acacia erioloba	942.4	60.3^{d}	169.8^{b}	47.2^{d}	456.3^{c}	391.8^{d}	324.8^{a}	635.1^{c}	69.0^{b}	19.1^{b}
Boscia albitrunca	939.9	75.6^{b}	253.3^{a}	18.0^{g}	369.2^{f}	292.2^{g}	128.5^{e}	656.7^{b}	53.5^{e}	18.5^{c}
Acacia haematoxylon	936.0	67.2^{c}	131.2^{e}	45.7^{d}	533.4^{a}	431.0^{b}	275.7^{b}	585.1^{d}	75.9^{a}	20.2^{a}
Olea europaea	938.2	60.9^{d}	74.9^{j}	29.0^{e}	355.6^{g}	327.8^{f}	131.3^{e}	679.6^{a}	47.2^{f}	18.1^{d}
Ziziphus mucronata	925.9	91.2^{a}	157.3^{d}	52.3^{c}	364.6^{gf}	291.3^{g}	166.6^{c}	655.5^{b}	65.4^{b}	17.9^{d}
Terminalia sericea	924.5	51.3^{e}	97.4^{i}	63.7^{b}	520.1^{b}	445.7^{a}	105.3^{f}	543.2^{e}	59.1^{d}	17.5^{d}
Rhus lancea	922.9	49.0^{f}	120.3^{h}	23.7^{f}	454.0^{c}	404.5^{c}	95.0^{g}	645.7^{bc}	61.3^{c}	17.6^{d}
Acacia karroo	920.4	69.1^{c}	124.7^{g}	73.2^{a}	427.2^{d}	351.8^{e}	133.3^{de}	669.0^{a}	75.0^{a}	18.5^{c}
Prosopis glandulosa	937.8	62.2^{d}	163.5^{c}	29.8^{e}	403.6^{e}	347.8^{e}	101.8^{f}	594.9^{d}	43.1^{f}	17.7^{d}
Acacia luederitzii	935.5	60.2^{d}	130.6^{f}	63.2^{b}	460.3^{c}	385.8^{d}	138.8^{d}	639.3^{c}	74.4^{a}	20.2^{a}
Mean	932.35	64.7	142.3	44.58	434.43	366.97	160.12	630.41	62.38	18.53
Range	920.4-942.4	49-91.2	74.9-253.3	18-73.2	355.6-533.6	292.2-445.7	95-324.8	543.2-679.6	43.1-75.85	17.5-20.2
SE		0.728	0	0.508	0.413	0.386	0.197	4.367	0.081	0
P		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Shrubs										
Acacia mellifera	922.2	84.1^{c}	203.4^{c}	40.3^{c}	373.6^{f}	302.1^{f}	80.1^{h}	623.2^{c}	52.9^{d}	17.7^{b}
Acacia hebeclada	931.4	62.9^{g}	233.0^{b}	34.0^{d}	401.2^{e}	390.0^{cd}	97.4^{f}	681.0^{b}	80.1^{ab}	18.1^{a}
Grewia flava	926.1	70.2^{f}	125.1^{h}	60.5^{b}	443.9^{d}	399.5^{c}	105.3^{e}	572.9^{d}	51.3^{d}	18.1^{a}
Dichrostachys cinerea	910.2	40.5^{h}	141.5^{e}	26.0^{e}	508.9^{a}	376.8^{d}	227.8^{d}	725.4^{a}	93.0^{a}	18.1^{a}
Hermannia burchelli	910.7	75.3^{e}	177.2^{d}	23.2^{f}	369.7^{f}	353.2^{e}	69.9^{i}	725.9^{a}	72.2^{b}	16.9^{c}
Lycuim cinereum	918.9	216.3^{a}	328.9^{a}	20.3^{g}	363.0^{f}	277.2^{g}	85.4^{g}	589.4^{d}	80.5^{ab}	12.6^{e}
Monechma genistifolium	908.2	209.5^{b}	83.8^{i}	33.9^{d}	494.2^{b}	465.1^{a}	266.2^{b}	381.5^{f}	48.6^{de}	13.8^{d}
Hermannia tomentosa	919.6	85.0^{c}	136.2^{f}	22.5^{f}	474.9^{c}	426.4^{b}	290.3^{a}	534.4^{f}	61.9^{c}	16.5^{c}
Monechma incanum	908.8	80.6^{d}	130.6^{g}	68.1^{a}	449.1^{d}	350.1^{e}	232.1^{c}	631.3^{c}	64.6^{bc}	16.9^{c}
Mean	917.34	102.71	173.3	36.53	430.94	371.15	161.58	607.22	67.3	16.5^{c}
Range	908.2-931.4	40.5-216.3	83.3-328.9	20.3-68.1	363.0-508.9	277.2-465.13	69.9-290.3	381.5-725.9	48.63-93.03	12.6-18.1
SE		3.53	0	0.289	0.352	0.499	0.14	6.136	0.187	0
P		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Parameters with different superscript across the columns are significantly different (P<0.05) for trees/shrub. Ash, OM =organic matter, CP = crud protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, NFC = non fibre carbohydrates, NDFN = neutral detergent fiber nitrogen and GE = gross energy

Table 2.3 Phenolic composition of browse species selected form the Kalahari region in g/kg DM

Scientific name	Total phenol	Non- tannin phenols	Total tannin	Condensed tannin	Hydrolysable tannin
Trees					
Acacia erioloba	182.4^{d}	37.1^{e}	145.4^{d}	67.5^{e}	77.9^{e}
Boscia albitrunca	24.7^{h}	15.6^{g}	9.1^{h}	2.2^{i}	6.96^{h}
Acacia haematoxylon	204.1^{c}	40.7^{d}	163.4^{c}	74.7^{d}	88.7^{cd}
Olea europaea	154.9^{f}	81.9^{b}	73.0^{g}	33.9^{h}	39.1^{g}
Ziziphus mucronata	136.2^{g}	56.8^{c}	79.4^{g}	44.2^{g}	35.1^{g}
Terminali sericea	223.4^{b}	116.5^{a}	106.8^{f}	42.5^{g}	64.3^{f}
Rhus lancea	226.6^{b}	29.6^{f}	197.0^{b}	88.1^{c}	108.8^{b}
Acacia karroo	217.2^{b}	16.5^{g}	200.7^{b}	103.5^{c}	97.2^{c}
Prosopis glandulosa	171.2^{e}	39.2^{de}	132.0^{e}	53.0^{f}	79.0^{de}
Acacia luederitzii	314.5^{a}	15.8^{g}	298.8^{a}	125.4^{a}	173.4^{a}
Mean	185.52	44.97	140.56	63.5	77.05
Range	24.7-314.5	15.6-116.5	9.1-298.8	2.2-125.4	6.69-173.4
SE	3.4	0.78	3.41	1.16	3.58
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Shrubs					
Acacia mellifera	54.8^{f}	24.1^{d}	30.6^{f}	5.3^{h}	25.3^{f}
Acacia hebeclada	30.5^{h}	14.4^{f}	16.1^{g}	3.2^{h}	12.9^{g}
Grewia flava	233.7^{c}	62.4^{b}	171.3^{c}	81.1^{c}	90.2^{c}
Dichrostachys cinerea	386.5^{a}	66.7^{a}	319.8^{a}	124.4^{a}	195.4^{a}
Hermannia burchelli	189.3^{d}	41.0^{c}	148.3^{d}	71.3^{d}	77.0^{d}
Lycuim cinereum	24.3^{h}	11.5^{g}	12.8^{g}	9.6^{g}	3.2^{h}
Monechma genistifolium	44.9^{g}	16.7^{e}	28.2^{f}	13.8^{f}	14.4^{g}
Hermannia tomentosa	97.3^{e}	25.2^{d}	72.1^{e}	18.6^{e}	53.4^{e}
Monechma incanum	307.6^{b}	25.0^{d}	282.5^{b}	119.0^{b}	163.5^{b}
Mean	152.09	31.89	120.19	49.59	70.59
Range	24.3-386.5	11.5-66.7	12.8-319.8	3.21-124..4	3.2-195.4
SE	3.08	0.55	2.9	0.73	2.88
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Parameters with different superscript across the columns are significantly different (P<0.05) for trees/shrub

Table 2.4 Mineral composition of trees and shrubs

Species	Ca (g/kg DM)	P(g/kg DM)	Mn(g/kg DM)	Fe(g/kg DM)	Zn (mg/kg DM)
Trees					
Acacia erioloba	19.29^{b}	0.75^{h}	2.08^{i}	10.86^{e}	17.49^{c}
Boscia albitrunca	7.45^{h}	0.90^{de}	4.40^{b}	7.12^{g}	20.50^{b}
Acacia haematoxylon	18.75^{c}	0.86^{f}	3.95^{c}	28.82^{a}	21.00^{b}
Olea europaea	16.97^{d}	1.01^{c}	2.72^{g}	6.24^{h}	14.52^{f}
Ziziphus mucronata	20.81^{a}	1.18^{b}	3.83^{d}	17.19^{b}	20.98^{b}
Terminalia sericea	15.21^{e}	0.87^{ef}	3.64^{e}	11.81^{d}	22.52^{a}
Rhus lancea	8.10^{g}	1.25^{a}	1.19^{j}	6.57^{h}	14.51^{f}
Acacia karroo	14.54^{f}	0.80^{g}	2.56^{h}	10.38^{f}	15.49^{e}
Prosopis glandulosa	15.62^{e}	1.04^{c}	2.92^{f}	6.40^{h}	20.43^{b}
Acacia luederitzii	15.15^{e}	0.93^{d}	5.70^{a}	15.53^{c}	16.51^{d}
Mean	15.19	0.96	4.09	12.09	18.4
Range	7.45-20.81	0.75-1.25	1.19-5.7	6.24-28.82	14.51-22.53
SE	0.133	0.01	0.028	0.126	0.257
P	<0.001	<0.001	<0.001	<0.001	<0.001
Shrubs					
Acacia mellifera	26.48^{a}	0.73^{h}	3.80^{e}	10.20^{f}	12.99^{f}
Acacia hebeclada	16.33^{e}	1.87^{b}	1.66^{g}	10.27^{f}	20.98^{d}
Grewia flava	21.81^{c}	1.05^{d}	4.03^{d}	11.43^{e}	14.51^{e}
Dichrostachys cinerea	10.32^{i}	0.98^{e}	4.02^{d}	12.52^{d}	12.98^{f}
Hermannia burchelli	13.04^{g}	1.56^{c}	2.45^{f}	13.53^{c}	22.99^{c}
Lycuim cinereum	25.13^{b}	3.54^{a}	9.54^{a}	29.63^{a}	21.46^{d}
Monechma genistifolium	15.62^{f}	0.86^{f}	5.78^{c}	11.35^{e}	14.52^{e}
Hermannia tomentosa	12.34^{h}	1.06^{d}	7.27^{b}	29.55^{a}	29.47^{b}
Monechma incanum	18.29^{d}	0.75^{g}	1.56^{h}	16.24^{b}	46.98^{a}
Means	17.71	1.38	4.98	16.08	21.88
Range	10.32-26.48	0.73-1.87	1.66-9.54	10.2-29.63	12.99-46.98
SE	0.133	0.005	0.031	0.255	0.187
P	<0.001	<0.001	<0.001	<0.001	<0.001

Parameters with different superscript across the columns are significantly different (P<0.05) for trees/shrub. Ca= calcium, P = phosphorus, Mn = manganese, Fe = iron, Zn = zinc

CHAPTER 3

Potential reduction of *in vitro* gas and methane production associated with the tannin composition of browse foliage from the Kalahari region

3.1 Abstract

In this study, leaves of 19 browse species commonly found in the Kalahari bush dune veldt were analysed for *in vitro* organic matter digestibility, volatile fatty acids, rumen ammonia, total gas and methane gas production in an *in vitro* incubation system. Samples of the tree and shrub forage species were incubated with and without polyethylene glycol 8000 for 48 hours in a gas production unit and measurement of total gas, methane, volatile fatty acids, ammonia nitrogen and *in vitro* organic matter digestibility were done. *Acacia luederitzii* and *M. incanum* showed the best potential to decrease methane production by up to 90% after 48 hours of incubation. The secondary components (tannins) of the browse species had no significant effect on *in vitro* organic matter digestibility and ammonia nitrogen concentrations, but volatile fatty acid, methane and gas production were decreased significantly.

Key words: browse, methane, digestibility, tannin

3.2 Introduction

Methane is among the most important greenhouse gases and its global atmospheric concentration has substantially increased over the last 150 years (Mascarelli, 2009). Livestock, especially ruminants, contribute to CH_4 emissions through enteric fermentation as well as fermentation which take place in the manure (IPCC, 2006). McCrabb & Hunter (1999) measured losses of gross energy intake by the ruminant of up to 12 % through enteric CH_4 fermentation. South African livestock production is based on a unique combination of commercial (intensive and extensive) and emerging and communal (subsistence) production systems. The levels of productivity and efficiency in these production systems vary greatly in certain areas and it is important to distinguish between them when calculating GHG emissions. Methane production in livestock is influenced by several factors other than population numbers, including the size and productivity of animals, level of feed intake, diet composition, digestibility and quality of forage, forage species and cultivar, as well as variation among animals (Scholtz *et al*., 2012). This is especially the case when animals use highly fibrous diets which are prevalent in the tropics. Inclusion of feeds containing plant secondary metabolites (PSM), such as saponins or phenols (especially tannins) in diets seems promising as a nutritional strategy to reduce CH_4 emissions from ruminants in the tropics, as many tropical plants are rich in PSM (Puchala

et al., 2005 & Goel *et al*., 2008). The abundance of PSM in tropical plants can be associated with the role that it plays when exposed to environmental stress (assumed to promote resistance against environmental stress) and damaged mechanically or environmentally (Vogt, 2010). The huge diversity in PSM structures may explain its variable potential and effects on methanogenesis and rumen function with observed responses depending on source, type and level of tannins (Waghorn & McNabb, 2003, Mueller-Harvey, 2006; Patra *et al*., 2006).

Condensed tannins (CT) are a diverse group of polymeric flavanols with multiple phenolic groups that chelate metal ions and form complexes with macro-molecules, such as proteins and polysaccharides (Schofield *et al*., 2001). Condensed tannins exhibit multiple biological activities in ruminants, including protein binding (Jones & Mangan, 1977), anthelmintic properties (Athanasiadou *et al*., 2001) and reduction in enteric CH_4 emission (Tedeschi *et al*., 2011). Results from previous studies suggested that feeding forages that contain bioactive CT to ruminants, generally effectively inhibits CH_4 produced during enteric fermentation (Woodward *et al*., 2001, Waghorn *et al*., 2002, Kamra *et al*., 2006, Huang *et al*., 2011; Pellikaan *et al*., 2011; Puchala *et al*., 2012). According to Field *et al*. (1989) and Tavendale *et al*. (2005), the extent of inhibition is related to the type of tannin, tannins of lower molecular weights (MW) like in oligomeric tannins appear to be more effective against methanogens than its monomeric precursors or tannins with a higher MW (Williams *et al*., 1983).

When investigating plants containing secondary compounds on herbivory production, including tannin rich browse, it is crucial to consider the effects of such plants on rumen microbial fermentation, using the *in vitro* gas production technique (Salem *et al*., 2007; Norman *et al*., 2010). Polyethylene glycol (PEG), a tannin binding agent, has the potential to reduce phenolic related anti-nutritive effects in browse plants by forming tannin–PEG complexes (Khazaal *et al*., 1996). It has been used successfully to mitigate adverse effects of secondary compounds on rumen fermentation and quantify indirectly the effect of tannin on various fermentation components by comparing with data from control (without PEG). Addition of PEG to high tannin browse species increased *in vitro* gas and methane production, ammonia N concentration and short chain fatty acid production, (Getachew *et al*., 2001; Salem *et al*., 2007). The objective of this study was to investigate variation between browse species, from the Kalahari bush dune veldt, in terms of gas production characteristics, forage digestibility, volatile fatty acid, NH_3-N and methane production. The single effect of tannins was also studied and quantified for each browse specie by incubating the browse plants with or without PEG. The gas production technique has been widely used for evaluation of the nutritive value of various types of tropical plants (Krishnamoorthy *et al*., 1995; Salem, 2005) and different classes of feeds (Getachew *et al*., 1998). Throughout this study the effects of secondary phenols on gas, methane and digestibility will be discussed.

3.3 Materials and methods

3.3.1 Sample collection and preparation

Samples of 19 edible browse forage species were collected from the Kalahari (S 26° 46.610' E 22° 34.557') area located in the Northern Cape Province of South Africa between March-April 2012. Emphasis was given to browse species commonly utilized by livestock in this area. The list of browse samples used for this study is indicated in Table 3.1. The plant samples were collected when the browse plants were at a medium maturity vegetative stage. During the sampling period, approximately 5 kg of fresh plant leaves and twinges (fine stems) were hand plucked from the browse species to be used in an *in vitro* study. These plant materials were dried first in a cool dark place. Thereafter, forage samples were sub-sampled and dried at 55 °C for 48 h in a forced air oven. Subsequently the samples were ground to pass through a 2 mm and 1 mm sieves in a Willey mill and was stored until used for analysis. These browse species were systematically divided into two categories as trees (with a height of >2 m) and shrubs (with a height <2 m). Accordingly, they were analysed, compared and discussed between and within the two broader groupings.

3.3.2 Determination of chemical composition of forage sample

Samples of the forage materials were analysed for DM, total ash, nitrogen, ether extract, total phenols (TP), total tannins (TT), condensed tannins (CT), hydrolysable tannins (HP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) non fibre carbohydrates, macro and micro minerals. Details of the procedures used in are described in section 2.3.2 (page 21).

3.3.3 *In vitro* gas production measurement

3.3.3.1 Collection of rumen fluid from donor sheep

The rumen fluid was collected from two rumen cannulated Merino weathers fed *ad libtum* on alfalfa hay which were kept on the Experimental farm of the University of Pretoria. Rumen fluid was always collected before the morning feeding. The rumen fluid was transferred into the incubator within an hour, and kept at a constant temperature of 39 ºC. Approximately 500 mL of the rumen fluid was collected from each donor animal, mixed, strained through four layers of cheesecloth and transferred to pre-heated thermos flasks. In the laboratory, the flasks were emptied into a volumetric flask while being purged with CO_2 to maintain anaerobic conditions (Grant & Mertens, 1992). After blending, the rumen fluid was transferred to a large glass beaker inside a 39 °C water bath being continuously purged with CO_2 and continuously stirred as recommended by Goering and Van Soest

(1970). Thereafter, the required volume of rumen fluid was added to the buffer solution in the respective incubation bottles; 15 mL of rumen fluid to 25 mL parts of buffer solution.

3.3.3.2 Buffer media preparation, sample incubation and gas measurement

The buffer solution, macro mineral solution and micro mineral solution were prepared as described by Goering and Van Soest 1970. The micro mineral solution was prepared with slight modification by replacing $MgSO_4.7H_2O$ with $MgCl_2.6H_2O$ to reduce the amount of SO_4 in the media as suggested by Mould *et al*. (2005) and stored in a dark glass bottles to maintain the stability of the solution. In the morning before commencement of the mixture of 1.3 L of rumen fluid, distilled water, rumen buffer solution, macro and micro mineral solutions were mixed with the tryptose and prepared with 0.1% (wt/vol) resazurin. A mass of 0.386 g of L-cysteine hydrochloride was weighed and directly added to the rest of the solution once all chemicals were dissolved. As soon as L-cysteine hydrochloride was added to the buffer solution it was placed in a 39°C water bath and CO_2 was bubbled through the solution until the buffer solution was clear, indicating that the solution was sufficiently reduced.

A semi- automated system was used to measure gas production through *in vitro* incubation at 39°C, according to the method described by Theodorou *et al.* (1994). The system consists of a digital data tracker (Tracker 220 series indicators, Omega Engineering, Inc., Laval, QC, Canada) connected to a pressure transducer (PX4200-015GI from Omega Engineering, Inc., Laval, QC, Canada) modified with needle tip. Approximately 400 mg of respective browse foliage sample was weighed into a 120 ml serum bottle. Then 40 mL of rumen fluid + the buffer medium was added under a stream of CO_2 to each serum bottles and then closed with a rubber stopper and a crimp seal cap. A needle of a medical syringe was inserted through the rubber stopper of each serum bottle for about 5 seconds to release a small amount of gas that might have built up and create starting point for incubation. All serum bottles were returned to the incubator and the rotary shaker was turned on at 120 rpm. Gas pressure was taken at 2, 4, 8, 12, 24, 48 and 72h. To quantify gas production derived from the culture medium and the ruminal inoculums, two blanks were included in every analysis. Two replicates of the same browse and four different cycles were executed for every browse sample studied. The pressure and volume values were registered and added to the values of the previous readings. Therefore, the cumulative pressure and volume of the fermentation gases could be obtained. However, fermentation was terminated after 72 h by removing the serum bottles from the incubator and placing them on ice. After opening the bottle pH readings were taken and the supernatants were collected and used for volatile fatty acids and ammonia-nitrogen analyses.

3.3.4 Short chain volatile fatty acids and ammonia-nitrogen analysis

The 5 mL supernatant serum bottle contents were centrifuged in Sorvall Centrifuge (SL-50 T, 8×50 ml) at 25,000rpm for 15 min at 4° C and a part of the supernatant was transferred to a

micro-centrifuge tube (capacity 1.5 mL) containing meta-phosphoric acid (250 g/L), (5 g/L) as internal standard. The samples were centrifuged at 10,000*g* for 5 minutes in a micro-centrifuge. The standard VFA mixture consisted of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids and was treated in the same manner as that for the sample. The VFA's in test sample were analyzed by the gas chromatography (GC) with FID analyzer and it was calibrated against the standard. The final concentration was reported after deducting the corresponding blank values.

For ammonia-N analysis 50 µl of a standard, supernatant sample and 0.1 N HCl (blank) was measured into test tubes in duplicates, using pipettes while 2.5 ml of phenol reagent and 2 ml of hypochlorite reagent were added and mixed. The tubes were then placed in a water-bath at 95°C for 5 minutes. The tubes were removed and cooled to between 25 and 30°C and then read on a spectrophotometer at 630 nm. The NH_3-N concentrations were reported as g/kg DM (Broderick & Kang, 1980).

3.3.5 Determination of *in vitro* digestible organic matter

The procedure used in this study was according to that of Tilley & Terry (1963), as modified by Engels & Van der Merwe (1967) who involved two digestion phases. During the first digestion phase, feed samples (200 mg) were incubated in triplicate under anaerobic conditions with rumen liquor for 48 h at 39° C with the inclusion of blanks and standards in every batch of incubation. This was followed by a 48 h acid pepsin digestion phase at 39° C under anaerobic conditions. Following the 96 hour incubation, the residual plant materials were collected and oven dried at 105°C for 12 hours. Ash contents were determined by combustion (550°C for 2 hours) (Engels & Van der Merwe, 1967). Metabolizable energy was determined by using the equation ME= 0.016*IVOMD suggested by McDonald *et al*. (2002) for roughages.

3.3.6 Methane production measurements

Methane production was measured from duplicate bottles incubated for each browse by taking gas samples at 2, 12, 24, and 48 h and analyzing the methane concentration using the SRI multiple gas #1 chromatography analyzer system. Gas produced from each bottle at various times was recorded and samples of the gas were taken using a Hamilton syringe and 1 mL of sampled gas produced was injected manually (pull and push method of sample injection) into the GC, which was calibrated with 100% CH_4. Two blanks were included for correction of CH_4 produced from the inoculum in each cycle and a total of two cycles were executed for each sample. Methane concentration that was measured at each time interval was related for total gas volume to determine its concentration (Tavendale *et al.,* 2005) and converted later into energy and mass values using 39.54 kJ/l CH_4 and 0.716 mg/mL CH_4 factors, respectively (Santoso *et al*., 2007).

3.3.7 Statistical analysis

Tree foliage gas, VFA, NH_3-N and methane concentrations were statistically analysed using the 'GLM' option of SAS (9.1), with methods of Steel & Torrie (1980), and differences among foliage species were determined using Duncan's multiple-range test (Duncan & Brant 1983). Correlation between CH_4 production and other studied parameters of the browse species e.g. (chemical composition, VFA, NH_3) were executed using Proc corr of SAS (9.1).

3.4 Results and discussion

3.4.1 *In vitro* gas production and tannin bioactivity

The *in vitro* gas production results for the incubated browses with or without PEG at 2, 12, 24 and 48 h production (mL/g DM) are summarized in Table 3.2. The results showed significant differences ($p< 0.05$) in the cumulative gas production (GP) among browses both in the presence and absence of PEG. Inclusion of PEG in fermentation of tropical browses resulted in a significant ($p<0.05$) increase of GP profile particularly in the browse forage specie with a high secondary phenolic component (Table 2.3 Chapter 2). There was no effect of PEG on the GP profile in *A. mellifera* (at 2 hr), *A. hebeclada* and *L. cinereum* (at 12 hr) due to lower concentrations of the phenolic content (example tannin) in such plants (Table 2.3 Chapter 2). Addition of PEG could overcome the adverse effects of high tannins on nutrient availability as indicated by cumulative GP because PEG has a high affinity towards the formation of PEG-tannin complexes which inactivates tannins (Kamalak *et al*., 2005; Salem, 2005). As observed in the current study for *R. lancea, A. luederitzii* and *M. incanum* inclusion of PEG during the incubation of tannin-rich plants led to an increase in GP of up to 100%. This has the potential to affect rumen fermentation. In this study differences in gas production between the forage leaves from different species could be due to the proportion and nature of its fibre concentration where methane and gas production increased with a more fibrous feed (Rubanza *et al*., 2003). The variation in gas production between forage could be due to genotypic characteristics relative to the type of secondary compound activity on digestibility (Muetzel & Becker, 2006; Salem *et al*., 2006).

3.4.2 *In vitro* CH_4 production

The volume of methane (Table 3.2) produced at 24 hours differed significantly ($p<0.05$) between the browse species, this was partly due to different concentrations of phenolic components. The result for CH_4 production at 2, 12 and 48 hours are also presented in Table 3.3, but was not discussed, it follows the same trend as was for CH_4 production at 24 hours. The plant species were divided into tree

and shrub species while comparisons were made within tree species and within shrub species, but not between trees and shrubs. In addition methane production at 24 hours with PEG and without PEG was compared for each specie and discussed. At 24 hours the highest amount of CH_4 was produced of incubation with and without the inclusion of PEG, among the tree forage by (*B. albitrunca*) and among the shrub forage by (*H. tomentosa*). Therefore, it could be partly due to the low condensed tannin components of both *B. albitrunca* (2.2 g/kg DM) and *H. tomentosa* (18.6 g/kg DM) which exhibit a small effect on methane reduction. The tree species *R. lancea* and *A. luederitzii* and the shrub species *D. cinerea,* and *M. incanum* had significantly decreased ($p<0.001$) methane production during the 24 h incubation period. This can be attributed partly to the phenolic components which inhibit methanogenesis. Therefore the cumulative methane production at 24 h of *in vitro* incubation was decreased by 83.5, 83.7, 94.2 and 96.4 % in *D. cinerea*, *R. lancea, M. incanum,* and *A. luederitzii* foliage samples, respectively. While the cumulated methane production at 48 h of *in vitro* incubation was decreased by 77.3, 62.2, 92.2 and 93.65 % in *D. cinerea R. lancea, M. incanum,* and *A. luederitzii* foliage samples, respectively, while *Z. mucronata* showed no decreases in CH_4 production after 48hours with or without PEG. The data show, *M. incanum* and *A. luederitzii* foliage has a long term effect on total methane production. This supports previous studies which reported that ruminal CH_4 production may be lower, when using diets containing phenols (Carulla *et al*., 2005; Puchala *et al*., 2005; Animut *et al*., 2008). This is in agreement with Hess *et al*. (2003), who reported that extract from CT-containing legumes have shown methanogensis toxicity. However, this is not always the case (Beauchemin *et al*., 2007; Oliveira *et al*., 2007) and such differences between studies may be related to the diversity in the structures of phenolic compounds, the activities of the individual phenolic sources, interaction with other compounds and the dosages administered (Makkar, 2003; Rochfort *et al*., 2008). From the data in Table 3.3 it can be concluded that the phenolic components decreased methane production in all the browse species. The higher the concentrations of total phenols, total tannins or condensed tannins the larger was the reduction in methane production. It is not certain which specific phenolic component has the biggest effect on methane production.

3.4.3 Volatile fatty acid and rumen ammonia-N production

The analysis of the concentrations of VFA's produced during digestion of carbohydrates in the rumen provides information to compare the nutritional value of ruminant feeds (Markantonatos *et al*., 2008). Gas production from a variety of feeds incubated *in vitro* has been closely related to the production of VFA based on carbohydrate fermentation (Getachew *et al*., 2002). Propionate contributes to the energy supply of the ruminants as the main gluconeogenic precursor. Ruminants absorb small quantities of glucose by the small intestine only (Getachew *et al*., 2002). The negative effect of CT on propionate yields has been reported *in vivo* (Waghorn *et al*., 1994) and *in vitro* (McMahon *et al*., 1999).

The concentration of volatile fatty acids (VFA's) and rumen ammonia-N in Table 3.4 differed significantly ($p<0.05$) between the trees and shrubs species. *Boscia albitrunca* and *A. melliferia* recorded the highest concentration of NH_3-N among the tree and shrub forages, while *A. karroo* and *M. incanum* had the lowest NH_3-N concentrations without the inclusion of PEG among tree and shrub forages respectively. When PEG was included, *A. karroo* and *L. cinereum* had the highest concentrations of NH_3-N among tree and shrub respectively. *Olea europaea* and *M. genistifolium* had the lowest NH_3-N concentrations among tree and shrub forages respectively. Addition of PEG increased the NH_3-N concentration of some browse species between 0-40 % indicating that PEG bounded with tannins and released nitrogen for degradation (Khazaal *et al*., 1996). The extent of the improvement in fermentation of *B. albitrunca* and *A. mellifera* browse species by addition of PEG probably depended on the level, as well as the nature, of the secondary compounds (Ebong, 1995).

The increase in NH_3-N concentrations in *B. albitrunca* and *A. mellifera* with addition of PEG could be partly due to increased CP degradability and/or poor synchronization between N and carbohydrate release in the rumen (Getachew *et al*., 2000b). The rapid release of ammonia-N that is not matched with the availability of fermentable carbohydrates in the rumen can lead to the accumulation of NH_3. The observed higher levels of ammonia-N when incubated with PEG suggest that the utilization of *A. luederitzii* and *M. incanum* browse leaves can be improved by the inclusion of PEG for browse which is high in tannins. The results of this study also showed no significant difference in NH_3-N concentration when PEG was added to *D. cinerea* and *H. burchelli* while the NH_3-N concentration decreased when incubated with PEG for browse species such as *B. albitrunca, A. mellifera* and *A. hebeclada* which have lower tannin concentrations.

The addition of PEG, which inhibits the activity of CT, increased the concentration of propionate (22% and 26% in *Z. mucronata* and *T. sericea*, respectively) which is consistent with the finding of Burggraaf *et al*. (2008) who also reported an overall increase in propionate concentration in rumen fluid when PEG was added. The total VFA concentration in mmol/L showed an increase in concentration with the addition of PEG, but the level of increase, was significantly ($p<0.05$) different between the different browse species in the current study. The overall percentage increases which ranged from 0-25 %, where *D. cinerea* and *Z. mucronata* showed the highest increase in VFA concentration, *P. glandulosa, A. mellifera* and *H. burchelli* showed slightly lower VFA concentrations with PEG incubation. The data obtained from the 19 browse species in this study confirmed that phenolic compounds decrease rumen production of VFA's as reported by Waghorn *et al*. (1994). This negative relationship between fermentation properties and phenolics was reported earlier by (Burggraaf *et al*., 2008).

The IVDOM values in this study varied, from poorly digested to highly digested (200-800 g/kg DM^{-1}). This is within the same range of values reported by Otsinya *et al*., (1999) and Rothauge *et al*. (2003) for digestibility. The low *in vitro* digestibility of dry matter and organic matter in *T. sericea, D. cinerea* and *R. lancea* could be due to higher NDF, lignin and phenolics concentrations which reduces

digestibility (Ammar *et al*., 2005). However, digestibility of tree leaves were adversely affected by secondary compounds such as, tannins, as shown in several studies (Peng *et al*., 2005; Rakhmani *et al*., 2005 and Salem *et al*., 2006). Condensed tannins inhibit microbial attachment to feed particles and caused significant detrimental effects on the microbial population and inhibiting rumen fermentation in varying degrees (Burggraaf *et al*., 2008).

It has been suggested that gas production at 24 h is proportional to the amount of actually digested carbohydrates at maintenance and is highly correlated to the ME concentration of feedstuffs (Giger-Riverdan & Sauvant, 2000). Salem *et al.* (2007) indicated that PEG increased the ME concentration of foliage of *Lotus* species and a variety of browse tree leaves. However, in this study the formula of McDonald *et al*. (2002) ME= (0.016*IVOMD)) for roughages was used to calculate the ME values, but was not reported in this study. Due to the non-significant effect of PEG on IVOMD in this study, it could not be confirmed that PEG increases the ME concentration. Differences in ME among browse species reflect variation in fermentable carbohydrates and available N among the browse species. While fermentable carbohydrates tend to elevate the rate of gas production, other factors decrease gas production by diverting carbon from gas to microbial protein (Menke & Steingass, 1988). According to Jones *et al*. (2000), PEG increases CP digestibility, but not *in vitro* digestible dry matter (IVDMD). In this study, PEG did not increase N utilization in the rumen or IVOMD, but increased VFA production in the rumen.

3.4.4 Loss of energy as CH_4

The amount of feed energy loss in form of methane by the various forage species is as shown in table 3.5. The forage from *A. karroo* and *A. luederitzii* and forage from *D. cinerea* and *M. incanum*, decreased the amount of organic matter (by between 13 and 18 g/kg when PEG was not added) that was used by methanogenic bacteria to produce methane. The amount of GE (MJ/kg) lost as methane was also calculated and the same browse forage species saved between 0.5 to 1 % GE, respectively when tannins were added. In this study, it was observed that a loss of gross energy intake through enteric CH_4 fermentation of the incubated browse species range from 0.06-1.65 % of GE intake, and was higher than the results reported by McCrabb & Hunter (1999). In all browse forage species, lower the amount of GE loss was associated with, the higher the amount of total tannin and condensed tannin content recorded. We can make a general assumption that methane production was decreased by either the total tannin or condensed tannin concentrations. As found in the study by Monforte-Briceno *et al*. (2005) and Tavendale *et al*. (2005), the action of CT on methanogenesis can be attributed to indirect effects, by reduced H_2 production and digestibility as well as by direct inhibitory effects on methanogens. The specific molecular weight and type of condensed tannin that decreased methane production was not studied during this trial. More detailed research on molecular weight and type of condensed tannin is necessary to identify the specific components involved.

3.4.5 Correlations between chemical composition, secondary compounds and *in vitro* digestibility, gas and methane production

The correlation between different nutritional components was investigated to determine the relationships between different components, such as chemical and phenolic composition and IVOMD, TVFA, NH_3-N, GP_{24} and CH_4 as shown in Table 3.1. From the results in this study, IVOMD is correlated with chemical composition such as crud protein, neutral detergent fibre and acid detergent fibre. Furthermore, in this study, total VFA's is correlated with NDF while NH_3-N is correlated with CP. Gas production was significantly ($p<0.001$) correlated with NDF and ADF while IVOMD, TVFA, NH3-N, GP_{24} and CH_4 concentrations are all significantly ($p<0.05$) correlated with total phenols, total tannins, condensed and hydrolysed tannins as presented in (Table 2.3). Other secondary compounds, such as saponins and essential oils, may have another mode of action on ruminal microorganisms. Saponins change cell membrane properties (Moss *et al*., 2000) and essential oils could change N metabolism of rumen micro-organisms and inhibit growth of bacteria (Mclntosh *et al*., 2003). Salam (2005) also reported a negative correlation between secondary compounds and *in vitro* gas production and DM degradability of *Acacia saligna* leaves incubated with inoculum of sheep, cattle and buffalo. The correlation results in this study are similar to those reported by Salem *et al.,* 2007 and Rubanza *et al*., (2003). These researchers also reported a significant ($p<0.05$) negative relationship between IVOMD, NH_3-N, total gas and CH_4 production with the phenolic compounds (total phenols, total tannins, condensed tannins, hydrolysed tannins) with *in vitro* degradability of legumes after 24 h of *in vitro* incubation. From the correlation table in this study it seems that total tannins and condensed tannins have the biggest effect on CH_4 production.

Table 3.1 Correlation between different fermentation products, nutritional and phenolic components

	IVOMD	TVFA	NH_3-N	GP_{24}	CH_4
Chemical composition					
Ash	0.00043	0.40475	0.30581	0.43446	0.38066
OM	0.00043	0.40475	0.30581	0.43446	0.38066
CP	0.66639*	0.28331	0.50042*	0.47026	0.08677
NDFN	0.0054	-0.31476	-0.2924	-0.3473	-0.46133
NDF	-0.74652*	-0.5932*	-0.5304	-0.67893*	-0.3723
ADF	-0.63965*	-0.38326	-0.34233	-0.50149*	-0.07653
ADL	-0.27028	-0.0922	0.04748	-0.18242	-0.00323
NFC	0.05584	0.01108	-0.01605	0.06941	-0.11949
Phenolic Components					
TP	-0.69493*	-0.5631*	-0.55093*	-0.73452*	-0.63429*
TT	-0.60308*	-0.4968*	-0.58449*	-0.7401*	-0.67445*
CT	-0.56258*	-0.47298*	-0.58643*	-0.73431*	-0.67601*
HT	-0.61957*	-0.50323*	-0.56955*	-0.72738*	-0.65773*

Chemical components, OM, organic matter; CP, crud protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; NFC, non-fibre carbohydrates; Phenolic components, TP, total phenols; TT, total tannins; CT, condensed tannins; HT, hydrolysed tannins; IVOMD, *in vitro* organic matter digestibility; TVFA, total volatile fatty acids; NH_3-N, rumen ammonia; GP_{24}, gas production; CH_4, methane. * = Significant ($P< 0.05$)

3.5 Conclusion

There were significant ($p<0.05$) variations in *in vitro* gas and methane production, VFA's, rumen ammonia-N degradability and GE, ME and DM digestibility among different browse species collected from Kalahari bush dune veldt. These variations were associated with the NDF, ADF and ADL concentrations and/or presence of tannins in the different browse species. Phenolic compounds have no significant effect on NH_3-N utilization or IVOMD in this study, but it negatively affected the concentration of VFA from OM. Tree forage (*R. lancea* and *A. luederitzii*) and shrub forage (*D. cinerea* and *M. incanum*) decreased methane and gas production between 70-90 %. *A. luederitzii* and *M. incanum* had a prolonged effect on CH_4 production. GE loss (measured by bomb calorie meter) was also improved without the inclusion of PEG, making more energy available that could be used to increase animal production. Tannin extracts from *A. luederitzii* and *A. karroo* could be used as a

dietary alternative to reduce enteric methane production. However, the practical utility of these plants, or combinations of these browse, needs further investigation using short-term and long-term *in vivo* studies on ruminant.

Table 3.2 Volumes (mL/g DM) of gas production from the studied browse and shrubs

	Hours of incubation							
	2h		12h		24h		48h	
Scientific name	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**
Trees								
Acacia erioloba	21.22^{b}_{1}	14.14^{b}_{2}	46.73^{d}_{1}	33.84^{d}_{2}	54.22^{cd}_{1}	42.57^{e}_{2}	68.22^{c}_{1}	50.34^{e}_{2}
Boscia albitrunca	24.54^{a}_{1}	11.93^{bc}_{2}	60.04^{b}_{1}	47.98^{b}_{2}	72.80^{a}_{1}	61.71^{b}_{2}	87.36^{a}_{1}	72.80^{b}_{2}
Acacia haematoxylon	12.90^{g}_{1}	10.82^{cd}_{1}	38.41^{f}_{1}	24.13^{f}_{2}	46.18^{f}_{1}	28.70^{g}_{2}	55.47^{f}_{1}	36.19^{g}_{2}
Olea europaea	12.62^{g}_{1}	13.59^{b}_{1}	40.63^{f}_{1}	40.49^{e}_{1}	56.16^{cd}_{1}	55.05^{c}_{1}	72.25^{cd}_{1}	64.90^{c}_{2}
Ziziphus mucronata	19.83^{c}_{1}	18.58^{a}_{1}	65.87^{a}_{1}	55.74^{a}_{2}	75.30^{a}_{1}	68.92^{a}_{2}	84.03^{a}_{1}	77.24^{a}_{2}
Terminalia sericea	14.56^{e}_{1}	9.98^{cd}_{2}	38.41^{f}_{1}	22.19^{f}_{2}	47.01^{f}_{1}	27.32^{g}_{2}	58.52^{ef}_{1}	35.92^{g}_{2}
Rhus lancea	13.17^{f}_{1}	8.32^{de}_{2}	50.34^{cd}_{1}	14.70^{g}_{2}	63.09^{b}_{1}	21.77^{h}_{2}	76.82^{b}_{1}	35.92^{g}_{2}
Acacia karroo	17.89^{d}_{1}	9.43^{cd}_{2}	52.00^{c}_{1}	28.84^{e}_{2}	58.10^{c}_{1}	36.75^{f}_{2}	61.85^{de}_{1}	46.18^{f}_{2}
Prosopis glandulosa	14.56^{e}_{1}	14.14^{b}_{1}	42.57^{ef}_{1}	41.05^{c}_{1}	48.40^{ef}_{1}	48.12^{d}_{1}	54.64^{f}_{1}	53.94^{d}_{1}
Acacia luederitzii	17.06^{d}_{1}	5.82^{e}_{2}	46.45^{de}_{1}	14.42^{g}_{2}	52.28^{de}_{1}	17.88^{i}_{2}	57.41^{f}_{1}	18.72^{b}_{2}
Mean	16.83	11.68	48.15	32.34	57.35	40.88	66.92	49.95
MSE	0.729	0.864	1.321	1.154	1.557	1.209	1.286	1.430
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Shrubs								
Acacia mellifera	12.06^{c}_{1}	10.82^{bc}_{1}	30.09^{e}_{2}	36.89^{d}_{1}	48.95^{d}_{1}	41.74^{c}_{2}	59.77^{c}_{1}	54.64^{b}_{2}
Acacia hebeclada	19.55^{a}_{1}	20.89^{a}_{1}	55.33^{a}_{1}	53.80^{a}_{1}	64.48^{a}_{1}	63.37^{a}_{1}	73.22^{a}_{1}	72.80^{a}_{1}
Grewia flava	8.18^{d}_{1}	5.55^{f}_{2}	43.40^{c}_{1}	22.19^{f}_{2}	56.72^{c}_{1}	34.25^{e}_{2}	67.95^{b}_{1}	50.34^{c}_{2}
Dichrostachys cinerea	15.39^{b}_{1}	9.15^{cde}_{2}	43.68^{c}_{1}	23.30^{f}_{2}	53.66^{c}_{1}	28.70^{f}_{2}	56.02^{c}_{1}	33.70^{e}_{2}
Hermannia burchelli	15.39^{b}_{1}	12.48^{b}_{2}	55.61^{a}_{1}	47.70^{b}_{2}	64.76^{a}_{1}	60.88^{a}_{2}	68.50^{b}_{1}	70.86^{a}_{1}
Lycuim cinereum	10.12^{d}_{1}	6.93^{ef}_{2}	27.60^{f}_{1}	27.46^{e}_{1}	35.08^{e}_{1}	31.76^{e}_{2}	45.34^{e}_{1}	39.24^{d}_{2}
Monechma genistifolium	9.29^{d}_{1}	9.43^{cd}_{1}	37.02^{d}_{1}	34.39^{d}_{2}	42.29^{d}_{1}	41.46^{d}_{1}	47.01^{d}_{1}	45.77^{cd}_{1}
Hermannia tomentosa	19.55^{a}_{1}	10.82^{bc}_{2}	50.61^{b}_{1}	40.21^{c}_{2}	60.60^{b}_{1}	53.11^{b}_{2}	67.67^{b}_{1}	68.36^{a}_{1}
Monechma incanum	15.11^{b}_{1}	7.49^{def}_{2}	53.94^{a}_{1}	23.85^{f}_{2}	65.59^{a}_{1}	29.26^{f}_{2}	71.83^{ab}_{1}	33.42^{e}_{2}
Mean	13.85	10.39	44.14	34.42	54.68	42.72	61.92	52.12
MSE	0.627	0.768	0.832	1.029	1.169	1.252	1.424	1.479
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means with different superscripts (letters) within a column are significantly (P<0.05) different
Means with different subscripts (numbers) in rows within each incubation time are significantly (P<0.05) different
No PEG denotes presence of tannin and PEG denotes absence of tannin

Table 3.3 Volumes (mL/g DM) of methane production from the studied browse and shrubs

	Hours of incubation							
	2h		12h		24h		48h	
Scientific name	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**
Trees								
Acacia erioloba	$0.83^{a}{}_{1}$	$0.35^{c}{}_{2}$	$6.90^{c}{}_{1}$	$3.78^{d}{}_{2}$	$15.73^{d}{}_{1}$	$11.30^{d}{}_{2}$	$24.88^{cd}{}_{1}$	$18.10^{d}{}_{2}$
Boscia albitrunca	$0.38^{d}{}_{1}$	$0.15^{d}{}_{2}$	$9.03^{b}{}_{1}$	$7.80^{b}{}_{1}$	$25.75^{a}{}_{1}$	$25.28^{a}{}_{1}$	$41.18^{a}{}_{1}$	$38.50^{a}{}_{1}$
Acacia haematoxylon	$0.53^{bc}{}_{1}$	$0.28^{c}{}_{2}$	$6.13^{c}{}_{1}$	$3.2^{e}{}_{2}$	$13.55^{e}{}_{1}$	$8.00^{e}{}_{2}$	$20.93^{e}{}_{1}$	$14.23^{e}{}_{2}$
Olea europaea	$0.38^{d}{}_{2}$	$0.55^{a}{}_{1}$	$3.53^{d}{}_{2}$	$6.88^{c}{}_{1}$	$20.23^{b}{}_{1}$	$19.95^{b}{}_{1}$	$27.68^{c}{}_{1}$	$24.63^{c}{}_{2}$
Ziziphus mucronata	$0.88^{a}{}_{1}$	$0.63^{a}{}_{2}$	$12.53^{a}{}_{1}$	$10.28^{a}{}_{2}$	$25.60^{a}{}_{1}$	$25.45^{a}{}_{1}$	$36.03^{b}{}_{1}$	$35.95^{b}{}_{1}$
Terminalia sericea	$0.65^{b}{}_{1}$	$0.30^{c}{}_{2}$	$6.28^{c}{}_{1}$	$2.63^{f}{}_{2}$	$13.35^{e}{}_{1}$	$7.18^{e}{}_{2}$	$21.90^{de}{}_{1}$	$12.28^{f}{}_{2}$
Rhus lancea	$0.38^{d}{}_{1}$	$0.10^{de}{}_{2}$	$7.45^{bc}{}_{1}$	$0.65^{h}{}_{2}$	$17.40^{c}{}_{1}$	$2.83^{g}{}_{2}$	$27.23^{c}{}_{1}$	$10.30^{g}{}_{2}$
Acacia karroo	$0.63^{b}{}_{1}$	$0.08^{de}{}_{2}$	$9.20^{b}{}_{1}$	$1.55^{g}{}_{2}$	$17.95^{c}{}_{1}$	$4.90^{f}{}_{2}$	$24.28^{cd}{}_{1}$	$6.95^{h}{}_{2}$
Prosopis glandulosa	$0.58^{b}{}_{1}$	$0.43^{b}{}_{2}$	$7.08^{c}{}_{1}$	$6.48^{c}{}_{1}$	$16.15^{d}{}_{1}$	$12.23^{c}{}_{2}$	$19.43^{f}{}_{1}$	$15.03^{d}{}_{2}$
Acacia luederitzii	$0.63^{b}{}_{1}$	$0.03^{e}{}_{2}$	$8.80^{b}{}_{1}$	$0.05^{i}{}_{2}$	$17.50^{c}{}_{1}$	$0.63^{h}{}_{2}$	$19.30^{e}{}_{1}$	$1.23^{i}{}_{2}$
Mean	0.583	0.288	7.690	4.330	17.320	11.773	26.280	16.718
MSE	0.018	0.108	0.223	0.073	0.523	0.128	0.426	0.181
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Shrubs								
Acacia mellifera	$0.40^{e}{}_{1}$	$0.28^{c}{}_{2}$	$5.23^{e}{}_{1}$	$3.75^{c}{}_{2}$	$14.08^{e}{}_{1}$	$9.63^{de}{}_{2}$	$23.73^{c}{}_{1}$	$17.93^{c}{}_{2}$
Acacia hebeclada	$1.03^{ab}{}_{1}$	$0.95^{a}{}_{2}$	$10.90^{c}{}_{1}$	$9.68^{b}{}_{1}$	$25.18^{b}{}_{1}$	$20.58^{b}{}_{2}$	$31.20^{b}{}_{1}$	$24.90^{b}{}_{2}$
Grewia flava	$0.30^{e}{}_{1}$	$0.13^{d}{}_{2}$	$7.85^{d}{}_{1}$	$3.30^{d}{}_{2}$	$19.30^{d}{}_{1}$	$11.30^{d}{}_{2}$	$29.10^{bc}{}_{1}$	$22.53^{bc}{}_{2}$
Dichrostachys cinerea	$0.70^{c}{}_{1}$	$0.10^{de}{}_{2}$	$8.38^{d}{}_{1}$	$1.03^{e}{}_{2}$	$18.10^{de}{}_{1}$	$2.98^{e}{}_{2}$	$18.58^{d}{}_{1}$	$4.20^{d}{}_{2}$
Hermannia burchelli	$0.60^{cd}{}_{1}$	$0.40^{b}{}_{2}$	$12.60^{b}{}_{1}$	$10.38^{a}{}_{1}$	$26.23^{b}{}_{1}$	$25.58^{a}{}_{1}$	$31.05^{b}{}_{1}$	$22.73^{bc}{}_{2}$
Lycuim cinereum	$0.03^{f}{}_{1}$	$0.03^{e}{}_{1}$	$0.43^{f}{}_{1}$	$0.20^{f}{}_{1}$	$0.63^{f}{}_{1}$	$0.73^{f}{}_{1}$	$6.65^{e}{}_{1}$	$2.75^{e}{}_{2}$
Monechma genistifolium	$0.50^{de}{}_{1}$	$0.45^{b}{}_{1}$	$10.13^{c}{}_{1}$	$9.85^{b}{}_{1}$	$20.88^{c}{}_{1}$	$16.83^{c}{}_{2}$	$23.68^{c}{}_{1}$	$17.73^{c}{}_{2}$
Hermannia tomentosa	$1.10^{a}{}_{1}$	$0.40^{b}{}_{1}$	$14.30^{a}{}_{1}$	$9.98^{b}{}_{2}$	$30.85^{a}{}_{1}$	$23.23^{ab}{}_{2}$	$33.08^{ab}{}_{1}$	$31.55^{a}{}_{1}$
Monechma incanum	$0.80^{bc}{}_{1}$	$0.08^{de}{}_{2}$	$14.65^{a}{}_{1}$	$0.60^{ef}{}_{2}$	$30.83^{a}{}_{1}$	$1.78^{fe}{}_{2}$	$38.18^{a}{}_{1}$	$2.95^{e}{}_{2}$
Mean	0.606	0.311	9.383	5.417	20.672	12.511	26.136	16.361
MSE	0.013	0.025	0.064	0.156	0.143	0.425	0.251	0.576
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different superscripts (letters) within a column are significantly (P<0.05) different
Means with different subscripts (numbers) in rows within each incubation time are significantly (P<0.05) different
No PEG denotes presence of tannin and PEG denotes absence of tannin

Table 3.4 Total and individual volatile fatty acid (mmol/L) concentration, in the supernatant after 72h incubation of 400mg DM of browses with or without poly ethylene glycol

	Acetic		Propionic		Iso butyric		Butyric		Valeric		Total VFA mmol/L	
Scientific name	***PEG***	***No PEG***	***PEG***	***No PEG***	***PEG***	***No PEG***	***PEG***	***No PEG***	***PEG***	***No PEG***	***PEG***	***No PEG***
Trees												
Acacia erioloba	66.06^{i}_{1}	62.29^{g}_{1}	18.91^{c}_{1}	18.90^{d}_{1}	2.45^{c}_{1}	2.38^{c1}	8.91^{h}_{1}	8.35^{e}_{2}	3.28^{f}_{1}	3.07^{c}_{2}	99.61^{g}_{1}	95.00^{g}_{1}
Boscia albitrunca	77.17^{c}_{1}	71.54^{c}_{2}	18.21^{cd}_{1}	18.01^{e}_{1}	2.42^{c}_{1}	2.42^{b}_{1}	13.00^{b}_{1}	11.80^{a}_{2}	3.13^{i}_{1}	2.96^{e}_{2}	113.92^{c}_{1}	106.73^{c}_{2}
Acacia haematoxylon	65.15^{j}_{1}	67.67^{e}_{1}	19.52^{c}_{1}	16.93^{f}_{2}	2.62^{b}_{1}	2.00^{e}_{2}	9.25^{g}_{1}	7.65^{g}_{2}	3.56^{c}_{1}	2.94^{g}_{2}	100.1^{g}_{1}	97.18^{e}_{1}
Olea europaea	71.81^{f}	68.80^{d}_{1}	21.66^{b}_{1}	21.83^{b}_{1}	2.20^{f}_{1}	1.96^{f}_{2}	10.86^{c}_{1}	10.87^{c}_{1}	3.21^{g}_{1}	3.00^{d}_{2}	109.74^{d}_{1}	106.46^{c}_{1}
Ziziphus mucronata	104.7^{a}_{1}	79.08^{a}_{2}	30.10^{a}_{1}	23.28^{a}_{2}	2.92^{a}_{1}	2.44^{a}_{2}	13.81^{a}_{1}	11.19^{b}_{2}	4.76^{a}_{1}	3.66^{a}_{2}	156.28^{a}_{1}	119.66^{a}_{2}
Terminalia sericea	69.23^{g}_{1}	57.85^{h}_{2}	18.72^{cd}_{1}	13.74^{g}_{2}	2.09^{h}_{1}	1.45^{j}_{2}	9.26^{g}_{1}	6.07^{i}_{2}	3.70^{b}_{1}	2.96^{f}_{2}	103.01^{f}_{1}	82.07^{i}_{2}
Rhus lancea	80.37^{b}_{1}	75.60^{b}_{1}	19.17^{c}_{1}	17.47^{f}_{2}	2.36^{d}_{1}	1.58^{i}_{2}	10.27^{d}_{1}	7.60^{g}_{2}	3.37^{e}_{1}	2.72^{h}_{2}	115.53^{b}_{1}	104.96^{d}_{2}
Acacia karroo	72.46^{e}_{1}	66.41^{f}_{2}	21.01^{b}_{1}	19.93^{c}_{1}	2.08^{h}_{1}	1.73^{g}_{2}	9.40^{g}_{1}	7.88^{f}_{2}	3.14^{h}_{1}	2.62^{i}_{2}	108.09^{e}_{1}	98.58^{e}_{2}
Prosopis glandulosa	73.46^{d}_{1}	75.24^{b}_{1}	20.59^{b}_{1}	20.58^{c}_{1}	2.23^{e}_{1}	2.36^{d}_{1}	8.87^{h}_{1}	9.35^{d}_{2}	3.39^{d}_{1}	3.40^{b}_{1}	108.54^{e}_{1}	110.92^{b}_{1}
Acacia luederitzii	66.97^{h}_{1}	55.33^{j}_{2}	18.46^{cd}_{1}	17.65^{g}_{1}	2.11^{g}_{1}	1.67^{h}_{2}	8.38^{i}_{1}	7.45^{h}_{1}	3.09^{j}_{1}	1.93^{j}_{2}	99.01^{g}_{1}	84.03^{h}_{2}
Mean	74.74	67.98	20.64	18.8^{3}	2.35	2.00	10.20	8.82	3.46	2.93	111.38	100.56
Shrubs												
Acacia mellifera	66.07^{h}_{2}	70.06^{g}_{1}	23.44^{b}_{1}	22.17^{b}_{1}	1.92^{h}_{2}	2.19^{e}_{1}	9.66^{d}_{1}	9.61^{b}_{1}	3.16^{e}_{1}	2.98^{c}_{2}	104.25^{h}_{1}	107.00^{e}_{1}
Acacia hebeclada	76.24^{d}_{1}	75.38^{e}_{1}	24.01^{a}_{1}	22.89^{a}_{1}	2.83^{a}_{1}	2.77^{a}_{1}	10.70^{b}_{1}	10.54^{a}_{1}	4.24^{a}_{1}	4.01^{a}_{1}	118.03^{b}_{1}	115.59^{b}_{1}
Grewia flava	74.81^{e}_{1}	72.39^{a}_{1}	17.62^{g}_{1}	16.90^{g}_{1}	2.27^{f}_{1}	2.02^{g}_{2}	8.95^{f}_{1}	7.63^{f}_{2}	3.08^{g}_{1}	2.78^{f}_{2}	106.72^{g}_{1}	101.71^{h}_{2}
Dichrostachys cinerea	76.43^{d}_{1}	57.08^{i}_{2}	21.03^{e}_{1}	17.15^{f}_{2}	2.36^{d}_{1}	1.36^{i}_{1}	8.62^{h}_{1}	5.64^{h}_{2}	3.41^{c}_{1}	2.24^{i}_{2}	111.84^{d}_{1}	83.47^{i}_{2}
Hermannia burchelli	73.91^{f}_{2}	76.15^{d}_{1}	18.24^{f}_{1}	17.38^{e}_{1}	2.31^{e}_{1}	2.22^{d}_{1}	9.43^{e}_{1}	8.98^{d}_{2}	3.13^{f}_{1}	2.95^{e}_{2}	107.03^{f}_{1}	107.69^{d}_{1}
Lycuim cinereum	81.63^{b}_{1}	77.68^{c}_{1}	16.16^{i}_{1}	16.88^{h}_{1}	2.31^{e}_{1}	2.31^{c}_{1}	5.36^{i}_{2}	6.39^{g}_{1}	2.45^{i}_{1}	2.45^{h}_{1}	107.91^{e}_{1}	105.72^{f}_{1}
Monechma genistifolium	72.46^{g}_{1}	73.67^{f}_{1}	17.28^{h}_{1}	16.07^{i}_{1}	2.14^{g}_{1}	2.12^{f}_{1}	8.82^{g}_{1}	8.50^{e}_{2}	2.84^{h}_{1}	2.76^{g}_{1}	103.54^{i}_{1}	103.12^{g}_{1}
Hermannia tomentosa	92.20^{a}_{1}	81.17^{a}_{2}	22.94^{c}_{1}	19.86^{d}_{2}	2.79^{b}_{1}	2.38^{b}_{2}	11.52^{a}_{1}	9.62^{b}_{2}	3.67^{b}_{1}	3.17^{b}_{2}	133.11^{a}_{1}	116.20^{a2}
Monechma incanum	77.44^{c}_{1}	77.93^{b}_{1}	21.16^{d}_{1}	21.98^{c}_{1}	2.42^{c}_{1}	1.81^{h}_{2}	10.53^{c}_{1}	9.37^{c}_{2}	3.21^{d}_{1}	2.97^{d}_{2}	114.76^{c}_{1}	114.07^{c}_{1}
Mean	76.80	73.50	20.21	19.03	2.37	2.13	9.29	8.48	3.24	2.92	111.91	106.06

Means with different superscripts (letters) within a column are significantly (P<0.05) different
Means with different subscripts (numbers) in rows within each incubation time are significantly (P<0.05) different
No PEG denotes presence of tannin and PEG denotes absence of tannin

Table 3.5 Loss of energy from the browse sample as methane

Scientific name	IVOMD		GE (MJ/kg)	CH4 g/kg DD		Methane per g/kg IVOMD		MJ/kg loss of GE as methane		Rumen NH_3 mg/100mL		pH	
	PEG	*No PEG*		*PEG*	*No PEG*	*PEG*	*No PEG*	*PEG*	*No PEG*	*PEG*	*No PEG*	*PEG*	*No PEG*
Trees													
Acacia erioloba	44.01^{d}_{1}	44.55^{c}_{1}	19.1	11.3^{f}_{1}	8.14^{d}_{2}	25.7^{g}_{1}	18.3^{de}_{2}	1.12^{d}_{1}	0.79^{f}_{2}	20.49^{f}_{2}	33.30^{b}_{1}	7.0	6.8
Boscia albitrunca	62.17^{a}_{1}	62.08^{a}_{1}	18.5	18.5^{a}_{1}	18.2^{a}_{1}	29.8^{c}_{1}	29.3^{a}_{1}	0.89^{f}_{1}	0.87^{d}_{1}	22.80^{b}_{2}	36.61^{a}_{1}	7.0	6.9
Acacia haematoxylon	30.96^{f}_{1}	30.88^{d}_{1}	20.2	9.76^{g}_{1}	5.76^{e}_{2}	31.5^{b}_{1}	18.7^{d}_{2}	2.06^{a}_{1}	1.22^{a}_{2}	21.66^{c}_{2}	25.11^{e}_{1}	6.9	6.9
Olea europaea	53.15^{b}_{1}	49.17^{bc}_{2}	18.1	14.6^{b}_{1}	14.4^{b}_{1}	27.4^{e}_{2}	29.2^{a}_{1}	0.93^{ef}_{1}	0.99^{b}_{1}	19.01^{j}_{2}	22.33^{g}_{1}	6.8	6.7
Ziziphus mucronata	63.17^{a}_{1}	64.69^{a}_{1}	17.9	18.4^{a}_{1}	18.3^{a}_{1}	29.2^{d}_{1}	28.3^{b}_{1}	0.83^{g}_{1}	0.80^{e}_{1}	20.73^{e}_{2}	29.22^{c}_{1}	6.8	6.8
Terminalia sericea	38.46^{c}_{1}	24.89^{d}_{2}	17.5	9.61^{g}_{1}	5.17^{f}_{2}	25.0^{h}_{1}	20.8^{c}_{2}	1.14^{d}_{1}	0.95^{bc}_{2}	20.16^{g}_{2}	22.83^{f}_{1}	6.9	6.9
Rhus lancea	36.66^{e}_{1}	23.02^{d}_{2}	17.6	12.5^{d}_{1}	2.04^{h}_{2}	34.2^{a}_{1}	8.85^{g}_{2}	1.64^{b}_{1}	0.42^{h}_{2}	21.03^{d}_{1}	18.82^{h}_{1}	6.7	6.7
Acacia karroo	48.78^{c}_{1}	56.13^{b}_{1}	18.5	12.9^{c}_{1}	3.53^{g}_{2}	26.5^{f}_{1}	6.29^{h}_{2}	1.00^{e}_{1}	0.24^{i}_{2}	26.91^{a}_{1}	16.06^{j}_{2}	6.8	6.8
Prosopis glandulosa	55.35^{b}_{1}	56.49^{b}_{1}	17.7	11.6^{e}_{1}	8.81^{c}_{2}	21.1^{i}_{1}	15.6^{f}_{2}	0.67^{h}_{1}	0.50^{g}_{2}	19.44^{i}_{2}	25.71^{d}_{1}	6.9	6.8
Acacia luederitzii	43.3^{d}_{1}	44.85^{c}_{1}	20.2	12.6^{cd}_{1}	0.45^{i}_{2}	29.1^{d}_{1}	1.01^{i}_{2}	1.36^{c}_{1}	0.05^{j}_{2}	19.96^{h}_{1}	17.16^{i}_{2}	7.0	6.9
Mean	47.6	45.68	18.53	12.47	8.48	26.2	18.56	1.02	0.72	21.22	24.72	6.88	6.82
P	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Shrubs													
Acacia mellifera	57.08^{c}_{1}	59.19^{cd}_{1}	17.7	10.1^{g}_{1}	6.93^{f}_{2}	17.8^{h}_{1}	11.7^{f}_{2}	0.55^{h}_{1}	0.36^{f}_{2}	21.46^{g}_{2}	29.89^{a}_{1}	6.9	6.9
Acacia hebeclada	61.76^{b}_{2}	61.06^{bc}_{1}	18.1	18.1^{bc}_{1}	14.8^{c}_{2}	29.4^{f}_{1}	24.3^{d}_{2}	0.86^{f}_{1}	0.71^{d}_{2}	22.43^{b}_{2}	26.17^{c}_{1}	6.9	6.9
Grewia flava	32.53^{g}_{1}	30.94^{g}_{2}	18.1	13.9^{e}_{1}	8.14^{e}_{2}	42.7^{c}_{1}	26.3^{c}_{2}	2.38^{a}_{1}	1.46^{a}_{2}	22.00^{d}_{2}	28.48^{b}_{1}	6.9	6.8
Dichrostachys cinerea	33.54^{g}_{1}	30.96^{g}_{2}	18.1	13.0^{f}_{1}	2.15^{g}_{2}	38.9^{d}_{1}	6.93^{g}_{2}	2.10^{c}_{1}	0.37^{f}_{2}	21.16^{h}_{1}	20.02^{h}_{1}	6.9	6.8
Hermannia burchelli	56.23^{c}_{1}	63.49^{b}_{1}	16.9	18.9^{b}_{1}	18.4^{a}_{1}	33.6^{e}_{1}	29.0^{b}_{2}	1.01^{e}_{1}	0.87^{c}_{2}	21.52^{f}_{1}	22.26^{f}_{1}	6.9	6.8
Lycuim cinereum	77.07^{a}_{1}	81.02^{a}_{1}	12.6	0.45^{h}_{1}	0.53^{i}_{1}	0.59^{i}_{1}	0.65^{i}_{1}	0.01^{i}_{1}	0.01^{h}_{1}	22.65^{a}_{2}	25.32^{d}_{1}	7.1	7.0
Monechma genistifolium	52.47^{d}_{1}	57.12^{d}_{1}	13.8	15.0^{d}_{1}	12.1^{d}_{2}	28.7^{g}_{1}	21.2^{e}_{2}	0.75^{g}_{1}	0.56^{e}_{2}	16.38^{i}_{2}	21.48^{g}_{1}	7.0	7.0
Hermannia tomentosa	40.9^{f}_{2}	47.34^{e}_{1}	16.5	22.2^{a}_{1}	16.7^{b}_{2}	54.3^{a}_{1}	35.3^{a}_{2}	2.19^{b}_{1}	1.43^{ab}_{2}	22.33^{c}_{1}	24.02^{e}_{1}	6.9	6.8
Monechma incanum	47.64^{e}_{1}	38.74^{f}_{2}	16.9	22.2^{a}_{1}	1.28^{h}_{2}	46.6^{b}_{1}	3.31^{h}_{2}	1.65^{d}_{1}	0.12^{g}_{2}	21.87^{f}_{1}	19.89^{i}_{1}	6.8	6.9
Mean	51.02	52.21	16.52	14.88	9.01	29.17	17.25	0.94	0.56	21.31	24.17	6.92	6.88
P	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Means with different superscripts (letters) within a column are significantly (P<0.05) different. Means with different subscripts (numbers) in rows within each incubation time are significantly (P<0.05) different. No PEG denotes presence of tannin and PEG denotes absence of tannin
(IVODM) = *in vitro* organic dry matter, CH4 = methane, ME = metabolisable energy, GE = gross energy and DD = Digestibile Dry Matter

CHAPTER 4

In vitro methane and gas production characteristics of *Eragrostis trichopophora* substrate supplemented with different browse foliage

4.1 Abstract

An *in vitro* gas production study was conducted to evaluate the potential of six browse species (high, medium and low condensed tannin concentrations) as antimethanogenic additives to *Eragrostis trichopophora* based substrate. The browse species studied were *Acacia luederitzii*, *Monechma incanum*, *Acacia erioloba*, *Acacia haematoxylon*, *Olea europaea* and *Acacia mellifera*. The edible forage dry matter of the browse species were incubated with *Eragrostis trichopophora* in a 30:70 (w/w) ratio by adding 40 mL of a buffered rumen fluid at 39 ^{0}C for 48 h. Gas and methane production at different time intervals after incubation were determined while the volatile fatty acid (VFA) concentration were recorded after 48 h. *Acacia luederitzii* and *Monechma incanum* foliage decreased methane production by more than 50%, but simultaneously decreased other parameters of rumen fermentation such as VFA concentration and ammonia-N.

Key words: browse, *in vitro*, methane, ammonia-N degradability, tannin

4.2 Introduction

Ruminants are major contributors to biogenic methane formation, and it has been estimated that reduction of methane formation from domesticated ruminants could contribute to stabilising atmospheric methane concentrations (Gibbs *et al*., 1989; Crutzen, 1995; Johnson & Johnson, 1995). Reducing enteric methane has become a focus of animal nutrition, especially in countries where agriculture is a major economic enterprise. There are currently no robust, reproducible and economically viable methods for reducing methane emissions from ruminants grazing on pastures. Manipulating the rumen microbial ecosystem to enhance digestibility of fibrous feeds, reduce methane emission and N excretion, and improve performance, are some of the most important goals for animal nutritionists.

However, researchers manipulating the rumen microbial ecosystem to enhance digestibility of fibrous feeds, and reduce methane emission and N excretion by ruminants have failed to find an effective chemical inhibitor of ruminal methane formation whose efficacy will persists for several days (Clapperton, 1977; Van Nevel & Demeyer, 1996). The only effective chemical inhibitor widely in use is ionophores, which inhibits the formation of free hydrogen by species that provides hydrogen to the methanogens (Nagaraja *et al.*, 1997), and subsequently decrease methane emissions by up to

25% (Van Nevel & Demeyer, 1996), but the overall effect of ionophores appears to be inconsistent. There is a need for feed additives with the potential to reduce ruminal methanogenesis. Extensive screening of plants and plant extracts that exhibit CH_4 reducing properties have been conducted (Kamra *et al*., 2006; Bodas *et al*., 2008; Garcia-Gonzalez *et al*., 2008; Soliva *et al*., 2008). Tannins are among the compounds considered promising in CH_4 reduction (Patra & Saxena 2010). In tropical herbaceous forages like trees and shrub species there are appreciable amounts of tannins and other phenolic compounds in their foliage which may reduce methane production (Martin *et al*., 2010).

However, the effectiveness of plants and plant extracts that have high levels of saponins, flavonoids and tannins, varies depending on its molecular weight, type and concentration of these compounds (Patra *et al*., 2006). Some *in vitro* studies combined additives with single substrates such as grain meals (Callaway & Martin, 1996; Carro & Ranilla, 2003; Pellikaan *et al*., 2011) or hay (Lourenco *et al*., 2008; Goel *et al*., 2009) to reduce methane production. Other studies used a mixed basal substrate, such as alfalfa hay (Wang *et al*., 2000; Busquet *et al*., 2005) or grass hay (Lila *et al*., 2003; Hu *et al*., 2005; Guo *et al*., 2008) combined with a concentrate to reduce methane. Few reports exist in which effects of additives were studied in combination with different substrates within a single experiment to reduce methane concentrations. Research to identify new compounds or novel uses for existing natural products to reduce methane is expensive, but is essential to identify new active compounds given the wide range of molecular diversity in these products (Borris, 1996). In this study *Eragrostis trichopophora* grass was incubated with different browse species with various levels of total tannins and condensed tannins to determine the effectiveness of these browse species in reducing methane production. In doing so we can possibly select browse species with the potential to decrease methane production extensively.

4.3 Materials and methods

4.3.1 Selection of the browse species

One kilogram of edible foliage sample of six browse species were sampled in the Kalahari (S 26° 46.610' E 22° 34.557') area situated in the Northern Cape Province of South Africa between March-April 2012. These species (*M. incanum*, *A. luederitzii*, *A. erioloba*, *A. haematoxylon*, *O. europaea* and *A. mellifera)* were analyzed (Chapter 2) and based on its tannin concentrations were selected to be used for this trail. Two browse species each representing a low, medium and high tannin concentration group, was selected for inclusion. These browse species were supplemented with the substrate *E. Trichopophora* in a 30:70 ratio (w/w).

4.3.2 *In vitro* gas production measurement

4.3.2.1 Collection of rumen fluid from donor sheep

The rumen fluid was collected from two rumen cannulated Merino weathers fed *ad libtum* on alfalfa hay which were kept on the Experimental farm of the University of Pretoria. Rumen fluid was always collected before the morning feeding. The rumen fluid was transferred into the incubator within an hour, and kept at a constant temperature of 39 ºC. Approximately 500 mL of the rumen fluid was collected from each donor animal, mixed, strained through four layers of cheesecloth and transferred to pre-heated thermos flasks. In the laboratory, the flasks were emptied into a flask while being purged with CO_2 to maintain anaerobic conditions (Grant & Mertens, 1992). After blending, the rumen fluid was transferred to a large glass beaker inside a 39 °C water bath being continuously purged with CO_2 and continuously stirred as recommended by Goering and Van Soest (1970). Thereafter, the required volume of rumen fluid was added to the buffer solution in the respective incubation bottles; 15 mL of rumen fluid to 25 mL parts of buffer solution.

4.3.2.2 Buffer media preparation, sample incubation and gas measurement

The buffer solution, macro mineral solution and micro mineral solution were prepared as described in Goering and Van Soest 1970. The micro mineral solution was prepared with slight modification by replacing $MgSO_4.7H_2O$ with $MgCl_2.6H_2O$ to reduce the amount of SO_4 in the media as suggested by Mould *et al*., (2005) and stored in a dark glass bottles to maintain the stability of the solution. In the morning before rumen fluid collection, a mixture of 1.3 L rumen buffer solution, macro and micro mineral solutions were mixed with the tryptose and prepared with 0.1% (wt/vol) resazurin. A mass of 0.386 g of L-cysteine hydrochloride was weighed and directly added to the rest of the solution once all chemicals were dissolved. As soon as L-cysteine hydrochloride was added to the buffer solution it was placed in a 39°C water bath and CO_2 was bubbled through the solution until the buffer solution was clear, indicating that the solution was sufficiently reduced.

A semi- automated system was used to measure gas production through *in vitro* incubation at 39°C, according to the method described by Theodorou *et al.* (1994). The system consists of a digital data tracker (Tracker 220 series indicators, Omega Engineering, Inc., Laval, QC, Canada) connected to a pressure transducer (PX4200-015GI from Omega Engineering, Inc., Laval, QC, Canada) with needle on the tip. Approximately 400 mg of respective browse foliage sample was weighed into a 120 ml serum bottle. Then 40 mL of rumen fluid + the buffer medium was added under a stream of CO_2 to each serum bottles and then closed with a rubber stopper and a crimp seal cap. A needle of a medical syringe was inserted through the rubber stopper of each serum bottle for about 5 seconds to release a small amount of gas that might have built up and create starting point for incubation. All serum

bottles were returned to the incubator and the rotary shaker was turned on at 120 rpm. Gas pressure was taken at 2, 4, 8, 12, 24, 48 and 72 h. To quantify gas production derived from the culture medium and the ruminal inoculums, two blanks were included in every analysis. Two replicates of the same browse and four different cycles were executed for every browse sample studied. The pressure and volume values were registered and added to the values of the previous readings. Therefore, the cumulative pressure and volume of the fermentation gases could be obtained. However, fermentation was terminated after 72 h by removing the serum bottles from the incubator and placing them on ice. After opening the bottle, pH readings were taken and the supernatants were collected and used for volatile fatty acids and ammonia-nitrogen analyses.

4.3.3 Short chain volatile fatty acids and ammonia-nitrogen analysis

The 5 mL supernatant serum bottle contents were centrifuged in Sorvall Centrifuge (SL-50 T, 8×50 ml) at 25,000*g* for 15 min at 4° C and a part of the supernatant was transferred to a micro-centrifuge tube (capacity 1.5 mL) containing meta-phosphoric acid (250 g/L), (5 g/L) as internal standard. The samples were centrifuged at 10,000*g* for 5 minutes in a micro-centrifuge. The standard VFA mixture consisted of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids and was treated in the same manner as that for the sample. The VFA's in test sample were analyzed by the gas chromotaphore (GC) with FID analyzer and it was calibrated against the standard. The final concentration was reported after deducting the corresponding blank values.

For ammonia-N analysis 50 µl of a standard, supernatant sample and 0.1 N HCl (blank) was measured into test tubes in duplicates, using pipettes while 2.5 ml of phenol reagent and 2 ml of hypochlorite reagent were added and mixed. The tubes were then placed in a water-bath at 95°C for 5 minutes. The tubes were removed and cooled to between 25 and 30°C and then read on a spectrophotometer at 630 nm. The NH_3-N concentrations were reported as g/kg DM (Broderick & Kang, 1980).

4.3.4 Determination of *in vitro* digestible organic matter

The procedure used in this study was according to that of Tilley & Terry (1963), as modified by Engels & Van der Merwe (1967) who involved two digestion phases. During the first digestion phase, feed samples (200 mg) were incubated in triplicate under anaerobic conditions with rumen liquor for 48 h at 39° C with the inclusion of blanks and standards in every batch of incubation. This was followed by a 48 h acid pepsin digestion phase at 39° C under anaerobic conditions. Following the 96 hour incubation, the residual plant materials were collected and oven dried at 105°C for 12 hours. Ash contents were determined by combustion (550°C for 2 hours) (Engels & Van der Merwe, 1967). Metabolizable energy was determined by using the equation ME= 0.016*IVOMD suggested by McDonald *et al*. (2002) for roughages.

4.3.5 Methane production measurements

Methane production was measured from duplicate bottles incubated for each browse by taking gas samples at 2, 12, 24, and 48 h and analyzing the methane concentration using the SRI multiple gas #1 chromatography analyzer system. Gas produced from each bottle at various times was recorded and samples of the gas were taken using a Hamilton syringe and 1 mL of sampled gas produced was injected manually (pull and push method of sample injection) into the GC, which was calibrated with 100% CH_4. Two blanks were included for correction of CH_4 produced from the inoculum in each cycle and a total of two cycles were executed for each sample. Methane concentration that was measured at each time interval was related for total gas volume to determining its concentration (Tavendale *et al*., 2005) and converted later into energy and mass values using 39.54 kJ/l CH_4 and 0.716 mg/mL CH_4 factors, respectively (Santoso *et al*., 2007).

4.3.6 Statistical analysis

Tree and shrub foliage nutrient, plant secondary compound, gas, VFA, NH_3-N and methane concentrations were statistically analysed using the 'GLM' option of SAS (9.1), with methods of Steel & Torrie (1980), and differences among foliage species were determined using Duncan's multiple-range test (Duncan & Brant 1983).

4.4 Results and discussion

4.4.1 Chemical composition

The chemical composition of *Eragrostis trichopophora* was analysed for NDF, ADF, ADL, CP, EE, OM, ADIN and NDIN. The values are presented in the Table 4.1. *Eragrostis trichopophora* had a low CP concentration while its NDF component is relatively high. The chemical composition of the browse species was presented earlier in Chapter 2, page 31.

Table 4.1: Chemical composition of *Eragrostis trichopophora* (g/kg) DM

Ash	OM	CP	EE	NDF	ADF	ADL	NDIN	CELLULOSE
33.10	919.70	34.30	9.47	794.90	477.70	67.90	21.00	409.80

OM = organic matter CP = crud protein EE = ether extract NDF = neutral detergent fiber ADF = acid detergent fiber ADL = acid detergent lignin NDIN- neutral detergent insoluble nitrogen, Cellulose calculated as (ADF-ADL)

4.4.2 *In vitro* enteric methane production

In the current study the in vitro methane production as shown in Table 4.3 indicates that after 24 h of incubation there was significant differences ($p<0.001$) between the different browse species that were used to supplement *E. trichopophora* substrate. Figure 4.1 and 4.2 represent the amount of methane being produced over 48 hours of incubation. The results obtained after 24 h incubation differed because of the different concentrations of phenolic components within the browse species. The total amount of methane produced at 24 h by *in vitro* incubation was decreased in *A. luederitzii* by 56.7 % and 18 % in *M. incanum*, respectively, due to the inhibitory effects of tannins on methane. The *O. europaea* and *A. mellifera* browse showed no effect of decreases on CH_4 production after 24 and 48 h. The cumulative methane production at 48 h of *in vitro* incubation was also decreased by 61.2 %, 43.6 %, 24.7 % and 14.4 % in *A. luederitzii, M. incanum, A. erioloba* and *A. haematoxylon*, respectively. The reduction in methane production in *A. luederitzii* and *M. incanum* samples may be attributed to its total tannin or condensed tannin concentrations, which may indicate direct inhibition of methanogenesis, fermentation of organic matter or its inhibitory effects on ciliate protozoa (Hess *et al*., 2003b).

The CT action on methanogenesis has been attributed partly to indirect effects of, reduced H_2 production and digestibility, and partly by direct inhibitory effects on methanogens (Monforte-Briceno *et al*., 2005, Tavendale *et al*., 2005). Hess *et al*. (2004) reported that *in vitro* methane production decreased when *Calliandra* tannins were supplemented with a tropical grass substrate as was observed when *A. luederitzii* and *M. incanum* was supplemented to *E. trichopophora*. This further confirms earlier reports that condensed tannins (CT) lower CH_4 emission by ruminants (Carulla *et al*., 2005; Puchala *et al*., 2005). During this study similar results were observed from species with higher condensed tannin concentrations that showed the best potential to reduce methane concentrations. Carulla *et al*. (2005) reported that when sheep were fed a mixture of *Lolium perenne* and *Trifolium pratense* or *M. sativa* was supplemented with 29 g CT/kg dietary dry matter (DM) of *Acacia mearnsii*, CH_4 emission was reduced by 130 kJ. However, a limited number of studies investigated the direct and indirect effects of plants secondary components on methane production in animals, and it is difficult to provide a comprehensive assessment at this stage about the size of decrease that might be realistically expected an *in vivo* research.

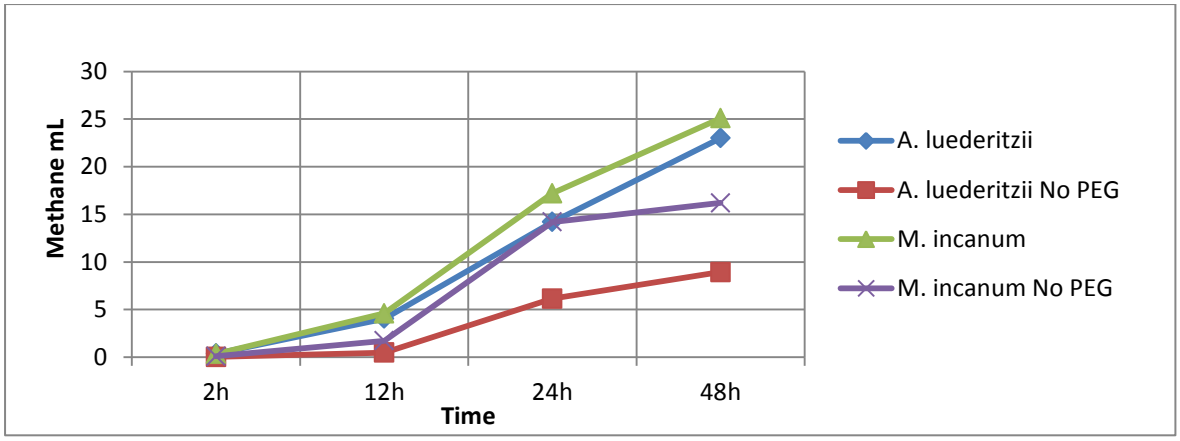

Figure 4.1 Methane production from high tannin concentration browse during 48 hour incubation. *A. luederitzii = Acacia luederitzii, M. incanum = Monechma incanum*

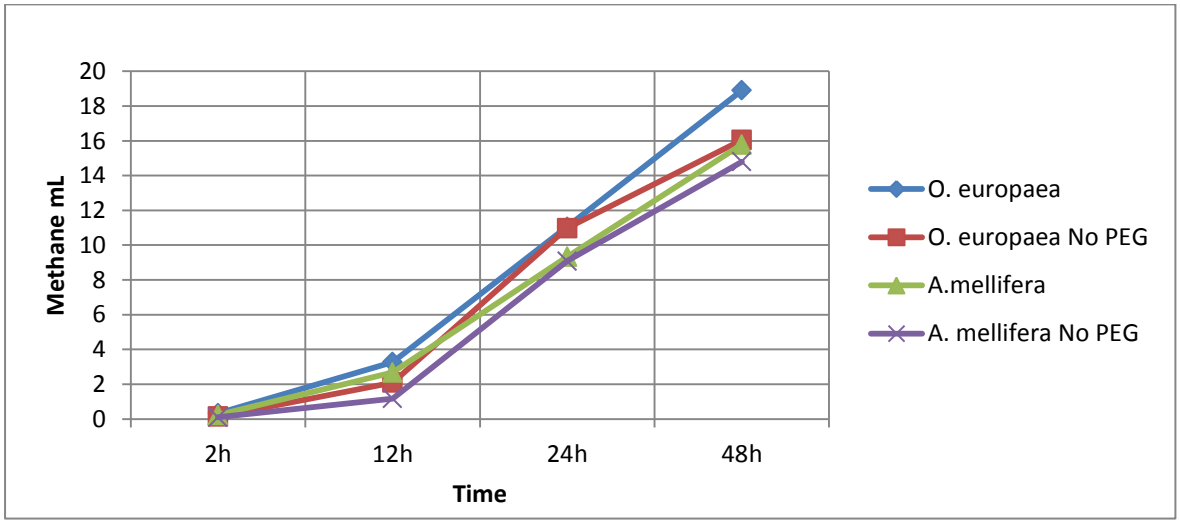

Figure 4.2 Methane production from low tannin concentration browse during 48 hour incubation. *O. europaea = Olea europaea* and *A. mellifera = Acacia mellifera*

4.4.3 *In vitro* gas production and *in vitro* organic matter digestibility

The *in vitro* gas production results for the incubated browses without or with PEG at 24 h production (mL/g DM) are summarized in Table 4.2. The results showed that with and without poly ethylene glycol, there were significant differences ($p<0.001$) among the different browses supplemented to the grass substrate, in terms of cumulative gas production (GP). When "no PEG" was included in all the browse-grass substrates, gas production was between 6 and 80 ml lower after 24 hours and 48 hours incubation. *A. luederitzii* decreased gas production by 30.54 % while *M. incanum, A. erioloba* and *A. haematoxylon*s samples decreased gas production between 6-18 % respectively, after 24 and 48 hours incubation, while *O. europaea* and *A. mellifera* showed no significant decreases in gas production at (24 and 48 hours), but Rodriguez *et al*. (2009) reported much higher volumes of total gas production at 24 hours (GP_{24}) compared to what was observed in this study which may be due to the lower digestibility of the browse grass-substrates. Figure 4.3 and 4.4

represent the gas production during 48 hours of incubation time for high and low tannin containing browse grass-substrates. There is a clear indication that fermentation was inhibited in the browse grass-substrate with high tannin concentrations when PEG was not added. This could be associated to the much lower digestibility (Table 4.4) of the browse substrates in this study compared to the browse species reported by Rodriguez *et al*. (2009). CH_4 production as shown in this study could be reduced by supplementing grasses with browse species with high tannin content, but also resulting in a reduction of DM digestibility.

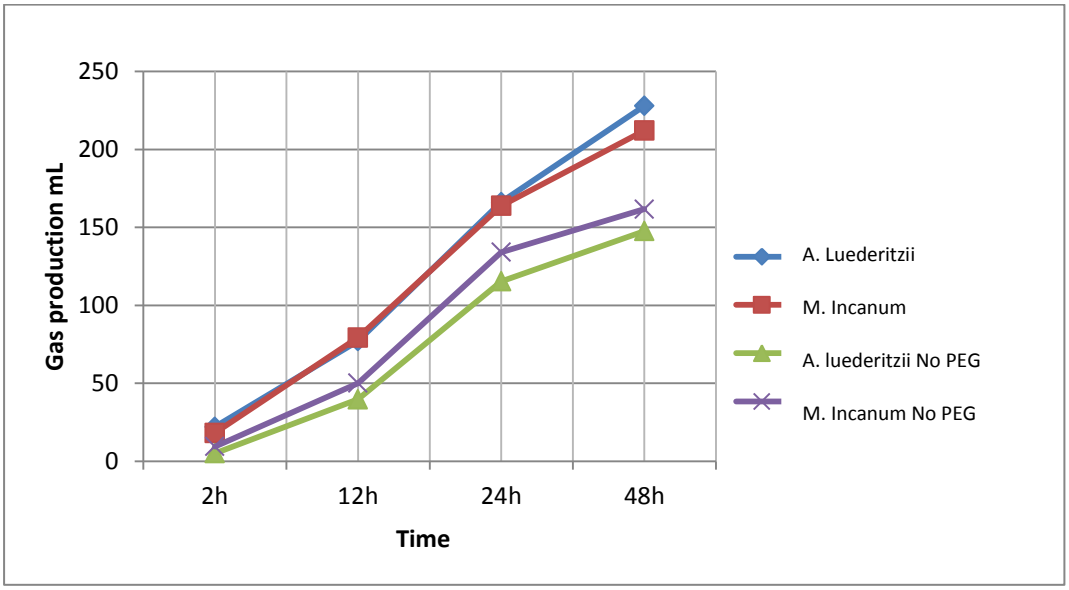

Figure 4.3 Gas production from browse with high tannin concentration during 48 hours incubation. *A. luederitzii = Acacia luederitzii, M. incanum = Monechma incanum*

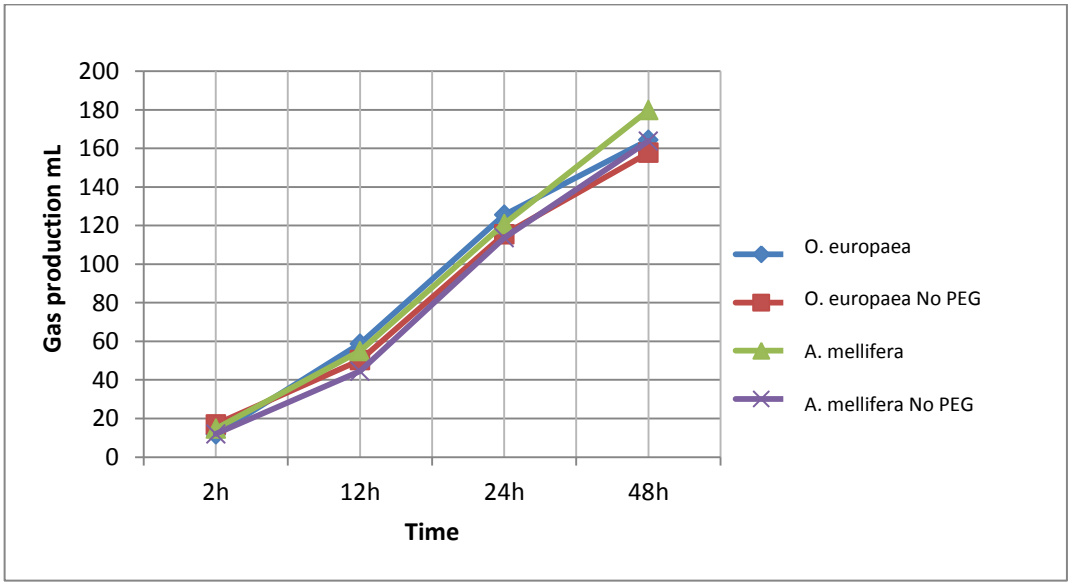

Figure 4.4 Gas production from browse with low tannin concentration during 48 hours incubation. *O. europaea = Olea europaea* and *A. mellifera = Acacia mellifera*

4.4.4 VFA concentration

The results from Table 4.4 indicates that the inclusion of PEG in *A. luederitzii*, *M. incanum*, *A. erioloba* and *A. haematoxylon* grass substrate resulted in increased total VFA production, while the browse grass-substrate *A. melliferia* and *O. europa* had a reduction in the total VFA produced when PEG was included (Table 4.4). The acetic, propionic, Iso butyric, butyric, and valeric concentrations differed among the browse species. Reports on the effects of CT on ruminal VFA concentration and composition vary among studies depending on dose and source of CT (Bhatta *et al*., 2005). In the study of Khiaosa-Ard *et al*. (2009) using a rumen simulation technique, a decrease in the acetate to propionate ratio was reported when CT extract from *Acacia mearnsii* was fed to animals. Similar results were reported in this study (See chapter 2, page 11). However the change in VFA production reported by Bhatta *et al*. (2009) are not similar to the results reported in this study. Acetate production has increased in this study when PEG was included in those browse-substrate species with high CT concentrations while browse substrate species with low CT concentrations showed a reduction in acetate production with PEG. Beauchemin *et al*. (2007) reported that increasing levels of tannins to 20 g/kg DM only tended to decrease ruminal total VFA concentration and decreased the acetate to propionate ratio in cattle.

In contrast to the studies of Carulla *et al*. (2005); Beauchemin *et al*. (2007), Khiaosa-Ard *et al*., (2009), reported that VFA concentrations remained unchanged, but that the molar proportion of propionate increased in sheep fed *A. mearnsii* containing CT, were the acetate to propionate ratio in this study also decreased when no PEG was included, but was not reported in Table 4.4. In general, molar ratios of the principal VFA are not changed by feeding CT containing forages (Waghorn & Shelton, 1997; Puchala *et al*., 2005), but its concentrations in rumen liquor is often reduced, probably a reflection of a larger rumen pool size and a slower rate of VFA production due to a slower rate of fibre digestion (Waghorn *et al*., 1994). The results from this study indicate that total tannins and condensed tannins cause an inhibitory effect on VFA production thus compromising digestibility.

4.4.5 Loss of energy as CH_4

As indicated in Table 4.5 *A. luederitzii* and *M. incanum* browse grass-substrates decreased the amount of feed energy converted to methane. The reduction of DM used by methanogenic bacteria ranged from 0 to 8.07 g/kg DM when PEG was not added. The amount of GE (MJ/kg) lost as methane was also calculated and is presented in Table 4.5. *Eragrostis trichopophora* substrate supplemented with *A. luederitzii* had an increase production of GE. The two browse species (*A. luederitzii* and *M. incanum*) had the highest total tannin and condensed tannin concentrations among the rest of browse substrate species used in this study (Chapter 2, page 32). Methane production was decreased by either the total tannin or condensed tannin concentrations in this study. According to Monforte-Briceno *et al*. (2005) and Tavendale *et al*., (2005), the action of CT on methanogenesis can be attributed to the

indirect effects of reduced H_2 production and digestibility, and by direct inhibitory effects on methanogens. Plant attributes that influence the amount of methane produced in a sample are those chemical components that increase its fermentation potential, such as high CP, GE, digestibility values and low ADL concentrations. In Table 4.6 methane production was expressed as a ratio of gas volume, VFA and IVOMD with or without PEG and the ratios were significantly ($p<0.001$) lower in substrates without PEG compared to the browse grass-substrates with PEG. The lower ratios for CH_4: GP_{24}, CH_4: VFA and CH_4: IVOMD that were observed for *A. luederitzii* is partly due to *A. luederitzii* having higher fermentation properties compared to the other browse grass-substrates, but is also related to the tannin concentrations that reduced CH_4 production.

4.5 Conclusion

The browse substrate species that differed in their tannin concentrations were incubated as a supplement to *Eragrostis trichopophora* hay in a ratio of 30:70, and their effectiveness to reduce methane production was assessed. From this study it can be concluded that *A. luederitzii* reduced methane production by more than 55% over 48 hour of incubation. The reduction in methane production recorded with the inclusion of *A. luederitzii*, however, comes at the expense of 11% reduction in overall digestibility. Browse species with high tannin composition could possibly be used as a natural alternative to reduce methane production. However, DM digestibility was lower in all browse grass-substrates, showing a negative relationship when supplementing grass species with browse species. Tannin extracts from *A. luederitzii* could possibly be used as a dietary alternative to reduce methane production. Further research with regard to the type of tannin (molecular weight, chemical interaction) needs to be executed to determine at what concentration tannins can be supplemented to reduce methane production without reducing digestibility or animal performance.

Table 4.2 Volumes (mL/g DM) of gas production from the studied browse and shrubs supplemented to grass hay at a ratio of 30:70 with or without polyethylene glycol

		Hours of incubation							
		2h		12h		24h		48h	
Species	**Tannin level**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**
Acacia luederitzii	High	$22.08^{a}{}_{1}$	$5.23^{d}{}_{2}$	$77.13^{ab}{}_{1}$	$39.78^{d}{}_{2}$	$166.23^{a}{}_{1}$	$115.45^{c}{}_{1}$	$227.93^{a}{}_{1}$	$147.68^{c}{}_{2}$
Monechma incanum	High	$18.18^{b}{}_{1}$	$9.53^{c}{}_{2}$	$79.38^{a}{}_{1}$	$50.10^{b}{}_{2}$	$163.88^{a}{}_{1}$	$134.15^{b}{}_{2}$	$212.10^{b}{}_{1}$	$161.73^{d}{}_{2}$
Acacia erioloba	Medium	$21.33^{a}{}_{1}$	$17.50^{a}{}_{2}$	$73.50^{b}{}_{1}$	$54.50^{ab}{}_{2}$	$140.95^{b}{}_{1}$	$131.43^{b}{}_{2}$	$188.55^{d}{}_{1}$	$160.33^{d}{}_{2}$
Acacia haematoxylon	Medium	$19.58^{ab}{}_{1}$	$13.00^{b}{}_{2}$	$78.08^{ab}{}_{1}$	$56.33^{a}{}_{2}$	$161.18^{a}{}_{1}$	$132.28^{b}{}_{2}$	$197.05^{c}{}_{1}$	$177.15^{b}{}_{2}$
Olea europaea	Low	$11.83^{d}{}_{2}$	$16.98^{a}{}_{1}$	$58.78^{c}{}_{1}$	$50.28^{b}{}_{2}$	$125.60^{c}{}_{1}$	$115.45^{c}{}_{2}$	$164.43^{f}{}_{1}$	$157.80^{cb}{}_{2}$
Acacia mellifera	Low	$14.73^{c}{}_{1}$	$12.00^{cb}{}_{2}$	$54.95^{c}{}_{1}$	$44.48^{c}{}_{2}$	$120.88^{c}{}_{1}$	$113.60^{c}{}_{2}$	$179.83^{e}{}_{1}$	$163.83^{cb}{}_{2}$
Eragrostis trichopophora		4.35^{e}	3.48^{d}	$57.2^{c}{}_{1}$	45.25^{c2}	$157.5^{a}{}_{1}$	$156.0^{a}{}_{1}$	$221.78^{a}{}_{1}$	$212.85^{a}{}_{2}$
MSE		0.388	0.34	0.663	0.623	1.846	1.004	1.005	1.332
P		<0.0001	<0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means with different superscripts (letters) within a column are significantly (P<0.05) different

Means with different subscripts (numbers) in rows within each incubation time are significantly (P<0.05) different

No PEG denotes presence of tannin and PEG denotes absence of tannin

Table 4.3 Volumes (mL/g DM) of methane production from the studied browse and shrubs supplemented to grass hay at a ratio of 30:70 with or without polyethylene glycol

		Hours of incubation							
		2h		12h		24h		48h	
Species	**Tannin level**	**PEG**	**No PEG**	**PEG**	**NO PEG**	**PEG**	**No PEG**	**PEG**	**NO PEG**
Acacia luederitzii	High	$0.38^{a}{}_{1}$	$0.03^{d}{}_{2}$	$4.05^{ab}{}_{1}$	$0.48^{d}{}_{2}$	$14.20^{c}{}_{1}$	$6.15^{d}{}_{2}$	$23.00^{c}{}_{1}$	$8.93^{d}{}_{2}$
Monechma incanum	High	$0.35^{a}{}_{1}$	$0.10^{bc}{}_{2}$	$4.60^{b}{}_{1}$	$1.73^{abc}{}_{2}$	$17.20^{ab}{}_{1}$	$14.20^{b}{}_{2}$	$25.08^{c}{}_{1}$	$16.20^{b}{}_{2}$
Acacia erioloba	Medium	$0.38^{a}{}_{1}$	$0.25^{a}{}_{2}$	$3.83^{bc}{}_{1}$	$2.13^{ab}{}_{2}$	$13.33^{cd}{}_{1}$	$10.88^{c}{}_{2}$	$18.85^{d}{}_{1}$	$14.20^{c}{}_{2}$
Acacia haematoxylon	Medium	$0.33^{ab}{}_{1}$	$0.13^{b}{}_{2}$	$3.95^{ab}{}_{1}$	$1.65^{bc}{}_{2}$	$12.28^{de}{}_{1}$	$10.53^{c}{}_{2}$	$18.55^{d}{}_{1}$	$15.88^{b}{}_{2}$
Olea europaea	Low	$0.33^{ab}{}_{1}$	$0.15^{b}{}_{2}$	$3.28^{cd}{}_{1}$	$2.10^{ab}{}_{2}$	$11.05^{ef}{}_{1}$	$10.98^{c}{}_{1}$	$18.90^{d}{}_{1}$	$16.05^{b}{}_{2}$
Acacia mellifera	Low	$0.23^{b}{}_{1}$	$0.10^{bc}{}_{2}$	$2.68^{de}{}_{1}$	$1.18^{c}{}_{2}$	$9.33^{f}{}_{1}$	$9.08^{c}{}_{1}$	$15.78^{c}{}_{1}$	$14.80^{cb}{}_{2}$
Eragrostis trichopophora		$0.10^{c}{}_{1}$	$0.05^{c}{}_{1}$	$2.80^{de}{}_{1}$	$2.28^{a}{}_{2}$	$18.78^{a}{}_{1}$	$17.63^{a}{}_{2}$	$39.93^{a}{}_{1}$	$33.95^{a}{}_{2}$
MSE		0.17	0.01	0.089	0.08	0.258	0.309	0.32	0.338
P		<0.0001	<0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means with different superscripts (letters) within a column are significantly (P<0.05) different

Means with different subscripts (numbers) in rows within each incubation time are significantly (P<0.05) different

No PEG denotes presence of tannin and PEG denotes absence of tannin

Table 4.4 Total and individual volatile fatty acid (mmol/L) concentration, in supernatant after 72h incubation of 400mg DM of browses with or without poly ethylene glycol

	Acetic		*Propionic*		*Iso butyric*		*Butyric*		*Valeric*		*Total VFA mmol/L*		*IVOMD*	
Species	**PEG**	**NO PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**
Acacia luederitzii	51.91^{e}_{1}	49.19^{d}_{2}	12.63^{e}_{2}	13.91^{c}_{1}	1.44^{e}_{1}	1.41^{d}_{1}	5.06^{f}_{1}	4.74^{f}_{2}	1.71^{e}_{1}	1.78^{c}_{1}	72.76^{f}_{1}	71.04^{f}_{2}	58.79^{a}_{1}	47.51^{d}_{2}
Monechma incanum	59.04^{a}_{1}	48.9^{d}_{2}	15.72^{a}_{1}	13.44^{d}_{2}	1.70^{b}_{1}	1.47^{c}_{2}	7.15^{a}_{1}	5.60^{e}_{2}	2.15^{a}_{1}	1.78^{c}_{2}	85.76^{a}_{1}	71.78^{e}_{2}	58.88^{a}_{1}	52.27^{a}_{2}
Acacia erioloba	56.21^{c}_{1}	55.84^{b}_{1}	15.16^{b}_{1}	14.92^{b}_{1}	1.76^{a}_{1}	1.77^{a}_{1}	6.41^{d}_{1}	6.26^{c}_{1}	2.18^{a}_{1}	2.17^{a}_{1}	81.72^{c}_{1}	81.07^{c}_{2}	50.31^{c}_{1}	48.19^{c}_{2}
Acacia haematoxylon	57.68^{b}_{1}	51.50^{c}_{2}	14.85^{c}_{1}	13.23^{e}_{2}	1.74^{a}_{1}	1.64^{b}_{2}	6.58^{b}_{1}	5.88^{d}_{2}	2.11^{b}_{1}	1.96^{b}_{2}	82.96^{b}_{1}	74.21^{d}_{2}	55.73^{b}_{1}	49.31^{b}_{2}
Olea europaea	50.61^{f}_{2}	55.74^{b}_{1}	14.93^{c}_{2}	15.82^{a}_{1}	1.50^{d}_{2}	1.62^{b}_{1}	6.15^{e}_{2}	6.50^{b}_{1}	1.88^{d}_{1}	1.93^{b}_{1}	74.53^{e}_{2}	81.61^{b}_{1}	45.63^{e}_{1}	43.38^{f}_{2}
Acacia mellifera	54.25^{d}_{2}	61.72^{a}_{1}	14.00^{d}_{2}	15.60^{ab}_{1}	1.57^{c}_{2}	1.79^{a}_{1}	6.51^{c}_{2}	7.29^{a}_{1}	1.96^{c}_{2}	2.16^{a}_{1}	78.29^{d}_{2}	88.55^{a}_{1}	47.01^{d}_{1}	45.40^{e}_{2}
Eragrostis trichopophora	62.13_{1}	56.98_{2}	15.26_{1}	13.54_{2}	1.63_{1}	1.42_{2}	6.20_{1}	5.82_{2}	1.83_{1}	1.76_{1}	87.05_{1}	79.52_{2}	54.74_{1}	54.4_{1}

Means with different superscripts (letters) within a column are significantly (P<0.05) different

Means with different subscripts (numbers) in rows within each incubation time are significantly (P<0.05) different

No PEG denotes presence of tannin and PEG denotes absence of tannin

IVOMD = in vitro organic matter digestibility and VFA = volatile fatty acid

Table 4.5 Loss of energy as methane (MJ/ g DM) from the browse sample supplemented to grass hay at a ratio of 30:70

		GE MJ/kg	CH_4 ml		CH_4 g/Kg		Methane per g/Kg IVOMD		MJ/Kg loss of GE as methane	
Species	**Tannin level**		**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**
Acacia luederitzii	High	20.2	14.2	6.15	10.22	4.43	17.39	9.32	$0.60^{a}{}_{1}$	$0.40^{b}{}_{2}$
Monechma incanum	High	16.9	17.2	14.2	12.38	10.22	21.03	19.56	$0.60^{a}{}_{2}$	$0.63^{a}{}_{1}$
Acacia erioloba	Medium	19.1	13.33	10.88	9.59	7.83	19.07	16.25	$0.72^{a}{}_{1}$	$0.64^{a}{}_{2}$
Acacia haematoxylon	Medium	20.2	12.28	10.53	8.84	7.58	15.86	15.37	$0.57^{b}{}_{2}$	$0.63^{b}{}_{1}$
Olea europaea	Low	18.1	11.05	10.98	7.96	7.9	17.43	18.22	$0.69^{a}{}_{2}$	$0.76^{a}{}_{1}$
Acacia mellifera	Low	17.7	9.33	9.08	6.71	6.53	14.28	14.39	$0.54^{b}{}_{2}$	$0.56^{b}{}_{1}$
Eragrostis trichopophora			18.78	17.63	13.52	12.69	24.7	23.33		

Means with different superscripts (letters) within a column are significantly (P<0.05) different

Means with different subscripts (numbers) in rows within each incubation time are significantly (P<0.05) different

No PEG denotes presence of tannin and PEG denotes absence of tannin

CH_4 = methane IVOMD = in vitro organic matter digestibility GE = gross energy

Table 4.6 Ratio of methane to some fermentation parameters (1:1) on a DM basis

		CH_4/Gas		CH_4/VFA		CH_4/IVODM	
Species	**Tannin level**	**PEG**	**No PEG**	**PEG**	**No PEG**	**PEG**	**No PEG**
Acacia luederitzii	High	$0.085424^{a}{}_{1}$	$0.05327^{b}{}_{2}$	$0.195162^{b}{}_{1}$	$0.086571^{b}{}_{2}$	$0.241538^{a}{}_{1}$	$0.129446^{b}{}_{2}$
Monechma incanum	High	$0.104955^{b}{}_{2}$	$0.105852^{a}{}_{2}$	$0.20056^{a}{}_{1}$	$0.197827^{a}{}_{2}$	$0.29212^{b}{}_{1}$	$0.271666^{a}{}_{2}$
Acacia erioloba	Medium	$0.094573^{a}{}_{1}$	$0.082782^{a}{}_{2}$	$0.163118^{a}{}_{1}$	$0.134205^{b}{}_{2}$	$0.264957^{a}{}_{1}$	$0.225773^{a}{}_{2}$
Acacia haematoxylon	Medium	$0.076188^{b}{}_{2}$	$0.079604^{b}{}_{1}$	$0.148023^{b}{}_{1}$	$0.141895^{a}{}_{2}$	$0.220348^{b}{}_{1}$	$0.213547^{b}{}_{2}$
Olea europaea	Low	$0.087978^{a}{}_{2}$	$0.095106^{a}{}_{1}$	$0.148262^{a}{}_{1}$	$0.134542^{a}{}_{2}$	$0.242165^{a}{}_{2}$	$0.253112^{a}{}_{1}$
Acacia mellifera	Low	$0.077184^{b}{}_{2}$	$0.07993^{b}{}_{1}$	$0.119172^{b}{}_{1}$	$0.102541^{b}{}_{2}$	$0.198468^{b}{}_{2}$	$0.20^{b}{}_{1}$

Means with different superscripts (letters) within a column are significantly ($P<0.05$) different

Means with different subscripts (numbers) in rows within each incubation time are significantly ($P<0.05$) different

No PEG denotes presence of tannin and PEG denotes absence of tannin

CH_4 = methane IVODM = in vitro organic dry matter and VFA = volatile fatty acid

CHAPTER 5

General conclusion

Nineteen browse (shrub and tree) species were selected from Kalahari-bush-dune-veldt in the Northern Cape, region of South Africa and their forage harvested during mid-vegetative stage to determine their chemical composition and nutritional value of the edible forage biomass (leaves and <2mm stem) and to assess the effects of their phenolic compounds (tannins) on methane and gas production, organic matter digestibility, rumen ammonia and volatile fatty acid production. Although the nutritive value of conventional feeds on animals have been studied extensively, much less information is available about the nutritive value of alternative feeds such as browses from the Kalahari-bush-dune-veldt. Browse species such as *B. albitrunca*, *R. lancea*, *L. cinereum* and *H. burchelli* may become an important source of nutrients for grazing animals under harsh conditions in the semi-arid Kalahari bush dune veldt, especially during the dry season when the quality and quantity of green herbage is limited. Data collected as part of this study showed that:

- Some of the shrub and tree species forage (*B. albitrunca*, *R. lancea*, *L. cinereum* and *H. burchelli*) could possibly be used as a supplement during times of drought, by virtue of having moderate CP values and low fiber content. The availability of these browse species is however limited, as low rainfall in the Kalahari-bush-dune-veldt is the most limiting factor for cultivation of the browse species in the area.
- Some of the browse species selected from the Kalahari region have showed good potentials to reduce methane production. *A. luederitzii* and *M. incanum* showed superior potential to decrease methane production, by up to 90 % after 48 h of incubation.
- The tree species *A. luederitzii* significantly ($p>0.001$) reduced the amount of CH_4 produced annually per animal unit (450kg) from 119.49 Kg CH_4 with PEG to 4.15 Kg CH_4 without PEG

while *M. incanum* reduced CH_4 production form 191.35 Kg CH_4 with PEG to 13.59 Kg CH_4 without PEG per annum, assumeing an average DM intake of 2.5% of body weight. Tannin extracts from *A. luederitzii* and *M. incanum* could possibly be used as a dietary alternative to reduce enteric methane production in ruminants.

- When extrapolated to animal level the plant secondary components (tannins) of the browse species had no significant ($p>0.001$) effect on IVOMD and rumen ammonia but VFA, methane and gas production was decreased significantly ($p>0.001$) when, *Acacia luederitzii* and *Monechma incanum* were used to supplement *Eragrostis trichopophora* grass at a ratio of 30:70 (browse: grass). They, however, decreased methane production by more than 50%, but at the expense of digestibility and VFA production.
- When tree species *A. luederitzii* was supplemented to *Eragrostis trichopophora*, it significantly ($p>0.001$) reduced the amount of CH_4 produced annually per animal unit from 71.41 Kg CH_4 with PEG to 38.72 Kg CH_4 without PEG based on the *in vitro* data. Out of 19 browse species tested during the *in vitro* study, *A. luederitzii* had the best potential to reduce enteric methane production, when used as a dietary alternative.

Therefore, it is recommended that an *in vivo* feeding trial should be done to verify the promising results from this *in vitro* study. In addition, the *in vivo* study is important to determine the actual nutrient degradability and availability of these browse species in order to further elaborate how methane production could be reduced without compromising nutrient digestibility.

CHAPTER 6

Critical evaluation of this study

6.1 Collection of browse species and chemical composition

Nineteen browse species were selected from the Kalahari bush dune veldt and their foliage harvested. These samples were collected from only one farm it may however not be the representative of the whole Kalahari bush dune veldt region. A description of the nutritional value of the different species could be better determined by taking foliage samples from more area to give a better representative of the Kalahari bush dune veldt. In this study, the samples were collected during mid-vegetative stage. The nutritional value of the browse species may differ within season and across season and plant samples should be collected and analysed over the different seasons. The possible use of these browse plants as a dietary alternative to reduce enteric methane production should be used to determine the effect on animal production. Acid detergent insoluble nitrogen concentration of the 19 browse species was not analysed to determine the actual crude protein available to the animals.

6.2 Quantifying tannins

Information on the type of tannin (chemical structure) present in the samples and found responsible for the reduction in methane production was not determined in this study. Therefore, the molecular weight of the different types of tannins from the different browse plants should be quantified, to determine what type of tannin is responsible for the reduction in methane production and reducing *in vitro* digestibility. In addition, plant species grown under well-defined environmental conditions in a glasshouse will also advance knowledge on the factors controlling tannin biosynthesis and concentration.

6.3 Poly ethylene glycol

The use of PEG 8000 during the *in vitro* gas production studies was aimed to inactivate tannins in order to quantify the effects of tannins on rumen fermentation. It is a common practise to use a ratio of 1:1 (sample: PEG) to determine the effects of tannins on digestibility, gas and methane production. However, it is not yet clear if the PEG in a ratio of 1:1 was able to bind all the tannins present in the browse species with higher tannin concentration, and make it inert. Generally such information has relevance not only for quantification of tannins but also for designing detanninification approaches, using PEG.

6.4 Continuous reduction in methane production

From the results in this study, it seems that *Acacia luederitzii* and *Monechma incanum* continuously decreased methane production up to 48 hours. However, it is not known if this reduction in methane production will persist after 48 hours and if so, for how long. Further research is required to determine the extent of the effectiveness as well as the duration of its effect in terms of reducing methane production over 48 hours.

6.5 Feeding trial

One of the limitations of the current study is that the results were not confirmed by an *in vivo* feeding trial making use of a methane chamber. This is necessary to determine the *in vivo* nutrient availability and fermentibility of these browse species. The potential gross energy loss from ruminants as enteric methane could potentially be reduced by supplementation of some browse species.

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