Biomass yield and nutritive value of *Stylosanthes scabra* accessions as forage source for Goats

by

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

DOCTOR OF PHILOSOPHY:

Animal Science

In the Faculty of Natural and Agricultural Sciences

Department of Animal and Wildlife Sciences

University of Pretoria

Pretoria

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October 2015

Declaration

I, **Thamsanqa Doctor Empire Mpanza,** declare that this thesis, which I hereby submit for the degree of PhD (Animal Production Management) at the University of Pretoria, is my own work, except where reference has been made, and it has not previously been submitted by me for the degree at this university or any other university or tertiary institution.

Candidate's signature :____________________

Mr TDE Mpanza (Thabekhulu, Mavundla, Siwela, Donda, Sandanezwe).

Dedication

This work is wholeheartedly dedicated to my lovely wife, Phumzile Pearl Mpanza (uma-Sibiya: Gazu, Sibiya, Ndaba, Gumede kaNdaba, Mgxebe, Khathi, Sotobe kaMampalala, S'khobobo kaMabhabhakazane).

Preface

This work was conducted by me (Mr TDE Mpanza) in the Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa. The study consists of three components, namely fieldwork, *in vitro* (laboratory trials) and *in vivo* (stall feeding) with the aim of evaluating *Stylosanthes scabra* accessions as alternative forage resources for small ruminants in South Africa.

This thesis is based on five experimental chapters, which are written as separate manuscripts for submission to peer-reviewed Journals:

1. **Thamsanqa DE Mpanza and Abubeker Hassen**. 2016. Adaptability and agronomic performance of *Stylosanthes scabra* accessions grown in a subtropical region of South Africa (to be submitted for publication in *Crop and Pasture Science Journal*).

2. **Thamsanqa DE Mpanza and Abubeker Hassen**. 2016. Nutritive value of forage from *Stylosanthes scabra* accessions grown in a subtropical region in South Africa (to be submitted for publication in *Crop and Pasture Science Journal*).

3. **Thamsanqa DE Mpanza, Abubeker Hassen, Edward F Donkin and William T Nzuza**. 2014. Relative preference for, palatability and intake of *Stylosanthes scabra* accessions adapted in Pretoria (published in parts in *Tropical Grasslands – Forrajes Tropicales volume 2, page 92-93*.).

4. **Thamsanqa DE Mpanza, Abubeker Hassen and Belete S Gemeda**. 2016. *In vitro* ruminal fermentation and digestibility of *Eragrostis* hay supplemented with forages of selected accessions of *Stylosanthes scabra* (to be submitted for publication in the *Asian Australasian Journal of Animal Science*).

5. **Thamsanqa DE Mpanza and Abubeker Hassen**. 2015. Partial replacements of *Stylosanthes scabra* forage for lucerne in total mixed ration diet of Saanen goats (published in *Tropical Animal Health and Production, volume* 47(7), *page 1391-1396: DOI 10. 1007/s11250-01500876-6*).

These studies were conducted with the aim of improving poor-quality forage during the dry season in smallholder systems through identification and strategic utilization of drought-tolerant forage legumes as alternative feed resources. Consequently, *Stylosanthes scabra* accessions were evaluated as potentially useful drought-tolerant forage for livestock production in South Africa. The thesis has a general introduction, in which the background of farming in South Africa is discussed. This is followed by the literature review (chapter 1) which covers the importance of livestock to smallholder subsistence farmers, challenges they face, alternative forages, and their integration in livestock farming. Chapter 2 examines fifteen accessions of *Stylosanthes scabra* received from International Livestock Research Institute (ILRI) in Ethiopia, for adaptability, yield performance and persistence in the subtropical climate of Pretoria, Gauteng, South Africa. Chapter 3 evaluates the nutritive value, anti-nutritional factors and fermentation of the adaptable and productive accessions based on laboratory analyses of forage samples. Chapter 4 evaluates the acceptability and preferences of five selected accessions of *Stylosanthes scabra* using Saanen goats. While chapter 5 assesses the effect of supplementing poor-quality grass hay with five selected *Stylosanthes scabra* accessions at two levels (15% and 30%) each on *in vitro* ruminal fermentation and their associative effects. Chapter 6 determines the effects of partial replacement of lucerne (alfalfa) forage in total mixed ration (TMR) with *Stylosanthes scabra* forage on intake, digestibility and nitrogen balance of Saanen goats. Lastly, general conclusions and recommendations and future research areas that should be addressed are discussed in Chapter 7.

This thesis is prepared in accordance with instructions to authors for publication of manuscripts in the *African Journal of Range and Forage Science*.

Acknowledgements

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

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

I also like to thank everybody who has contributed to the success of this study. First I am indebted to the National Department of Agriculture, Forestry and Fisheries (DAFF), South Africa for covering my living expenses during the study period. I would like also to thank Gauteng Department of Agriculture and Rural Development (GDARD) for partly covering the research costs. Additional research funding was made available from the National Research Foundation (NRF).

My sincere thanks go to Dr Abubeker Hassen for his trust in me and willingness to work with me as his PhD student. I wish to express my sincere gratitude to him for moral support and mentoring.

My gratitude goes to Hatfield Experimental Farm, University of Pretoria, for allowing me to use a section of the farmland to establish an evaluation trial of *Stylosanthes scabra* accessions. I would like to thank the Department of Animal and Wildlife Sciences, especially Mrs C Swanepoel and the staff, for their support in preparing and successfully conducting the stall feeding trials with Saanen goats at the small stock unit, and also the nutrition laboratory staff for their help in all lab analyses. Your contributions made this study possible and achievable. May God Almighty bless you all. I am also grateful to Dr A Jorge of the International Livestock Research Institute (ILRI) in Ethiopia for providing me with the *Stylosanthes scabra* seeds. I would also like to thank GDARD project team members in particular Prof EF Donkin, Dr MS Thantsha and Mr WT Nzuza for their contribution during the planning or implementation of this study. I also thank Dr BS Gemeda for his assistance with some *in vitro* trials and statistical analyses of data.

I wish to express my sincere and deepest gratitude to my wife, Phumzile Pearl Mpanza for tremendous support, love and sacrifices. You are a God-given partner. I want also to thank the support from my sister-in-law Mrs ZZ Nhlozi with her husband Mr S Nhlozi. May God bless you.

I will not forget to thank God for a number of people who encouraged me in my childhood so that I grew up focused and determined in life. “Indeed *umuntu ngumuntu ngabantu*.” Everything I am today is because of you all. “It takes a village to raise a child in Africa” (anonymous) (*kwazise phela ingane ingeyomphakathi*). Last I would like to thank my lovely parents, Mr MB Mpanza kaKhambi lembangi, kaGwaz’ingubo, kaNokhenkana and Ms HVC Linda kaMalakhiya, kaBhayi.

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List of abbreviations

a: immediate fermentable fraction

ADF: acid detergent fibre

ADFd: acid detergent fibre digestibility

ADG: average daily gain

ADL: acid detergent lignin

ANF: anti-nutritional factors

ANOVA: analysis of variance

AOAC: association of official analytical chemists

a.s.l: above sea level

AUCC: animal use care committee

b: Insoluble but slowly fermentable fraction

$BW^{0.75}$: metabolic body weight

c: rate of fermentation

C: carbon

Ca: calcium

CEC: cations exchange capacity

CIAT: Centro Internacionale de Agricultura Tropical

CP: crude protein

CPd: crude protein digestibility

C:N: carbon to nitrogen ratio

CO_2: carbon dioxide

CV: coefficient of variation

^{o}C: degrees Celsius

DAFF: Department of Agriculture, Forestry and Fisheries

DM: dry matter

DMd: dry matter digestibility

DMI: dry matter intake

DMRT: Duncan's multiple range test

DoA: Department of Agriculture

ED: effective degradability

EZ: ecological zone

GDARD: Gauteng Department of Agriculture and Rural Development

GDP: gross domestic product

GJ: gigajoles

GLM: general linear model

GV: gas volume

H_2SO_4: sulphuric acid

ILCA: International Livestock Centre for Africa

ILRI: International Livestock Research Institute

IVOMD: *in vitro* organic matter digestibility

K: potassium

LER: land equivalent ratio

LSD: least significant difference

ME: metabolizable energy

MEY: metabolizable energy yield

mg: milligram

µmol: micromole

$MgSO_4.7H_2O$: magnesium sulphate anhydrous

$MgCl_2.6H_2O$: magnesium chloride anhydrous

MJ: megajoule

ml: milliliter

mm: millimitter

N: nitrogen

NDF: neutral detergent fibre

NDFd: neutral detergent fibre degradability

NRC: National Research Council

NTP: non-tannin phenols

OM: organic matter

OMd: organic matter digestibility

P: phosphorus

PGP: potential gas production

pH: potency of hydrogen

PVPP: polyvinyl-polypyrrolidone

RCD: randomized complete design

RCBD: randomized complete block design

RPI: relative preference index

SAS: statistical analysis system

SCFA: short chain fatty acid

SD: standard deviation

SEM: standard error of mean

SSA: sub-Saharan Africa

t ha^{-1}: ton per hectare

TCT: total condensed tannins

TMR: total mixed ration

TP: total phenols

TT: total tannins

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Abstract

Shortage of quality feed is the major constraint in livestock production, particularly under smallholder subsistence farmer's conditions in sub-Saharan Africa. Livestock production under smallholder subsistence farmers depends predominantly on communal grazed natural pastures, which are often inadequate in both quantity and quality, particularly during the dry season. In order to improve livestock production in these areas, shortage of feed must be addressed in terms of quantity and quality. *Stylosanthes scabra* (Vog.) accessions were evaluated for adaptability, agronomic performance, persistency and nutritive value in subtropical climate of Pretoria as potential forage source for ruminant animals.

Several experiments (including field trials, laboratory experiment and stall feeding trials) were conducted at Hatfield Experimental Farm, University of Pretoria, South Africa. In the field trial, 15 accessions of *Stylosanthes scabra* were evaluated for adaptability and agronomic performance. The persistence of *Stylosanthes scabra* accessions under rain-fed conditions over three years showed their adaptability to the study area. However, three of the accessions, namely 9281, 11595 and 11604, were consistently superior in terms of biomass yield over the three-year period (2012 to 2014). Thus, from the biomass production point of view, these three accessions are recommended for future use as legume forage. Promising accessions identified in terms of adaptability and productive parameters were further evaluated for nutritive value parameters by determining their chemical composition, phenolic compound concentration, *in vitro* organic matter digestibility and *in vitro* gas production

characteristics. Generally, the adaptable accessions have more than 17% DM crude protein (CP) content with relatively low neutral detergent fibre (NDF) ranging from 29.9 to 70.1% DM and very low total phenols ranging from 5.5 to 11.4 g kg^{-1} DM, total tannins ranging from 2.4 to 8.4 g kg^{-1} DM and total condensed tannins ranging from 0.3 to 3.4 g kg^{-1} DM. Thus, they were highly digestible with the range of 66 to 79%. This indicates that these accessions can be utilized as supplementary forage and nitrogen sources to complement poor-quality forage.

Five *Stylosanthes scabra*, selected from the promising accessions, were in a preference and palatability study using Saanen goats. Generally, the five accessions were acceptable and palatable to goats. However, there was a significant ($P < 0.05$) difference in terms of intake and preferences. Accession 11604 was the most preferred, while accession 11255 was the least preferred. Five accessions that were used in palatability study were further used to evaluate their effect to poor-quality grass hay when supplemented at two levels (15% and 30%). The response to supplementation was measured by monitoring *in vitro* ruminal fermentation, associative effects and degradability of NDF. Supplementing poor-quality grass hay with *Stylosanthes scabra* accessions improved grass hay fermentation. This study showed that 30% supplementation level with accession 11604 led to a positive associative effect with grass hay and also improved NDF degradability. Consequently, this accession was recommended for future use as a forage supplement to poor-quality basal diet.

The inclusion of *Stylosanthes scabra* forages in total mixed rations (TMR) for Saanen goats as a partial replacement of lucerne (alfalfa) forage did not significantly ($P > 0.05$) affect the nutritive value; nor did it affect the animals' performance in terms of intake, nutrient digestibility and nitrogen retention. This means that *Stylosanthes scabra* can be used to partly replace alfalfa without compromising the nutritive value of the TMR diet. The overall outcome is that accession 11604 is recommended for use as supplementary feed resource. However, further study is required to determine suitable means of integrating this accession in the farming system in order to converts this forage into high-quality animal products (meat, milk, wool, etc).

GENERAL INTRODUCTION

Background

About 80% of South African agricultural land is suitable for extensive livestock production (DoA 2003). However, it is not clear how much of this land is available to communal farmers in relation to the numbers of livestock they own. Communal farmers own approximately 65% of the livestock in South Africa (DoA 2003), but production is very poor. This could be the result of a number of factors, which include poor management, the prevalence of diseases, inadequate nutrition, inadequate supply and shortage of grazing land. Thus, the contribution of these livestock to the gross domestic product (GDP) is minimal.

Livestock farming in communal areas depends on natural vegetation as a main source of feed throughout the year. Therefore, fluctuations in both forage quantity and quality pose major challenges to livestock production (Scogings et al. 2004; Dziba et al. 2007). These variations in forage quantity and quality in turn lead to over grazing of communal areas owing to lack of appropriate grazing management and over utilization of natural vegetation (Fuhlendorf and Engle 2001; Masafu 2006; Sultan et al. 2008). Consequently, this amounts to further reduction of both forage yield and quality from natural pastures (Mapiye et al. 2008). In this regard browsers may augment forage by ingesting leaves and seedpods from plants (Or and Ward 2003). Local trees and shrubs have been used as forage for animal in areas such as Zululand, KwaZulu-Natal, South Africa (Kunene et al. 2003; Mpanza et al. 2009), Tanzania (Komwihangilo et al. 1995, 2001) and Cambodia (Keopaseuht et al. 2004). However, some browsed plant species contain anti-nutritional factors and/or plant secondary metabolites (Robins and Brooker 2005; Iason 2005; Nguyen et al. 2005). These anti-nutritional factors (ANF) include tannins, proteinase inhibitor, cyanogens, mimosine and indospicine (Hassen 2006). The presence of these compounds in plant material reduces foraging efficiency depending on the level (Makkar 2003b; Hassen et al. 2008). Anti-nutritional factors have been reported to influence browsing behaviour, species preference, and intake of forage materials by browsers (Ganqa et al. 2005; Nyamukanza et al. 2010; Mkhize et al. 2011).

The survey study conducted in Zululand, KwaZulu-Natal, South Africa, as with other sub-Saharan African farmers, revealed that livestock mortality is more frequent in communal areas, particularly during the dry season (Kunene et al. 2003; Mpanza 2007). This could be related to a number of factors such as parasites, poor management and inadequate nutrition (Sebsibe 2006; Vatta 2007). Feed shortages in particular have been reported to result in slow growth and low reproductive performance, and eventual production drops (Kanani et al. 2006; Ajayi et al. 2007).

Increasing aridity in South Africa, which is characterized by low rainfall, has a negative impact on vegetation composition and biomass production from natural pastures (O'Connor and Kikker 2004). Since the available feed is of poor-quality, livestock production under smallholder subsistence farmer conditions is often low, due to lack of affordable protein supplementation. Therefore, alternative fodder crops should be introduced that are drought tolerant with good biomass yield and acceptable nutritive value. Consequently, *Stylosanthes scabra* (Vog.) was identified for further evaluation as an alternative forage resource for ruminants. *Stylosanthes scabra* is an important pasture legume for seasonally dry and frost-prone tropical and subtropical environments (Hall et al. 1995). It is characterized as a shrubby, erect legume that produces moderately high biomass yield and is drought tolerant (Akinlade et al. 2008). Therefore, the agronomic and nutritive value evaluation of *Stylosanthes scabra* accessions were conducted in order to identify adaptable superior accessions that could be utilized during the dry season by small ruminants kept under smallholder farming conditions as potential forage and nitrogen supplements to improve poor-quality roughage utilization.

CHAPTER 1

Literature review

1.1. Livestock farming and its importance to smallholder farmers

Livestock farming is a common practice by pastoralists and smallholder subsistence farmers in communal areas in sub-Saharan Africa (SSA). However, the major constraint they face is low productivity. This could be influenced by factors such as high stocking rates over marginal grazing land, poor grazing management and poor feed quality. Often livestock farming in communal areas is practised on marginal and shrinking grazing land because of high population (human and livestock) in communal areas. Natural pastures constitute major feed resources for animals in communal areas, but are characterized by low crude protein (CP) content, which is often below 7%, with high fibre, particularly during the dry season. Forage with CP content below the threshold level (7%) is known to restrict rumen microbial activity, and consequently results in poor digestibility of the feed materials (Hariadi and Santoso 2010). According to Ogunbosoye and Babayemi (2010), feed scarcity and poor quality are major reasons for poor livestock performance under smallholder subsistence farmers in communal areas.

About half (50%) of the population of South Africa are considered poor (Terreblanche 2002) and of these 17.5 million people are living below the poverty threshold (Thornton et al. 2002). About 10.6 million of these people are smallholder subsistence farmers, who keep livestock and live in the communal areas (Thornton et al. 2002). Thus, livestock make a substantial contribution to their livelihoods by serving as sources of food and income (Mekoya 2008; Mabe et al. 2010; Castel et al. 2010). Smallholder subsistence farmers often keep more than one animal species, such as cattle, goats, sheep and chicken to fulfil a multipurpose role in the household (Perry et al. 2002; Homann et al. 2007). However, the importance of these animals varies from community to community.

Livestock farming systems in developing countries range from extensive pastoral systems, which are dominated by smallholder subsistence producers and semi-subsistence production, to large-scale commercially oriented industrial production systems (McDermott et al. 2010). Farming operations in South Africa are subdivided into two main categories, namely commercial and small-scale farming (Vatta 2007). For farmers with small land holdings, livestock also contribute large proportion of their income (Kunene et al. 2003; Mabe et al. 2010). This is because the sale of animals provides a proportion of annual cash income and capital assets of households (Miller and Photakoun 2008). According to Kunene et al. (2003)

and Machethe (2004), livestock contribute between 20 – 30% of the household income. Therefore, livestock is an important and integral component of farming systems, which contributes greatly to agricultural and rural development (Bembridge 1988).

Small ruminants (goats) are known to play a crucial socio-economic role in rural areas (Mamabolo and Webb 2005). In these areas, womans are among the most resource-poor smallholder subsistence farmers (Mamabolo and Webb 2005). They contribute substantially to the livelihoods of rural people especially those of poor and medium resource endowment (Rymer et al. 2002; Homann et al. 2007). Goats are regarded as good sources of meat, milk and milk products such as cheese and yoghurt (Anaeto et al. 2010). Therefore, goats in communal areas fulfil multiple roles as they meet farmers' needs (Haenlein and Ramirez 2007). Their adaptable character under harsh environment conditions makes them superior to other small ruminants. For example, they are found in drier areas of the Eastern Cape, South Africa, which is dominated by shrubs and bushes (Erasmus 2000), and are able to convert plant material into edible animal protein, which is useful to humans (Vatta 2007; Homann et al. 2007).

1.2. Challenges of livestock production in sub-Saharan Africa

According to Ben Salem and Smith (2008) and Ragvhuvansi et al. (2007), livestock production in arid and semi-arid regions, especially under smallholder subsistence communal systems is subjected to a number of challenges which includes fluctuation of both forage quantity and quality, poor management of grazing and prevalence of diseases. These challenges vary according to area, geographical location and country (Kosgey 2004). Of the many challenges so far documented, this study focuses on forage fluctuation. Therefore, it is important to give a brief overview of this factor since it has an impact on livestock production.

Livestock production, particularly ruminants, in the arid and semi-arid regions of sub-Saharan Africa depends solely on rangeland for forage production (Abusuwar and Ahmed 2010). Consequently, natural pastures play a significant role of providing important and cost-effective feed resources for livestock kept by smallholder subsistence farmers. Natural pasture tends to be high in energy and protein content in its early stages of growth (i.e. vegetative stage) particularly sweat veld type. However, as pasture plant matures palatability and digestibility decline, therefore poor management of grazing land in rural/communal area lead to miss utilization of these feed resources (Figure 1.1). Forage quality varies over the growing season and declines as the forage matures (Licitra et al. 1997). In the wet season, grasses grow faster, especially tropical grass species (C_4 grasses). Thus, they reach maturity more quickly. Consequently, during the dry season forage quality declines (Ajayi et al. 2007; Abusuwar and Ahmed 2010). This decline is the result of reduction of protein and soluble sugar content, while fibre content increases and negatively affects palatability, intake and digestibility (Gwaze et al. 2009; Moyo et al. 2012). Digestibility is positively correlated to crude protein (CP) content and negatively correlated to crude fibre (CF), acid detergent lignin (ADL), acid detergent fibre (ADF) and neutral detergent fibre (NDF) (Minson 1982; Jung et al. 1997). As a result, the shortage of good quality feed during the dry season hinders livestock production in tropical areas (Dziba et al. 2007; Ragvhuvansi et al. 2007; Ben Salem and Smith 2008), which result in weight loss and increasing mortality of animals (Van Rooyen and Homann-Kee Tui 2009).

The composition of pasture in the communal grazing areas is diverse, but inferior in terms of botanical composition and biomass production (Licitra et al. 1997). This could be influenced by poor management of grazing areas and poor knowledge of grazing capacity. The botanical composition of pastures influences the nutritive value of the feed by affecting the chemical composition, intake and digestibility. Hence, the nutritive value of pastures in communal areas is not guaranteed to sustain the nutritional requirement of animals. Because of over-dependence on low-quality forages and inadequate feed supply from natural pastures, livestock production is poor (Nyoka et al. 2004; Baloyi et al. 2006; Anele et al. 2011). Thus, seasonal fluctuations in forage yield and quality from natural pasture have been regarded as major constraints to livestock production in tropical and subtropical regions (Solorio Sánchez and Solorio Sánchez 2002; Tuwei et al. 2003; Van et al. 2005). In communal areas, grazing is the most common feeding system for livestock production by smallholder farmers (Homann et al. 2007). Therefore, an understanding of grazing capacity would help smallholder subsistence farmers to develop management plans for grazing areas, which would lead to sustainable use of natural pastures.

Figure 1.1: Communal grazing area located in sweat veld type in rural areas of Eastern Cape, South Africa (photo was taken by James Moorcroft 2015).

To understand the concept of forage fluctuation as one of the factors that affect livestock production under smallholder subsistence farmers in sub-Saharan Africa, it is important to elaborate the conditions in which livestock farming is taking place. The biggest challenge is the rapid increase in density of population and livestock, which leads to increasing demand for food and feed (Kabirizi et al. 2006; Macharia et al. 2011). This results in an increase in rural resettlement and cultivation (Nyoka et al. 2004), which subsequently decreases the per-capita land available for grazing (Ajayi et al. 2011). Thus, livestock farming in communal areas is practised in marginal or shrinking grazing land. This leads to overgrazing because of the high stocking rate. According to Moyo et al. (2012) in communal areas, grazing management (i.e. grazing type and stocking rate) is a major challenge. Communal rangelands in sub-Saharan Africa are supporting livestock at a level that is far above the recommended stocking rate. A high stocking rate favours the establishment of invasive plant species (bush encroachment), which could lead to a reduction in grazing capacity. A typical example is a communal grazing area that has been encroached by *Dichrostychys cinerea* (Figure 1.2). Continuous cultivation and overgrazing are common phenomena in communal areas in sub-Saharan Africa and lead to soil erosion and depletion of soil fertility (Sengul 2003; Ayisi and Mpangane 2004; Njarui et al. 2004). Low soil fertility, especially lack of nitrogen (N), and soil erosion result in low crop yield and poor-quality forage from natural pastures/rangelands (Kariuki et al. 1999; Njarui et al. 2004). Therefore, an alternative feed resource is needed to improve livestock production.

Figure 1.2: Communal grazing area encroached by *Dichrostachys cinerea* trees in Rust de Winter, North of Pretoria (photo was taken by Thami Mpanza 2012)

1.3. Alternative forage resources

1.3.1. Fodder trees, shrubs and herbaceous legumes

The scarcity of good-quality forage for animals in tropical and subtropical regions has resulted in non-conventional local trees and shrubs being considered as feed for animals, particularly during the dry season (Lascano et al. 2003; Hassen 2006; Kabi and Bareeba 2008). This is because they continue to produce green foliage, even during the dry season (Paterson et al. 1998). Thus, trees and shrubs have long been considered important fodder for animals, particularly when the quantity and quality of natural pastures have declined (Nguyen et al. 2005). Leguminous tree species such as *Leucaena leucocephala, Gliricidia sepium, Sesbania* to name just a few have been used as protein supplements and as chemotherapeutics in ruminant animals (Reyes Sánchez et al. 2006; Vatta 2007; Kabi and Bareeba 2008). When compared with forages from natural pastures, tree and shrub species contain enough minerals and nutrients to fulfil the nutritional requirements of ruminant animals (Ba et al. 2005; Gwaze et al. 2009; Fadiyimu et al. 2010). However, the establishment of trees is slow and may take more than one season before the forage is available for use. Table 1.1 shows the chemical composition of some commonly used trees, shrubs and herbaceous species that have been used as feed supplements. Generally, leguminous species are known to maintain moderate to high levels of protein in leaves, even during the dry season (Franzel et al. 2003) in contrast with natural pastures, which at this time are characterized by low protein content. On the other hand, shrub species grow relatively quickly compared with trees and thus fodders from these shrubs could alleviate the feed challenges that face smallholder farmers (Kamalak et al. 2004).

Table 1.1: Chemical composition of trees and shrubs commonly used as non-conventional feed for animals

Species	Type	DM	Composition (g kg^{-1} DM)						References
		(%)	ADF	NDF	CP	Ca	K	P	
Leuceana leucocephala	Tree	90.6	228.0	336.0	252.7	14.8	10.6	2.8	Aletor and Omodara 1994; Soltan et al. 2012
Gliricidia sepium	Tree	86.4	296.0	425.0	219.2	7.4	24.9	4.3	Aletor and Omodara 1994; Asaolu et al. 2011
Cajanus cajan	Tree	93.1	-	-	185.6	27.1	5.8	5.1	Aletor and Omodara 1994
Moringa oliefera	Tree	95.6	263.5	404.0	267.5	11.0	15.0	4.3	Asaolu et al. 2011
Leucaena diversifolia	Tree	-	318.8	346.1	162.6	9.0	5.0	2.0	Walker 2012
Vigna unguiculata	Herb	93.1	233.0	403.0	212.0	25.1	16.1	2.0	Anele et al. 2011
Zapoteca tetragona	Shrub	-	176.2	276.7	234.9	17.1	-	3.3	Bansi et al. 2014
Manihot esculenta	Shrub	88.8	446.0	573.0	193.0	-	-	-	Sath et al. 2012
Lablab purpureus	Herb	94.7	473.0	774.0	251.0	-	-	-	Geleti et al. 2013
Stylosanthes guianensis	Herb	95.9	403.0	715.0	237.0	-	-	-	Geleti et al. 2013
Desmodium intortum	Herb	95.0	406.0	649.0	229.0	-	-	-	Geleti et al. 2013
Centrosema pascuorum	Herb	87.4	363.7	493.7	236.8	-	-	-	Mokoboki et al. 2002
Macroptilium bracteatum	Herb	86.3	371.3	505.8	259.6	-	-	-	Mokoboki et al. 2002
Macroptilium gracile	Herb	88.2	374.6	445.1	249.8	-	-	-	Mokoboki et al. 2002
Vigna oblongifolia	Herb	88.6	335.0	418.3	241.5	-	-	-	Mokoboki et al. 2002
Butryospermum paradoxum	Tree	95.8	321.0	476.0	146.3	12	-	1.1	Njidda 2010
Leptadenia lancifolia	Herb	95.8	317.0	412.0	166.5	11	-	3.1	Njidda 2010

1.3.2. *Stylosanthes* as source of forage

1.3.1.1. Brief history of *Stylosanthes*

Stylosanthes is a genus that belongs to the sub-tribe *Stylosanthinae*, tribe *Aeschynomenae*, sub-family *Papilioniodae* and family *Leguminosae*. The genus consists of about 40 species, most of which are native to South America, and a few (*Stylosanthes erecta*, *Stylosanthes fruticosa* and *Stylosanthes suborbiculata*) are native to Africa (Schultze-Kraft and Keller-Grain 1992). The genus is classified into two groups, *Stylosanthes* and *Styposanthes*, *Stylosanthes* is a diploid species (2n = 2x = 20), whereas *Styposanthes* is a polyploidy; hence the genus has both diploid (2n = 2x = 20) and polyploid (2n = 40, 60) species (Maass and Sawkins 2004; Chandra et al. 2011). *Stylosanthes* species are versatile and productive tropical pasture legumes that are used in a range of agricultural systems under various ecological zones. A number of studies have been conducted on *Stylosanthes*, and that has led to the development of a number of cultivars that have been released for commercial purposes. *Stylosanthes* is known to grow in poor-quality soil, which is characterized by low nitrogen and phosphorus contents and also acidic. Hence, the genus is adapted to tropical and subtropical conditions (Little and Agyemang 1992). *Stylosanthes* is known to be tolerant of drought and can produce relatively high biomass yield under different ecological zones (Chandra 2009). The genus in Asian countries is used as cut-and-carry fodder, a cover crop to provide forage, green manure to stabilise soil erosion and a cash crop (Chakraborty 2004). However, in Africa, particularly West Africa, it is used as cut-and-carry, a protein bank, a fallow crop and hay (Mohamed-Saleem 1992). The genus produces forage with high protein content, but very low phosphorus concentration (Little and Agyemang 1992).

1.3.1.2. Introduction of *Stylosanthes* in Africa

Stylosanthes is regarded as the most economic and significant pasture and forage legume in the tropical regions (Cameron and Chakraborty 2004). Consequently, as an attempt to improve livestock nutrition, it was introduced in West Africa in the 1960s after several new improved cultivars were developed in Australia (Hanson and Heering 1992). Three species of *Stylosanthes*, namely *Stylosanthes hamata*, *Stylosanthes guianensis* and *Stylosanthes humilis*, were used to select material with improved feed quality during the dry season (Agishi and de Leeuw 1986). *Stylosanthes* has fitted successfully to the dry land of African agriculture, particularly because of its drought tolerant characteristics (Chandra 2009). Based on the merits of *Stylosanthes* genus in improving feed quality and soil fertility, an evaluation of some major species was conducted by the International Livestock Centre for Africa (ILCA), now International Livestock Research Institute (ILRI), with the aim of determining their adaptability, agronomic potential and nutritional quality in a wide range of environmental conditions and agro-ecological zones in West Africa (Table 1.2) (Hanson and Heering 1992). On the other hand, Table 1.3 shows the number of accessions per species that were found to be adaptable in different agro-ecological zones in West Africa, and thus were harvested and stored at ILRI and Centro Internacional de Agricultura Tropical (CIAT) for further evaluation. The environmental adaptation of these species was used to determine the area in which they could be used (Hanson and Heering 1992).

Table 1.2: Number of accessions of major species evaluated in ILRI (then ILCA) zonal research programmes in West Africa

Stylosanthes species	Agro-ecological zone				
	Semi-arid (Niger)	Semi-arid (Mali)	Sub-humid	Humid	Total
S. capitata	0	6	10	0	16
S. fruticosa	11	0	18	2	31
S. guianensis	6	12	145	6	169
S. hamata	3	8	447	2	460
S. humilis	0	5	9	0	14
S. macrocephala	0	1	3	0	4
S. scabra	0	5	18	8	31
S. viscosa	0	0	0	2	2

Source: Hanson and Heering (1992)

Table 1.3: Number of accessions of major species of *Stylosanthes* collected in agro-ecological zones and stored in ILRI and CIAT gene banks

	Agro-ecological zone					
Stylosanthes species	Arid	Semi-arid	Sub-humid	Humid	Unknown	Total
S. capitata	1	32	86	70	119	308
*S. fruticosa**	2	55	78	2	84	221
S. guianensis	0	46	380	675	444	1545
S. hamata	68	127	76	25	125	421
S. humilis	3	22	64	66	72	227
S. scabra	12	154	257	223	135	781
*S. viscose**	4	48	125	60	38	275

* These species originate in Africa

Source: Hanson and Heering (1992)

1.3.1.3. Performance of *Stylosanthes* species in Africa

The growing interest in crop-livestock production in sub-Saharan Africa has led to the use of a leguminous trees, shrubs and herbaceous species. Hence, mixed crop-livestock farming systems in the tropics and subtropics have become a common practice due to a number of factors including climate change, increasing population density, shifting demographics, and changes in household income (Pengelly et al. 2004). Thus, *Stylosanthes* legume has been a forage of interest in Africa for pasture improvement, particularly West Africa. This is based on the merits of the genus, which include high yield of protein per hectare (Little and Agyemang 1992). This genus has the capacity to produce herbage with protein content that is adequate for animal production although it is low in phosphorus (Little and Agyemang 1992). According to Mohamed-Saleem and de Leeuw (1992), the genus *Stylosanthes* has provided ample germplasm for a wide variety of agro-ecological situations in the tropics. Hence, studies have been conducted in various parts of African countries, particularly in West Africa, to test its adaptability and agronomic performance under various ecological zones. The biomass yield performance of *Stylosanthes* species that have been evaluated in African countries is shown in Table 1.4.

Table 1.4: Biomass production of *Stylosanthes* species evaluated in various ecological zones in Africa

Stylosanthes species	Cultivar	DM[1] yield (t ha^{-1})	Country	Study sites	EZ[2]	References
Stylosanthes hamata	Verano stylo	5.3	Nigeria	Kurmin biri	Sub-humid	ILCA[3] 1988
Stylosanthes guianensis	Cook	2.5	Ethiopia	Soddo	Sub-humid	ILCA 1988
Stylosanthes guianensis	Graham	2.0	Ethiopia	Soddo	Sub-humid	ILCA 1988
Stylosanthes guianensis	Endeavour	2.5	Ethiopia	Soddo	Sub-humid	ILCA 1988
Stylosanthes scabra	Seca	4.5	Ethiopia	Soddo	Sub-humid	ILCA 1988
Stylosanthes scabra	Fitzroy	4.7	Ethiopia	Soddo	Sub-humid	ILCA 1988
Stylosanthes fruticosa	-	0.4	Zimbabwe	Makoholi	Semi-arid	Clatworthy 1986
Stylosanthes guianensis	Schofielf	0.4	Zimbabwe	Grasslands	Semi-arid	Clatworthy 1986
Stylosanthes guianensis	Schofielf	1.5	Zimbabwe	Henderson	Semi-arid	Clatworthy 1986
Stylosanthes guianensis	Oxley	1.5	Zimbabwe	Grasslands	Semi-arid	Clatworthy 1986
Stylosanthes guianensis	Oxley	0.5	Zimbabwe	Makoholi	Semi-arid	Clatworthy 1986
Stylosanthes humilis	-	0.5	Zimbabwe	Makoholi	Semi-arid	Clatworthy 1986
Stylosanthes guianensis	Schofielf	6.1	Nigeria	Shika	Sub-humid	Agishi and de Leeuw 1986
Stylosanthes humilis	Townsville	4.7	Nigeria	Shika	Sub-humid	Agishi and de Leeuw 1986

[1] Dry matter
[2] Ecological zones
[3] International Livestock Centre for Africa

Table 1.4 continued

Stylosanthes hamata	Accessions*	3.3	Ethiopia	Soddo	Sub-humid	Larbi et al. 1992
Stylosanthes scabra	Accessions*	1.7	Ethiopia	Soddo	Sub-humid	Larbi et al. 1992
Stylosanthes capitata	-	5.7	Nigeria	Ile-Ife	Humid	Akinola 1991
Stylosanthes guianensis	Cook	9.7	Nigeria	Shika	Sub-humid	Akinola 1991
Stylosanthes guianensis	Endeavour	5.3	Nigeria	Shika	Sub-humid	Akinola 1991
Stylosanthes guianensis	Oxley	3.3	Nigeria	Shika	Sub-humid	Akinola 1991
Stylosanthes guianensis	Schofield	5.3	Nigeria	Shika	Sub-humid	Akinola 1991
Stylosanthes guianensis	Cook	6.4	Nigeria	Ile-Ife	Humid	Akinola 1991
Stylosanthes guianensis	Endeavour	5.2	Nigeria	Ile-Ife	Humid	Akinola 1991
Stylosanthes hamata	Verano stylo	6.0	Nigeria	Shika	Sub-humid	Akinola 1991
Stylosanthes hamata	Verano stylo	7.9	Nigeria	Ile-Ife	humid	Akinola 1991
Stylosanthes humilis	Gordon	3.4	Nigeria	Shika	Sub-humid	Akinola 1991
Stylosanthes humilis	Paterson	2.3	Nigeria	Shika	Sub-humid	Akinola 1991
Stylosanthes scabra	Fitzroy	9.6	Nigeria	Ile-Ife	Humid	Akinola 1991
Stylosanthes scabra	Seca	7.2	Nigeria	Ile-Ife	Humid	Akinola 1991
Stylosanthes guianensis	Accession*	3.8	Cameroon	Wakwa	Humid	Yonkeu et al. 1992
Stylosanthes capitata	46009a[#]	1.5	Cameroon	Wakwa	Humid	Yonkeu et al. 1992
Stylosanthes capitata	460096[#]	1.7	Cameroon	Wakwa	Humid	Yonkeu et al. 1992
Stylosanthes fruticasa	-	2.6	Cameroon	Wakwa	Humid	Yonkeu et al. 1992

* Different lines of the same species; [#] Centro Internacional de Agricultura Tropical accession number

The International Livestock Centre for Africa (ILCA) conducted studies to identify cultivars/accessions that can be adaptable to and productive in West and East Africa. Thus, the centre conducted studies in Ethiopia (East Africa) (Larbi et al. 1992) and Nigeria (West Africa) (Tarawali et al. 1992) in the late 1980s to early 1990's. The study in Ethiopia tested the agronomic performance of 18 accessions of the species *Stylosanthes guianensis* in terms of biomass production and protein yield. The commercial cultivar Cook was used for comparisons (Larbi et al. 1992). Findings of this study showed that half of the tested accessions were adaptable to and productive in an acidic soil at Soddo research site in Ethiopia, and the forage was of good quality with a protein concentration ranging from 98 to 114 g kg^{-1} (Table 1.5).

Table 1.5: Dry matter yield and crude protein concentration of *Stylosanthes guianensis* accessions on an acidic soil at Soddo, Ethiopia

Stylosanthes guianensis accessions (ILCA[4] no)	Country of origin	Dry matter yield (t ha^{-1} DM)	Crude protein (g kg^{-1} DM)
11737	Brazil	8.25	108
11776	Brazil	8.03	108
6995	Zimbabwe	6.96	111
11765	Brazil	6.64	99
11876	Colombia	6.43	102
11840	Venezuela	6.33	98
11878	Brazil	5.92	103
11732	Colombia	5.88	109
11733	Colombia	5.72	114

Source: Larbi et al. (1992)

[4] International Livestock Centre for Africa

In West Africa, ILCA conducted a study using a stepwise sequence of evaluation stages to identify the best performing species in the sub-humid zone of Nigeria. This type of study showed a full evaluation of the species, which had undergone various stages of testing, and the best performing species were selected for the next stage (Tarawali et al. 1992). These stages included the preliminary stage (focus on general growth parameters); agronomic stage (focus on biomass yield and protein content); multi- locational testing (focus on biomass yield from different sites); sward stage (focus on dry matter yield, nutritive value, seed production, drought tolerance, disease incidence and regeneration); grazing stage (focus on animal response after supplemented with fodder banks); and multiplication stage (seed production potential). Agronomic evaluation of *Stylosanthes hamata* accessions conducted by ILCA over three years in the sub-humid zone of Nigeria showed that biomass increased in the second year and dropped in the third year, with mean CP ranging from 109 g kg^{-1} DM to 141 g kg^{-1} DM (Table 1.6). Table 1.7 shows the performance of *Stylosanthes hamata* and *Stylosanthes scabra* in terms of biomass yield when they were evaluated on different study sites.

Table 1.6: Dry matter yield and crude protein concentration of *Stylosanthes hamata* accessions evaluated in sub-humid zone of Nigeria

Stylosanthes hamata accessions (ILCA[5] no.)	Dry matter (kg ha^{-1})			Crude protein mean (g kg^{-1} DM)
	1989	1990	1991	
15868	3.0	5.1	5.0	118
15876	3.1	4.8	4.3	112
15861	3.0	3.2	3.5	113
15959	1.8	3.5	2.9	136
15908	1.3	3.5	3.2	126
15895	1.9	3.3	1.9	141
15924	1.4	3.2	2.4	109
15932	1.5	3.4	2.0	108
15892	1.8	2.9	2.0	133
15936	1.2	2.8	1.6	122
15938	0.8	1.7	1.0	112
15926	1.0	1.6	1.1	104
Verano	1.2	1.3	0.7	123
15925	0.6	0.6	1.4	108

Source: Tarawali et al. (1992)

[5] International Livestock Centre for Africa

Table 1.7: Dry matter yield (kg ha^{-1}) of accessions tested in various sites in Nigeria

Study site	*Stylosanthes hamata* cv Verano				*Stylosanthes scabra* ILCA[6] 441			
	1988	1989	1990	1991	1988	1989	1990	1991
Makurdi	4345	8071	5739	3858	9327	21825	5337	2215
Jos	927	1425	3057	131	2594	11855	907	1258
Bauchi	5102	2447	1890	747	4441	2140	36	0
Rano	1050	1652	2636	697	n/p[1]	n/p	n/p	n/p
Maiduguri	910	5927	2220	1577	n/p	n/p	n/p	n/p
Katsina	98	1507	n/a[2]	n/a	431	3304	n/a	n/a

[1] Not planted; [2] not available

Source: Tarawali et al. (1992)

1.3.1.4. *Stylosanthes* in livestock production

The genus *Stylosanthes* is the most economical and significant pasture and forage legume in tropical regions of the globe. Consequently it has been utilized in various feeding systems, ranging from freshly cut fodder to dry leaf meal supplements (Cameron and Chakraborty 2004). The species has thus contributed significantly to animal nutrition in the tropics and subtropics. In developing countries, the species has been used by resource-poor farmers as animal feed (Phaikaew et al. 2004). Improved animal production from *Stylosanthes* pasture technology has increased the financial viability of cattle grazing in the tropics, and provided greater management options to meet consumer demands for higher-quality products (Hall and Glatzle 2004). This species was introduced to complement the poor-quality native grass pastures that dominate tropical and subtropical regions of the world. Because of its merits, it is used to feed livestock, including cattle, sheep, pigs, goats and poultry (Hall and Glatzle 2004). A weight gain of 760 g per head per day was recorded for cattle grazing mixed pastures of Seca and Verano with native *Sorghum plumosun, Chrysopogon fallax* and *Aristida* species, compared with 430 g per head per day on native grass pasture in the dry season (Hall and Glatzle 2004). According to a study conducted in the sub-humid zone of Nigeria, goats on stylo-based pasture gained 1 kg more weight than those on fallows under the same stocking rate of four does per hectare (Ikwuegbu et al. 1992). Horro bulls that grazed *Stylosanthes guianensis* over sown natural pasture had high weight gain compared

[6] International Livestock Centre for Africa

with Horro bulls that were supplemented with *Leucaena leucacephala* hay (Lemma and Hassen 1995).

1.4. Integration of forage legumes as an intercrop in smallholder farming conditions

Intercropping is a practice in which two or more crops are grown together on the same space, planted at the same time or different times, but in the same growing season. This practice has been traditional for centuries all over the world, particularly for resource-poor smallholder subsistence farmers as a means of reducing risk and producing more crops, which enhances food production (Ajayi et al. 2011; Lithourgidis et al. 2011; Eskandari 2011). Consequently, this practice resulted in the intensification of production with effective use of natural resources that could lead to higher biomass yield, better quality forage and improved animal production.

1.4.1. Biomass yield of grass-legume intercrop

Natural pasture is the main feed resource in communal areas and thus contributes substantially to livestock production, whereas insufficient forage production and poor-quality forage during the dry season are the major constraints (Berhan 2006). Grass-legume intercropping increased the quantity and quality of forage (Baba et al. 2011; Mahapatra 2011; Idris et al. 2012). The effects of intercropping legumes with grass species on biomass yield production in various ecological zones are summarized in Table 1.8. In the humid areas, intercropping grass with legume increased dry matter yield between 22% and 54% more than their monoculture counter parts (Ezenwa and Aken'ova 1998). However, other studies such as Mwangi and Wambugu (2003) and Olanite et al. (2004) did not show significant differences in dry matter yield between monoculture and grass species intercropped with forage legume. This conflicting result could be explained by a number of factors, which include variation in ecological zone, compatibility of mixtures, proportions of grass and legumes in the mixture, and the period in which the mixture was evaluated. According to Mwangi and Wambugu (2003), better intercropped dry matter yield is influenced by effective utilization of natural resources.

Table 1.8: Effects of intercropping legumes with grass species on dry matter yield (t ha^{-1}) in various ecological zones in Africa

Grass /Legume intercrops	Grass alone	Intercrops	LER[7]	EZ[8]	Increase (%)	References
Panicum maximum + *Stylosanther guianensis*	1.57	2.35	-	Humid	49.08	Ajayi et al. 2007
Panicum maximum + *Aeschynomene histrix*	1.57	2.22	-	Humid	40.81	Ajayi et al. 2007
Panicum coloratum + *Stylosanthes guianensis*	7.42	8.34	1.24	Highland	1.34	Berham 2005
Chloris gayana + *Trifolium prantese*	5.86	6.19	1.24	Highland	3.51	Berham 2006
Panicum maximum + *Capsicum pubescenis*	12.91	14.52	1.40	Humid	7.56	Baba et al. 2011
Cinchrus ciliaris + *Stylosanthes humilis*	3.78	7.61	-	Savanna	153.67	Shehu and Akinola 1995
Cinchrus ciliaris + *Stylosanthes hamata*	4.09	8.18	-	Savanna	90.68	Shehu and Akinola 1995
Panicum maximum + *Stylosanthes humilis*	3.72	6.66	-	Savanna	71.65	Shehu and Akinola 1995
Panicum maximum + *Stylosanthes hamata*	4.24	8.79	-	Savanna	135.66	Shehu and Akinola 1995
Bromus inermis + *Medicago sativa*	4.30	14.49	1.28	Semi-arid	236.98	Gökkus et al. 1999
Bromus inermis + *Trifolium pratense*	4.47	13.28	1.16	Semi-arid	197.09	Gökkus et al. 1999
Chrysopogon fulvus + *Stylosanthes hamata*	5.24	6.73	-	Humid	28.44	Ram and Parihar 2008
Ppennisetum. purpureum + *Desmodium intortum*	10.75	11.65	-	Sub-humid	8.37	Kabirizi et al. 2006
Pennisetum purpureum + *Macraptilium atropurpureum*	10.75	11.18	-	Sub-humid	4.30	Kabirizi et al. 2006
Pennisetum purpureum + *Capsicum pubescenis*	10.75	10.89	-	Sub-humid	1.26	Kabirizi et al. 2006
Panicum maximum + *Cajalus canjan*	9.8	19.00	-	Savannah	93.88	Alalade et al., 2014
Panicum maximum + *Stylosanthes hamata*	9.8	37.00	-	Savannah	227.55	Alalade et al., 2014

[7] Land equivalent ratio
[8] Ecological zones

1.4.2. Forage quality of grass-legume intercrop

Shortage of soil nutrients in tropical and subtropical regions of Africa is high. In particular, soil nitrogen and phosphorus are the most common limiting nutrients (Ayisi and Mpangane 2004; Mpangane et al. 2004; Sierra and Nygren 2006). Consequently, biomass production under such soil conditions is poor, and is characterized by low protein content, which is often below the threshold level of 7% which is required for normal intake and rumen functioning (Ikhimioya 2008). Thus, leguminous plants improve soil fertility by symbiotic nitrogen fixation and subsequent excretion of nitrogen by nodules for immediate use by companion crops before the release of nitrogen from decaying leaf residues and roots (Scherer-Lorenzen et al. 2003; Lithourgidis et al. 2011). Therefore, the advantage of intercropping legumes with tropical grass species is that legumes ameliorate soil nitrogen content, which becomes available to companion grasses. Grass-legume mixtures are a means of improving productivity without additional investment (Baba et al. 2011). Thus, intercropping grass with legume species boosts the biomass yield of forage and improves the quality of grass forage, as shown in Table 1.9.

Table 1.9: Effects of intercropping legumes with grass species on forage quality in various ecological zones of Africa

Types	EZ[9]	Composition (g kg^{-1} DM)						References
		CP	NDF	ADF	Ash	Ca	P	
Chrysopogon fulvus (monocrop)	Humid	56.0	831.5	557.1	-	-	-	Ram and Parihar 2008
Chrysopogon fulvus + *Stylosanthes hamata*	Humid	63.9	781.9	543.2	-	-	-	Ram and Parihar 2008
Pennisetum purpureum (monocrop)	Sub-humid	74.0	657.0	-	-	2.3	2.2	Kabirizi et al. 2006
Pennisetum purpureum + legume	Sub-humid	84.0	631.0	-	-	3.3	2.5	Kabirizi et al. 2006
Pennisetum purpureum (monocrop)	Semi-arid	81.4	647.8	451.9	159.7	4.5	2.9	Njoka-Njiru et al. 2006
Pennisetum purpureum + *Stylosanthes scabra*	Semi-arid	99.6	632.3	435.7	149.7	4.0	2.9	Njoka-Njiru et al. 2006
Pennisetum purpureum + *Macroptilium atropurpureum*	Semi-arid	96.4	628.1	439.1	148.4	4.1	3.1	Njoka-Njiru et al. 2006
Panicum maximum (monocrop)	Humid	76.3	-	-	184.0	1.5	1.1	Ajayi et al. 2007
Panicum maximum + Verano *stylo*	Humid	88.5	-	-	218.0	2.5	7.6	Ajayi et al. 2007
Panicum maximum + *Aeschynomene histrix*	Humid	85.0	-	-	182.0	2.5	4.0	Ajayi et al. 2007
Panicum maximum (monocrop)	Humid	68.7	647.8	499.7	-	-	-	Ojo et al. 2013
Panicum maximum + *Lablab Purpureus*	Humid	93.5	628.1	460.3	-	-	-	Ojo et al. 2013
Panicum maximum (monocrop)	Savannah	71.1	739.8	528.1	-	1.4	2.1	Alalade et al. 2014
Panicum maximum + *Cajanus canjan*	Savannah	82.2	713.2	515.2	-	2.1	2.4	Alalade et al. 2014
Panicum maximum + *Canavalia ensiformis*	Savannah	92.7	699.6	500.0	-	2.1	2.5	Alalade et al. 2014
Panicum maximum + *Stylosanthes hamata*	Savannah	108.9	685.4	499.7	-	2.7	3.1	Alalade et al. 2014

CP: crude protein; NDF: neutral detergent fibre; ADF: Acid detergent fibre; Ca: calcium; P: phosphorus.

[9] Ecological zone

1.4.3. Livestock production from grass-legume intercrops

Besides the challenge of feed scarcity (discussed earlier), sometimes the available feed is of low-quality, and is inadequate to supply what the animal requires. Inadequate quantity and quality of feed supply to livestock lead to low production (Anele et al. 2011). Livestock in developing countries are dependent on poor-quality feed that is often low in digestible nutrients (Baloyi et al. 2006). This is noticeable during the dry season, when available feed resources from natural pastures and crop residues constitute over 90% of animal feed. At this time of the year natural pasture is at its maturity, hence is characterized by low protein, and low digestible nutrients (Ondiek et al. 1999) with high lignocellulosic compounds that are poorly digested. Such feed limit intake and results in lower animal performance (Undi et al. 2001; Seresinhe and Pathirana 2008).

However, owing to climatic variations and poor grazing management, natural pastures are changing in terms of quantity and quality of forage. Climatic variations affect forage quality negatively owing to alterations in botanical composition, biomass production and nutritive value which are considered critically important in animal production. Hence, shortages of forage in quantity and quality are common phenomena in communal areas, particularly during the dry season (Phiri et al. 2007; Sath et al. 2012). Thus, livestock production under such conditions is subject to low performance. Poor condition of livestock in the tropics is explained by inefficient digestion and utilization of nutrients absorbed from low-quality feed (Fadiyimu et al. 2010). According to Baloyi et al. (2006), feeding low-quality feed to animals' leads to sub-optimal productivity in terms of milk, meat and other products.

Natural pastures must be supplemented to improve livestock production. More importantly, supplementation with nitrogen and energy sources is essential to achieve a reasonable level of animal production during the dry season (Ondiek et al. 1999). However, supplementation with concentrates is not an option because of high costs. Hence, concentrates are scarcely utilized in developing countries by smallholder subsistence farmers (Olafadeham et al. 2014). On the other hand, the use of trees, shrubs and herbaceous forage legumes as supplementary feed for livestock is well documented (Granum et al. 2007; Fadiyimu et al. 2010; Thang et al. 2010). Forage legumes from trees and shrubs have great potential to serve as feed resources for ruminants managed by smallholder livestock farmers (Idowu et al. 2013), because they have a high level of protein content with reasonable levels of minerals and vitamins (Idowu et al. 2013).

Supplementation of low-quality feed with forage legumes increased voluntary feed intake (Pen et al. 2013) and improved animal performance in terms of daily weight gain (Akinlade et al. 2002; Abdulrazak et al. 2006; Ondiek et al. 2013) and milk production (Baloyi et al. 2006; Mupenzi et al. 2009). Bamikole et al. (2001) reported 41% increase in daily weight gain of goats fed intercrop of guinea grass and *Stylosanthes hamata* as compared with goats fed guinea grass alone. Bastida Garcia et al. (2011) reported a percentage increase of 72% in nitrogen retention of sheep fed 25% *Pisum sativum* L. hay as supplement. Baloyi et al. (2006) reported a 14% and 8% increase in milk production from Saanen goat supplemented with cowpea and *Stylosanthes scabra* forages, respectively. The contribution of forage legumes as supplements to animals that are fed on poor-quality feed are illustrated in Table 1.10.

Table 1.10: Effects of forage legume supplementation on intake, digestibility, daily weight gain and percentage increase

Ingredient	Animal species	Intake (g day^{-1})	Increase (%)	DMD[10] (%)	Increase (%)	ADG[11] (g day^{-1})	Increase (%)	References
Rhodes grass	Goats	474	-	49.8	-	26	-	Ondiek et al. 1999
Maize brand	Goats	545	15.0	60.8	-	27	-	Ondiek et al. 1999
Rye grass + *Glicidia sepium*	Goats	604	27.4	58.1	16.7	43	65.4	Ondiek et al. 1999
Maize brand + *Gliricidia sepium*	Goats	565	19.2	58.4	-3.9	69	155.6	Ondiek et al. 1999
Urea treated maize stover	Goats	486	-	51.4	-	-	-	Aregheore and Perera 2004
Maize stove + *Erythrina variegata*	Goats	728	49.8	67.2	30.7	-	-	Aregheore and Perera 2004
Maize stover + *Gliricidia sepeum*	Goats	714	46.9	63.7	23.9	-	-	Aregheore and Perera 2004
Maize stover + *Leucaena leucocephala*	Goats	746	53.5	66.0	28.4	-	-	Aregheore and Perera 2004
Rice straw + Para grass	Cattle	3466	-	63.9	-	155	-	Sath et al. 2012
Rice straw + Para grass + 15% Cassava	Cattle	3727	7.5	60.5	-5.3	251	61.9	Sath et al. 2012
Rice straw + Para grass + 30% Cassava	Cattle	4125	19.0	60.4	-5.5	469	202.6	Sath et al. 2012
Rice straw + Para grass + 40% Cassava	Cattle	4225	21.9	58.5	-8.5	492	217.4	Sath et al. 2012
Elephant grass	Sheep	682.7	-	56.6	-	-	-	Bansi et al. 2014
Elephant grass + *Zapoteca tetragona*	Sheep	795.2	16.5	62.2	9.9	-	-	Bansi et al. 2014
Maize stover	Sheep	48.5	-	50.0	-	-	-	Undi et al. 2001
Maize stover + *Stylosanthes guainensis*	Sheep	78.4	61.6	53.2	6.4	-	-	Undi et al. 2001
Maize stover + *Macroptilium atropurpureum*	Sheep	75.9	56.5	51.1	2.2	-	-	Undi et al. 2001
Maize stover + *Capsicum pubescens*	Sheep	92.9	91.5	53.4	6.8	-	-	Undi et al. 2001

[10] Dry matter digestibility
[11] Average daily gain

1.5. Problem statement

The performance of *Stylosanthes* in terms of biomass production and persistence in East and West Africa is well documented. However, little appears to have been done in the subtropical climate of South Africa. Although a preliminary study was conducted by Rootman et al. (2004) in Limpopo on species such as *Stylosanthes scabra* in the semi-arid tropical climate of the province, there is still a dearth of information about the performance of this species in subtropical climatic conditions which are often characterized by frost in winter. In the previous study (Rootman et al. 2004) *Stylosanthes scabra* was compared with other species to determine most adaptable. In the present study various accessions of *Stylosanthes scabra* were selected, based on passport data (agroecological similarity of original site of collection with subtropical climate in South Africa) and earlier study site in similar agroclimatic zones. The selected accessions were further evaluated for adaptability and performance in the subtropical climate of Pretoria, northern Gauteng, South Africa.

1.6. Objectives

1.6.1. General objective

The general objective of this study was to identify drought and frost tolerant alternative forage legumes that can be used as a source of roughage and protein supplement to improve poor-quality feed utilization by small ruminants, using goats as the model animal.

1.6.2. Specific objectives

The specific objectives of the research were:

- To evaluate the adaptability, growth performance, biomass yield and nutritive value of *Stylosanthes scabra* accessions under subtropical conditions of Pretoria, Gauteng
- To study the palatability of the promising *Stylosanthes scabra* accessions to Saanen goats
- To determine the effect of supplementing poor-quality grass hay with *Stylosanthes scabra* accessions on *in vitro* ruminal fermentation and digestibility of forage based diets
- To determine the effects of different levels of *Stylosanthes scabra* forage in total mix ration as partial replacement of lucerne on dry matter intake, digestibility and nitrogen retention of Saanen goats

1.7. Hypotheses

In this study, the following hypotheses were formulated and various field and laboratory experiments were undertaken to test these hypotheses.

- Ho: There is no variation among the accessions of *Stylosanthes scabra* in terms of their adaptability, growth performance and nutritive value under the climatic and edaphic conditions of Pretoria.
- Ho: Accessions of *Stylosanthes scabra* do not vary in terms of their palatability in Saanen goats.
- Ho: Supplementing poor-quality grass hay with selected *Stylosanthes scabra* accessions does not affect *in vitro* ruminal fermentation and digestibility of forage based diets.
- Ho: Replacing lucerne (alfalfa) hay with *Stylosanthes scabra* forage in a TMR diet of goats does not affect intake, digestibility and nitrogen balance.

CHAPTER 2

Adaptability and agronomic performance of *Stylosanthes scabra* accessions grown in a subtropical region of South Africa

Abstract

Poor production of livestock by smallholder subsistence farmers is associated mainly with inadequate supply and poor-quality of forage. Thus, to augment livestock production, forage quantity and quality must be improved. This study evaluated the adaptability and agronomic performance of *Stylosanthes scabra* accessions in the subtropical climate of Pretoria. The experiment was conducted at Hatfield Experimental Farm, University of Pretoria. Fifteen accessions of *Stylosanthes scabra* were grown in 2 x 3 m plots as treatments in a randomized complete block design with three replicates. These accessions were evaluated for adaptability traits by scoring pest and disease incidences, as well as frost tolerance in winter. In addition, the accessions were evaluated in terms of plant height, canopy spread, tillering capacity, plant survival, and dry matter yield. The persistence of most of *Stylosanthes scabra* accessions over three years showed their adaptability to the study area under rain-fed conditions. In general, biomass yield in the second year (5.3 t ha^{-1} DM) was higher than in the establishment year (4.9 t ha^{-1} DM), whereas in the third year the biomass yield declined to 4.3 t ha^{-1} DM. In addition the crude protein contents of these accessions were bove 18% DM. Although most accessions were found to be adaptable, these three accessions (9281, 11595 and 11604) tended to increase biomass production over the three years period, and thus are recommended for use as alternative forage legume. However, further studies are required to evaluate these accessions in terms of their seed production potential and to determine how best these forages could be integrated into the feeding systems of target farmers to improve small ruminant production.

Keywords: Crude protein yield, Fodder crops, Legumes, Livestock productivity

2.1. Introduction

In tropical and subtropical regions of sub-Saharan Africa (SSA), livestock contribute substantially to the livelihoods of resource-poor smallholder subsistence farmers. However, these livestock depend predominantly on natural pastures and crop residues that are of poor-quality (Akinola et al. 2010; Rebero and Mupenzi 2012). Inadequate supply and poor-quality of the forage are major constraints for livestock production (Ogunbosoye and Babayemi 2010; Anele et al. 2011), as the nutrients are not sufficient to satisfy the needs of animals (Mendieta-Ariaca et al. 2009). Hence, animals often resort to browse trees and shrubs to compensate nutrient deficiency (Mpanza et al. 2009).

Therefore, to improve livestock production in tropical and subtropical regions, particularly those kept by smallholder subsistence farmers, sustainable solutions must be found for seasonal deficiencies of quality feed. To achieve this, it is necessary to systematically search and evaluate alternative fodder crops to identify superior accessions that survive the climatic and the edaphic conditions of target areas and produce moderately high forage of acceptable quality. Consequently, drought and frost-tolerant forage legume *Stylosanthes scabra* (Vog.) accessions were evaluated as a potential alternative forage source for small ruminants in the subtropical area of Pretoria, Gauteng.

Stylosanthes scabra (Vog.) is a perennial shrubby legume, which originates from tropical areas of South America, mainly Brazil, Colombia and Venezuela (Schultze-Kraft et al. 1984). This legume is adaptable to tropical and subtropical areas of poor-quality (infertile) and acidic soil. It can grow in semi-arid areas with annual precipitation of 325 mm. Thus, it is regarded as one of the most important species for drier regions (Pathak et al. 2004). The species grows up to 1.2 m and produces moderately high biomass yield, ranging from 1 to 10 t ha^{-1} DM as sole pasture, while when intercropped with grasses it produced from 2 to 7 t ha^{-1} DM (Akinlade et al. 2008, www.tropicalforages.info). Therefore, *Stylasanthes scabra* is an important pasture legume for seasonally dry tropical and subtropical environments (Hall et al. 1995). However, it is less known in South Africa as a forage legume that could be utilized as supplementary feed for livestock during the dry season or for pasture improvement and would subsequently enhance livestock production by smallholder subsistence farmers under semi-arid conditions. The objective of this study was to evaluate the adaptability and herbage yield of fifteen *Stylosanthes scabra* accessions in the subtropical climate of Pretoria, Gauteng, South Africa. Hypothesis of this study is that there is no variation among the accessions in terms of their adaptability and biomass yield production.

2.2. Materials and methods

2.2.1. Location

The experiment was conducted at Hatfield Experimental Farm, University of Pretoria, which is located geographically at 25^{o}44'30" S, 28^{o}15'30" E, at an elevation of 1370 metres above sea level (asl). The study area has two distinctive seasons: the dry season (March – September) and the rainy season (October – February), with warm and humid conditions in summer, while winter is dry, cold and sunny. Mean maximum monthly temperature in summer remain above 29°C and the minimum monthly temperature remains above 12°C, whereas in winter, particularly in June, the maximum and minimum temperatures drop to 22°C and 4°C, respectively. This is a summer rainfall area with a mean precipitation of 674 mm, however, during the study period annual rainfall was slightly higher (683.9 and 698.5 mm) in 2012 and 2014, respectively while in 2013 it was slightly lower (532.1 mm) than the mean precipitation. Monthly rainfall data and 20-year long-term averages of rainfall are presented in Figure 2.1. Long term rainfall data were obtained from Pretoria Eendracht station (0513314C9) weather records held by South African Weather Services.

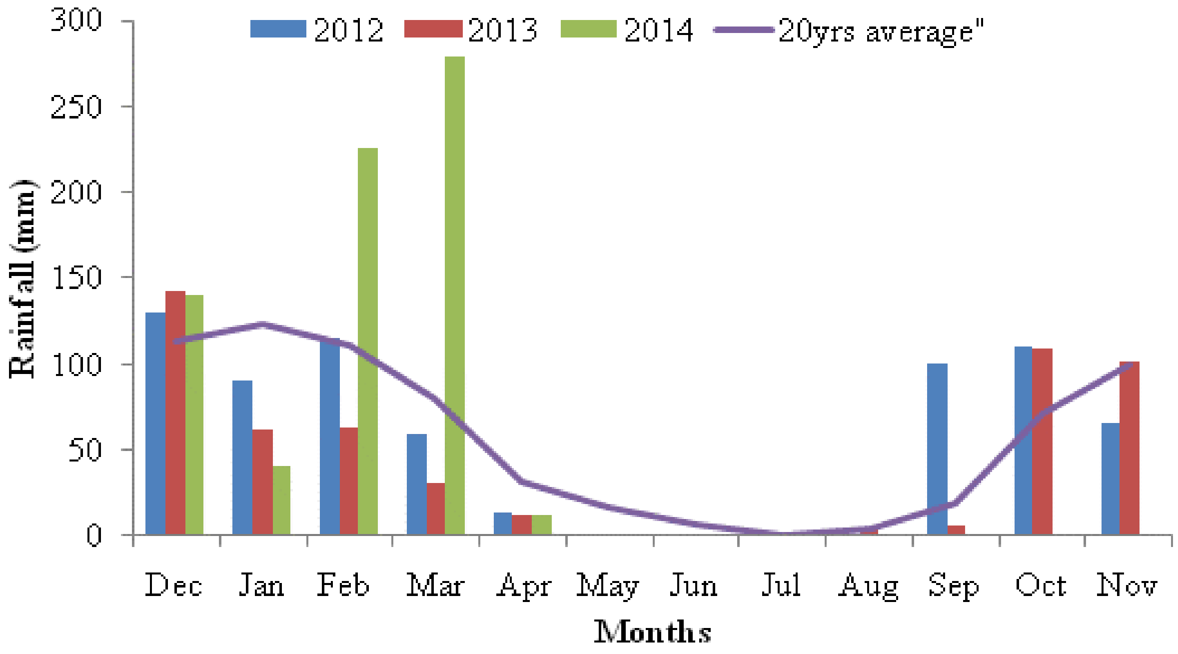

Figure 2.1: Monthly rainfall during experimental period and averages of the last 20 years

2.2.2. Experimental treatment and design

Fifteen accessions of *Stylosanthes scabra* were obtained from the International Livestock Research Institute (ILRI) gene bank in Ethiopia (Table 2.1). However, owing to the small quantity of seeds received per accession (about 20 g), the seeds were initially sown in seedling trays, and vigorous seedlings were transplanted to the field plots when they were 10 cm high.

Table 2.1: Description of *Stylosanthes scabra* accessions that were evaluated in this study

Accession (ILRI[12] no.)	Collector's name	Country collected	Weight of 1000 seeds
140	William R	Brazil	1.89 g
170	Quintero B	Ecuador	3.50 g
441	William R	Brazil	1.00 g
9268	Burt R	Brazil	2.94 g
9281	NI[¥]	NI	7.63 g
11252	Schultze-Kraft R., Coradin L	Brazil	2.13 g
11255	Schultze-Kraft R., Coradin L	Brazil	1.53 g
11591	Patino V.M., da Silva U	Brazil	1.81 g
11592	Patino V.M., da Silva U	Brazil	236 g
11595	Schultze-Kraft R., Coradin L	Brazil	2.02 g
11604	Univ. Federal de Bahia	Brazil	2.10 g
11625	Patino V.M., da Silva U	Brazil	11.34 g
12555	NI	NI	NI
15784	Schultze-Kraft R., et al.	Brazil	1.54 g
15795	NI	Brazil	1.45 g

Source: ILRI forage diversity gene bank, Ethiopia. [¥] NI no information available.

Prior to transplanting, the land was cleared and prepared to a fine tilth. Initial soil samples were taken at two soil depths (0 – 20 cm and 20 – 40 cm) for soil nutrient analysis. Soil at the experimental site is classified as sandy clay loam (Table 2.2). The treatments (accessions) were planted in a randomized complete block design (RCBD) with three replications. Each block had all fifteen accessions as treatments. Plots measured 3 x 2 m, and each had eight rows with twelve seedlings per row, maintained at a space of 0.25 m between and within rows (Figure 2.2). A spacing of 0.5 m was maintained between plots within a block, and blocks were set 1 m apart. Weeds were hand removed from these plots to reduce competition.

[12] International Livetock Research Institute

Table 2.2: Chemical properties of soil at the study site at Hatfield Experimental Farm, University of Pretoria

Properties	Soil Depth (cm)	
	0 – 20	20 – 40
pH (H_2O)[a]	6.39	6.21
Total N (%)	0.02	0.02
Carbon (%)	0.46	0.52
C:N ratio	23.17	23.50
Available P (ppm)[b]	5.86	1.98
Cations[c] (cmol kg^{-1})		
Ca	1.94	1.92
Mg	1.05	1.03
K	0.13	0.12
Na	0.04	0.05
CEC[1]	3.47	3.84
Soil texture[d] (%)		
Sand	70.67	66.0
Silt	5.33	6.0
Clay	24.0	28.0

[a]1:5 soil:water, [b] Extractant Bray 1, [c] Ammonium acetate method, [d] near infrared determination.

[1] Cations exchange capacity

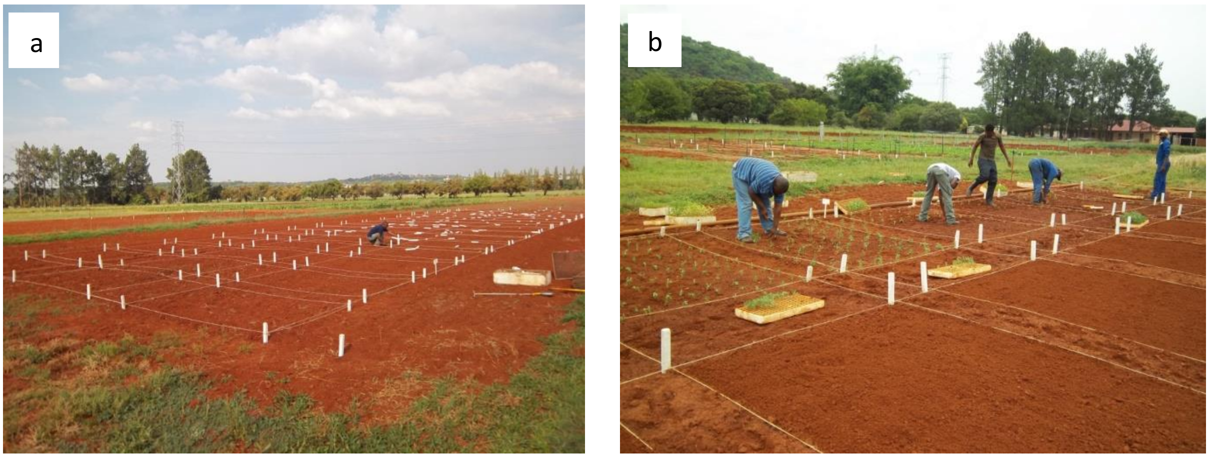

Figure 2.2: a) Plot preparation and treatment layout in the field; b) transplantation of *Stylosanthes scabra* accession seedlings in the respective field plots

2.2.3. Data collection

The accessions were observed for signs of pests and disease incidences and frost tolerance were recorded in order to monitor adaptability and performance. The growth parameters that were recorded included: plant height, canopy spread, tillering capacity, and plant survival. Plant height and canopy spread were recorded by measuring the tallest part of plant and widest canopy diameter of five randomly selected plants per plot. Tillering was recorded by counting new tillers from five randomly selected plants per plot after cutting (Tarawali et al. 1995). To estimate biomass yield, plants were harvested (whole plot) at 5 cm above the soil surface when they attained the 100% flowering stage (Figure 2.3). Wet mass of the forage was recorded immediately after cutting each plot, and two subsamples of approximately 1000 g each were taken, and oven dried at 60°C to consistent weight for 72 h to estimate the dry matter yield. Dried subsamples were separated into leaves and stems, and weighed separately in order to estimate leaf to stem ratios of these accessions. Later, leaves and edible stems (< 3 mm) were mixed and milled together to pass through a 1 mm sieve and used to determine the crude protein concentration, using standard procedures (AOAC 2000). Crude protein concentration was used to estimate the crude protein yield of these accessions, as described by Schroeder (2013). Data on adaptability traits and biomass yield production were collected over three years to determine the adaptability, persistence and yield performance of the studied accessions.

Figure 2.3: Plots of *Stylosanthes scabra* harvested at 100% flowering stage of growth

2.2.4. Statistical analysis

Data on yield performances of year 1 and year 3 and also crude protein yield were square root transformed, but untransformed data were used for the means. The recorded data were subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure of Statistical Analysis System, version 9.0 (SAS 2002) for randomized complete block designs to study the effect of treatment. Where F-value showed significance in relation to the accessions, means separation was done using least significant difference (LSD) at $P < 0.05$.

2.3. Results

2.3.1. General observation

In the establishment year (first year of evaluation), all accessions were vigorous and performed well. However, small black insects (pest susceptible) were noticed in some individual plants (see Figure 2.4). Nevertheless, they did not damage the plant or spread to others, identification and classification of pests that was observed were beyond the scope of this study. None of the accessions could survive frost in winter, so all leaves were shaded in winter (May – July). However, most accessions sprouted again in spring, except accession 11591.

Figure 2.4: Incidence of pest attacks on *Stylosanthes scabra* plants observed during establishment year

2.3.2. Growth parameters

Data on growth characteristics (i.e. plant height, canopy spread, tillering capacity, survival and leaf to stem ratio) of the *Stylosanthes scabra* accessions are presented in Table 2.3. Plant height varied significantly among the accessions ($P < 0.05$), ranging from 22.0 cm to 41.5 cm. The shortest was accession 15795, while the tallest was 11591. There was significant ($P < 0.05$) variation in canopy spread diameter which ranged between 31.6 cm for accession 9281 and 43.6 cm for accession 9268. Accession 15795 had the highest (19.9) tillering capacity, while accession 11625 had the lowest (11.1), with a significant ($P < 0.05$) variation among the accessions. Plant survival after winter varied significantly ($P < 0.05$), though it was above 85% in most accessions, the highest value being recorded for accession 11252 (98%) and the lowest (41%) for accession 12555.

Table 2.3: Growth parameters of *Stylosanthes scabra* accessions evaluated at Hatfield Experimental Farm.

Accession (ILRI[13] no)	Plant height[14](cm)	Canopy spread[15](cm)	Tillering capacity[16]	Days to 100% flowering[17]	%survival after 3yrs[18]
140	34.8^{bc}	34.1^{bcd}	14.1^{bcd}	56.0^{cd}	94.8^{a}
170	43.9^{a}	39.7^{ab}	12.3^{bcd}	63.7^{abc}	94.5^{a}
441	41.3^{ab}	32.9^{bcd}	13.3^{bcd}	61.0^{cd}	86.4^{a}
9268	39.2^{abc}	43.6^{a}	12.3^{bcd}	53.0^{d}	85.4^{a}
9281	35.8^{bc}	31.6^{d}	14.9^{b}	56.3^{cd}	95.1^{a}
11252	33.3^{c}	39.4^{abc}	13.5^{bcd}	61.5^{cd}	98.1^{a}
11255	32.8^{c}	39.5^{abc}	14.7^{bc}	58.5^{cd}	89.0^{a}
11591	41.5^{ab}	39.9^{ab}	13.9^{bcd}	70.7^{ab}	None
11592	34.5^{c}	32.1^{cd}	13.3^{bcd}	71.3^{ab}	88.5^{a}
11595	36.8^{bc}	43.2^{a}	12.3^{bcd}	56.3^{cd}	95.8^{a}
11604	34.1^{c}	36.9^{abc}	11.6^{cd}	60.3^{cd}	95.7^{a}
11625	35.4^{bc}	33.8^{bcd}	11.1^{d}	72.0^{a}	89.9^{a}
12555	32.4^{c}	39.93^{ab}	14.3^{bc}	59.3^{cd}	41.3^{b}
15784	34.3^{c}	38.3^{abc}	11.7^{cd}	63.0^{bc}	93.0^{a}
15795	22.0^{d}	35.3^{bcd}	19.9^{a}	56.7^{cd}	88.3^{a}
SEM	2.34	2.57	1.05	3.03	5.11

Means within a column with different letters in superscript differ significantly ($P < 0.05$). SEM; Standard error of mean

[13] International Livestock Research Institute
[14] It was recorded from ground surface to the tip of longest plant part
[15] It was recorded from the diagonal spread of plant (widest spread of plant)
[16] It was recorded by counting new tillers after each cutting
[17] It was recorded by counting the number of days plants have taken to attain 100% flowering stage after cutting
[18] It was recorded by counting plants that were still surviving after 3 years

2.3.3. Biomass yield

Biomass production over three years of the *Stylosanthes scabra* accessions are presented in Table 2.4. Biomass was estimated at the 100% flowering stage, in order to get the highest biomass yield. Biomass yield annual means of *Stylosanthes scabra* showed a significant variation ($P < 0.05$) over three years of evaluation. Of a total of 15 accessions evaluated for adaptability, about 87% showed persistence over three years under rain-fed conditions. Accession 11591 produced the highest yield (6.7 t ha^{-1} DM) in the establishment year. However, this accession was not able to recover after a period of low temperatures and frost in winter. Thus, it was not adaptable to subtropical climate conditions in Pretoria, South Africa. Biomass yield increased slightly in the second year for most accessions, except accessions 441 and 11255, whereas accession 11252 remained the same. In the third year, the biomass yield for most of these accessions dropped, the highest rate of drop being 254% for accession 9268. However, yield from accessions 9281, 11595 and 11604 increased throughout the study from establishment year to the third year of evaluation. Regardless of the accession, slightly higher average dry matter yield was recorded in year 2, whereas significantly lower average dry matter yield was recorded in year 3.

Table 2.4: Biomass yield of *Stylosanthes scabra* accessions over three years

Accessions (ILRI[19] no)	Biomass yield (t ha^{-1}DM)			
	Year 1	Year 2	Year 3	Mean
140	4.2^{bc}_{A}	5.3^{b}_{A}	5.3^{ab}_{A}	4.9
170	$4.9^{bc}_{A,B}$	5.6^{ab}_{A}	3.6^{bc}_{B}	4.7
441	5.3^{abc}_{A}	$5.0^{b}_{A,B}$	3.8^{abc}_{B}	3.5
9268	4.4^{bc}_{B}	6.3^{a}_{A}	1.8^{c}_{C}	4.1
9281	4.6^{bc}_{A}	5.0^{b}_{A}	5.4^{ab}_{A}	5.0
11252	5.4^{abc}_{A}	5.4^{ab}_{A}	4.5^{abc}_{A}	5.1
11255	5.8^{ab}_{A}	5.4^{ab}_{A}	3.3^{bc}_{B}	4.8
11591	6.7^{a}	-	-	-
11592	5.2^{abc}_{A}	5.6^{ab}_{A}	3.3^{bc}_{B}	4.7
11595	5.0^{abc}_{A}	5.3^{b}_{A}	6.6^{a}_{A}	5.6
11604	4.8^{bc}_{A}	5.2^{b}_{A}	5.4^{ab}_{A}	5.1
11625	4.8^{bc}_{A}	5.3^{b}_{A}	4.5^{abc}_{A}	4.9
12555	4.4^{bc}_{A}	5.1^{b}_{A}	2.9^{bc}_{B}	4.1
15784	3.9^{c}_{B}	5.6^{ab}_{A}	4.7^{ab}_{A}	4.7
15795	4.6^{bc}_{A}	5.3^{b}_{A}	3.3^{bc}_{B}	4.4
SEM	0.14	0.28	0.78	
Year effects	4.9_{A}	5.3_{A}	4.3_{B}	

Means within a column with different letters in superscript differ significantly ($P < 0.05$)

Means within a row with different letters in subscript differs significantly ($P < 0.05$)

SEM; Standard error of mean

[19] International Livestock Research Institute

2.3.4. Quality of *Stylosanthes scabra* accessions

Leaf to stem ratio, crude protein (CP) concentration and CP yield of *Stylosanthes scabra* accessions for the first year performance are presented in Table 2.5. There was a significant ($P < 0.05$) variation among the accessions in terms of leaf to stem ratio, CP concentration and CP yield. The proportions of leaf to stem were above 1 in most accessions, except for 9268, 170, 11255, 11591 and 11592, where significant differences ($P < 0.05$) were observed compared to the rest of *Stylosanthes scabra* accessions. The highest (226.6 g kg^{-1} DM) CP concentrate was recorded for accessions 11591 and the lowest (188.1 g kg^{-1} DM) for accession 15795. Regardless of its high leaf to stem ratio accession 15795 has the lowest CP values compared to other accessions. However, the leaves for this accession and others (e.g. 11252) are relatively light green (Figure 2.5). Moreover this figure also shows the greenness of *Stylosanthes scabra* which strongly relate with the nitrogen content in leaves. Accession 11252 and 15795 observed to be lighter in colour as compared with accession 11592. The crude protein content of these accessions was above 18%. That is 2.6 times the threshold level of 7% essential to meet the maintenance requirement of animals. Crude protein yield varied among these accessions, the highest (152.6 t ha^{-1}) being recorded in accession 11591 and the lowest (81.4 t ha^{-1}) for accession 15784.

Table 2.5: Leaf to stem proportion, crude protein content and protein yield of *Stylosanthes scabra* accessions evaluated at Hatfield Experimental Farm

Accession (ILRI[20] no.)	Leaf to stem ratio[21]	Crude protein ($g\ kg^{-1}$ DM)	Crude protein yield ($t\ ha^{-1}$)[22]
140	1.2^{ab}	199.4^{de}	0.8^{b}
170	0.8^{bc}	219.0^{ab}	1.1^{ab}
441	1.1^{abc}	211.1^{bc}	1.1^{ab}
9268	1.0^{abc}	221.7^{a}	1.0^{b}
9281	1.2^{ab}	199.1^{de}	0.9^{b}
11252	1.1^{ab}	193.4^{efg}	1.0^{ab}
11255	0.9^{bc}	198.1^{def}	1.2^{ab}
11591	0.9^{bc}	226.6^{a}	1.5^{a}
11592	0.7^{c}	212.5^{bc}	1.1^{ab}
11595	1.1^{ab}	200.7^{de}	1.0^{b}
11604	1.1^{abc}	196.8^{efg}	0.9^{b}
11625	1.2^{ab}	189.7^{fg}	0.9^{b}
12555	1.2^{ab}	206.5^{cd}	0.9^{b}
15784	1.2^{ab}	210.7^{bc}	0.8^{b}
15795	1.3^{a}	188.1^{g}	0.9^{b}
SEM	0.13	0.29	0.07

Means within a column with different letters in superscript differ significantly ($P < 0.05$)

SEM; Standard error of mean

[20] International Livestock Research Institute

[21] It was estimated by weighing leaves and stems separately and dividing leaves weights by stem weights

[22] Crude protein yield was estimated as described in Schroeder 2013

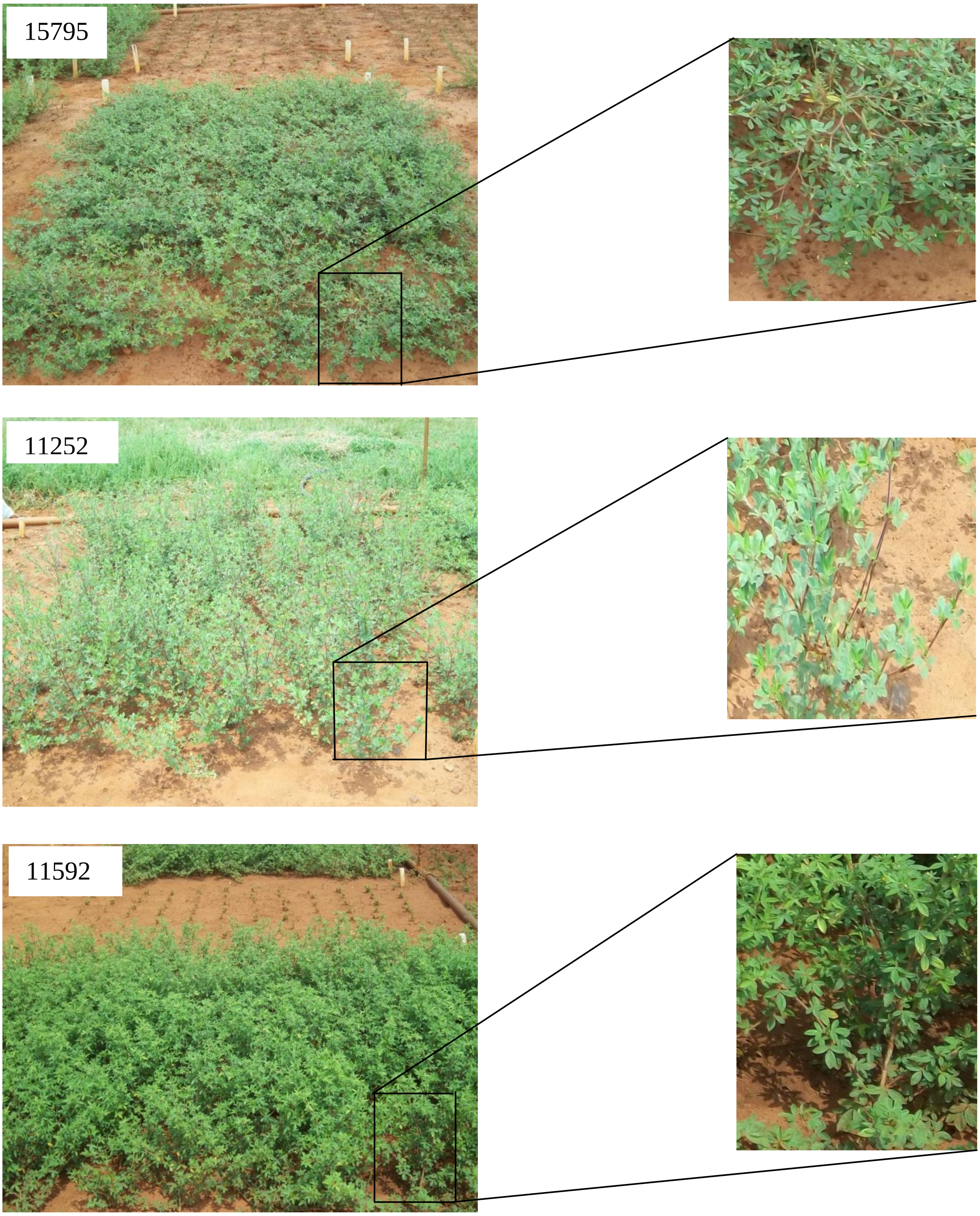

Figure 2.5: Variation in leafiness and greenness of some *Stylosanthes scabra* accessions that were evaluated for adaptability in Pretoria.

2.4. Discussion

The main objective of this study was to identify *Stylosanthes scabra* accessions that were adaptable to, persistent and productive in the subtropical climate of Pretoria, northern Gauteng, South Africa. Differences in adaptability and plant performance that was observed among these accessions could be associated with genetic variation among the accessions.

This study showed that *Stylosanthes scabra* accessions tested under rain-fed conditions in northern Gauteng were persistent over three years. The survival rate of most accessions were above 80%, except for accession 12555 which had less than 50%, and accession 11591 did not tolerate the winter conditions. According to Ciotti et al. (1999), the persistence of a crop is determined by longevity of the perennial plants and seedling regeneration. Therefore, the persistence of most *Stylosanthes scabra* accessions in terms of re-growth and survival confirms its adaptability to the growing conditions of Pretoria, which are often characterized by the presence of frost. The study area is typified by low soil carbon and low nitrogen concentration, and the soil is slightly acidic (Table 2.2). The performance of *Stylosanthes scabra* in such climatic and soil conditions is consistent with findings of Rootman et al. (2004), who reported that *Stylosanthes scabra* was persistent in the semi-arid tropical climate of Limpopo. Other researchers (such as Akinola et al. 2010) reported that *Stylosanthes scabra* is adaptable to a wide range of climatic and edaphic environments.

Although the accessions were found to be adaptable and productive in the study area, they all could not withstand cold and frost in winter (May - July) and none remained green during this period. Nevertheless, a majority re-grew in spring (August/September). This confirms the results of Hall et al. (1995), who reported that *Stylosanthes* resprouted after it was weathered in winter. Because these accessions could not tolerate cold and frost in winter, under subtropical conditions it is more appropriate to cut and conserve the forage material in the growing season than to leave the plants as a fodder bank for dry season feeding.

Among the *Stylosanthes scabra* accessions evaluated in the present study, fourteen produced above 4 t ha^{-1} DM of biomass, without any input (irrigation and/or fertiliser). Dry matter yield recorded in the present study for the first year was higher than the 1.5 t ha^{-1} DM recorded by Akinlade et al. (2008) in the savannah zone of Nigeria and the 2.4 t ha^{-1} DM recorded by Ciotti et al. (1999) in the sub-humid zone of Argentina. *Stylosanthes scabra* was established poorly from seeds, hence, this affected biomass production, particularly in the establishment year (Akinalade et al. 2008). However, in the present study seedlings were produced in seedling trays, and vigorous seedlings were transplanted to field plots to improve plant establishment and population density per plot. Therefore, this could be attributed to the high biomass production recorded in this study, in contrast with earlier studies. The productivity of the accessions in terms of biomass production was further monitored for three years. Generally, they tended to increase dry matter yield in the second year but in the third year a significant drop in biomass production was recorded in six of these accessions (170, 441, 9268, 11255, 11592 and 12555. On the other hand, accessions 9281, 11595 and 11604 tended to increase dry matter yield production over three years (Table 2.4).

Accession 15795 observed to be more of a creeper, whereas other accessions were erect (see Figure 2.5). On the other hand accession 11592 had bigger stem in relation to accessions 15795 and 11252, respectively, hence it had the lowest leaf to stem ratio. In addition, the greenness of *Stylosanthes scabra* strongly relate with the nitrogen content in leaves. The lighter green colour that is observed in accession 11252 and 15795 as compared with accession 11592 (Figure 2.5) may indicate lower nitrogen content in leaves, as the greenness of leaf most often relates to the nitrogen content of leaves. Hence some of the accession (e.g. 15795) recorded lower CP value, regardless of the observed relatively high leaf to stem ratio (Table 2.5). According to Netto et al. (2005) nitrogen content of the plant is one of the factors that affect leaf greenness. Leaf greenness indicates chlorophyll concentration and is strongly linked with the nitrogen content in leaf since most of leaf nitrogen is contained in chlorophyll molecules (Peterson et al. 1993). However, crude protein content of these accessions was above the threshold level of 7%, which is required for normal intake, and functioning of the rumen (Ikhomoya 2008). In fact the protein contents recorded in this study are in the range that is required for lactating dairy cows (Poppi and McLennan 1995). This indicates that these accessions can be used as supplementary source of protein and forage for low-quality forage diets.

2.5. Conclusion

The present study showed that most of the *Stylosanthes scabra* accessions were adaptable to and productive in the subtropical climate of Pretoria. The ability to achieve an average biomass yield of 4.9 ton of dry matter per hectare without any input is vital in environments such as in the northern region of Gauteng. The observed high crude protein content indicates that the accessions are suitable for utilization as supplementary forage to serve as good-quality roughage and as protein source to supplement poor-quality forage. However, accessions 9281, 11595 and 11604 were superior in terms of their biomass yield potential over the three year assessment period. Thus, these accessions are recommended for possible biomass production under resource-poor smallholder subsistence farmer's conditions. However, further study is required to determine the seed production potential of these accessions.

CHAPTER 3

Nutritive value of forage from *Stylosanthes scabra* accessions grown in a subtropical region of South Africa

Abstract

Twelve accessions of *Stylosanthes scabra* grown under rain-fed conditions in Pretoria, Gauteng, South Africa, were evaluated for their potential nutritive values, *in vitro* organic matter digestibility, and kinetics of fermentation. Forage material of each accession was harvested at 100% flowering stage. Subsamples were taken to determine chemical composition, anti-nutritional factors, *in vitro* organic matter digestibility (IVOMD) and ruminal fermentation. The content of crude protein (CP), neutral detergent fibre (NDF) and anti-nutritional factors such as total phenols, total tannins and total condensed tannins were significantly ($P < 0.05$) different among accessions, except ash content. These accessions differed ($P < 0.05$) in terms of gas production, but were similar ($P > 0.05$) in the rate of fermentation and effective gas production. These accessions were significantly ($P < 0.05$) different in terms of IVOMD and metabolizable energy (ME). Generally, CP value of 20.3 % DM was above the minimum concentration level of 7% CP essential to meet the requirement of ruminant animal for maintenance purpose. Whereas ME value of 9.8 MJ kg^{-1} DM was in the range of 8.8 MJ kg^{-1} DM and 13.4 MJ kg^{-1} DM which is adequate to meet daily requirement of animal for maintenance and production purposes. On the other hand fibre content (NDF) was below the upper limit (60%) which reduces intake by ruminant. High CP content, IVOMD and ruminal fermentation indicates the potential of these accessions to be strategically used as supplement to poor-quality forage during the dry season. Although most accessions tested in this study showed good nutritive value however, accessions 140, 9281, 11595 and 11604 are recommended for use as forage supplements based on their metabolizable energy yield values.

Keywords: Anti-nutritional factors; Chemical composition; *In vitro* digestibility; Kinetics of fermentation

3.1. Introduction

Natural pastures and crop residues provide major feed resources for animals kept by smallholder subsistence farmers (Rebero and Mupenzi 2012). However, these feed resources are characterized by low CP content, and are seldom sufficient to meet animal nutritional requirements for maintenance and production (Mendieta-Araica et al. 2009). Protein and fermentable metabolizable energy (ME) levels of feeds are often low, and these affect palatability and intake of feed (Kordestany and Ebne-Abbasi 2012). Consequently, the utilization of such feed material by animals leads to loss of body weight and condition, reduced reproductive performance, and an increase in mortality rate (Ajayi et al. 2005).

To enhance animal production performance in sub-Saharan Africa, feed deficiencies must be corrected in terms of quantity and quality to improve intake and digestibility of poor-quality forage. Dietary deficiency correction could increase feed degradation in the rumen, and thus improve animal metabolic capacity to use energy. Consequently, voluntary intake and productivity would increase (Al-Masri 2013). The inclusion of fodder legumes in poor-quality forage diets improves the overall protein content and digestibility of feed, and thus enhances livestock production performance (Kordestany and Ebne-Abbasi 2012; Ajayi et al. 2005). Forages from shrubs, trees and herbaceous legumes have been widely used as nitrogen, energy and mineral sources (Bamikole et al. 2004; Kumara-Mahipala et al. 2009).

Stylosanthes scabra is regarded as a suitable tropical legume in Australia in terms of forage production and as a supplementary feed. Improved live weight and increased stocking rates have been reported on steers grazing *Stylosanthes scabra* pastures (Hall and Glatzel 2004). The inclusion of *Stylosanthes scabra* in animal feed improved milk production in Africa (Baloyi et al. 2006; Mupenzi et al. 2009). Chapter 2 showed that most accessions tested were adaptable and productive with the average biomass yield ranging from 3.5 to 5.6 t ha^{-1} DM. The objective of this study was to evaluate the potential nutritive value and digestibility of the adaptable and productive accessions in Pretoria, by determining their chemical composition, anti-nutritional factors, *in vitro* organic matter digestibility and kinetics of fermentation of the edible forage material.

3.2. Materials and methods

3.2.1. Location

The study area is located at 25°44′30″ S, 28°15′30″ E; 1370 m above sea level. Its soil profile shows that carbon (C) percentage ranges from 0.41 to 0.60, total nitrogen from 0.019 to 0.026, and phosphorus from 1.26 to 6.55 in the soil depth of 0 – 40 cm. Soil texture is characterized by a high proportion of sand, ranging from 64% to 72%, with a small proportion of clay (24% and 28%) and silt (4% and 8%). Thus, it is classified as sandy clay loam soil. The soil is slightly acidic with a pH ranging from 5.86 to 6.68 (further details were indicated in chapter 2, section 2.2.1).

3.2.2. Plant material and growing conditions

Forage samples of 12 accessions found to be adaptable and productive in subtropical climate of Pretoria were harvested at 5 cm above soil on attaining 100% flowering stage. Two subsamples (≈ 1000 g) of forage were taken per plot, dried over 72 h in an oven set at 60°C. Accessions that survived winter and produced above 3 t ha^{-1} DM in the third year were regarded as adaptable and productive. The edible forage samples that are consumable to animals (leaves and stem of less than 3 mm in diameter) were separated and milled to pass through a 1 mm screen and stored in airtight plastic containers. These samples were later used to determine chemical composition, anti-nutritional factors, *in vitro* digestibility, and kinetics of fermentation.

3.2.3. Chemical composition analysis

The chemical composition of the forage material was determined using the standard procedures of AOAC (2000). Dry matter (DM) content was determined by drying the samples at 105°C for 16 hours and then igniting them in a muffle furnace at 550°C for 4 hours to determine ash. The nitrogen content of the forage was measured by the Dumas technique, from which CP was calculated as N x 6.25 (AOAC 2000). Ankom fibre analyser was used to determine neutral detergent fibre (NDF) content, according to Van Soest et al. (1991). An *in vitro* digestible organic matter (IVOMD) was determined as organic matter loss after 96 hours of incubation, according to the Tilley and Terry (1963) procedure as modified by Engels and Van der Merwe (1967).

3.2.4. Anti-nutritional factors

Anti-nutritional factors such as total phenols (TP), total tannins (TT) and total condensed tannins (TCT) were determined according to the procedure of Makkar (2003a). Following this procedure, TP and TT were then determined by a modified Folin-Ciocalteu method using polyvinyl-polypyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols (NTP). Butanol-HCl-iron procedure was used to determine condensed tannins. Both TP and TT were calibrated against tannic acid solution as a standard, and thus values were expressed as tannic acid equivalents, whereas TCT was expressed as leucocyanidin equivalent.

3.2.5. Rumen fluid collection, buffer preparation and *in vitro* gas production

Two South African Merino sheep (males) fitted with permanent rumen cannula were used as rumen fluid donors. Donor sheep were fed alfalfa hay daily, at *ad libitum,* as basal forage with free access to water. Rumen fluid of approximately 450 ml per animal was collected before morning feeding. Both fluids were mixed and strained through four layers of cheese-cloth into pre-warmed thermos flasks and transported to the laboratory within 20 minutes. On arrival the rumen fluid was purged with carbon dioxide (CO_2) to maintain the anaerobic conditions.

The buffer, micro and macro mineral solutions were prepared as described by Goering and van Soest (1970). However, the macro-mineral solution was modified slightly by replacing magnesium sulphate ($MgSO_4.7H_2O$) with magnesium chlorite ($MgCl_2.6H_2O$) to reduce sulphate in the medium as recommended by Mould et al. (2005). These solutions were prepared a day before hand, and kept in separate containers in a 4°C fridge. Resazurin solution was prepared by dissolving 100 mg resazurin in 100 ml distilled water, and kept in a dark container in a fridge because of light sensitivity. Before the start of the experiment in the morning, an appropriate amount of distilled water was measured and a small portion was used to dissolve the tryptone. All solutions were measured as described by Goering and Van Soest (1970), and mixed together with distilled water in conical flasks and blended with a magnetic stirrer for few minutes, placed in a water bath (39°C), and purged with CO_2 until light pinkish in colour. Appropriate amounts of L-cysteine hydrochloride and sodium sulphite were weighed and added directly to the rest of the solution and flushed with CO_2 untill the pinkish colour turned colourless, which indicated the complete removal of oxygen.

Samples of approximately 400 mg per accession were weighed into 120 ml serum bottle in duplicate. An amount of 40 ml (15 ml rumen fluid and 25 ml solution) of buffered rumen fluid was dispensed per serum bottle. Each bottle was sealed with rubber stopper and aluminium seal caps immediately after loading. Bottles were incubated in a continuous shaking incubator at a rate of 120 revolutions per minute (rpm) set to 39°C with gas volume (GV) recorded at 2, 4, 8, 12, 24, 48, 72 and 96 hours post incubation. Gas volume was measured by inserting a 23 gauge (0.6 mm) needle attached to a pressure transducer connected to a visual display. The average volume of gas produced from blanks (containing buffered rumen fluid only) was subtracted from the volume of gas produced from the incubated sample. Incubations were performed in three runs conducted in different weeks with two replicates per run. Gas volume produced was plotted against incubation time, and the Ørskov and McDonald (1979) non-linear equation was used to estimate gas production characteristics.

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

Where *GV* is the total gas volume (ml 400 mg^{-1} DM) at time t; *b* is the insoluble but slowly fermentable fraction (ml); and *c* is the rate constant of gas production per hour.

3.2.6. Calculations and statistical analysis

Effective gas production (EGP) was estimated using the Ørskov and McDonald (1979) equation, assuming the flow rate constant (k) of 0.05 h^{-1}. EGP = b*c / (k + c). Since 400 mg of samples was used in this study for *in vitro* gas production, the recorded gas volume after 24 hours' incubation was adjusted to 200 mg to estimate ME and short chain fatty acid (SCFA) values as per the equations of Menke et al. (1979) and Menke and Steingass (1988). Therefore, Menke et al. (1979) equation was used to calculate ME, while Menke and Steingass (1988) equation was used to calculate SCFA.

ME (MJ kg^{-1} DM) = 2.2 + 0.136GV + 0.057CP + 0.002859CP^2.

SCFA (µmol g^{-1} DM) = 0.0239GV – 0.0601.

Where: ME = metabolizable energy, GV = net gas volume at 24 hours (ml 200 mg^{-1} DM) and CP = crude protein (%).

The metabolizable energy values that were calculated for each accession were further used to estimate the metabolizable energy yield (MEY) of each adaptable and productive accession. Metabolizable energy yield of accessions were calculated as the product of dry matter yield (Table 2.4) and metabolizable energy value (Table 3.3).

Metabolizable Energy Yield (GJ ha^{-1}) = Dry matter yield x Metabolizable energy

The NLIN procedure of SAS (2002) was used to fit non-linear regression model of *in vitro* incubation times. Data on metabolizable energy yield of year 1 and year 3 were log transformed for normality, but untransformed data was used for the means. Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS 2002). Where significant differences occurred the F-test means were separated using Duncan's new multiple range test (DMRT).

3.3. Results

3.3.1. Plant chemical composition and anti-nutritional factors

The chemical composition and anti-nutritional factors of *Stylosanthes scabra* accessions adapted to the subtropical climate of Pretoria are presented in Table 3.1. The variations for ash were not different ($P > 0.05$) among the accessions, whereas there were significant ($P < 0.05$) differences between accessions in terms of CP and NDF concentration. The CP concentration of the forage materials ranged from 189.7 g kg^{-1} DM in accession 11625 to 230.9 g kg^{-1} DM in accession 170. Fibre (NDF) concentration ranged from 345.3 g kg^{-1} DM in accession 11255 to 559.2 g kg^{-1} DM in accession 170.

There were significant ($P < 0.05$) differences among accessions in terms of TP, TT and TCT concentration. The TP concentration ranged from 5.7 g kg^{-1} DM in accession 15795 to 9.8 g kg^{-1} DM in accessions 11255 and 11592. The total tannins (TT) ranged from 2.4 g kg^{-1} DM in accession 15784 to 5.6 g kg^{-1} DM in accession 11595. There were three accessions (140, 9281 and 15795) in which total condensed tannins were not detected. Among those accessions that had a detectable level, TCT concentration ranged from 0.5 g kg^{-1} DM in accession 170 to 3.1 g kg^{-1} DM in accession 11252.

Table 3.1: Chemical composition and phenolic compounds of *Stylosanthes scabra* accessions adaptable to subtropical climate of Pretoria

Accessions (ILRI[23] no)	Chemical composition (g kg^{-1} DM)					
	Ash	CP	NDF	TP	TT	TCT
140	92.3	196.5^{b}	501.7^{ab}	6.7^{c}	3.2^{ab}	nd
170	93.5	230.9^{a}	559.2^{a}	6.9^{c}	4.5^{ab}	0.5^{f}
441	97.5	206.7^{b}	539.2^{ab}	7.8^{bc}	3.4^{ab}	1.9^{cd}
9281	88.3	195.3^{b}	469.0^{ab}	6.8^{c}	4.2^{ab}	nd
11252	86.5	193.4^{b}	439.9^{ab}	8.8^{ab}	3.5^{ab}	3.1^{a}
11255	82.5	198.0^{b}	345.3^{b}	9.8^{a}	3.5^{ab}	2.3^{bc}
11592	84.8	210.9^{ab}	511.1^{ab}	9.8^{a}	4.4^{ab}	2.3^{bc}
11595	86.8	211.7^{ab}	483.3^{ab}	8.5^{b}	5.6^{a}	2.9^{ab}
11604	94.5	197.9^{b}	498.0^{ab}	6.9^{c}	3.0^{b}	1.0^{ef}
11625	82.5	189.7^{b}	353.9^{b}	7.8^{bc}	3.2^{ab}	1.3^{de}
15784	80.0	212.9^{ab}	529.7^{ab}	7.2^{c}	2.4^{b}	1.8^{cd}
15795	76.3	196.3^{b}	446.7^{ab}	5.7^{d}	2.6^{b}	nd
SEM	7.60	6.87	68.08	0.36	0.75	0.25

Means within a column with different letters in superscript differ significantly ($P < 0.05$).
CP: crude protein; NDF: neutral detergent fibre; TP: total phenols; TT: total tannins; TCT: total condensed tannins, SEM: Standard error of mean. nd: not detected

[23] International Livestock Research Institute

3.3.2. *In vitro* gas production characteristics

Figure 3.1 shows the trend for *in vitro* gas production over 96 h of incubation, which represents ruminal fermentation of accessions of *Stylosanthes scabra* forages over time. The cumulative gas production increased as the time of incubation advanced to 12 h, but from 24 h to 72 h it tended to increase at a slower rate whereas after 72 h it remained constant. The variations in terms of gas production among these accessions across the incubation period were significantly ($P < 0.05$) different.

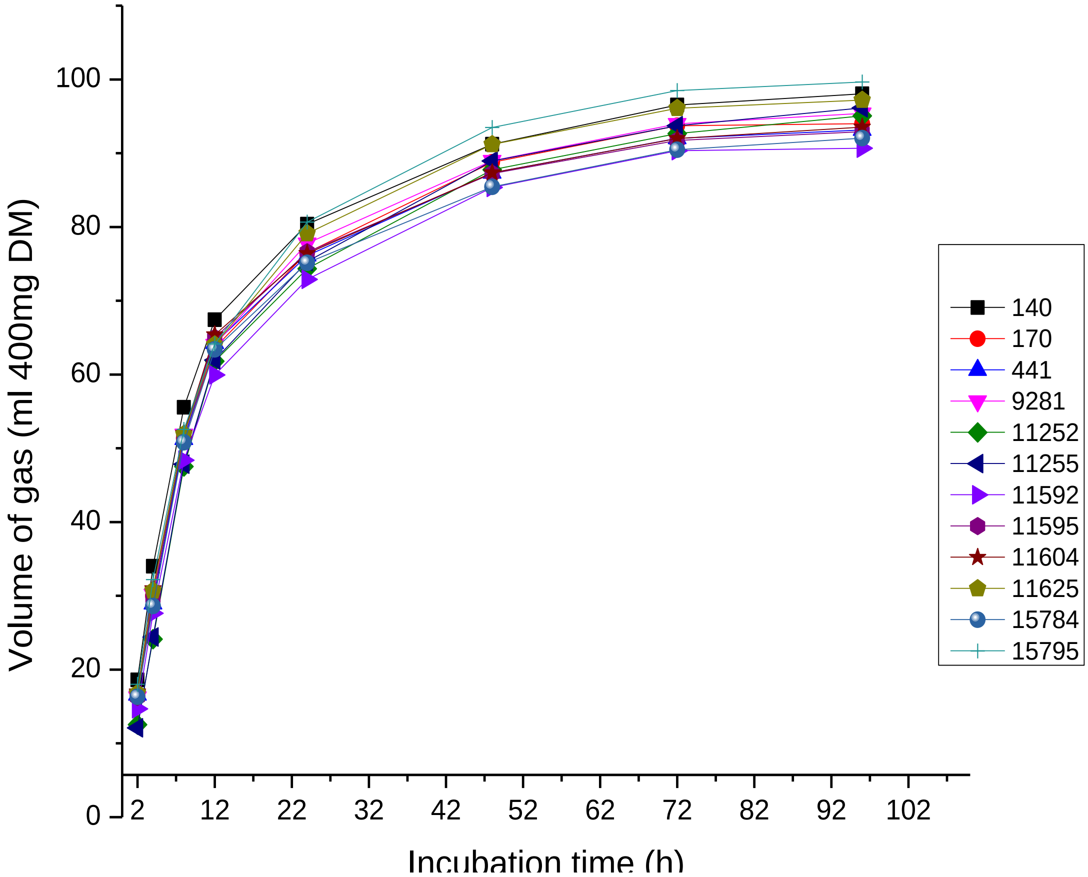

Figure 3.1: Gas production (ml 400 mg^{-1} DM) pattern of accessions of *Stylosanthes scabra* adaptable to subtropical climate of Pretoria

Table 3.2 shows the gas production characteristics, which represents those associated with the fermentation of gas production from slowly fermentable fraction (b); rate of gas production (c); and effective gas production (EGP) of *Stylosanthes scabra* accessions grown in Pretoria. However, significant ($P < 0.05$) differences among these accessions were observed only on gas production, whereas the rate of fermentation and effective gas production did not differ ($P > 0.05$).

Table 3.2: *In vitro* gas production characteristics of *Stylosanthes scabra* accessions adaptable to subtropical climate of Pretoria

Accession (ILRI[24] no)	b (ml 400 mg^{-1})	c (h^{-1})	EGP (ml 400 mg^{-1})
140	90.2^{ab}	0.097	59.0
170	88.8^{ab}	0.091	56.9
441	88.6^{ab}	0.096	57.8
9281	90.2^{ab}	0.093	58.1
11252	89.2^{ab}	0.086	59.0
11255	90.8^{ab}	0.086	59.8
11592	86.2^{b}	0.087	54.5
11595	82.0^{b}	0.099	58.9
11604	87.8^{ab}	0.098	57.9
11625	91.4^{a}	0.088	57.8
15784	87.3^{ab}	0.097	57.2
15795	91.7^{a}	0.084	56.7
SEM	3.79	0.0099	1.76

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

[24] International Livestock Research Institute

3.3.3. Feeding values

In vitro dietary evaluations of the *Stylosanthes scabra* accessions were assessed by determining IVOMD as described in section 3.2.3. However, ME and SCFA of forages were calculated using the equations of Menke et al. (1979) and Menke and Steingass (1988), respectively. The variation in net gas production (GV_{24h} ml 400 mg^{-1} DM) of *Stylosanthes scabra* was not significant ($P > 0.05$), but *in vitro* organic matter digestibility (IVOMD) varied significantly ($P < 0.05$) among accessions (Table 3.3). Accession 9281 had the highest IVOMD value, while accession 11592 had the lowest. There were significant ($P < 0.05$) differences among the accessions in terms of ME, while the difference in terms of SCFA was not significant ($P > 0.05$).

Table 3.3: *In vitro* dietary evaluation of *Stylosanthes scabra* accessions adaptable to subtropical climate of Pretoria

Accessions (ILRI[25] no)	*In vitro* parameters			
	GV_{24h} (ml 400 mg^{-1})	IVOMD (% DM)	ME (MJ kg^{-1} DM)	SCFA (µmol g^{-1} DM)
140	80.4	75.6^{a}	9.9^{ab}	0.9
170	76.5	72.3^{abc}	10.2^{a}	0.9
441	76.1	69.7^{bc}	9.8^{ab}	0.8
9281	77.8	76.5^{a}	9.7^{ab}	0.9
11252	75.6	69.8^{bc}	9.5^{b}	0.8
11255	74.9	71.6^{abc}	9.5^{b}	0.8
11592	72.9	67.4^{c}	9.6^{ab}	0.8
11595	76.6	68.4^{c}	9.9^{ab}	0.9
11604	76.4	71.8^{abc}	9.6^{ab}	0.9
11625	79.1	71.1^{abc}	9.7^{ab}	0.9
15784	75.1	71.4^{abc}	9.8^{ab}	0.8
15795	80.7	74.7^{ab}	9.9^{ab}	0.9
SEM	2.45	1.62	0.20	0.03

Means within a column with different letters superscript differ significantly ($P < 0.05$).
GV: gas volume; IVOMD: in vitro organic matter digestibility; ME: metabolizable energy; SCFA: short chain fatty acid; SEM, Standard error of mean.

Metabolizable energy yield of *Stylosanthes scabra* accessions per year and mean over three years are presented in Table 3.4. There were significant ($P < 0.05$) variations observed among the accessions in terms of metabolizable energy yield (MEY). Generally MEY increased in the second year and decreased in some accessions in the third year. However, an interesting trend of increasing MEYs over three year was noticed on these accessions 140, 9281, 11595 and 11604. The general means value of MEYs ranged from 42.6 GJ ha^{-1} to 55.5 GJ ha^{-1} for accessions 15795 and 11595, respectively.

[25] International Livestock Research Institute

Table 3.4: Estimated values of metabolizable energy yield content of *Stylosanthes scabra* accessions adaptable to subtropical climate of Pretoria

Accessions (ILRI[26] no)	Metabolizable energy yield (GJ ha^{-1})[27]			
	Year 1	Year 2	Year 3	Mean
140	41.5^{ab}	50.9^{ab}	54.4^{ab}	48.9^{ab}
170	50.4^{ab}	58.2^{a}	32.7^{b}	47.1^{ab}
441	46.8^{ab}	49.5^{ab}	41.2^{ab}	45.8^{ab}
9281	43.6^{ab}	47.7^{b}	55.3^{ab}	48.9^{ab}
11252	51.3^{ab}	51.6^{ab}	42.4^{ab}	48.4^{ab}
11255	55.3^{a}	51.0^{ab}	31.5^{b}	45.9^{ab}
11592	50.0^{ab}	54.1^{ab}	31.9^{b}	45.4^{ab}
11595	49.5^{ab}	51.6^{ab}	65.3^{a}	55.5^{a}
11604	44.4^{ab}	49.4^{ab}	51.8^{ab}	48.5^{ab}
11625	46.6^{ab}	53.3^{ab}	49.2^{a}	49.7^{ab}
15784	32.1^{b}	55.1^{ab}	47.0^{ab}	44.7^{ab}
15975	43.5^{ab}	51.3^{ab}	32.9^{ab}	42.6^{b}
SEM	0.20	2.84	0.20	3.15

Means within a column with different letters superscript differ significantly ($P < 0.05$).
SEM, Standard error of mean.

[26] International Livestock Research Institute
[27] Gigajoles per hectare

3.4. Discussion

During the long dry season, forage resources in tropical and subtropical regions of sub-Saharan Africa (SSA) are inadequate to support animal production (Mendieta-Araica et al. 2009). The available feed materials are characterized by high fibre with low protein content, consequently they are poorly digested (Mendieta-Araica et al. 2009). To improve the utilization of these poor-quality forages, they must be supplemented with a high protein feed resource that would supply ruminal degradable protein (Kaitho et al. 1998). In this regard, the forage materials of *Stylosanthes scabra* that were tested in the subtropical climate of Pretoria were found to be of good quality, with high CP concentration ranging from 178 to 232 g kg^{-1} DM (Table 3.5). The CP content of all accessions was above the critical threshold level (7%), which is necessary for normal intake of dry matter and rumen functioning of animal (Ikhimioya 2008). The CP values reported in this study were also above 11%, which is considered adequate for growing beef cattle (Valarini and Possenti 2006). In fact they were within a range of 16% to 20%, which is acceptable for lactating dairy cow (Poppi and McLennan 1995; Schingoethe 1998). Crude protein concentration of accessions 140 and 441 reported in this study were above the values of 147 g kg^{-1} DM and 150 g kg^{-1} DM, respectively, reported for the same accessions by Akinlade et al. (2008) planted in the derived savannah zone of Nigeria. However, this variation could be because of differences in soil chemical composition and the climatic conditions in which the accessions were grown. The NDF content of accessions tested in Pretoria was below the upper limit of 60%, above which dry matter intake is depressed (Meissner et al. 1991). However, the presence of anti-nutritional factors such as tannins in the forage material can have both, beneficial and detrimental effects on animals, depending on the type and level of tannin (Njidda and Nasiru 2010). The concentration of tannins reported in this study for *Stylosanthes scabra* accessions, however, was far below the critical value of 90 g kg^{-1} DM, above which dry matter intake and digestion are depressed in goats (Nastis and Malachek 1981). Similarly, the total condensed tannins value recorded in this study was far lower than the critical value of 50 g kg^{-1} DM, above which it becomes detrimental to animals (Barry and McNabb 1999). Therefore, the total tannin and condensed tannin concentration of *Stylosanthes scabra* forages reported in this study are within a safe range for animal consumption. Consequently, when forages of *Stylosanthes scabra* are fed to animals it should not negatively affect intake and digestibility of nutrients.

Accessions of *Stylosanthes scabra* did not differ in terms of gas production at various times of incubation. Gas production is a reflection of fermentation, which is influenced by a number of factors, such as fibre content, presence of secondary plant metabolites, and potency of rumen fluid (Babayemi et al. 2004). The trend of gas production illustrated in Figure 3.1 shows that the forage material of *Stylosanthes scabra* accessions was highly fermentable in the early hours (2 to 12 h) of incubation. However, as incubation times advanced, the rate of fermentation slowed down. The relatively high mean gas volume (98 ml 400 mg^{-1}) recorded in the present study for these accessions could be associated with the high CP content, low NDF and anti-nutritional factors (Table 3.1). According to Njidda and Nasiru (2010), a high level of rumen degradable protein in feed enhances microbial multiplication, which in turn determines the extent of fermentation. The tannin concentration reported in this study for each accession is very low to negatively affect fermentation, in contrast with a minimum value of 20 g kg^{-1} DM that cause a minimal effect on fermentation (Getachew et al. 2008). Thus, *Stylosanthes scabra* forages were highly digestible and this is in line with the high EGP and mean gas production rate (c) constant recorded in Tables 3.2 and 3.4. According to Abegunde et al. (2011), the rate at which feed and its constituents are degraded in the rumen is as important as the extent of digestion. Values of the rate of fermentation recorded in this study are within the range of values recorded by Ajayi and Babayemi (2008) for *Stylosanthes guianensis*.

In vitro gas production provides a useful basis from which energy value (e.g. metabolizable energy), organic matter digestibility and short chain fatty acid can be predicted (Getachew et al. 2004). Amount of organic matter substrate fermented and short chain fatty acid produced upon fermentation are influenced by protein, fibre and phenolic contents of the forage (Babayemi et al. 2009). Metabolizable energy values calculated in this study were within a range of values required to meet the daily maintenance and production of ruminants (Table 3.3). According to Ajayi et al. (2010), metabolizable energy value between 8.8 MJ kg^{-1} DM to 13.4 MJ kg^{-1} DM is adequate to meet the daily maintenance and production requirements of ruminants. The ME value reported in this study were far above the 2.32 MJ kg^{-1} DM recommended for a confined goats with body weight of 10 kg (Steele 1996), and slightly above 8.37 MJ kg^{-1} DM value recommended for dry ewes (NRC 1985). The metabolizable energy values reported for *Stylosanthes scabra* in the present study were also higher than for *Avena sativa* L. reported by Kafilzadeh and Heidary (2013), but within the range of values recorded by Abas et al. (2005) for alfalfa hay. According to EL-Waziry (2007) feed potential is assessed by evaluating the CP, cell wall components, IVOMD and ME contents of the feed material. The MEY indicate the potential metabolizable energy yield of the accessions which is also critical important if these accessions can be used in feed formulation. According to Neimeläinen et al. (2001) metabolizable energy yield is batter unity than dry matter yield in comparing crop productivity, however, the utilization of that crop as feed depends on ME concentration of crop among others. Generally these accessions had relatively good nutritive value, which shows their potential and suitability as a supplementary feed source for ruminant fed poor-quality forage diets.

3.5. Conclusion

This study demonstrated that *Stylosanthes scabra* forages contain relatively high levels of protein with low levels of anti-nutritional factors and fibre content (NDF). Thus, they had good fermentation attributes. In other words, forage materials from *Stylosanthes scabra* accessions grown in Pretoria are suitable as protein supplements for low-quality forages, and might provide enough energy as a roughage source. Although most accessions were of reasonable quality, accessions such as 140, 9281, 11595 and 11604 are recommended for further use as forage supplementation because of their metabolizable energy yield.

CHAPTER 4

Relative preference for, palatability and intake of *Stylosanthes scabra* accessions adapted in Pretoria

This chapter was published in-parts in:

Thamsanqa DE Mpanza, Abubeker Hassen, Edward F Donkin and William T Nzuza .2014. Relative preference for, palatability and intake of *Stylosanthes scabra* accessions adapted in Pretoria. *Tropical Grasslands – Forrajes Tropicales*, 2: 92−93. www.tropicalgrasslands.info

Abstract

Preference for palatability and intake of five selected *Stylosanthes scabra* accessions were evaluated using Saanen goats (48.7 kg ± 2.78). Five fresh branches of accessions were mounted on a foraging board in a cafeteria system, and offered to each goat for 30 minutes per day. Goat behaviour in terms of frequent visit and time spent on browsing each accession was recorded. Accessions were analysed for chemical composition and *in vitro* gas production. Significant variations ($P < 0.05$) were observed in ash concentration and total extractable phenols. There was significant variation ($P < 0.05$) in animal behaviour, dry matter intake and preference index. Preference rankings of these accessions were in the following order: 11604 > 11595 > 11252 > 9281 > 11255. Although accessions were acceptable to Saanen goats, 11252, 11595 and 11604 were the most preferred. Thus, further research is necessary to determine how these accessions would influence digestibility and animal performance when used as a roughage source or protein supplement for goats fed on poor-quality forage.

Keywords: Acceptability, Browsing behaviour, Forage legumes, Goats, Tannins

4.1. Introduction

Poor nutrition and limited feed supply for livestock led to weight loss, low reproductive potential and illness, due to increased susceptibility to parasites and disease (Kanani et al. 2006; Ajayi et al. 2007; Birteed et al. 2011). To overcome these problems, the quantity and quality of feed materials for animals must be improved. The introduction of forage legumes as potential forage sources could be an option to improve feed quality. This is because forage legume supplementation would boost feed quality by providing protein, vitamins and minerals to poor-quality feed materials (Bamikole et al. 2004), and the product is cheaper than concentrate supplementation.

In earlier studies, *Stylosanthes scabra* accessions were evaluated for adaptability and performance in the subtropical climate of Pretoria, Gauteng , South Africa, and were found to be adaptable and productive (chapter 2). Forage material of these accessions was found to be of good quality when judged for chemical composition and ruminal fermentation characteristics (chapter 3). However, the palatability of these accessions to animals is not known. Therefore, among the adapted and productive accessions, five were selected based on their dry matter yield potential as well as their chemical composition, with the aim of determining their acceptability to and preference for Saanen goats.

4.2. Materials and methods

4.2.1. Experimental setup

Five Saanen goats (48.7 kg ± 2.78) were used in the study and were individually housed in eight square metre pens. Permission to use animals for the study was granted by the Animal Ethics Committee of the University of Pretoria (reference no EC085-12). Five accessions of *Stylosanthes scabra* were selected from fifteen accessions that had been evaluated earlier for adaptability and performance in Pretoria. These accessions were 9281, 11252, 11255, 11595 and 11604, according to International Livestock Research Institute (ILRI) classification numbers. Five branches of different *Stylosanthes scabra* forage accessions were harvested daily and mounted on a foraging board in a cafeteria system, thus permitting free access of animals to the forages of their choice (Figure 4.1). The *Stylosanthes scabra* forages were exposed to one goat at a time for 30 minutes per day in the morning. The goats were offered alfalfa hay as a basal diet after the trial and it was removed at 18:00 for overnight starving.

Figure 4.1: a) *Stylosanthes scabra* forages mounted on foraging board, and b) goat eating *Stylosanthes scabra* forages

4.2.2. Data collection and analysis

After ten days of adaptation period, data were collected for five consecutive days. Individual animals were observed throughout from a five metre distance. At this range, it was expected that there would not be any interference with the animal, and behaviours such as frequency of visit and time (seconds) spent per visit for each accession were recorded. Each accession was weighed before and after its exposure to the animal and the weight was converted to dry matter (DM) by determining the moisture content of the feed after being removed. Daily relative preference index (RPI) was calculated as described by Larbi et al. (1993). The accessions were ranked according to dry matter intake and RPI values, and the one with highest intake and RPI was regarded as being the most preferred.

Subsamples of each accession were taken, dried in the oven set to 60°C for 72 hours, milled through a 1 mm sieve, and used for chemical composition analysis. Dry matter (DM) content was determined by drying the sample at 105°C for 16 hours, and then the sample was ignited in a muffle furnace at 550°C for 4 hours for ash determination (AOAC 2000). Nitrogen content was measured by Dumas technique, from which crude protein (CP) was calculated as N x 6.25 (AOAC 2000). An Ankom fibre analyser was used to determine neutral detergent fibre (NDF) content according to Van Soest et al. (1991). *In vitro* organic matter digestibility (IVOMD) was determined according to Tilley and Terry (1963) procedure as modified by Engels and Van der Merwe (1967). Determination of phenolic compound, which include total phenols (TP) and total tannins (TT) were conducted according to the procedure of Makkar (2003a).

About 400 mg of dried and milled samples were incubated *in vitro* with rumen fluid in serum bottles of 120 ml in duplicates in two separate runs following the procedure of Menke and Steingass (1988). (Details on rumen fluid collection and preparation of solution are described in section 3.2.4 of chapter 3). Aliquots (40 ml), consisting of 15 ml rumen fluid and 25 ml buffer solution, were dispensed into serum bottles, which had previously been warmed at 39°C and flushed with carbon dioxide gas (CO_2). Serum bottles were sealed with butyl rubber stoppers and aluminium crimp seals, and incubated in a continuously shaking incubator at a rate of 120 revolutions per minute (rpm) set to 39°C. Gas volume (GV) was recorded at 2, 4, 8, 24 and 48 hours post incubation by inserting a 23 gauge (0.6 mm) needle attached to a pressure transducer that was connected to a visual display. The average volume of gas produced from blanks (that is, from buffered rumen fluid without substrate) was subtracted from the volume of gas produced from the incubated samples. The net gas volume produced was then plotted against incubation time to fit the equation of Ørskov and McDonald (1979) as described below.

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

where *GV* is the total gas volume (ml 400 mg^{-1} DM) at time t, *b* is the insoluble but slowly fermentable fraction (ml) and *c* is the rate constant of gas production hour.

Data on frequent animal visit to an individual plant and time spent on each plant were log transformed, but untransformed data were used for the means. Data were subjected to analysis of variance for a balanced change over design with five periods using the General Linear Models Procedure of SAS (2002). Where F- ratio showed a significant difference ($P < 0.05$), Duncan multiple range test was used to separate means.

4.3. Results

4.3.1. Chemical composition and *in vitro* organic matter digestibility

The chemical composition, phenolic compounds and organic matter digestibility of the five *Stylosanthes scabra* accessions are presented in Table 4.1. These accessions generally were significantly different ($P < 0.05$) in terms of ash, NDF, TP, TT and IVOMD, but not significant ($P > 0.05$) in DM and CP concentration. These five accessions were highly digestible with the *in vitro* organic matter digestibility percentage ranging from 68% to 74%.

Table 4.1: Forage chemical composition, phenolic compounds and *in vitro* organic matter digestibility of accessions

Accession (ILRI[28] no)	DM (%)	Chemical composition (g kg^{-1} DM)					IVOMD (% DM)
		Ash	CP	NDF	TP	TT	
9281	92.3	96.9^{a}	184.1	418.7^{b}	1.9^{e}	1.2^{b}	74.2^{a}
11252	91.8	93.6^{a}	177.5	439.9^{ab}	2.6^{b}	1.0^{c}	71.8^{ab}
11255	92.3	80.1^{b}	182.8	334.3^{c}	2.9^{a}	1.1^{b}	71.6^{abc}
11595	92.4	94.4^{a}	185.4	483.4^{ab}	2.5^{c}	1.6^{a}	69.8^{bc}
11604	92.3	102.7^{a}	181.7	499.3^{a}	2.0^{d}	0.9^{d}	68.4^{c}
SEM	0.41	3.44	4.96	21.2	0.02	0.03	0.97

Means within a column with different letters in superscript differ significantly ($P < 0.05$)

DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; TP: total phenols; IVOMD: *in vitro* organic matter digestibility

SEM: Standard error of mean

4.3.2. *In vitro* gas production

The *in vitro* gas production characteristics of the five *Stylosanthes scabra* accessions are presented in Table 4.2. There were no significant ($P > 0.05$) differences in gas production and gas production kinetics among *Stylosanthes scabra* accessions throughout the incubation period.

[28] International Livestock Research Institute

Table 4.2: *In vitro* gas production (ml 400 mg^{-1} DM) and gas production kinetics of five *Stylosanthes scabra* accessions

Accession (ILRI[29] no)	Incubation hours					PGP (ml 400 mg^{-1})	c (h^{-1})
	2 h	4 h	8 h	24 h	48 h		
9281	16.1	29.8	53.2	80.0	91.1	91.4	0.107
11252	12.3	23.7	48.4	75.2	88.4	91.4	0.096
11255	11.8	23.9	48.6	76.2	89.6	93.0	0.097
11595	16.1	29.4	53.2	78.9	89.4	89.8	0.111
11604	16.8	30.6	53.4	78.7	89.5	88.7	0.110
SEM	2.44	2.99	2.38	2.03	1.79	4.11	0.0088

PGP: potential gas production; c: rate of gas production

SEM, Standard error of mean

4.3.3. Animal behaviour

Table 4.3 shows the frequency of animal visits to *Stylosanthes scabra* accessions. Generally there were significant differences ($P < 0.05$) in the frequency of animal visits with the following ranking order: 11604 > 11595 > 11252 > 9281 > 11255. In addition, daily frequent animal visits to accessions varied significantly ($P < 0.05$), except on day five (Table 4.3). Accession 11604 was the most frequently visited except on day four and five. On day four and five, the most frequently visited accessions were 11252 and 11255, respectively, although the difference observed on day five was not significant.

[29] International Livestock Research Institute

Table 4.3: Frequency of animal visits to *Stylosanthes scabra* accessions

Accession (ILRI[30] no)	Days					Mean
	1	2	3	4	5	
9281	34.6[bc]	11.2[c]	19.6[c]	20.6[ab]	38.4	24.9[bc]
11252	12.8[c]	28.4[bc]	19.2[c]	49.6[a]	39.4	29.9[bc]
11255	13.4[c]	12.8[c]	17.6[c]	12.8[b]	41.8	19.7[c]
11595	40.2[ab]	43.4[ab]	48.8[b]	25.2[ab]	31.2	37.8[ab]
11604	68.6[a]	76.2[a]	75.2[a]	32.8[ab]	39.0	58.4[a]
SEM	0.43	0.40	0.36	0.56	0.37	0.21

Means within a column with different letters in superscript differ significantly ($P < 0.05$)
SEM, Standard error of mean

Table 4.4 shows the mean time spent (seconds) per visit by Saanen goats browsing five accessions. There was a significant difference (P < 0.05) in daily time spent on browsing accessions except on day five, when there was no significant difference ($P > 0.05$). Generally, goats spent most time in browsing accession 11604 and least time on accession 11255. On day four and five, the goats spent most of the time browsing accessions 11252 and 11255, respectively, although the difference observed on day five was not significant.

Table 4.4: Daily mean times (seconds) spent per 30 minutes by goats browsing *Stylosanthes scabra* accessions

Accession (ILRI no)	Days					Mean
	1	2	3	4	5	
9281	386[ab]	106[c]	196[c]	248[ab]	292	245.6[bc]
11252	124[b]	284[bc]	192[c]	482[a]	386	293.6[bc]
11255	126[b]	122[c]	174[c]	110[b]	410	188.4[c]
11595	386[ab]	432[b]	488[b]	242[ab]	310	371.6[b]
11604	670[a]	754[a]	750[a]	314[ab]	390	575.6[a]
SEM	0.62	0.57	0.38	0.77	0.55	0.28

Means within a column with different letters in superscript differ significantly ($P < 0.05$)
SEM, Standard error of mean

[30] International Livestock Research Institute

The intake of five *Stylosanthes scabra* accessions during observation and their relative preference indices are presented in Table 4.5. High significant variation (P < 0.05) was observed among these accessions in terms of average daily forage intake. The same trend was observed in relative preference index (RPI). Thus accession 11604 was ranked the most consumed and preferred accession by Saanen goats.

Table 4.5: Relative intake, preference and ranking of five *Stylosanthes scabra* accessions forages fed to Saanen goats

Accession (ILRI[31] no)	Average daily intake (g day^{-1} DM)	Relative preference index (%)	Preference ranking
9281	63.37^{b}	40.49^{b}	4
11252	95.27^{ab}	60.90^{ab}	3
11255	52.20^{b}	35.53^{b}	5
11595	112.20^{ab}	67.04^{ab}	2
11604	139.27^{a}	84.44^{a}	1
SEM	0.243	0.247	

Means within a column with different letters in superscript differ significantly (P < 0.05)
SEM, Standard error of mean

4.4. Discussion

Generally, the crude protein concentration of *Stylosanthes scabra* forages used in this study was above the critical threshold level of 7%. This level is the minimum requirement that supports normal intake and rumen functioning (Ikhimioya 2008). All five accessions used in the study were regarded as having intermediate to high nutritional quality, because the CP values were above 17% (Table 4.1). The neutral detergent fibre was below the upper limit of 60% (Meissner et al. 1991), which is known to restrict the intake of forage by animals. Similarly the tannin concentrations of these forages were lower than the critical level of 9%, which is known to affect intake and digestion in goats (Nastis and Malachek 1981). This indicates their potential use as supplements to a basal diet of poor-quality feed resources in order to improve digestibility by the animal.

[31] International Livestock Research Institute

The high gas production observed in the present study from the five incubated accessions (Table 4.2) indicates the digestibility of the forage materials. Gas production is a reflection of fermentation, and is influenced by fibre content, presence of plant secondary metabolites, and potency of rumen fluid (Njidda and Nasiru 2010). These accessions included in this study had relatively low fibre (NDF) and tannins concentrations with high crude protein concentration, and this might have contributed to the observed high gas production (Table 4.2). According to Njidda and Nasiru (2010), high protein in feed enhances microbial multiplication, and may influence the extent of fermentation. This is in accordance with the high rate of gas production that was recorded in the present study (Table 4.2). According to Abegunde et al. (2011), the rates at which feed or its constituents are degraded in the rumen are as important as the extent of digestion. Therefore, the high rate of gas production concurs with high *in vitro* organic matter digestibility (Table 4.1). This could be because more organic matter is available for fermentation.

The frequency of visits of an animal to each accession corresponds with the time spent by the animal browsing that accession (Table 4.3 and Table 4.4). Therefore, all accessions were acceptable to Saanen goats. However, acceptability varied significantly. The basis of this difference is not clear, because nutritionally these plants were more or less comparable in nutritive value (Table 4.1). The accession 11595 was the second most frequently visited and browsed tended to have higher total extractable tannins. On the other hand, accession 11604 appeared to have lower *in vitro* organic matter digestibility while it contained the lowest total extractable tannins and the higher NDF (Table 4.1). However, according to Osuga et al. (2008), the nutritional significance of tannins depends on their biological activity. Thus, the trend that is observed in Table 4.3 and Table 4.4 could be possibly attributed to differences in biological activity of the tannins among the accessions. Unfortunately this was not considered in this study.

Generally all these accessions were browsed by Saanen goats, and thus were acceptable and palatable to animals. The dry matter intake and relative preference index indicated that accessions 11252, 11595 and 11604 were the most preferred while accessions 9281 and 11255 were the least preferred. However, accession 11604 tended to be the highest in terms of dry matter intake and relative preference index whereas accession 11255 tended to be the lowest. Results that are presented in Table 4.3 show that accession 11604 was the most frequently visited. Hence, this accession was the most preferred among others (Table 4.4). However, in Kalioa et al. (2006) most frequent visited plant was not the most preferred plant.

4.5. Conclusion

Chemical composition, phenolic compound, *in vitro* organic matter digestibility and *in vitro* gas production showed the potential nutritional value of *Sthylosanthes scabra* accessions, but the feeding value depends on acceptability and preference by animals when used as roughage source and/or supplemented to low-quality feed. This study showed that all five *Stylosanthes scabra* accessions were acceptable and palatable to Saanen goats. However, three accessions were highly preferred, namely 11604, 11595 and 11252. Accession 11604 tended to be the highest among the three. Thus, further study is required to determine how these preferred accessions could influence digestibility and animal performance when used as roughage source or protein supplement to goats fed on poor-quality forage.

CHAPTER 5

In vitro ruminal fermentation and digestibility of *Eragrostis* hay supplemented with forages of selected accessions of *Stylosanthes scabra*

Abstract

This study evaluated the effects of supplementing poor-quality *Eragrostis trichophora* grass (hereafter grass hay) with five selected *Stylosanthes scabra* accession forages on *in vitro* fermentation and gas production. Grass hay was bought from the local market and *Stylosanthes scabra* accessions were harvested at 100% flowering stage from the field trial at the University of Pretoria. Grass hay and accessions were oven dried, milled and analyzed for chemical composition. *In vitro* gas production of grass hay, accessions and supplemented grass hay were recorded with a semi-automated system. Generally, supplementing poor-quality grass hay with *Stylosanthes scabra* accessions improved the fermentation of grass hay. However, only accession 11604 at thirty per cent supplementation level showed a positive associative effect with grass hay; hence it influenced NDF degradability. Consequently, this accession is recommended for future use as a forage supplement for poor-quality basal diet. However, the feeding value of this accession must be determined in terms of dry matter intake, digestibility and animal performance.

Keywords: Associative effect; Degradability, Poor-quality forage, Vog

5.1. Introduction

Tropical grass species are important and cost-effective feed resources, which play a vital role in livestock production under smallholder subsistence farmers. However, high fluctuations in the quantity and quality of feed affect livestock performance negatively (Ajayi et al. 2005; Anele et al. 2011). Forage quality varies over the growing season and declines as the plant matures (Licitra et al. 1997). The increase in fibre content subsequently leads to poor digestibility, low digesta flow, and thus low feed intake (Valarini and Possenti 2006; Moyo et al. 2012). This cheaper feed resource may lead to extra baggage of methane production due to high fibre content, particularly at maturity (Migwi et al. 2013). Enteric fermentation of fibres in the rumen leads to 4 - 12% gross energy loss as methane (Alford et al. 2006).

Palatability and intake of forage materials are influenced by protein, metabolizable energy and fibre level (Njidda and Nasiru 2010; Kordestany and Ebne-Abbasi 2012). Therefore, the supplementation of rumen degradable nitrogen and fermentable energy is essential to improve palatability, intake and rumen fermentation that would improve animal production (Ondiek et al. 1999). According to Hariadi and Santoso (2010), low levels of protein content (below 7%) restrict rumen microbial activity and thus reduce feed digestibility, which is true of most tropical feed resources. On the other hand, optimum to high levels of protein in feed enhances microbial multiplication, which in turn determines the extent of *in vitro* fermentation of feed material (Njidda and Nasiru 2010).

Concentrate supplementation is not practically achievable by smallholder susbsistence farmers in sub-Saharan Africa owing to the high costs involved. Therefore, smallholder susbsistence farmers should regard forage legumes as forage and protein sources. Because of the crude protein content (above 17%) and high fermentability of *Stylosanthes scabra* forages grown in Pretoria, South Africa (Mpanza et al. 2014), these could be utilized as protein sources to augment poor-quality feed. Supplementing this forage to poor-quality grass hay would improve the protein content and probably enhance fermentation, depending on other factors such as fibre content and anti-nutritional factors. The objective of this study was to determine effects of supplementing *Stylosanthes scabra* accession forages on *in vitro* ruminal degradation of neutral detergent fibre and gas production characteristics of poor-quality *Eragrostis trichophora* grass hay.

5.2. Materials and methods

5.2.1. Plant materials and chemical composition analysis

Five *Stylosanthes scabra* accessions (International Livestock Research Institute (ILRI) accession numbers 9281, 11252, 11255, 11595 and 11604) were harvested at 5 cm above the ground from a field trial that has been going on for the past three years at Hatfield Experimental Farm, University of Pretoria. *Stylosanthes scabra* accessions were harvested at 100% flowering stage of growth. The harvested samples were oven dried at 60°C for 72 hours. *Eragrostis trichophora* hay (hereafter grass hay) was purchased from the local market. Forage materials (*Stylosanthes scabra* and grass hay) were ground to pass through a 1 mm sieve in a Willy mill, and stored in airtight plastic containers for subsequent analysis of chemical composition and *in vitro* gas production studies.

Stylosanthes scabra and grass hay were analysed separately for chemical composition following standard procedures. Dry matter (DM) content was determined by drying the sample at 105°C for 16 hours. Then the samples were ignited in a muffle furnace at 550°C for 4 hours for ash determination, following the procedure of AOAC (2000). Nitrogen content was measured by Duma's technique, from which crude protein (CP) was calculated as N x 6.25 (AOAC, 2000). The method that is described by Mertens (2002) was used to determine the NDF content, exclusive of ash, sodium sulphite and heat-stable amylase were used in NDF determination. Acid detergent fibre (ADF) was determined by the method described by Raffrenato and Van Amburgh (2011) and acid detergent lignin (ADL) was determined as described by (Goering and Van Soest 1970).

5.2.2. Treatment setup

Sixteen treatments were formulated for the *in vitro* ruminal fermentation, and degradability studies. These were i) sole grass hay (unsupplemented grass), ii) five individual *Stylosanthes scabra* accession forages, and iii) ten with grass hay supplemented with two levels (15% and 30%) of forage from each accession.

5.2.3. Rumen fluid collection and buffer preparation

Two South African Merino sheep (males) fitted with permanent rumen cannula were used as rumen fluid donors. Donor sheep were fed alfalfa hay daily *ad libitum*, as basal forage with free access to water. Rumen fluid of approximately 450 ml per animal was collected before morning feeding. Both fluids were mixed and strained through four layers of cheese-cloth into pre-warmed thermos flasks and transported to the laboratory within 20 minutes. On arrival the rumen fluid was purged with carbon dioxide (CO_2) to maintain the anaerobic conditions.

The buffer, micro and macro mineral solutions were prepared as described by Goering and van Soest (1970). However, the macro-mineral solution was modified slightly by replacing magnesium sulphate ($MgSO_4.7H_2O$) with magnesium chlorite ($MgCl_2.6H_2O$) to reduce sulphate in the medium as recommended by Mould et al. (2005). These solutions were prepared a day before hand, and kept in separate containers in a 4°C fridge. Resazurin solution was prepared by dissolving 100 mg resazurin in 100 ml distilled water, and kept in a dark container in a fridge because of light sensitivity. Before the start of the experiment in the morning, an appropriate amount of distilled water was measured and a small portion was used to dissolve the tryptone. All solutions were measured as described by Goering and Van Soest (1970), and mixed together with distilled water in conical flasks and blended with a magnetic stir for few minutes, placed in a water bath (39°C), and purged with CO_2 until light pinkish in colour. Appropriate amounts of L-cysteine hydrochloride and sodium sulphite were weighed and added directly to the rest of the solution and flushed with CO_2 until the pinkish colour turned colourless, which indicated the complete removal of oxygen.

5.2.4. Incubation of test feed and gas measurement

Prior to incubation, each pure treatments (i.e. unsupplemented grass hay, five *Stylosanthes scabra* accessions and grass hay supplemented with accession at two levels each) were weighed (≈0.4 g) into 120 ml serum bottles in three replicates per run. Aliquots (40 ml) consisting of 15 ml rumen fluid and 25 ml buffer solution, were dispensed into the serum bottles previously warmed at 39°C and flushed with CO_2. Serum bottles were each sealed with a butyl rubber stopper and aluminium crimp seals. To release gas that might have built up at the start, small needles (0.6 mm) were inserted in rubber approximately for 5 second, and serum bottles were incubated in a continuous rotary shaking incubator at a rate of 120 rotations per minute (rpm), set to 39^{o}C with gas volume (GV) recorded at 2, 4, 8, 12, 24, 48 and 72 hours post incubation. Gas volume was measured by inserting a 23 gauge (0.6 mm) needle that was attached to a pressure transducer, which was connected to a data tracker with a visual display. Two runs were conducted in different weeks with three replicates per run.

For each run three blanks were included, which contained the buffered rumen fluid without substrate. The average volume of gas produced from blanks was subtracted from the volume of gas produced from the incubated treatments. The gas volume was plotted against incubation time, and Ørskov and McDonald (1979) equation was used to estimate gas production characteristics.

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

where *GV* is the total gas volume (ml 400 mg^{-1} DM) at time t; *a* is the immediately fermentable fraction (ml); *b* is the insoluble, but slowly fermentable fraction (ml); and *c* is the rate constant of gas production per hour.

5.2.5. Thirty-hour neutral detergent fibre degradation

To determine the 30h *in vitro* neutral detergent fibre degradability, the method proposed by Goering and Van Soest (1970) was used. Rumen fluid was collected from two South African Merino sheep donor. Approximately 500 mg of each treatment was weighed in duplicate per run, and transferred into 120 ml serum bottles. There were two runs, each with two replicates and two blank samples, which contained only buffered rumen fluid without substrate. Serum bottles were filled with 40 ml buffered solution, which consisted of 15 ml rumen fluid and 25 ml buffer solution. After which bottles were closed with rubber stoppers, and incubated in the *in vitro* water bath set at 39°C for 30 hours under constant CO_2 positive pressure. After 30 hours of incubation, each treatment was used to determine NDF according to the method of Mertens (2002).

5.2.6. Calculations and statistical analysis

Effective gas production (EGP) was estimated using Ørskov and McDonald (1979) equation, assuming the flow rate constant (k) of 0.05 h^{-1}. EGP = b*c / (k + c). To determine the fermentation and digestion kinetics of gas production, the (NLIN) procedure of SAS (2002) was used to fit the non-linear regression model to *in vitro* incubation data. To determine NDF degradability, NDF after incubation was subtracted from the NDF of samples without incubation. The procedure of Niderkom et al. (2011) was used to determine the associative effects between grass hay and *Stylosanthes scabra* accessions at different levels of supplementation on fermentation. Metabolizable energy (ME) was calculated with the equation of Menke et al. (1979), while short chain fatty acid (SCFA), and organic matter digestibility (OMD) were calculated with the equations of Menke and Steingass (1988). Gas production values at 24 h were adjusted to 200 mg in order to fit the equations when calculating ME, SCFA and OMD.

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 2.2 + 0.136GV + 0.057CP + 0.002859CP^2$$

$$\text{OMD (\%)} = 14.88 + 0.889GV + 0.45CP + 0.651XA$$

$$\text{SCFA (}\mu\text{mol g}^{-1}\text{ DM)} = 0.0239GV - 0.0601$$

Where GV is net gas volume at 24 hours; CP is the crude protein (%); and XA is Ash (%)

Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS 2002). Where significant differences occurred, the means were separated by Duncan's new multiple range test (DMRT).

5.3. RESULTS

5.3.1. Chemical composition of grass hay and *Stylosanthes scabra* accessions

Table 5.1 shows the chemical composition of grass hay and *Stylosanthes scabra* accessions. Crude protein concentration of grass hay was very low (34 g kg^{-1} DM), while fibre concentrations were very high (NDF 813.4 g kg^{-1} DM and ADF 475.8 g kg^{-1} DM). The value of neutral detergent fibre in grass hay was more than the values recorded for *Stylosanthes scabra* accessions, while CP concentration of *Stylosanthes scabra* accessions ranged from 178 g kg^{-1} DM to 185 g kg^{-1} DM. These values are more than 5 times the value found in grass hay. Accession 11595 contained high acid detergent lignin (ADL) as compared with other accessions. *Stylosanthes scabra* accessions contained relatively low total tannins with the lowest value of 0.9 g kg^{-1} DM recorded for accession 11604 and the highest value of 1.6 g kg^{-1} DM for accession 11595, while tannin was not detected in grass hay.

Table 5.1: Chemical composition of *Eragrostis trichophora* grass hay and *Stylosanthes scabra* accessions

Treatments	DM (%)	Composition (g kg^{-1} DM)					
		CP	NDF	ADF	ADL	Ash	TT
Grass hay	93.7	34.3	813.4	475.8	65.4	33.1	nd
9281	92.9	184.1	330.8	282.9	42.4	96.9	1.22
11252	92.2	177.5	349.7	300.6	42.6	93.6	1.02
11255	92.3	182.8	349.7	292.9	43.5	80.1	1.14
11595	92.4	185.4	300.3	273.9	63.4	94.4	1.64
11604	92.5	181.7	347.1	316.4	41.3	102.7	0.87

DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; TT: Total tannins.

nd, not detected.

5.3.2. *In vitro* gas production of grass hay and *Stylosanthes scabra* accessions

Table 5.2 shows gas production due to *in vitro* fermentation of poor-quality grass hay and *Stylosanthes scabra* accessions. Generally, *Stylosanthes scabra* accessions produced significantly ($P < 0.05$) higher gas volume than grass hay throughout the incubation period. There was a significant ($P < 0.05$) difference in the volume of gas production among the accessions during the first four hours of incubation. However, as the incubation time advanced the difference was not significant ($P > 0.05$). The volume of gas production from the accessions in the first two hours of incubation was three times higher than that of grass hay, and the difference decreased as incubation times advanced. However, in the first twelve hours, accessions produced more than double the amount that was recorded in grass hay.

Table 5.2: *In vitro* gas production (ml 400 mg^{-1} DM) of poor-quality grass hay and *Stylosanthes scabra* accessions

Treatments	Incubation period							
	2h	4h	8h	12h	24h	30h	48h	72h
Grass hay	5.5^{d}	11.3^{c}	18.0^{b}	26.5^{b}	44.1^{b}	56.4^{b}	75.2^{b}	86.5^{b}
9281	19.8^{a}	34.9^{a}	49.5^{a}	60.5^{a}	74.2^{a}	83.6^{a}	94.4^{a}	100.5^{a}
11252	17.0^{bc}	32.7^{b}	47.8^{a}	59.0^{a}	72.3^{a}	81.7^{a}	92.5^{a}	99.3^{a}
11255	17.3^{bc}	33.7^{ab}	48.2^{a}	59.2^{a}	72.5^{a}	81.6^{a}	92.1^{a}	98.5^{a}
11595	17.8^{b}	33.6^{ab}	49.6^{a}	60.6^{a}	73.5^{a}	82.7^{a}	93.5^{a}	100.3^{a}
11604	16.9^{c}	32.2^{b}	48.3^{a}	59.7^{a}	73.3^{a}	82.1^{a}	92.3^{a}	98.4^{a}
SEM	0.28	0.49	0.76	0.87	0.82	0.97	1.04	1.22

Means within a column with different letters in superscript differ significantly ($P < 0.05$).
SEM, Standard error of mean

5.3.3. Effect of supplementing poor-quality grass hay with *Stylosanthes scabra* accessions

The effects of the level of *Stylosanthes scabra* accession supplementation to the poor-quality grass hay are shown on Table 5.3. Supplementing grass hay with 15% forage of *Stylosanthes scabra* accessions significantly ($P < 0.05$) improved grass hay fermentation up to 48 h of incubation compared with grass hay incubated alone. However, supplementing grass hay with 15% of accession 11604 only showed a significant ($P < 0.05$) improvement in fermentation from 4 to 12h of incubation. There was a significant ($P < 0.05$) difference between grass hay in terms of gas production when supplemented by accessions at 15% level. Further increase in the level of supplementation of the accession to 30% led to a significant ($P < 0.05$) improvement in grass hay fermentation. There was a significant ($P < 0.05$) difference between accessions in terms of gas volume produced from grass hay supplemented by accessions at 30% level. Increasing supplementation level of accession 11604 to 30% led to the highest improvement of grass hay fermentation as compared with other accessions at the same level of supplementation.

Table 5.3: Effects of supplementing poor-quality grass hay with *Stylosanthes scabra* accessions on *in vitro* gas production (ml 400 mg^{-1} DM)

Treatments	Incubation period							
	2h	4h	8h	12h	24h	30h	48h	72h
	15% supplementation level							
Grass hay	5.5^{d}	11.3^{d}	18.0^{c}	26.5^{c}	44.1^{c}	56.4^{b}	75.2^{b}	86.5^{a}
Grass hay+15% 9281	7.3^{bc}	14.6^{ab}	21.8^{a}	30.5^{a}	47.4^{ab}	59.0^{a}	76.7^{ab}	86.9^{a}
Grass hay+15%11252	7.8^{ab}	14.8^{ab}	22.2^{a}	30.9^{a}	47.3^{ab}	58.9^{a}	76.9^{ab}	87.3^{a}
Grass hay+15%11255	7.9^{a}	15.2^{a}	22.6^{a}	31.3^{a}	48.4^{a}	60.1^{a}	78.0^{a}	88.6^{a}
Grass hay+15%11595	7.2^{c}	14.4^{b}	21.9^{a}	30.0^{a}	46.6^{b}	58.4^{a}	76.8^{ab}	87.5^{a}
Grass hay+15%11604	5.5^{d}	12.8^{c}	19.9^{b}	28.1^{b}	44.6^{c}	56.4^{b}	74.8^{b}	85.6^{a}
SEM	0.17	0.20	0.34	0.53	0.41	0.56	0.77	1.01
	30% supplementation level							
Grass hay	5.5^{e}	11.3^{d}	18.0^{d}	26.5^{d}	44.1^{d}	56.4^{d}	75.2^{cd}	86.5^{b}
Grass hay+30% 9281	9.9^{c}	18.2^{b}	26.4^{b}	34.9^{b}	50.8^{b}	61.9^{b}	77.5^{b}	87.1^{b}
Grass hay+30%11252	8.8^{d}	16.2^{c}	23.7^{c}	31.9^{c}	48.5^{c}	58.7^{c}	73.7^{d}	83.5^{c}
Grass hay+30%11255	9.3^{d}	16.8^{c}	24.3^{c}	32.6^{c}	48.7^{c}	58.9^{c}	75.3^{cd}	84.9^{bc}
Grass hay+30%11595	10.7^{b}	18.3^{b}	25.6^{b}	34.2^{b}	51.1^{b}	61.3^{b}	77.2^{bc}	86.6^{b}
Grass hay+30%11604	14.4^{a}	22.6^{a}	31.3^{a}	40.0^{a}	57.4^{a}	67.8^{a}	83.0^{a}	92.1^{a}
SEM	0.18	0.20	0.33	0.44	0.52	0.53	0.70	0.92

Means within a column with different letters in superscript differ significantly ($P < 0.05$)
SEM, standard error of mean

5.3.4. Gas production parameters of grass hay, *Stylosanthes scabra* accessions and supplemented grass hay

There were significant ($P < 0.05$) variations in gas production parameters between grass hay and *Stylosanthes scabra* accessions (Table 5.4). Increasing the supplementation level of accession 11604 from 15% to 30% led to a better fermentation of poor-quality grass hay (Table 5.4). Generally, the grass hay had a significantly ($P < 0.05$) high b-value (insoluble but slowly fermentable fraction) compared with *Stylosanthes scabra* accessions, while there was no significant ($P > 0.05$) difference in terms of b-value among the accessions. Supplementing grass hay with *Stylosanthes scabra* accessions at 15% level did not affect the insoluble but slowly fermentable fraction of grass hay, whereas at 30% supplementation level reduced significantly ($P < 0.05$) the insoluble but slowly fermentable fraction. The rate of fermentation (c-value) was significantly ($P < 0.05$) lower for grass hay compared with *Stylosanthes scabra* accessions. However, supplementing grass hay with *Stylosanthes scabra* accessions at 30% level significantly ($P < 0.05$) improved the fermentation rate (c-value). There was a significant ($P < 0.05$) difference between grass hay and accessions in terms of potential gas production and effective gas production.

Table 5.4: *In vitro* gas production kinetics of grass hay, *Stylosanthes scabra* accessions and supplemented grass hay

Treatments	b (ml)	c (h^{-1})	EGP(ml 400 mg^{-1})
Grass hay	106.4^{a}	0.024^{g}	34.4^{b}
9281	84.9^{b}	0.058^{b}	45.7^{a}
11252	85.8^{b}	0.059^{b}	46.5^{a}
11255	84.4^{b}	0.061^{b}	46.3^{a}
11595	85.7^{b}	0.061^{b}	47.1^{a}
11604	85.8^{b}	0.064^{a}	48.1^{a}
Grass hay+15% 9281	99.4^{a}	0.027^{ef}	34.7^{b}
Grass hay+15%11252	99.9^{a}	0.026^{efg}	34.3^{b}
Grass hay+15%11255	100.9^{a}	0.027^{ef}	35.1^{b}
Grass hay+15%11595	101.7^{a}	0.025^{fg}	34.3^{b}
Grass hay+15%11604	101.9^{a}	0.025^{fg}	34.0^{b}
Grass hay+30% 9281	91.8^{b}	0.030^{cd}	34.6^{b}
Grass hay+30%11252	89.8^{b}	0.029^{d}	33.5^{b}
Grass hay+30%11255	91.8^{b}	0.028^{de}	33.4^{b}
Grass hay+30%11595	91.5^{b}	0.029^{d}	34.0^{b}
Grass hay+30%11604	91.2^{b}	0.033^{c}	36.0^{b}
SEM	2.19	0.0008	1.02

Means within a column with different letters in superscript differ significantly ($P < 0.05$).

SEM, standard error of mean

5.3.5. Associative effects between grass hay and *Stylosanthes scabra* accession

In general, supplementing poor-quality grass hay with *Stylosanthes scabra* accessions highlighted the presence of significant ($P < 0.05$) associative effects between grass hay and some of the accessions (Table 5.5). Accession 11595 at supplementation level of 15% showed a negative associative effect with grass hay, while at 30% inclusion it showed a positive associative effect for only first four hours of incubation. On the other hand, accession 11604 when supplemented at 15% level showed a negative associative effect with grass hay. However, increasing supplementation level to 30% led to a positive associative effect throughout the incubation period.

Table 5.5: Associative effects (%) on gas production of grass hay supplemented with *Stylosanthes scabra* accessions

Treatments	Incubation periods							
	2h	4h	8h	12h	24h	30h	48h	72h
Grass hay+15% 9281	-3.5^{d}	-1.7^{d}	-3.9^{cd}	-3.6^{cd}	-2.4^{cd}	-2.5^{cd}	-1.7^{c}	-1.9^{cd}
Grass hay+15%11252	+8.4^{c}	+2.4^{bc}	-1.4^{bc}	-1.6^{bc}	-2.1^{c}	-2.2^{c}	-1.2^{bc}	-1.3^{bc}
Grass hay+15%11255	+9.7^{c}	+3.6^{b}	+0.4^{b}	-0.5^{b}	+0.2^{b}	-0.1^{b}	+0.4^{b}	+0.3^{b}
Grass hay+15%11595	-1.0^{d}	-1.2^{cd}	-3.8^{cd}	-5.2^{de}	-3.9^{cd}	-3.2^{cde}	-1.4^{c}	-1.2^{bc}
Grass hay+15%11604	-22.6^{e}	-11.4^{f}	-11.9^{e}	-10.9^{f}	-8.0^{e}	-6.4^{f}	-3.7^{d}	-3.1^{de}
Grass hay+30% 9281	+1.2^{d}	-1.0^{cd}	-3.9^{cd}	-5.0d^{e}	-4.4^{d}	-4.1^{de}	-4.2^{d}	-3.9^{e}
Grass hay+30%11252	-2.8^{d}	-9.3^{ef}	-12.0^{e}	-12.0^{f}	-7.7^{e}	-8.4^{g}	-8.2^{e}	-7.5^{f}
Grass hay+30%11255	+1.0^{d}	-7.4^{e}	-11.1^{e}	-11.1^{f}	-8.0^{e}	-8.5^{g}	-6.9^{e}	-6.5^{f}
Grass hay+30%11595	+16.8^{b}	+2.1b^{c}	-6.5^{d}	-6.7^{e}	-3.4^{cd}	-4.6^{e}	-4.3^{d}	-4.4^{e}
Grass hay+30%11604	+61.3^{a}	+28.9^{a}	+15.5^{a}	+9.8^{a}	+8.7^{a}	+5.7^{a}	+3.3^{a}	+2.3^{a}
SEM	2.22	1.20	1.19	0.91	0.71	0.58	0.60	0.58

Means within a column with different letters in superscript differ significantly ($P < 0.05$)

Negative or positive signs mean negative or positive effect of *Stylosanthes scabra* accessions on influencing fermentation of poor-quality grass hay as supplements.

SEM, standard error of mean

5.3.6. Feeding values of grass hay, *Stylosanthes scabra* accessions and supplemented grass hay

The dietary value of grass hay, *Stylosanthes scabra* accessions and supplemented grass hay were assessed by estimating the organic matter digestibility, metabolizable energy and short chain fatty acid of the incubated substrates (Table 5.6). Organic matter digestibility of grass hay was significantly (P < 0.05) lower in comparison with that of *Stylosanthes scabra* accessions. There was a significant (P < 0.05) variation of organic matter digestibility among these accessions. On the other hand, the inclusion of *Stylosanthes scabra* as supplements to poor-quality grass hay significantly (P < 0.05) improved organic matter digestibility of grass hay by 7.4% and 16.7%. Grass hay had a significantly (P < 0.05) low ME value compared with *Stylosanthes scabra* accessions. Metabolizable energy value among *Stylosanthes scabra* accessions varied significantly (P < 0.05). Generally, supplementing poor-quality grass hay with *Stylosanthes scabra* accessions improved ME concentration of grass hay by 7.0% and 16.7%. *Stylosanthes scabra* accessions showed significantly (P < 0.05) high SCFA in comparison with grass hay. Thus supplementing grass hay with *Stylosanthes scabra* accessions improved SCFA concentration by 7.8% and 19.6%.

Table 5.6: Feeding values of grass hay, *Stylosanthes scabra* accessions and supplemented grass hay

Treatments	OMD[32] (% DM)	ME[33](MJ kg^{-1} DM)	SCFA[34] (µmol g^{-1} DM)
Grass hay	38.2^{h}	5.4^{i}	0.46^{f}
9281	62.5^{a}	9.3^{a}	0.83^{a}
11252	61.1^{b}	9.0^{c}	0.80^{a}
11255	60.5^{b}	9.1^{bc}	0.81^{a}
11595	62.0^{a}	9.2^{ab}	0.82^{a}
11604	62.3^{a}	9.2^{ab}	0.82^{a}
Grass hay+15% 9281	41.3^{f}	5.8^{g}	0.51^{de}
Grass hay+15%11252	41.2^{f}	5.8^{g}	0.50^{de}
Grass hay+15%11255	41.6^{f}	5.9^{g}	0.51^{de}
Grass hay+15%11595	40.9^{f}	5.8^{g}	0.49^{e}
Grass hay+15%11604	40.1^{g}	5.6^{h}	0.47^{f}
Grass hay+30% 9281	44.4^{d}	6.3^{e}	0.54^{c}
Grass hay+30%11252	43.3^{e}	6.1^{f}	0.51^{de}
Grass hay+30%11255	43.2^{e}	6.1^{f}	0.52^{d}
Grass hay+30%11595	44.5^{d}	6.3^{e}	0.55^{c}
Grass hay+30%11604	47.5^{c}	6.7^{d}	0.63^{b}
SEM	0.28	0.04	0.007

Means within a column with different letters in superscript differ significantly ($P < 0.05$)
SEM, standard error of the mean

[32] Organic matter digestibility
[33] Metabolizable energy
[34] Short chain fatty acid

5.3.7. Thirty-hour *in vitro* neutral detergent fibre degradability and ratios of cell wall contents

Table 5.7 shows the neutral detergent fibre degradability and ratios of cell wall contents of treatments. The neutral detergent fibre degradability of *Stylosanthes scabra* accessions was significantly ($P < 0.05$) higher than that of poor-quality grass hay. There was a significant ($P < 0.05$) difference among the *Stylosanthes scabra* accessions in terms of NDF degradability. For most of these accessions, supplementing poor-quality grass hay with *Stylosanthes scabra* accessions did not significantly improve NDF degradability, irrespective of the level of supplementation. However, there is one exception in which accession 11604 at 30% level of supplementation significantly ($P < 0.05$) improved NDF degradability of supplemented grass hay by 15%.

The expression of ADF on an NDF basis indicates the amount of ADF in the NDF concentration of treatment samples. In this study for example *Stylosanthes scabra* accessions contain more than 80% ADF in the detected NDF, while grass hay contains just below 60% of ADF in the detected NDF. However, including 30% forage of *Stylosanthes scabra* accessions as supplements significantly ($P < 0.05$) increased ADF concentration on NDF in grass hay. Similarly, the expression of ADL in NDF basis indicates the percentage of ADL within the NDF. *Stylosanthes scabra* accessions had significantly ($P < 0.05$) higher ADL concentration on an NDF basis in comparison with grass hay. There was a significant ($P < 0.05$) positive correlation between *in vitro* gas production and *in vitro* NDF degradability of substrates after 30 hours incubation (Figure 5.1).

Table 5.7: Thirty-hour *in vitro* neutral detergent fibre degradation and ratios of cell wall components of grass hay, *Stylosanthes scabra* accessions, and supplemented grass hay

Treatments	*iv*NDFd[35](%DM)	(ADF/NDF) x 100	(ADL/NDF) x 100
Grass hay	19.3^{e}	58.5^{f}	8.0^{i}
9281	39.0^{b}	85.6^{b}	12.8^{b}
11252	42.9^{a}	87.6^{ab}	12.4^{bc}
11255	36.0^{c}	83.8^{b}	12.4^{bc}
11595	35.4^{c}	91.2^{a}	21.1^{a}
11604	35.6^{c}	91.2^{a}	11.9^{cd}
Grass hay+15% 9281	19.3^{e}	62.0^{def}	9.4^{gh}
Grass hay+15%11252	19.5^{e}	61.0^{ef}	8.8^{h}
Grass hay+15%11255	19.2^{e}	62.1^{def}	9.5^{fgh}
Grass hay+15%11595	20.8^{de}	61.7^{def}	11.2^{e}
Grass hay+15%11604	20.1^{de}	60.0^{ef}	11.5^{de}
Grass hay+30% 9281	19.1^{e}	65.8^{cd}	9.6^{fg}
Grass hay+30%11252	19.1^{e}	66.0^{cd}	7.6^{i}
Grass hay+30%11255	20.6^{de}	63.5^{cde}	9.1^{gh}
Grass hay+30%11595	20.0^{de}	66.8^{c}	10.1^{f}
Grass hay+30%11604	22.2^{d}	63.7^{cde}	7.6^{i}
SEM	0.76	1.34	0.23

Means within a column with different letters in superscript differ significantly ($P < 0.05$)
SEM, standard error of mean

[35] *In vitro* Neutral detergent fibre digestibility

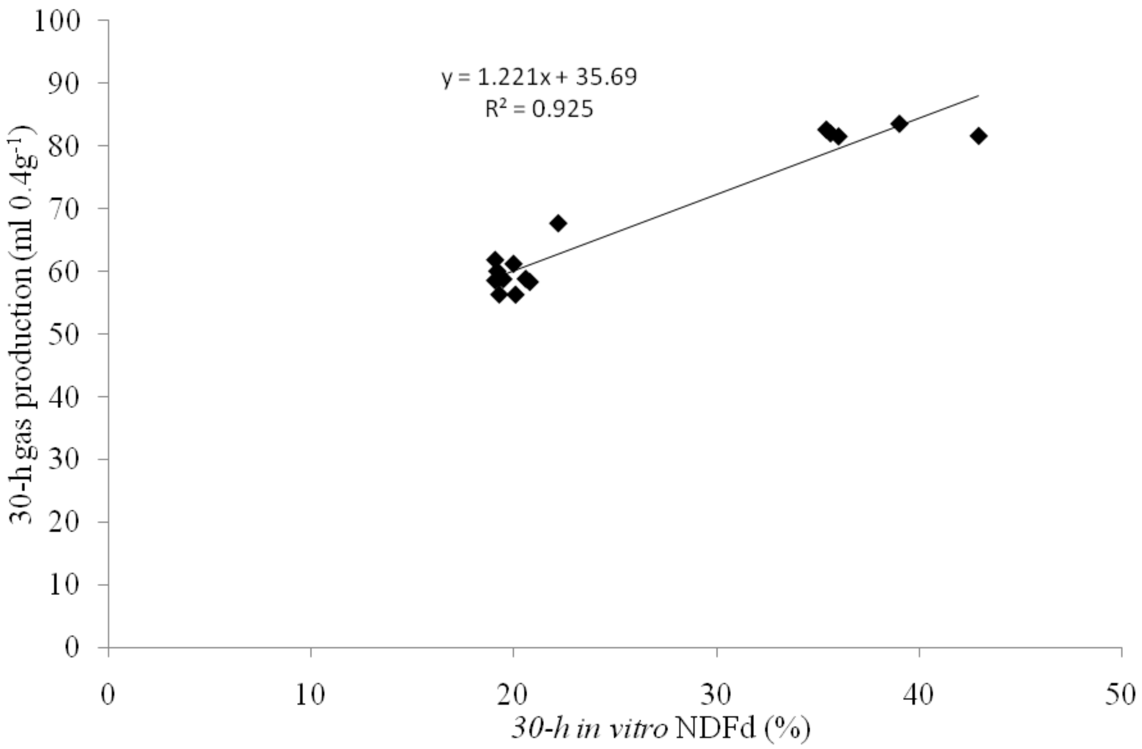

Figure 5.1: Scatter plot relationships between *in vitro* gas production and neutral detergent fibre degradability

5.4. Discussion

The crude protein content of the grass hay used in this study was far below the minimal threshold value (7%) required for normal functioning of the rumen (Ikhamioya 2008). On the other hand, *Stylosanthes scabra* accessions included in the study had above 17% of crude protein content. The CP values of these accessions were in the range of value required to meet the requirements of lactating dairy cows (Poppi and McLennan 1995). The ADF and NDF values recorded in this study for grass hay were above the ADF and NDF ranges of C-4 grass reported by McDonald et al. (2002) and Mertens (2002). However, ADF values for these accessions were lower than the range of values recorded by Mertens (2002) for *Medicago sativa*. Neutral detergent fibre level in the range of 60 to 65% is known to depress intake (Meissner et al. 1991; Van Soest et al. 1991). This study recorded an NDF value above this limit for grass hay, while that of the *Stylosanthes scabra* accessions was below this threshold level. Acid detergent lignin value for grass recorded in this study was higher than that of the accessions. This is inconsistent with findings of Van Soest (1982), who reported that generally legumes contain high lignin than grasses. The level of tannin reported for the

Stylosanthes scabra accessions in the study was very low, compared with a threshold value of 20 g kg^{-1} DM reported to have a minimal effect on gas production (Getachew et al. 2008).

In vitro gas production is a good parameter from which digestibility, fermentation and microbial protein synthesis of the tested feed by rumen microbes can be predicted (Sommart et al. 2000; Sallam 2005). In this regards, *Stylosanthes scabra* accessions were highly fermentable compared with grass hay when they were incubated individually. This could be attributed to high crude protein, coupled with low fibre concentrations in *Stylosanthes scabra* accessions compared with grass hay (Table 5.1). High fermentation of *Stylosanthes scabra* accessions in the first 24 hours of incubation indicated the quick digestibility of the forage compared to grass hay. According to Akinfemi et al. (2009), gas production is regarded as an indicator of carbohydrate degradation. Generally; supplementation of *Stylosanthes scabra* accessions to poor-quality grass hay provided rapid fermentable carbohydrate and rumen degradable nitrogen, thus fermentation was improved. Consequently, increasing the supplementation level of these accessions led to a significant (P < 0.05) improvement of grass fermentation throughout the incubation period (Table 5.3).

According to Tagliapietra et al. (2014), when different feeds are mixed in a diet, they do not behave separately from each other. Hence, they show interactions defined as associative effects. Associative effects occur when digestion of one feedstuff in the mixture is not independent of another (Niderkorn and Baumont 2009). In this study the supplementation of poor-quality grass hay with *Stylosanthes scabra* accessions led to associative effect. However, an interesting positive associative effect was observed for some of these accessions (e.g. 11604) when poor-quality grass hay was supplemented with 30% of forage level. This study showed that supplementing *Stylosanthes scabra* accessions to poor-quality grass hay improved the OMD and ME of grass hay. This could be associated with improved gas production observed in Table 5.3. However, the ME values recorded in this study were below the value of 8.4 MJ kg^{-1} DM, which is recommended for dry ewes (NRC 1985). The short chain fatty acid recorded in this study (Table 5.6), particularly that of supplemented grass hay showed that, *Stylosanthes scabra* inclusion as supplement to poor-quality grass hay improved energy. According to Mako et al. (2011), short chain fatty acid value indicates the presence of energy in the feedstuff. The significant highest SCFA value observed when grass hay was supplemented with 30% forage level of accession 11604 could indicate that, at this level the accession provided sufficient energy for digestion. This could explain the improvement that was noted in terms of NDF degradability when 30% forage of accession 11604 was supplemented to poor-quality grass hay. Degradability of NDF is an essential component of forage quality as it determines dry matter intake of the feed (Kafilzadeh and Heidary 2013). Therefore, this study showed that increasing accession 11604 level of supplementation to 30% would improve the nutritive value of poor-quality grass hay and enhance digestibility. The relationship between *in vitro* gas production and *in vitro* NDF degradability (Figure 5.1) indicated the important role that *Stylosanthes scabra* accessions could play on improving the nutritive value of poor-quality grass hay. According to Van Soet (1994), the degradability of NDF directly influences the nutritive value of the feed material.

5.5. Conclusion

Tropical grass species are cost-effective available feed resources for livestock kept by smallholder subsistence farmers in sub-Saharan Africa. However, this material cannot always meet the nutritional requirements of animals for maintenance, particularly at the maturity stage owing to low nutritive value. This study showed that supplementing poor-quality grass hay with 30% forage of some *Stylosanthes scabra* accessions improved fermentation. A positive associative effect between accession 11604 and grass hay indicated that up to 30% of this accession is adequate to improve fermentation of poor-quality grass. Hence, this accession is recommended as a supplementary source of forage and nitrogen to improve poor-quality grass hay fermentation and hence utilization. However, this accession must be systematically evaluated to determine its feeding value by monitoring intake, digestibility and animal performance using digestibility and growth performance trials.

CHAPTER 6

Partial replacements of *Stylosanthes scabra* forage for lucerne in total mixed ration diet of Saanen goats

This chapter is published in:

Thamsanqa Doctor Empire Mpanza and Abubeker Hassen. 2015. Partial replacements of *Stylosanthes scabra* forage for lucerne in total mixed ration diet of Saanen goats. *Tropical Animal Health and Production*, 47(7): 1391-1396, (DOI 10.1007/s11250-015-0876-6).

Abstract

The inclusion of *Stylosanthes scabra* cv. Vog forage in the total mixed ration (TMR) as partial replacement of lucerne (alfalfa) was evaluated for its effects on voluntary feed intake, nutrient digestibility and nitrogen balance in Saanen goats. Three experimental diets were formulated having 0% Vog (T1), 15% Vog (T2) and 30% Vog (T3) as partial replacements of lucerne forage in the TMR diet for goats. Eighteen Saanen goats of about seven months old were divided into three groups of six animals per group. Each group was randomly assigned to one of the three dietary treatments in a complete randomized design and the study lasted for a period of 21 days. Partial replacement of lucerne with *Stylosanthes scabra* forage did not affect the nutritive value of TMR diet. The variation that was observed in terms of composition among the treatment diets was not statistically significantly ($P > 0.05$). Animals that were fed 15% Vog recorded higher voluntary dry matter and nutrient (organic matter and fibres) intake but the difference was not statistically significant ($P > 0.05$) compared with the other treatments. Nutrient digestibility and nitrogen balance were not significantly different across the three diets. The lack of significant differences in feed intake, nutrient digestibility and nitrogen utilization following the inclusion of Vog in the TMR suggests that *Stylosanthes scabra* forage could partially replace lucerne in the TMR diet of goats. However, there is a need for a total replacement of Lucerne with *Stylosanthes scabra* forage in TMR diet of goats.

Keywords: *In vitro* ruminal fermentation, Feed intake, Lucerne, Nitrogen balance

6.1. Introduction

Poor livestock production in developing countries, particularly under smallholder subsistence farmer's condition, is attributed to over-dependence on low digestible, poor quality and inadequate feed supply from natural pastures. Sometimes, feed from natural pastures, especially at maturity, cannot even meet the maintenance requirements of the animals. To address this situation, fodder trees, shrubs and herbaceous legumes have been used as supplementary feed for ruminants (Fadiyimu et al. 2010; Abegunde and Akinsoyinu 2011; Barakat et al. 2013). Forage legumes generally contain high protein, minerals and vitamins (Idowu et al. 2013). Hence, they are often used as protein sources to correct the protein deficiency of natural pastures (Tufarelli et al. 2010). Incorporating fodder legumes into ruminant diet as supplementary feed has been noted to improve feed efficiency and feed intake (Mendieta-Araica et al. 2009; Pen et al. 2013) and also improved animal performance in terms of milk production in Saanen goats (Baloyi et al. 2006) and in Ankole cow (Mupenzi et al. 2009). Leguminous trees, shrubs and herbs could easily be grown by smallholder subsistence farmers, and their inclusion in animal's diet could reduce the overall feeding cost (Ososanya et al. 2013).

Stylosanthes scabra cv. Vog. is a hardy, erect shrubby legume, which produces moderate to high biomass yield with relatively good nutritive value (Akinlade et al. 2008). The erect and shrubby nature of this legume, along with drought-tolerant characteristics, makes the species suitable for dry areas (Chandra 2009). This legume has been evaluated for its adaptability and agronomic performance over a period of three years under rain-fed conditions in the subtropical climate of Pretoria, South Africa, and was found to be adaptable and productive (Mpanza et al. 2013). Furthermore, it was tested for acceptability, preference and palatability with Saanen goats and was found to be acceptable and palatable (Mpanza et al. 2014). This shows the potential use of this species as supplementary forage for livestock under smallholder subsistence farmers in South Africa. The inclusion of alternative legume forages in the total mixed ration of animals as protein source helps to reduce feed costs by replacing expensive concentrates as protein source (Olafadehan et al. 2014). *Stylosanthes scabra* forage contains more than 17% crude protein with low levels of tannins when grown in Pretoria, South Africa (Mpanza et al. 2014). Thus it has the potential to replace lucerne (alfalfa) in total mixed ration of goats as both forage and protein source. The objective of the present study was to investigate the effects of partial replacement of lucerne forage by *Stylosanthes* *scabra* on the voluntary feed intake, digestibility and nitrogen balance of Saanen goats fed total mixed ration.

6.2. Materials and methods

6.2.1. Location

The experiment was carried out at the small stock unit, Hatfield experimental Farm, University of Pretoria, which is located at 25°44'30" S, 28°15'30" E, at an elevation of 1370 metres above sea level. The study area has two distinctive seasons: a dry season (March – September) and a rainy season (October – February) with warm and humid conditions in summer, while winter is dry, cold and sunny. Mean annual summer rainfall of this area is 674 mm (Hassen 2006).

6.2.2. Forage material and treatments

Sufficient quantities of *Stylosanthes scabra* forage were harvested from screening trial plots that had been established for three years at Hatfield Experimental Farm, University of Pretoria. *Stylosanthes scabra* accessions were harvested at about 100% flowering stage of growth in order to obtain maximum biomass production. Harvested forages were shade dried over a week, but because of the small quantities of forage from each accession, they were mixed together in order to have enough forage material for the study. Other feed ingredients were purchased from the local market. Dried *Stylosanthes scabra*, lucerne forages and *Eragrostis* hay were chopped with a hammer-mill to pass through 25 mm diameter sieve, and were thoroughly mixed with the concentrates to avoid feed selectivity.

Stylosanthes scabra forage was used to partially replace lucerne forage in the traditional TMR diet referred to as an orthodox diet. Thus three dietary treatments were formulated in which lucerne was replaced by 0, 15% and 30% of *Stylosanthes scabra* forage on dry matter bases (Table 6.1). A computer software developed by Langston University Goat Research and Extension programs (2000), was used to formulate the diets using the on-line link to the program. Each level of *Stylosanthes scabra* inclusion was referred as treatment, thus there were three dietary treatments (T1, T2 and T3, respectively). Each treatment was replicated six times.

Table 6.1: Ingredients of total mixed rations for experimental treatments

Ingredient (% of total diet)	Dietary treatments		
	T1	T2	T3
Eragrostis hay	20.0	20.0	20.0
Lucerne hay	20.0	17.0	14.0
Stylosanthes scabra hay	-	3.0	6.0
Salt	0.5	0.5	0.5
Sodium bicarbonate	0.4	0.4	0.4
Limestone, calcium carbonate	1.2	1.2	1.2
Full fat soya roast	1.2	1.2	1.2
Molasses meal	2.0	2.0	2.0
Cotton seed	4.0	4.0	4.0
Wheat bran	5.0	5.0	5.0
Sunflower Oil Cake	7.0	7.0	7.0
Hominy chop SA	18.0	18.0	18.0
Maize ground	21.0	21.0	21.0
Vitamin premix[1]	0.4	0.4	0.4

[1] (18,000 iu/lb A, 3,920 iu/ lb D, 2.45 iu/lb E)

T1= 0% Seca; T2=15% Seca and T3=30% Seca replacing lucerne hay in TMR diet

6.2.3. Animals and their feeding

Eighteen healthy male Saanen goats of about seven months old, with an average weight of 29.6 ± 3.27 kg, were used in the study for three weeks. Goats were distributed in a complete randomized design with three dietary treatments and six replicates per treatment. The permit to use animals was granted by the Animal Use and Care Committee (AUCC) of the University of Pretoria (reference no. EC085-12). Animals were adapted to experimental diets for two weeks. To reduce the period of stay in metabolic cages, the first week of adaptation was done in open pens and second week done in metabolic cages (Figure 6.1). This was followed by seven days of data collection in which feed intake, feed refusal, total faecal and urine voided were collected, weighed and recorded, and representative samples were taken for subsequent lab analysis.

Figure 6.1: Saanen goats in metabolic cage during the study period

6.2.4. Data collection and chemical analysis

Following two weeks of adaptation, data on feed intake, urine and total faecal output were collected for seven consecutive days. For voluntary feed intake estimation, feed offered and refusal were recorded daily per animal, and representative daily samples were taken. Faecal bags were used to collect daily faecal output per animal and two samples were taken, one was used for daily dry mater output and the other one was stored in a freezer for later chemical analysis. Urine was collected in a plastic container, containing 20 ml of 10% sulphuric acid (H_2SO_4) to keep the pH below 4 in order to prevent the escape of ammonia. The volume of urine was measured daily, diluted with water to 5 litres (to prevent corrosiveness of ammonia) and then a sample of 100 ml was taken and stored in a freezer for later nitrogen analysis. Daily samples that were taken from feed, faeces and urine were pooled at the end of seven days of collection and representative subsamples were taken in duplicate per animal for chemical analysis.

Feed and faecal samples were then analysed in duplicate for dry matter (DM), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF) and nitrogen concentration. Feed samples were also analysed for *in vitro* organic matter digestibility (IVOMD), calcium (Ca) and phosphorus (P) concentration. Proximate composition was analysed using standard procedure according to AOAC (2000) while fibre contents (NDF and ADF) were determined according to Van Soest *et al*. (1991). *In vitro* digestible organic matter was determined following Tilley and Terry (1963) procedure as modified by Engels and Van der Merwe (1967).

6.2.5. Statistical analysis

Data were analysed by analysis of variance (ANOVA) using the general linear model (GLM) of Statistical Analysis Systems, software version 9.0 (SAS 2002). The experimental diet and random error were included in the model. Where F value showed significance difference for the treatment effect means were separated by using Duncan's multiple range test.

6.3. Results

The chemical composition of these experimental diets, lucerne and *Stylosanthes scabra* forages are presented in Table 6.2. Lucerne hay which is traditionally used in TMR diet for goats was of medium quality with a crude protein (CP) content of about 17%. *Stylosanthes scabra* forage used in this study had a CP value slightly higher (18.6%) than lucerne. There were no significant differences in the ADF and NDF contents of lucerne and *Stylosanthes scabra* forages. Partial replacement of lucerne with *Stylosanthes scabra* forage in the total mixed ration did not affect the chemical composition of diets. However, the CP levels in the dietary treatments were above the critical level that supports the intake and normal functioning of a rumen. The differences in CP, IVOMD, ash, ADF and NDF were not significant ($P > 0.05$) across these treatments.

Table 6.2: Chemical composition of dietary treatments and legume forages

Composition[&]	Dietary treatments[#]			Legume forages	
	T1	T2	T3	*Lucerne* hay	Seca[36] hay
CP (g kg^{-1} DM)	136.8	134.3	135.7	170.3	185.6
Ash (g kg^{-1} DM)	71.7	74.4	83.8	94.7	86.7
ADF (g kg^{-1} DM)	281.8	283. 7	290.3	327.8	319.9
NDF (g kg^{-1} DM)	417.2	423.8	443.9	415.7	447.9
IVOMD (% DM)	70.8	69.8	69.0	-	-
Ca (% DM)	0.7	0.8	0.8	-	-
P (% DM)	0.4	0.4	0.4	-	-

[&] CP: crude protein; ADF: acid detergent fibre NDF: neutral detergent fibre; IVOMD: *in vitro* organic matter digestibility; Ca: calcium; P: phosphorus.

[#] T1: 0% Vog; T2: 15% Vog; T3: 30% Vog of lucerne hay in TMR diet

Voluntary dry matter intake of the experimental diets fed to the Saanen goats are presented in Table 6.3. Partial replacement of lucerne with *Stylosanthes scabra* at 15% and 30% levels did not significantly ($P > 0.05$) affect the voluntary dry matter and nutrient intake of Saanen goats. Animals fed 15% Vog seemed to have a higher intake, while those on 30% Vog had the lowest, but the difference was not statistically significant.

[36] *Stylosanthes scabra*

Table 6.3: Body weight, dry matter and nutrient intakes of Saanen goats fed total mixed rations with or without *Stylosanthes scabra* forages

Parameters	Dietary treatments*			SEM[37]	*P* value
	T1	T2	T3		
Number of animal	6	6	6		
Initial body weight (kg head^{-1})	31	29	29	1.4	-
Final body weight (kg head^{-1})	34.4	33.7	33.1	1.26	-
Feed intake (g head^{-1} day^{-1})	1372	1402	1330	27.8	0.1830
Feed intake¥ (g kg^{-1} W$^{0.75}$ day^{-1})	94.7	103.1	97.4	5.93	0.6057
	Nutrients intake (g head^{-1} day^{-1})				
Organic matter intake	1273	1300	1218	59.2	0.6213
Crude protein intake	188.6	187.4	180.6	8.18	0.7586
NDF intake	570.5	594.1	590.5	28.34	0.8200
ADF intake	386.0	397.7	386.0	19.38	0.8862

¥ Feed intake per metabolic body weight

* T1: 0% Vog; T2: 15% Vog; T3: 30% Vog of lucerne hay in TMR diet

Partial replacement of lucerne by *Stylosanthes scabra* had no significant effects ($P > 0.05$) on nutrient digestibility (Table 6.4). The dry matter, CP, ADF, and OM digestibility seemed to vary among thr treatments diet however, the variation was not statistically significant.

[37] Standard error of mean

Table 6.4: Effects of partial replacement of lucerne with *Stylosanthes scabra* on nutrients digestibility of Saanen goats

Digestibility[@] (%)	Dietary treatments[$]			SEM[38]	*P* value
	T1	T2	T3		
DM	66.9	64.9	63.0	1.07	0.3435
OM	70.0	68.3	67.9	1.45	0.5671
CP	66.1	65.0	65.4	1.55	0.8894
NDF	50.4	49.5	51.9	2.26	0.7374
ADF	46.0	45.5	44.6	2.47	0.9264

[@] DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; OM: organic matter.

[$] T1: 0% Vog; T2: 15% Vog; T3: 30% Vog of lucerne hay in TMR diet

The daily nitrogen intake of the Saanen goats was not significantly affected by the replacement of lucerne with *Stylosanthes scabra* forage in the TMR diet across all levels. However, the nitrogen intake seemed to be the lowest for 30% Vog compared with the other two treatments (Table 6.5). Excretion of nitrogen through faeces was not significantly affected by treatment diet. However, animals fed 15% Vog diet had slightly higher nitrogen excretion in contrast with the other two treatments. Urinary nitrogen values seemed to be slightly higher for animals fed on 30% TMR diet, though the difference was not statistically significant ($P > 0.05$) when compared with the other two treatments. Saanen goats fed dietary treatments with different levels (15% or 30%) of *Stylosanthes scabra* forage in TMR had a positive nitrogen balance. Although goats that were fed 30% Seca diet seem to have slightly lower nitrogen retention, the observed difference was not statistically significant when compared with the other two treatments.

[38] Standard error of mean

Table 6.5: Effects of partial replacement of lucerne with *Stylosanthes scabra* on nitrogen retention in Saanen goats

Parameter (g head^{-1} day^{-1})	Dietary treatments[&]			SEM[39]	*P* value
	T1	T2	T3		
Number of animal	6	6	6		
Total nitrogen intake	30.2	30.0	29.0	1.31	0.7586
Nitrogen excreted in faeces	10.3	10.6	10.0	0.80	0.9001
Nitrogen excreted in urine	5.4	5.4	5.7	0.64	0.9595
Total nitrogen excreted	15.7	16.0	15.7	1.20	0.9771
Total nitrogen retained	14.5	14.0	13.2	0.89	0.5886

[&] T1: 0% Vog; T2:15% Vog and T3: 30% Vog of lucerne hay in TMR diet

6.4. Discussion

The primary objective of this study was to assess the potential of using *Stylosanthes scabra* forage as partial replacement of lucerne in total mixed ration formulated for Saanen goats. The nutrient profile of *Stylosanthes scabra* (Table 6.2) shows that the forage has a good nutritive value, which should result in good productive performance. The protein content of Vog forage used in the present study was above 11%, which is adequate for growing beef cattle (Valarini and Possenti 2006), and was in the range that is required to support a lactating dairy cows (Poppi and McLennan 1995).

In the present study, the voluntary feed intake of dietary treatments was similar across the treatments. This is inconsistent with the findings of Schnaider et al. (2014). Nutrient intake for DM, OM, CP, NDF and ADF were similar in all dietary treatment groups; consequently the respective *in vivo* digestibility of these nutrients was similar. These results also agree with the reports of Shi et al. (2014) and Sath et al. (2012). Even though NDF content seemed to be slightly higher, the level was still below the upper limit of 60%, capable of reducing dry matter intake (Meissner et al. 1991). Besides the levels of NDF and ADF, Mahgoub et al. (2005) reported that increasing level of non-conventional feed ingredient lead to the reduction of apparent nutrient digestibility. However, this may not be generalised to all non-conventional feeds due to their differences in nutritive values.

[39] Standard error of mean

Intake of CP decreased slightly, and this could be related to the CP concentration of dietary treatments. On the other hand, the excretion of nitrogen tended to increase in faecal and urine in treatment 2 and 3 diets. This could be attributed to the probability that nitrogen intake exceeded the requirements of goats in all treatments. Previous study indicated that *Stylosanthes scabra* forage contained a relatively low level of tannins (ranged 0.87 to 1.64 g kg^{-1} DM) (Mpanza et al. 2014). Although in this study tannin content was not determined, one could expect an increase in tannin intake with increasing levels of *Stylosanthes scabra* forage inclusion in the TMR diet. This could partly explain the trend that is observed in nitrogen balance. However, dietary treatment had no significant effect on some of the measured responses, including intake, digestibility and nitrogen retention.

6.5. Conclusion

The inclusion of *Stylosanthes scabra* forage in the TMR diet as partial replacement of lucerne did not show any significant effects in terms of dry matter and nutrient intake, digestibility and nitrogen utilization. This means that up to 30% of lucerne can be safely replaced with *Stylosanthes scabra* forage without compromising the nutritive value of the TMR diet of Saanen goats. However, this study evaluated only up to 30% inclusion level and thus future studies should assess the possibility of replacing a higher proportion of lucerne with *Stylosanthes scabra* forage. However, these benefits need to be quantified also in terms of animal productive performance and cost benefit.

CHAPTER 7

General conclusions and recommendations

7.1. General conclusions

The purpose of this study was to identify alternative forage legumes that are drought and frost tolerant, which could be used by communal smallholder subsistence farmers in the subtropical areas of Gauteng, South Africa. *Stylosanthes scabra* accessions were evaluated to identify accession(s) that could be utilized as potential alternative forage sources for supplementation of poor-quality feed. Consequently five experiments were conducted at Hatfield Experimental Farm, University of Pretoria, which included field evaluation, *in vitro* study and stall feeding. The overall results from this study showed that accession 11604 was found suitable for use as a supplementary forage source of protein and roughage for animals fed on poor-quality diets.

The screening trial was conducted to identify accessions that were adaptable and productive under rain-fed condition in the subtropical climate of Pretoria, northern Gauteng, South Africa. *Stylosanthes scabra* accessions were persistent over a three-year period of evaluation with relatively good biomass yield production under rain-fed condition. This indicates their adaptability to this climatic condition. Generally, the promising *Stylosanthes scabra* accessions produced a mean forage yield of 3.5 to 5.6 t ha^{-1} DM. These accessions had satisfactory nutritive value (high protein concentration, and adequate metabolizable energy and low in phenolic compounds) with good digestibility of organic matter. Generally, the digestible organic matter of these accessions was above 65% and the crude protein content ranged from 17% to 20%. This suggests that the promising accessions could be strategically used as supplemental feed sources to improve poor-quality forage utilization by small ruminants.

Acceptance of the selected promising *Stylosanthes scabra* accessions indicated that the edible forage material from *Stylosanthes scabra* was palatable to animals and thus could be utilized as a feed resource. Howover, the preference ranking indicated some accessions were preferred more than others. It was not clear weather preferences were influenced by chemical composition or the fermentability of the forage material. Results from this study showed that all five selected accessions were acceptable and palatable to Saanen goats. However, animal behaviour in terms of intake and preference indicated that accession 11604 tended to be the most consumed, thus it was highly preferred while accession 11255 was ranked least in order of preference by Saanen goats.

The effect of supplementing poor-quality grass hay (with CP < 4%) with varying levels of selected *Stylosanthes scabra* accessions indicated that they improved performance in terms of ruminal fermentation for the first 30 h. Thus, supplementing poor-quality grass hay with *Stylosanthes scabra* accessions increased the protein content of the diet to the level that microbes were able to meet their requirements and thus ferment the feed efficiently. However, supplementation of poor-quality grass hay with 30% forage of accession 11604 resulted in a positive associative effect between grass hay and the accession. Additionally, NDF degradability of grass hay was improved when supplemented by this accession (11604) at 30% level. This means that supplementing poor-quality grass hay with accession 11604 at 30% level may reduce the retention period of poor-quality feed in the rumen, and thus may improve feed intake of the poor-quality forage.

The effect of replacing alfalfa forage partially with the various levels of *Stylosanthes scabra* forage in the total mixed ration did not affect dry matter intake, nutrient digestibility and nitrogen balance when fed to goats. This means that alfalfa could be partially replaced by *Stylosanthes scabra* forage without compromising the nutritive value of total mixed ration (TMR) for Saanen goats.

7.2. Recommendations

The results of this study showed that *Stylosanthes scabra* is adaptable to and productive in the subtropical climate under rain-fed conditions of Pretoria, though some of the accessions could not withstand frost in winter. Thus, these results need to be extrapolated with caution to other agroecological areas in South Africa, because these accessions were evaluated only in one site over three years. Thus there is a need to undertake multi-location evaluation of the promising accessions to come up with accessions that may be able to produce reasonable biomass yield in wider agroecological areas. Furthermore, to find out accessions that would be suitable for specific agroecological areas in South Africa, such future work should include assessment of the promising accessions for nitrogen fixing ability in grass-legume intercropping and suitable methods of integration of accessions into the farming system in order to use the superior accessions for future pasture improvement programmes.

This study showed that *Stylosanthes scabra* could produce a relatively high biomass yield (3.5 to 5.6 t ha^{-1} DM) with good nutritive value (high in crude protein, highly digestible, adequate metabolizable energy, low in phenolic compounds and fibres). Most of these accessions were acceptable and palatable to goats as model animals that were used. These attributes indicate the suitability of using forage from *Stylosanthes scabra* as a supplementary feed source to improve poor-quality forage utilization by animals. However, further research is necessary for strategic use of the forage material in the diet of animals under communal and smallholder production systems in South Africa. Such research would help to establish the level at which production of livestock would be improved, particularly under smallholder subsistence farmers, who at the moment can not afford commercial feed supplements. Further research is required to determine how the benefits of using promising accession (ILRI no. 11604) identified in this study could be converted into animal product, for example how it could affect milk and meat production and quality.

The *in vitro* trial conducted in this study showed that 30% *Stylosanthes scabra* accessions supplementation to poor-quality grass hay improved fermentation. The positive associative effect between poor-quality grass hay and 30% level of accession 11604 supplementation resulted in better fermentability of the grass hay throughout the incubation period. Additionally, this accession (11604) at 30% supplementation level improved NDF degradability of grass hay. Consequently, supplementing this accession at 30% level might reduce the retention period of poor-quality feed in rumen of animal and perhaps increase dry matter intake. Thus, it is recommended that an *in vivo* trial should be conducted which would help to assess the effects of supplementing this accession to poor-quality forage on digestibility and retention period. The study would also assist in knowing how the forage of this accession would affect the enteric methane emission from ruminants fed poor-quality feed. Poor-quality feed stay longer in the rumen, and that leads to an increase of greenhouse gases production, particularly methane and nitrous oxide.

This study also showed that *Stylosanthes scabra* forage could replace up to 30% of alfalfa forage in the total mixed ration of goats without compromising the nutritive value of TMR. However, it is recommended that a higher proportion of replacement should be tested to determine how that would affect the nutritive value of TMR, and animal productive performance and moreover, to calculate the cost benefits of replacing alfalfa with *Stylosanthes scabra* forage.

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