Use of inert markers to predict diet composition, forage intake, digestibility and passage rate in sheep

By

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A dissertation submitted in fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE (ANIMAL SCIENCE)

In the

School of Agriculture, Earth and Environmental Sciences

Discipline of Animal & Poultry Science

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

May 2019

Pietermaritzburg, South Africa

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Declaration

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Dedication

This thesis is dedicated to my late brother Sisa Mabhala and Uncle Khoza Lizo Pepeta “Tatomncinci u-Marhuphuza” who didn’t live long enough to witness this achievement.

Acknowledgements

I would like to pass my deep acknowledgments to the following people and organizations who made it possible for me to finish this thesis through their guidance, moral and financial support.

Supervisors: Prof. Ignatius Verla Nsahlai and Prof. Abubeker Hassen for their noble supervision and constructive criticism throughout the study and for grooming me to be an independent person within academic research grounds and in general.

Academic staff from Animal Science Department: Professor Michael Chimonyo who financial assisted for veterinary check-ups of the experimental animals before the commencement of the experiment.

Technical staff: Miss Sithembile Ndlela, Mr Ntuthuko Mkhize and Mrs Nomandla Baca for their technical support regarding laboratory analysis. Special thanks to Miss Sthembile Ndlela for being always up to date with the orders of chemicals especially for analysis of *n*-alkanes and long chain alcohols which were difficult to purchase and took long time to arrive. It was not an easy process at all, I respect her patience.

Academic staff from other Departments: Prof. Fanie van Heerden from the department of organic chemistry at the University of KwaZulu-Natal for endless consultations, which led to deep insight of possibilities and difficulties associated with analysis of *n*-alkanes and long chain alcohols.

Financial assistance: Collaborative Postgraduate Training programme of the National Research Foundation (NRF) for funding the whole project and the University of KwaZulu-Natal (UKZN) Postgraduate bursary (P029) for funding the first year of the study.

Staff at Ukulinga research farm: Mr Sam Khumalo (Ankela) and Mrs Masefo Mokoma for their assistance in providing material that was needed for the study and for taking care of experimental animals.

Animal & Poultry Science final year students: Mr Nkosinathi Ngema (matyper esakeni, umadlokovu), Miss Thandeka Shabane and Miss Kayise Nkosi (Gupta, God fearing) for their assistance with data collection. You turned the farm into a comedy show, it was really helpful as a relief.

Colleagues: Dr Sylvester Fomum (Yes-baba), Mr Siyabonga Bhiya (Nkabi), Mr Fortune Thabethe (F-man), Mr Daniel Serummula (Kunzima) Mr Rasheed Adebayo (Rumen fill), Abd'quadri-Abojukoro Aderonke (Madam) who provided a conducive working space during the write up of the thesis. Special thanks to Mr Mehluli Moyo (Makhotha lento) for his assistance from the commencement of the study till the end.

Family and Friends: Mr Zamindlela Pepeta, Mrs Abegail Pepeta, Mrs Thozama Mdiya, Miss Abongile Pepeta, Mr Ayavuya Pepeta (Ingwenya), Mr Loyiso Pepeta, Miss Mbedzi Munaka (Skat), Mr Sinolutho Kauta, Mr Siphesihle Motwana and Bonke Madikizela who always provided moral support whenever I felt like everything was too much. Popza crew: Nhlanhla Mdlala (Chamack), Mlungisi mnyandu (Ganyan), Eric Majola (Phusu), Mondli Manqele (Skito) and Mfanelo Gabela (Sgora)

List of abbreviations

ADF	acid detergent fibre
ADL	acid detergent lignin
AIA	acid insoluble ash
BS	bean straw
CP	crude protein
Cr_2O_3	chromium (III) oxide
DM	Dry matter
DMI	dry matter intake
ED_{DM}	effective ruminal degradability
MADF	modified acid detergent fibre
FR	faecal recovery
GC-MS	gas chromatography-mass spectroscopy
GIT	gastrointestinal tract
GPS	global positioning system
GT	Grazing time
HEM	Hemicellulose
HG	hind gut
HG_l	hindgut liquid particles
HGs	hindgut solid particles
IADF	insoluble acid detergent fibre
INDF	insoluble neutral detergent fibre
k	ruminal outflow rate
K_l	fractional passage rate of liquid particles
K_s	fractional passage rate of solid particles
LH	lucerne hay
LCOHs	long chain alcohols
MS	maize stover
NDF	neutral detergent fibre
Obs	Observed
OF	oesofageal fistulated
P_D	potential degradability

Pred	Predicted
RGT	relative grazing time
RR	reticulo-rumen
RR_l	reticulo-rumen liquid particles
RRs	reticulo-rumen solid particles
SS	sorghum stover
TMS	trimethylsilyls
$TTMRT_l$	liquid total tract mean retention time
$TTMRT_s$	solid total tract mean retention time

Thesis output

Manuscripts under review in peer-reviewed scientific journals

1. Pepeta B.N, Moyo M and Nsahlai I.V. Determination of proportion of feed consumed as an index for diet selection in sheep. South African Journal of Animal Science.

Abstract presentations

1. Pepeta B.N, Moyo M and Nsahlai I.V. Determination of feed preference as index for feed preference. 50th Congress of South African Society of Animal Science. 18th to 21st of September 2017. Port Elizabeth, Eastern Cape Broad walk conference centre.
2. Pepeta B.N, Moyo M and Nsahlai I.V. Determination of feed preference as index for feed preference. 26th of October 2017. College of Agriculture, Engineering and Science Postgraduate research day. Westville campus.

Manuscripts under preparation

1. Pepeta B.N, Moyo M, Hassen A and Nsahlai I.V. Use of biomarkers to predict diet composition, forage intake, digestibility and diet selection in grazing sheep: Review
2. Pepeta B.N, Moyo M, Hassen A and Nsahlai I.V. Effect of animal stocking rate on dry matter intake, botanical composition, digestibility and passage rate of diet selected by sheep.

General abstract

The mechanisms that regulate intake and composition of selected diets in ruminants are complex and vary among animals of the same species and cross species. These are governed by highly variable aspects, which range from animal factors to physio-chemical properties of feeds. Understanding how ruminants select their diets is imperative to improve their utilisation of feed resources regarding the diversity of plant species that can be used as their sources of feed. The objectives of the study were to: (1) determine the effect of group feeding and removal of dietary ingredient (*Sorghum bicolor*) on diet selection, nutrient and total dry matter intake, and digestibility in choice-fed sheep; (2) asses the effect of animal stocking rate on dry matter and nutrient intake, botanical composition, nutrients selected, total tract digestibility and passage rate of diet consumed by sheep; and (3) predict dry matter and nutrient intake, botanical composition, nutrients selected and total tract digestibility using inert markers. Twelve sheep (mean weight: 29.7 ± 4.63 kg) were assigned to three treatments. In treatment one, five feeds were fed to sheep fed as a group of 9 sheep (G). In the second treatment, five feeds were fed to sheep penned in isolation (I) and in the last treatment, four feeds with sorghum stover (SS) removed were fed to sheep penned in isolation (R). There were five experimental feeds: veld hay (VH), sorghum stover (SS) and maize stover (MS) fed *ad-libitum*, and Lucerne hay (LH) and bean straw (BS) fed at restriction levels of 0.15 and 0.35 kg/day per sheep, respectively, in a group or individually fed sheep. Diet compositions were similar (p >0.05) between sheep fed individually with or without SS. Similarity in proportion of these dietary ingredients consumed between R and I may be due to less selection of SS; therefore, its removal did not significantly influence consumption and selection of other dietary ingredients. Group feeding of sheep relative to individual feeding with similar dietary ingredients influenced selection of SS. Sheep fed individually had lower intake levels of SS. Establishment of a dominance hierarchy in group-fed sheep may have caused dominant animals to feed on poor quality stovers just to prevent sheep lower in the hierarchy from eating resulting in high consumption of stovers. Fifteen sheep (mean body weight 46.5 ±3.3 kg) were blocked by weight into four groups and each sheep was randomly allocated to four stocking rates (treatments) of 1, 2, 4 and 8 sheep per pen and fed: MS, SS, and VH. All feeds were fed on separate feeding troughs *ad-libitum*. To evaluate the effect of animal stocking rate (SR) on passage rate of digesta, one sheep each from stocking rates one (SR1) and two (SR2) animals per pen and two sheep each pair

from stocking rates of four (SR4) and eight (SR8) animals per pen were randomly selected and dosed with Ytterbium (particulate) and cobalt-ethylenediamine tetraacetic acid (Co-EDTA; liquid) markers. An optimisation procedure was used to predict diet selection by minimising the sum of the squared discrepancies between the proportional concentration of markers (acid insoluble ash: AIA, modified acid detergent fibre: MADF, and acid detergent lignin: ADL) in faeces (A) and their proportional concentration in dietary components (E) (MS, SS and VH), corrected for faecal recoveries of markers. Fractional passage rate (liquid and particulate) from both the rumen and in the hind gut, mean retention time, and total mean retention times across treatments were similar (p >0.05). Similarly, intake of dietary ingredients, nutrients (crude protein: CP, neutral detergent fibre: NDF and acid detergent fibre: ADF), total dry matter intake and composition of diets selected were not different across treatments. Selectivity index factors of diets selected were all within the range of 1.56-3.80, which reflected that animals were able to retain the diets they selected long enough in the gastro intestinal tract (GIT) for efficient digestion. Total tract digestibility and mineral intake (Ash) differed (p <0.05) in relation to animal stocking rate. Sheep in SR2 had the highest digestibility and consequently increased dry matter intake. Predicted dry mater intake and total tract digestibility of a diet selected by sheep were less sensitive to correction of incomplete faecal recovery of the markers and they tended to be similar to observed dietary parameters. Therefore, inert markers can be used to predict several components of a diet selected by grazing sheep and other classes of ruminants.

Key words: diet selection, passage rate, prediction, space allowance, stovers,

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Chapter 1

General introduction

1.1 Background

Ruminants in pastoral feeding systems are dependent on forages as a dietary source to meet their nutritional requirements (Bocquier and González-García, 2010). On the other hand, most commercial feeding systems generally feed high quality forages supplemented with concentrates, leading to high production levels as a result of animals consuming adequate quantities of required nutrients. The amount of poor-quality forages consumed by ruminants in rangelands even if available *ad libitum*, still limit the animals from achieving their genetic potential in performance because of their inherent poor quality and conditions. This leads to reduced intake due to low rate of degradation, slow rate of clearance in the rumen and poor quality of most tropical roughages. Nonetheless, pasture can be able to provide substantial proportion of the nutritional requirements of ruminants. The digestive tract of ruminants is capable of utilising roughages through pre-gastric fermentation resulting in production of energy for growth, reproduction, production (i.e. milk, meat and wool or cashmere) and maintenance (Duncan and Poppi, 2008). However, there is limited, if any available literature on plant species selection choices made by these ruminants in rangelands. Furthermore, the extent to which quality and rates of passage of digesta of selected diets by these ruminants changes in relation to animal stocking rate is also unknown. This makes it difficult to know which supplementary feeds to use in addition to grazing to come up with sustainable feeding practices.

Efficient utilisation of available feed resources for ruminants by animal nutritionists or farmers depends on the quality and quantity of the herbage that can be consumed. Quality is evaluated by two indices: through rates of rumen degradation and chemical composition (nutritional content) of each dietary component and then using proportions consumed to estimate composition of diet consumed. However, it is difficult to determine the quantity and composition of herbage consumed by rangeland ruminants because selection choices vary in relation to differences in forage species availability, forage quality (Sollenberger and Vanzant, 2011), forage spatial distribution (Laca, 2008) and animal factors (Arnold, 2017). Free-foraging ruminants in natural pastures and forests are also distributed spatially and

temporally to different degrees making their distribution an essential contributing factor on the degree of forage and nutrient intake, selection choices and subsequent diet composition (Ganskopp and Bohnert, 2009). Rates of passage of digesta influences the gut fill of an animal, which subsequently regulates quantity of forage consumed. Generally, animals eat to meet their nutritional requirements. Animal stocking rate has a profound influence on plant selection choices made by grazing animals because the availability of plant species changes and declines over time; it also fluctuates seasonally, being the lowest in the cold-dry season in tropical and subtropical regions. Animals then compete for limited amount of better-quality forages available. Therefore, studying the rate of passage and chemical composition of herbage consumed under different animal stocking rates becomes imperative.

Different methods have been proposed to estimate diet composition and intake in free-ranging ruminants and these methods have limitations, which include their low accuracy, time consuming and disturbance to the normal foraging behaviour of animals (Li *et al*., 2015). Of the promising methods, the use of inert markers have received most attention and have been shown to be applicable and accurate in estimating diet composition and intake (Lin *et al*., 2012). Plant *n*-alkanes and long chain alcohols are both found in epicuticular wax produced by plants, and they are classically made up of aliphatic lipid compound mixtures with a chain length ranging from 21 to 36 carbon atoms (Dove and Mayes, 1996; Elwert and Dove, 2005; Dove, 2010). Abundance and pattern of these wax compound varies within plant parts and between plant species (Ferreira *et al*., 2017). On the other hand, inert markers used to estimate digestibility include acid insoluble ash (AIA), acid detergent lignin (ADL), indigestible acid detergent fibre (IADF), indigestible neutral detergent fibre (INDF), and modified acid detergent fibre (MADF). These markers have been successfully used in several studies but the differences in their recoveries remain a typical setback in using them to accurately estimate intake, diet composition and digestibility (Kanani, 2012). These markers are all recovered in faeces to varying extents and they neither affect nor be affected by the gastro intestinal tract of animals. These attributes offer a great opportunity to use these markers to estimate several components of a diet selected in nutritional studies (Heublein *et al*., 2017).

1.2 Research question

Does diet selection, diet composition (nutritive value and digestibility), passage rate of digesta and feed intake remain the same when sheep are fed at different animal stocking rates either with the same (all) dietary ingredient choices or with one dietary ingredient choice absent from the offered choices?

1.3 Justification

Dry matter intake and diet composition (nutritive value and digestibility) of forages are important determining factors of performance and nutritional status in ruminants. These are governed by the rate of passage of digesta and the degradability of feed stuffs in the rumen. Achieving accurate estimates of such diet attributes allows the prediction of the amount of nutrients available for animals to use in a consumed diet. This will also allow farmers to predict the carrying capacity of their pastures and will improve the utilization of pre-existing and other potential alternative feed resources (e.g. crop residues and concentrates).

Understanding the nutritive value of preferred and selected dietary components by ruminants is important for rangelands and semi-natural grasslands management and facilitates the choice and amount of supplement feeds (protein and energy) required to achieve sustenance and optimal production on different seasons and during periods of nutritional stress throughout the year.

1.4 Objectives:

1.4.1 The broad objective of the study was to determine the effect of animal stocking rates on diet selection, diet composition (nutritive value and digestibility), passage rate and intake in sheep when conditions under which they graze are simulated, by exploiting roughages as dietary feed ingredient choices. Assess the preferred straws for use as supplementary feeds, to ameliorate the impact of winter dry season on different feeding systems.

The Specific objectives:

1. Determine the effect of group feeding and removal of dietary ingredient (*Sorghum bicolor*) on diet selection, nutrient and total dry matter intake, and digestibility in choice-fed sheep.

2. Determine the effect of animal stocking rate on dry matter and nutrient intake, botanical composition, nutrients selected, total tract digestibility and passage rate of diet consumed by sheep.
3. Predict dry matter and nutrient intake, botanical composition, nutrients selected and total tract digestibility using inert markers.

1.5 Hypotheses

The hypotheses tested were that:

1. Group feeding and removal of dietary ingredient (*Sorghum bicolor*) both will influence diet selection, nutrient and total intake, and digestibility in choice-fed sheep.

2. Animal stocking rate will influence botanical composition and quality selected, dry matter and nutrient intake, total tract digestibility and passage rate of a diet consume.

3. Inert markers can be used to predict the dry matter and nutrient intake, botanical composition, nutrients selected and total tract digestibility using concentration of inert markers in plant and faecal samples.

Chapter 2

Literature review

2.1. Introduction

Nutritional status of grazing sheep does not only influence selection choices but also the composition of diet and the quantity of herbage they consume. Differences in plant distribution, number of plant species and animals in the ecosystem are key factors that affect the dynamic distribution, flow of nutrients and minerals, through ingestion and egestion of faeces (Schnyder *et al.,* 2010). In addition, plant biomass of rangelands, flow of nutrients and performance of grazing animals are negatively affected by deposition of heavy metals and radionuclides pollutant loads (Ali *et al*., 2013). Inadequate nutrition is the primary constraint to production of sheep reared in Sub-Saharan Africa because natural grasslands and crop residues are the substantial feed resources (McDowell, 1988), which are inherently deficient in nutrients (Teferedegne, 2000). Improved production performance for grazing or indoor animals relying on poor quality roughage, is largely dependent on adequate nutrient intake achieved through supplementation of poor-quality pastures and crop residues with nutrient dense concentrate feeds. Commercial supplementary feeds are not readily available and affordable to most resource-limited farmers. Therefore, there is a need for cost-effective alternative supplement feeds to combine with or replace commercial supplements (Breman and De Wit, 1983). Crop residues treated with either sodium hydroxide (Dass and Kundu, 1994), anhydrous ammonia (Gotlib *et al*., 1977) or urea (Yadav and Yadav, 1989) have a potential to be alternatives to commercial supplements to sustain animals at moderate production levels. Thus, crop residues may be an essential feed resource for small ruminants during the dry season when feed availability and quality are low.

There is limited literature on factors that affect plant selection choices and the extent of their consumption by animals under natural conditions. The effects of animal stocking rate, spatial distribution of watering points, feed and seasonal fluctuation on feed intake and chemical composition of selected diets by ruminants in rangelands are not well understood. It is also difficult to choose and quantify crop residues to use in addition to grazing. Hence, this necessitates research on crop residues preferred ruminants given at *ad libitum* level of consumption, to establish efficient feeding systems.

Most tropical grasslands are characterised by low nutritional quality and quantity of forage as a result of erratic rainfall during the hot-wet and cold-dry seasons (Salem and Smith, 2008). Over-cultivation, climate change and overgrazing in sub-Saharan Africa reduces both availability and quality of available forages (López-Bermúdez and García-Gómez, 2006). Theoretically, most plants in pastures fail to recover after intensive defoliation in response to excessive animal stocking rates and low resting interval because of depleted energy reserves in plants (Nefzaoui and Salem, 2000). Consequentially, this leads to low feed intake patterns that subsequently decrease animal productivity; be it low milk yield, slow growth, poor carcass quality, poor wool production or high mortality rates.

Understanding the nutritive value and intake of selected dietary components by sheep is important for managing different feeding systems and facilitates the choice and quantity of supplement feeds required for maintenance and optimal growth of animals. Grazing is the most common and economical system of feeding for all classes of ruminants (Van Soest, 2018). Identifying and improving pre-existing methods of predicting intake, composition of diet selected and factors affecting intake in sheep grazing in rangelands is of importance. Determining how much can be supplemented to meet animal requirements depends on the ability to predict composition and quantity of selected diet and animal requirements. In addition, the ability to estimate composition (chemical and botanical) and intake of a selected diet are integral for understanding the influence of grazing and browsing sheep on plant biomass and nutrient intake levels derived from consumed forages and browse species in the grazed area (Galyean and Gunter, 2016). The interaction of chemical composition of diets selected, rates of passage and digestibility of diets, and feed preference are interrelated in regulating intake in ruminants (Doan and Guo, 2019). These serve as important tools in evaluating animal performance in the dynamic ecosystem under which animals graze. These variables are also direct indices used in predicting animal performance in relation to selected diet making them useful in modelling of animal performance. Hence, there is a need to improve existing methods and develop alternatives for predicting dry matter intake, diet composition (botanical) and digestibility of selected diets of free-grazing animals.

This review aims to assess the potential use of inert markers to estimate diet composition, forage intake, supplement intake, digestibility and diet selection in sheep grazing on rangelands. Factors affecting diet selection, and the methods used in estimating the amount of herbage consumed are also discussed.

2.2. Markers and their uses

Markers are components that are either administered orally or are naturally and intrinsically available in the consumed feedstuffs. These feed entities should pass certain criterion to be regarded as markers. They should be: indigestible, inert, not bulk, neither affect nor be affected by the gastro intestinal tract (GIT) and its microbial population (Velasquez, 2017), mix and remain uniformly distributed in digesta (Faichney, 1975; Owen and Hanson, 1992; Pellikaan *et al*., 2013). Internal markers that are readily available in feedstuffs and external markers that are synthetic and administered orally through dosing or inserted in a cannula are both recovered in faeces (Marais, 2000). External markers have been successfully used to estimate faecal output and passage rate of digesta whereas internal markers have been used to estimate digestibility (Giráldez *et al*., 2004). These measurements are subsequently used to estimate intake (Dove and Mayes, 2006). There is no marker that fulfils all requirements of an ideal marker which are: full recovery in faeces, physical attributes that are: not distinct to that of a consumed diet, does not disturb the animal (Dove and Mayes, 2006) and the functioning of normal digestive system.

In vitro methods have been used to estimate digestibility instead of these markers because of their failure to fulfil the ideal marker requirements. However, *in vitro* methods do not take into consideration individual animal variation and supplement diet effects when estimating digestibility (Goetsch *et al*., 2010). Plant cuticular wax compounds were shown to circumvent such shortcomings.

2.3. Plant cuticular wax compounds

The surface of most browse species or forage resources contain a layer of epicuticular wax classically made up of aliphatic lipid compound mixtures (Duncan *et al*., 1999; Charmley and Dove, 2008; Dove, 2010). The bundance and composition of these wax compounds varies within plant parts and between plant species (Ding and Long, 2009). Roots have the least concentrations of these compounds, whereas floral parts and leaves have the highest (Dawson *et al*., 2000; Roumet *et al*., 2006). Cuticular wax compounds have been used as internal markers to determine diets selected by grazing ruminants. With the variability in composition and distribution of these compounds in different parts of the plant, floral parts,

leaves and whole plant, these different fractions should be analysed separately for these plant epicuticular compounds to know the selected parts of the plant by grazing and browsing herbivores. Fraser *et al*. (2006) reported that sheep selected different plant parts, showing preference for leaves than stem and shoot, as it would be expected under natural pasture conditions (Grant *et al*., 1987; Fraser and Gordon, 1997) suggesting that separate analyses of different parts of the plant would give better estimates of diets selected by ruminants.

Frequently used plant cuticular compounds are shown in **Table 1**. *N*-alkanes (C_{21}- C_{35}) are the prevalent class of these compounds and they are frequently used in nutritional studies of sheep and other classes of ruminants and non-ruminants as bio-markers (Hameleers and Mayes, 1998). About 10 % of these markers have even-numbered chain length with the rest being odd numbered. The most prevalent *n*-alkanes are the ones with 29, 31 and 33 carbon atoms (Dawson *et al*., 2000). Alkenes are mostly odd numbered mono-enes and they are prevalent in floral parts of plants, with chain lengths ranging from 23 to 33 carbon atoms (Roument *et al*., 2006). Alkenes are the other class of hydrocarbons with potential use as markers (Dove and Mayes, 2006; Charmley and Dove, 2008). No reported studies have been done to estimate diet selection using alkenes, thus validation of these is needed before they can be regarded as additional class of markers in nutritional studies. One of the major challenges associated with the use of alkenes is that they tend to be impure when running analyses using gas chromatography-mass spectroscopy (GC-MS), suggesting the need for additional effective analytical procedures to be able to declare them as suitable additional markers to conventional markers (*n*-alkanes) (Ali *et al.*, 2004). This makes alkenes less preferred as markers for use in animal nutrition studies.

However, long chain alcohols (LC-OHs) have been assessed and validated as markers for predicting diet composition (Ali *et al*., 2004; Ali *et al*., 2005). They are analysed following the same procedure as *n*-alkanes with few more additional analytical steps and they are reported to improve the accuracy of predicted diet composition (Bugalho *et al*., 2002; Kelman *et al*., 2003). Additional analytical steps are crucial to form trimethylsilyls (TMS), which are less stable than alcohol acetates, making these more suitable for GC-MS to yield better mass spectra distinct to other classes of aliphatic compounds. Therefore, they are suitable additional markers to *n*-alkanes over alternative plant cuticular wax compounds.

Table 2. 1 Main properties of plant cuticular wax compounds used as markers in nutritional studies

Class	C-length	Properties	Comments	References
***N*-alkanes**	C_{21}-C_{37}	Odd numbered C-chains	Highly common	Ali *et al*. 2005
Branched alkanes	C_{28}-C_{32}	Iso- and ante-iso-branched chain	Rare	Dove and Mayes 2006
1st-alcohols	C_{20}-C_{34}	Saturated even numbered chain	Common in high concentrations	Ashton, 1998
2nd - alcohols	C_{29}	Odd-Chained, mainly C_{29}	High concentration in conifer leaves	Dove and Mayes 2006

C-length – carbon chain length, 1st - primary alcohols, 2nd - secondary alcohols

2.4. Methods of estimating botanical composition of selected diets

2.4.1. Utilisation technique

Utilisation technique is one of the most primitive methods used in the assessment of diets consumed by grazing herbivores (McInns *et al*., 1983). This approach points out where and to what extent the rangeland is utilised. The biomass of the range is measured before and after the range is grazed by ruminants. Frequency and time of utilisation of plant species in the grazed range remains unanswered by this approach. However, combining the use of inert makers, video recording devices and the utilisation technique can modify and improve the quantification of the frequency and time of feeding on the rangelands. Regrowth after defoliation and invasion of the range by other animals rather than those of interest could negatively influence the accuracy of utilisation technique and subsequently reduce the accuracy and credibility of the results (Cook and stoddart, 1953). Therefore, these should be taken into consideration as underlying factors affecting the accuracy of utilisation technique in estimating botanical composition of diet selected by grazing ruminants.

Studies comparing samples obtained from fistula and utilisation data revealed no agreement between these two techniques (Laycock *et al*., 1972; McInnis, 1976). These differences may be attributed to sampling time, because ruminants chew the cud and the composition of utilised forage in the range is extrapolated from what is left in the range after utilisation and

does not account for effect of rumination. Data from the fore-mentioned studies and the one by Cook and Stoddart (1953) revealed that when plants are actively growing, and they are utilised by more than one grazing species; there is a limit to the use of the utilisation method in determining botanical composition of selected diets.

2.4.2. Direct observations

Direct observation of animals while grazing and cage plot procedures are used to evaluate botanical components of diets consumed (McInns *et al*., 1983). Direct observation is the visual assessment of range plant species in comparison with values of hand clipped forage species, whereas cage plot method is principally based on comparing the amount of herbage inside the cage with the amount outside after animals have grazed the area (Barnes, 1976). Direct observation of animals requires no sophisticated equipment. Identification of plant species consumed is the key drawback associated with this approach because animals graze continuously, and successive sample collection becomes biased. Besides, it is difficult to approach untamed animals close enough to do accurate observation of what they are foraging, and it even becomes hard to locate them (McInns *et al*., 1983). However, tame animals can be used for close observation. On the other hand, only one animal can be observed by one observer at a given time, while diet selection is a complex concept involving constant interaction between animals (Pieretti *et al*., 2006) with the likelihood of reducing the accuracy of this technique.

Video recording tools in conjunction with global positioning system (GPS) can be used to locate the site of animals where they are foraging and their faeces as well. Managing animal stocking rate and animal distribution are crucial factors to consider when using video recording devices because they might not cover all the grazed range resulting in missing animals outside the covered range and some animals may be difficult to distinguish from each other. Remote sensing devices have been validated by Leyequien *et al*. (2007) in assessing animal distribution. Bite size and time spent grazing have also been used to get an insight of quantitative information of grazing animals (Holechek *et al*., 1982). Time spent grazing has an ability to be used as an index of species preference and/or importance of plant species in the diet consumed. Grazing time (GT_i) of i^{th} species in the range is defined as the product of relative grazing time (RGT_i), proportion of the observation time spent grazing on

the range (G) and the total time spent by animals in the range (T). Where: RGT_i is the ratio of time spent grazing i^{th} species to the total time spent on all species grazed in the range.

$$GT_i = RGTi \times G \times T \quad \text{Eqn (1)}$$

Free *et al*. (1971) reported results of diet composition from direct observation method, which were more consistent with oesophageal fistulated (OF) animals than cage plot method (Laycock *et al*. 1972), suggesting that direct observation can be combined with other techniques to improve the accuracy of determining diet composition.

The complexities in distinguishing between hedonic and active grazing, makes it difficult to quantify and predict actual intake of each plant species consumed in the range. The experience of the observer and the number of animals grazing in the range may also hugely impact the accuracy and precision of direct observation method. The stage of development of the plant and the number of plant species in the grazed area could also affect the accuracy of this method, because as plant species ages it gets easier to identify (Free *et al*., 1971) as opposed to younger stages of growth of the plant. Similarly, when there are few numbers of plant in the grazed area as opposed to when there is a cumbersome number, it becomes easy to identify and distinguish consumed plant species by grazing animals.

2.4.3. Stable carbon isotopes

Plants follow two photosynthetic pathways: C_3 and C_4 (Van der Merwe, 1982). The ratios of ^{13}C and ^{12}C carbon atoms in plant tissues differ depending on the type of plant and the photosynthetic pathway. Plants that follow C_4 photosynthetic pathway are comprised of legumes, forbs and browse whereas tropical C_3 plants consist of grasses. Composition of consumed dietary components is evaluated either on oesophageal samples (Coates *et al*., 1987), animal tissues (Tieszen *et al*., 1983) or faecal samples (Jones *et al*., 1979). This method cannot distinguish plants (at species level) that constitute a consumed diet. However, the method could be used to determine the proportion of tropical C_4 and tropical C_3 plants classically in diet selected by ruminants grazing in rangelands. The contribution of legumes (C_4) towards dietary nitrogen supply is important in most feeding systems. There are few C_4 photosynthetic pathway-based plants in temperate regions (Mayes and Dove, 2000). Therefore, this method is more practically applicable to tropical regions. Estimates based on animal tissues such as fur, mohair and wool could be used to determine the ratio of C_3 and C_4 plants in a diet selected by animals (Dove and Mayes, 1996). However, differences in the

degree of digestion (degradability) of different feeds and on different animals are key factors for accurate estimation of diet composition. Plants with high digestibility potential are often underestimated when faecal samples are used because of the amount of carbon reaching faecal level (Mayes and Dove, 2000).

2.4.4. Inert markers

When diet composition is estimated using inert markers, profiles of these markers in faeces are compared with profiles of dietary components to quantify the proportions of dietary components consumed. Faecal collection should be timed for marker concentration stability when using external marker (commonly dotriacontane), to get representative samples which increases the accuracy of plant cuticular wax compounds-based estimates of diet composition (Valiente *et al*., 2003). On the other hand, modified acid detergent fibre (MADF), acid detergent lignin (ADL), indigestible neutral detergent fibre (INDF), indigestible acid detergent fibre (IADF), indigestible acid detergent nitrogen (IADN) and acid insoluble (IAI) do not require any dosing of animals. Therefore, they do not require time to stabilize in faeces, which enhances effective potential markers to predict the botanical composition of diets selected by free ranging animals. There are several methods and software tools used to compare these profiles. Theoretically, when the number of inert markers is equal to or more than dietary components the diet composition can be estimated using simultaneous equations (Merchant, 1996). When animals are grazing natural pastures characterised by complex vegetation for an animal to choose from, this become compromised because it is difficult to solve the equations, and these predict "nonsensical" diets (Newman *et al*., 1995; Barcia *et al*., 2007). However, the least-square optimisation method becomes more useful than matrix equations which can be solved manually. Several studies using automated softwares have managed to get accurate results (Dove and Moore, 1995; Newman *et al*., 1995; Barcia *et al*., 2007). Fundamentally, these automated computer-based algorithms minimise the squared difference between the concentrations of the observed plant cuticular markers in faeces and the concentrations arising from consumed diet. That is,

$$\sum_{i=1}^{n}[A-E]^2 = \sum_{i=1}^{n}[\frac{Fi}{Ft} - \frac{a*D1i+b*D2i+c*D3i+\cdots z*Dni}{a*D1t+b*D2t+c*D3t+\cdots z*Dnt}]^2$$

Where actual concentration of alkane *i* in faeces is denoted as (F_i), dietary components are D1, D2 and D3, quantities of dietary component D1, D2 and D3 are *a, b* and *c*, respectively. Concentration of alkane *i* in dietary components D1, D2 and D3 are denoted as $D1_i$, $D2_i$ and $D3_i$, respectively. Equation (2) can be applied by using proportion of marker concentration of individual dietary component in faeces from total faecal marker concentration (Mayes *et al*., 1994). Proportions of dietary components D1, D2 and D3 can be calculated from their absolute amounts using the following equation: where (*a*) is the dietary proportion of component D1.

$$D1 = \frac{a}{a+b+c} \qquad \text{Eqn (2)}$$

Proportions of *a*, *b* and *c* together sum up to a total of one kilogram of faeces. This information can also be used to determine whole diet digestibility, using the following equation,

$$\text{Diet digestibility} = \frac{(a+b+c)-1}{a+b+c} \qquad \text{Eqn (3)}$$

Fraser *et al*. (2006) described the use of *n*-alkanes approach as a method with no limited number of dietary components to be identified in the consumed diet. Conversely, Bugalho *et al*. (2002) reported that the limitation in using *n*-alkanes as markers for determining diet composition of sheep is the small number of *n*-alkanes available in comparison to numerous dietary components available for an animal to choose from, thereby, limiting its use in distinguishing complex dietary components. The number of *n*-alkanes should be equal or more than plant species in the consumed diet. There is limited number of dietary components possible to detect in a diet consumed by animals, since pastures with complex plant species have limited number of *n*-alkanes distinctive enough and available in sufficient quantities to be used as markers. Therefore, adding other classes of inert markers (MADF, ADL, AIA, INDF, IADF, IADN and LC-OHs) is essential to increase the accuracy of such approach by increasing the number of markers to be used in relation to generally complex botanical components of rangelands.

N-alkanes and LC-OHs showed a clear discrimination between species of a consumed diet with no misclassification of species in the diet consisting of clover, *Lotus* (*L. corniculants*

and *L. penduculatus*) and grass species (*Phalaris aquatic* and *Austrodanthonia richardsonii*) (Kelman *et al*., 2003). When *n*-alkanes were used alone, they predicted 18 ± 1.1 % of *Lotus* in the diet consumed by sheep, whereas when *n*-alkanes and alcohols were used in combination they predicted 26 ± 1.2 %. The actual concentration of *Lotus* in the sward was (31%). Therefore, the combination of LC-OHs and *n*-alkanes approach could increase the accuracy of estimating diet composition. Hence, the proof of the concept of using inert markers in rangelands is imperative, where animals are fed in a simulated natural environment by providing several different feeds from *ad libitum* to restricted feeding levels at different animal stocking rates. In a controlled simulated environment, it would be possible to compare the observed and the predicted botanical composition of selected diet. The use of inert markers in predicting diet composition in rangeland would be determined by the result from the proof of such concept.

2.4.5. Microhistogical procedures

This method is based on the use of microscope to identify plant fragments in faecal samples, stomach contents and oesophageal extrusa (Holecheck *et al*., 1982). Proportion of fragments found in faeces from consumed browse/forage species is then used to determine diet composition and preference. Stomach content method requires the slaughter of the animal to identify plant species available in the stomach, which are then used to determine the diet selected by the animal. Oesophageal fistula is invasive, animals are cut open at the oesophagus to get samples of bolus. Microhistological methods can be replaced with the combination of inert markers and utilization techniques to ensure that all plant components found in these samples are included in the estimation of diet composition. Plant components with similar concentrations of specific markers still have an opportunity of being distinguished by additional markers and they can be detected in consumed diet, consequently improving the accuracy of estimating diet composition.

Most wildlife researchers use the stomach content analysis method in large herbivores to estimate proportion of browse and forage species in the consumed diet. An important drawback of this method is the difference in degree of degradability of the consumed plant species, which alters the proportions of the original consumed diet found in the stomach when the animal is slaughtered (Vavra and Holechek, 1980; Holechek *et al*., 1982). Some dietary components maybe chewed and digested beyond recognition. Analysis of stomach

contents do not provide information about where and when the animal ingested the forage. Stomach contents are evaluated by: listing number of plant species found in the sample, measuring the volume, weight and the frequency of occurrence of each plant species (Chamrad and Box, 1964).

Wilson *et al*. (1977) proposed a trocar method as an alternative approach rather than slaughter of animals. Animals are tranquilized, cut open and samples are taken using a trocar, the wound is then sewn up. Overdosage of the sedative, wound infections and illnesses are some of the major possible causes of death of many animals subjected to this surgical procedure, therefore, endangered species should not be subjected to this method. Dead animals killed by wild carnivores and animals culled or slaughtered for consumptions are the ones that could be used to analyse the stomach contents. Hence, validation and improvement of the accuracy of the use of inert markers in predicting diet composition can be achieved through this approach. This could be achieved by sampling the digesta at faecal level and comparing its marker concentrations with plant samples from where the animal was grazing; the results are then be validated by data from stomach contents.

Fistulated animals have been used in domesticated herbivores to estimate diet composition (Leury *et al*., 1999). Oesophageal fistula is often preferred over rumen fistula because samples collected from the rumen are contaminated with rumen contents making it difficult to analyse for diet botanical composition. Samples should be collected immediately after an animal swallows because when samples are collected from the rumen they are likely to be chewed and degraded beyond recognition. Dove *et al*. (2000) and Dove and Mayes (2005) reported that there is enough data to prove that test animals and fistulated animals select the same diet. If this is the case, then concentration of a marker recovered from faeces of test animals and oesophageal extrusa from fistulated animals should be the same. Dove *et al*. (2000) considered examining the concept in castrate sheep and grazing ewes. Results were the same with those obtained by Leury *et al*. (1999) in sheep foraging on senescing grassland, where test animals and fistulated animal preferred the same diet or forage species. With regards to reliability of fistulated animals in determining diet composition, direct sampling from the mouth after a tamed animal has taken a few bites to get representative samples of what an animal selected in rangelands can replace the use of fistulated animals.

Faecal analysis received more attention than alternative methods of predicting composition of diet consumed by sheep in the late 1950s and early 1980s because of the exceptional advantages it possesses, and has been extensively discussed (Croker, 1959; Anthony and Smith, 1974; Scotcher, 1979; Holechek *et al*., 1982). Analysis of faecal samples for diet composition and selection does not interfere with natural behaviour of foraging animals. This approach is the most relevant and non-invasive to wild animals. The size of the faecal sample is limited by the quantity of faeces and how frequently animals defecates. However, there are serious setbacks associated with faecal analysis and this has been extensively discussed elsewhere (Smith and Shandruk, 1979; Sanders *et al*., 1980; Ward, 1981). These include: accuracy of the method in estimating diet composition because proportion of diet components found in faeces is often not the same as in consumed diet, because the rate of passage and degradation differs for different classes of plants across and within animal species. Preference indices of forage species cannot be determined, because the site where the forage was consumed cannot be identified. The approach is laborious; thus, the overall problem of this method is accuracy.

Anthony and Smith (1974) pointed out that 15 faecal samples and 50 rumen samples of grazing deer gave the same level of precision (Holecheck *et al*., 1982), and this was attributed to samples collected from animals grazing on the same location and season. Other studies (Free *et al*., 1971; Dunnet *et al*., 1973; Kessler *et al*., 1981) revealed that known diet contents were not in agreement with contents determined by faecal analysis (Holechek *et al*., 1982). However, Hansen (1971) described good consistency between ingested diet composition and faecal composition. On the other hand, Casebeer and Koss (1970) found close relationship between stomach contents and faecal material composition on four different seasons (i.e. winter 97.3%, spring 99.8%, summer 99.3% and autumn 99.6%) when using cattle, wildebeest and zebra even though other diet components were over or under estimated depending on the season and animal-animal variation. Johnson and Pearson (1981) reported high consistence (r = 0.99) between oesophageal fistulated and faecal content samples from cattle, with the trend for herbaceous plant species other than grass being underestimated and this could have been caused by high digestibility of these herbaceous plants due to high protein content compared to grasses. There are few studies comparing known diet contents with contents determined by faecal analysis. Therefore, this calls for further verification based on the accuracy of faecal analysis technique in predicting known diet composition in sheep. However, this approach can be used in conjunction with

other methods like inert markers to ensure that each species in the diet is identified without including any invasive alternative methods. Fragments not found in faeces can be traced by inert markers whether they were not consumed, or they were not detected, resulting in accurate estimates of diet botanical composition.

2.5. Estimation of forage intake

Estimation of total forage intake, proportion of plant species and plant parts consumed in a diet by rangeland herbivores is useful in predicting nutrient intake, animal performance and estimating grazing pressure put upon preferred plants by herbivores within an ecosystem (Provenza *et al*., 2015). Time spent grazing and bite size, which are derived from behavioural data, have been widely used in sheep grazing in natural grasslands to estimate feed intake (Decandia *et al*., 2000) and approaches encompassing water and/or sodium turnover (Silanikove *et al*., 1987; Dove and Mayes, 2005). The relationship between intake, digestibility and subsequent faecal output has been effectively used to estimate intake. The simple and yet fundamental equation in animal nutrition contains components (digestibility and faecal output) that are difficult to accurately determine in animals grazing in native grasslands (Dove, 2010).

That is, the relationship among (I) intake, diet digestibility (D), and faecal output (F), relationship is:

$$I = \frac{F}{1-D} \qquad \text{Eqn (4)}$$

Digestibility is measured on animals fed in a controlled environment where direct measurements of faecal output and intake can be estimated for subsequent estimation of digestibility. This digestibility value is then used in estimating intake of animals foraging in pastures using Eqn (4). There are problems associated with the estimation of faecal output and digestibility (Langlands, 1987; Dove and Mayes, 2005). The equation uses digestibility which is also calculated from intake to estimate intake. Faecal outputs are separate for each animal with a single digestibility value calculated from test animals which is extrapolated for intake estimation to all animals grazing a natural pasture. Representative estimates of digestibility are of more concern because erroneous digestibility when overestimation could

cause inflation in the estimated intake (Dove, 2010). However, the use of inert markers can improve the estimation of components used to estimate intake. Concentration of a marker in faeces and a feed or a diet are used to estimate digestibility using Eqn (6) (from 2.6.2.2), which mitigates the error of using a single digestibility value measured on animals fed in a different feeding system to that of interest where intake is measured.

2.5.1 Factors affecting forage intake

2.5.1.1 Rumen fill

Voluntary dry matter intake of forages is limited by distention of the gut, and load of digesta in the rumen (Allison, 1985). Rate of disappearance of digesta in the reticulo-rumen is negatively correlated to the bulkiness of the feed consumed and water content of the feed. Feeds that are fermented and evacuated from the rumen at fast rates are less filling because they reside and occupy space in the rumen for a short period of time (Aitchison *et al*., 1986). The slow fermented fibrous fractions of the feed, dominantly the neutral detergent fibre (NDF), are implicated as fraction of feed stuff with lengthy filling effect (Mertens, 1987). On the other hand, when the actively growing plants with dry matter content of less than 20% are consumed, the volume of the water in the rumen increases (Decruyenaere and Stilmant, 2009). Regardless of the high digestibility of young plants, the intake decreases due to the filling effect of the water in the rumen (Decruyenaere and Stilmant, 2009). Minson (1963) reported increased intake in sheep when the physical form of a feed was altered to leave the rumen relatively fast. Boiling *et al*. (1967) also found the same response of improved intake in relation to finely milling feed, which subsequently increase rate of evacuation of digesta in the rumen. Therefore, bulkiness of the feed regulates intake in ruminants. Diets with high NDF take longer time to pass the rumen because the animal regurgitates and spend longer time to finely chewing the feed before it evacuates from the rumen. This leads to reduced time spent eating and ultimately reduced intake. Hence, physical alteration of the feed influences intake.

2.5.1.2 Protein content

Generally, animals when given a choice, select different proportions of feeds with the aim to meet their protein requirements (Kyriazakis, 2003). Protein is first deaminated to remove

the excess nitrogen fraction. The deamination process demands energy, thus there is no benefit in consumption of excess protein by animals when the requirements have been met (Forbes, 2007). Protein increases intake by stimulating digestibility through its associative effect attributed to intensification of microbial activity and subsequently passage rate (Elliott *et al*., 1967; Kartchner, 1980; Allison, 1985). Therefore, intake can be improved by supplementing foraging with protein-rich feedstuffs.

2.5.1.3 Dietary fibre

Fibre, mainly neutral detergent fibre (NDF) and acid detergent fibre (ADF), is a plant fraction with low solubility in specific solvents (Knudsen, 2001) and is slowly digested compared to starch in the rumen (Jung and Allen, 1995). Fractions of detergent fibre are negatively correlated to voluntary dry matter intake (Jung and Allen, 1995). Van Soest (1965) reported a high negative ($p < 0.05$) correlation between voluntary dry matter intake (VDMI) and NDF ($r = - 65$) compared to ADF ($r = -53$) on sheep feeding on grasses and legumes. Osbourn *et al*. (1974) and Reid *et al*. (1988) found negative correlation between NDF and VDMI in sheep feeding on grasses and legumes. However, ruminants essentially need a diet composed of roughages to a certain extent for optimal rumen functioning and microbial protein synthesis. Fibrous fraction of a diet or a feed necessitates prolonged chewing, resulting in secretion of saliva which has a fairly neutral pH and serves as a buffering agent in the rumen. Optimum ruminal pH levels (6.7-7) allow conducive environmental conditions for ruminal microbial species to improve degradation of consumed forages by ruminants. Ruminal microbes adhered to solid and liquid fraction of digesta during evacuation of digesta from the rumen to subsequent stomach chambers with low pH levels (pH 3.5-5.5) and are digested and absorbed as microbial protein by the animal. Thus, consideration should be taken regarding the fibre content of supplementary feedstuffs because fibre content of a diet selected by sheep influences rumen fill due to bulk density and the space they occupy in relation to space available in the rumen over time. Minson (1967) reported increased rate of passage of digesta and subsequent intake in sheep due to grinding and pelleting compared to normal form of *Digitaria decumbens* (Pangola grass). Therefore, physical bulk density and chemical composition manipulation of the feed by pelleting and grinding or chemical treatment (i.e. urea treatment) can improve intake of poor-quality roughages.

2.5.1.4 Dietary supplementation

Much of the documented work has been focusing on animal performance pointers, which include body weight gain, milk yield and wool production responses in relation to dietary supplementation. However, voluntary feed intake is of prime importance because the performance indicators depend on it, which in turn is governed by several interacting aspects. Energy and protein content of the diet consumed by ruminants influences intake (Vazquez and Smith, 2000; Kyriazakis, 2003). Protein improves intake though improvement of digestibility which can be attributed to intensification of microbial degradation rates and subsequently, rate at which digesta is evacuated from the rumen. On the other hand, Elliot *et al*. (1967) found that feeding a dietary supplement with readily available energy suppresses intake in sheep. This may be due to the fact that sheep are normally able to generate energy from roughages and when supplemented with diet that is able to meet the energy requirements of the animal, volatile fatty acids (VFAs) increase in the blood and satiety is consequently reached and there is no need for further grazing.

2.6 Methods for estimating feed intake

Estimating intake of grazing animals is a major challenge compared to indoor animals. However, there is a number of techniques that have been developed to estimate intake indirectly by estimating the digestibility and faecal output using a single or a combination of internal and/or external markers with a different success rate and accuracy levels.

2.6.1 Estimation of intake using n-alkanes

The alkane technique for estimating forage intake has been well validated in studies using housed sheep (Duncan *et al*., 1999; Dove and Olian, 1998; Vulich *et al*., 1991; Lewis *et al*., 2003) and goats (Valiente *et al*., 2003). When animals are dosed with synthetic even numbered alkane of adjacent chain length with an odd numbered alkane intrinsically available in the plant, and recovered to similar extent, it becomes easy to estimate faecal output. Errors arising from incomplete recoveries cancel out when using Eqn (5). Plant alkanes measure digestibility as an internal marker (*i*) then synthetic orally dosed even numbered alkane measures faecal output as an external marker (*j*). Digestibility and faecal output are not measured separately for alkane pair (*i* and *j*) and consequently intake is then calculated using these components, as:

$$I = \frac{D_j}{\frac{F_J \times R_I}{F_I \times R_J} \times (H_i - H_j)}$$ Eqn (5)

Where: D_j is the marker dose rate of synthetic alkane *j*, F_i and F_j are the concentration of alkanes *i* and *j* in faeces, H_i and H_j are marker concentration of alkanes *i* and *j* in herbage, R_i and R_j are faecal recoveries of alkanes *i* and *j*. Absolute concentrations are not important rather the ratio of alkane faecal concentrations. This equation also accounts for possibility of readily having orally dosed synthetic alkane *j* in herbage consumed by the animal (H_j).

Dosing with short-chain saturated hydrocarbons resulted in excretion curves of *n*-alkanes with low amplitude, consequently reducing precision and accuracy of developing meaningful faecal *n*-alkane parameters for determining proportions in the diet. These errors could be carried over to the estimation of intake (Duncan *et al*., 1999). *N*-alkanes with long C-chain length result in more accurate results approaching true value of interest than *n*-alkanes with short C-chain length (Hilburger, 2017). When these hydrocarbons are used as markers, gas chromatography mass spectrometer allows both plant and dosed marker to be analysed simultaneously, which circumvent bias and analytical error (Forbes, 2007). Double alkane-based technique is superior compared to *in-vitro* based methods of estimating digestibility for intake estimation using chromium sesquioxide (Cr_2O_3) in both cattle and sheep (Malossini *et al*., 1996; Dove *et al*., 2000).

2.6.2. Estimation of digestibility

Errors arising from incorrect estimation of digestibility can reduce the accuracy of intake estimation using Eqn. (4), especially when the diet digestibility is overestimated, because the denominator will be small, and the intake will be inflated (Langlands, 1987). One of the possibilities is that when animals are being supplemented, they are-under fed because of overestimated intake which would result in reduced animal performance evaluated through low milk yield, poor carcass quality, low conception rates and low average daily gain. However, the use of double adjacent *n*-alkanes to estimate intake can circumvent individual animal variation in estimating digestibility and therefore overcome the issue of inaccurate estimation of intake.

2.6.2.1. Estimating in vitro digestibility

In vitro digestibility studies assume that diet from oesophageal fistulated (OF) animals can be used to represent diet of test animals (Dove, 1998). However, samples from OF animals are collected within a short period of time (minutes) of grazing whereas grazing time of test animals may take from a few days up to weeks before sample collection. Besides, diet samples from OF animals may differ to that of test animals because of surgical procedure previously performed to OF animals, and difference in sex, age, physiological status, breed and management. In view of these, Dove *et al*. (2000) argued that it is difficult to test how valuable the concept of using data from OF to test animals. However, use of alkanes ameliorate the problem of faecal output and digestibility because they are determined simultaneously, reducing possible analytical errors finally improve intake estimates.

2.6.2.2. Estimating digestibility using internal markers

Internal markers used to estimate digestibility include but not limited to: AIA, ADL, IADF, INDF and MADF. These markers have been successfully used but the differences in their recoveries have been a typical setback in using them to accurately estimate intake, diet composition and digestibility (Kanani, 2012) Diet digestibility is calculated from relevant concentration of an indigestible marker in the diet and faeces, as:

$$D = 1 - \frac{C_d}{C_f} \qquad \text{Eqn (6)}$$

Where: D is the digestibility, C_d is the concentration of marker in the diet and C_f is the concentration of the marker in faeces.

The technique of analyzing *n*-alkanes in faeces and forages have been used by Ordakowski *et al*. (2001) in horses and by Mayes *et al*. (1986) in sheep to determine digestibility and dry matter intake. *N*-alkanes are more accurate than conventional markers (AIA, ADL, IADF, INDF and MADF) due to low feacal recoveries of conventional markers than *n*-alkanes. Despite that, these markers have a potential for estimating digestibility of a diet selected by grazing sheep. Results obtained from these markers can be regressed with those estimated using *n*-alkanes to obtain standard equation. These markers are relatively cheaper and easy to analyse compared to *n*–alkanes. Peiretti *et al*. (2006) reported high variability in

digestibility when *n*-alkanes were used as internal markers. In contrast, Kelman *et al*. (2003) found that alkanes provided clear and better estimates of diet digestibility and diet composition. Generally, the error arising from assumed recovery correction for these *n*-alkanes is less than 3% (Kelman *et al*., 2003). Alkane C_{33} or C_{31} are used for determining digestibility as they are intrinsic component of dietary components and have high faecal recoveries of about 95% (Mayes and Lamb, 1984).

2.6.2.3 Estimating faecal output

Faecal collection from grazing herbivores is difficult and animals can be disturbed during sample collection. Harness faecal bags are mostly limited to males because samples from females are often contaminated with urine leading to increased irritation to animals due to increased faecal weight. As such, only data from males is used for both sexes and that could introduce bias due to differences in physiological and reproductive status. Metabolic harness specifically for female sheep was developed in the late 1970s to achieve separate collection of faecal and urinary excretions. However, the separator was found to be prone to blockage by faeces sometimes, losing some of the faeces, necessitating design modifications (Michell, 1977). Therefore, external markers have an advantage because there is no need for total faecal collection. These markers are consistently administered for 12-14 days twice a day and faecal collection is done on the last 4-6 days when faecal marker should have reached steady concentration in faeces (Duncan *et al*., 1999.). On the other hand, Ferreira *et al*. (2007) concluded that three to five days are sufficient for administered commercial alkane (C_{32}) to stabilize in faeces. Therefore, faecal sampling either by rectal grab or by picking them up from the ground is efficient after 3 to 5 days have elapsed since the day of dosing to predict diet intake and composition when using *n*- alkanes.

Identity of the animal defecating needs to be known for the later method to be applied and contamination of faeces by alkanes from the soil needs to be taken into consideration as a limiting factor. The rate of passage of a diet consumed dictates the time for markers stability in faeces. Sampling should be preferably done in the morning when digesta from all feeds consumed the previous day should have reached the end of the GIT. In wild herbivores grazing in the forest and that are not familiar with human intervention, rectal faecal grab is difficult, if possible. Video recording devices may be used in wild animals to identify the source and the place of faecal samples. The video technique may be restricted in an area

where animals are scattered in such a way that some cannot be reached by the camera. However, several video recording devices may be stored in different regions of the grazed area for a given time and technique can overcome the setback of direct observation of animals, which may disturb their normal foraging behavior.

Intra ruminal controlled device and hard-shell gelatin capsule are used to administer chromium sequioxide, which have high faecal recoveries in goats (Kababya *et al*., 1998) and sheep (Langlands, 1987) than other faecal markers that are used in ruminant nutrition studies. Difficulty in analysis of this marker as chemically distinct entities (offered or/and consumed diet), leads to inaccurate estimates of faecal output.

Alkane dosing frequency and faecal sampling frequency both affect the validity of using rectal grab samples as representative samples for total faeces (Vulich *et al*., 1991; Sibbald *et al*., 2000). To overcome these, three samples for three times for each animal provides enough information to determine across and within day variation (Valiente *et al*., 2003). Dove *et al*. (2000) showed that dosing twice on daily basis with the aim of overcoming diurnal variation patterns in faecal alkane ratios was successful as shown in **Table 2** or sampling twice a day could not detect the variation and therefore the dosed and intrinsic alkane ratios were both similarly unaffected. However, faecal concentrations of chromium from grab samples were lower than in total faeces although dosing frequency for chromium was the same as for alkanes (Dove and Mayes, 1996). Faecal output is determined using the following equation:

$$FO\ (kg\ DM\ day^{-1}) = \frac{mr\ (mg\ day^{-1})}{fc\ (mg\ kg^{-1}\ DM)} \qquad \text{Eqn (7)}$$

Where, *FO* is the faecal output, mr is the marker dose rate and fc is the faecal marker concentration.

Table 2. 2 Regression relationship for concentrations of various components dosed twice a day in rectal grab samples of faeces (*y*) from grazing sheep in relation to the concentrations in total faeces (x)

Component	Regression slope[A]	Different from y = x?
Nitrogen	0.997±0.0111	*NS*
Ash	0.985±0.074	*NS*
Herbage alkanes		
C27	0.990±0.0119	*NS*
C29	1.004±0.0104	*NS*
C31	1.003±0.0053	*NS*
C33	0.939±0.0410	*NS*
Dosed alkanes		
C28	0.968±0.0244	*NS*
C32	0.975±0.0215	*NS*
Chromium	0.904±0.0228	P<0.01
Chromium[B]	0.879±0.0191	P<0.001

[A]All regressions constrained through zero, since *y*-intercept of unconstrained regression did not differ significantly from zero. [B]Chromium concentration in the rectal grab samples against expected chromium concentration in total faeces based on chromium dose and known faecal output (the relationship between actual and expected chromium concentration in total faeces did not differ ($y = x$). (*NS*) Not significant (Adopted from Dove *et al*., 2000)

2.7. Estimation of supplementary intake

The cheapest source of nutrients available for herbivores grazing in rangelands is browse and herbage. During the dry season when there is low forage availability and of usually of poor-quality, sheep are generally fed supplement diets to mitigate nutritional stress, reach production target and sustenance. Cereal straws are the cheapest supplement available in most feeding systems in Sub-Sahara Africa and they are characterised by low protein and high fibre contents, which are associated with low digestibility (Anderson, 1978; Kebede, 2006). Understanding of their associative nature with available forage in the natural grasslands is a key aspect to improving voluntary dry matter intake of grazing animals.

2.7.1. Use of plant cuticular wax compounds to estimate supplement intake

The method of estimating diet composition described in *1.4.5* may be used to get proportion of supplement in the diet. As such, whole diet intake can be estimated using equation (5) if animals are dosed with an even-chain hydrocarbon commonly dotriacontane (C_{32}). Animal performance can be predicted easily when the intake of the supplementary feed is known

and the interaction between the supplement and other dietary components is known. The supplementary feed needs to have or be labelled with a distinctive alkane profile (Elwert and Dove, 2005). For a supplement feed/diet to be coated with external source *n*-alkanes, two scenarios exist. Supplemented diet or feed to be treated with an external source of *n*-alkanes, for instance beeswax it is when the supplement feed/ diet does not have sufficient *n*-alkane concentration to be detected as a distinct feed source to allow estimation of diet composition (Dove and Olian, 1998; Dove, 2010).When roughage-based supplementary feeds are used with distinct and known alkane profile there is no need to treat with external source of hydrocarbons for manipulation of *n*-alkane levels because there are sufficient levels of natural hydrocarbons in the supplement permitting estimation of diet composition (Elwert and Dove, 2005). When diet composition is determined, diet intake can be partitioned into its constituents. Therefore, supplement intake can be determined. Diet digestibility can be estimated from this approach while considering forage-supplement interaction and individual animal difference on digestion capability. Errors in estimating whole diet intake and supplement intake can arise if diet composition is not accurately estimated.

2.7.2 Use of supplement intake to estimate forage intake

For known diet composition, intake of all dietary components can be estimated provided that one dietary component has a distinctive alkane profile with adequate concentrations fed in known amounts. Studies have shown that feeding a known amount of a supplement have a potential to estimate diet intake in sheep grazing in natural environments without dosing with even chain hydrocarbons (Dove and Olian, 1998; Dove *et al*., 2002; Elwert and Dove, 2005; Charmley and Dove, 2008).

Animals grazing in rangelands are usually fed on additional diet component, this component is generally a concentrate and it can be fed in known amounts. Dove and Olian (1998) reported that if the supplement does not contain a distinctive *n*-alkane profile or content it can be coated or labelled with an external source of known alkane profile (e.g. beeswax). This approach offers an advantage because it does not require dosing of animals, which prevents any disturbance of natural foraging behaviour of animals. This technique requires estimation of faecal recoveries to be used to estimate diet composition as the first step, then faecal samples can be collected directly from the rectum and there is no need for total feacal collection.

Beeswax is generally added to the supplement (Dove *et al*., 2002; Elwert and Dove, 2005; Charmley and Dove, 2008). Briefly, the supplement is coated with 1.22% (e.g. 610 g of beeswax to 50 kg of supplement feed) of finely grated beeswax previously dissolved in 2.5 L *n*-heptane with moderate heating to synthesise *n*-alkane profile. Octacosane (C_{28}) is added to the solution to make a final concentration of 250-300 mg/kg of organic matter. Supplementary feed is then sprayed with the solution and mixed thoroughly for uniform distribution of the coating solution. Cereal based supplements generally contain low levels of cuticular alkanes (Charmley and Dove, 2008). Therefore, there is a need to subject the feed to external sources of alkanes (octacosane and beeswax). Proportions of diet components are then used to calculate intake of each diet component in consumed diet. The intake for a given dietary component was calculated, as:

$$I_{dc} = I_s \times (P_{dc} / P_s) \qquad \text{Eqn (8)}$$

Where I_{dc} is the intake for a given dietary component, I_s is the intake of a supplement, P_{dc} is the proportion of a given dietary component and P_s is the Proportion of supplement with known amount in the diet.

2.8 Summary

It is concluded that *n*-alkane based estimates are more accurate than alternative techniques of estimating intake, because these compounds take into consideration differences in individual animal intake and interaction between forage and supplement diet offered. When *n*-alkanes are used alone, they can discriminate between a fewer number of species than in a diet usually consumed by goats and sheep grazing in communities consisting of complex plant species. This is caused by limited number of *n*-alkanes available and their quantities on consumed plant species in the diet. Conventional markers (AIA, ADL, INDF, IADF and MADF) in combination with *n*-alkanes and long chain alcohols have a potential in determining diet composition. Intake levels of supplement diet can be used to determine intake of the whole diet without dosing the animals with synthetic even numbered *n*-alkane.

Chapter 3

Determination of proportion of feed consumed as an index for diet selection in sheep

Abstract

The study determined the effect of reducing dietary ingredients and group feeding on proportion of feed consumed (diet selection), total intake, and digestibility in choice-fed sheep. There were five experimental feeds: veld hay (VH), sorghum stover (SS) and maize stover (MS) fed *ad-libitum*, and Lucerne hay (LH) and bean straw (BS) fed at restriction levels of 0.15 and 0.350 kg/day per sheep, respectively, in a group or individually fed sheep. Twelve sheep (mean weight 29.7 ± 4.63 kg) were assigned to three treatments. In treatment one, five feeds were fed to sheep fed as a group of 9 sheep (G). In the second treatment, five feeds were fed to sheep penned in isolation (I) and in the last treatment, four feeds with sorghum stover removed were fed to sheep penned in isolation (R). Proportion of BS, VH, LH and MS did not differ between sheep in G and I, however sheep in I consumed less (p <0.04) SS in their selected diet than sheep in G. Diet compositions were similar (p >0.05) in the proportion of BS, VH, LH, and MS between sheep in R and I. Similarity in proportion of these dietary ingredients consumed between sheep in R and I may be due to less selection of SS; therefore, its removal did not sufficiently influence consumption and selection of other dietary ingredients. Feeds with low crude protein (CP), high acid detergent fibre (ADF) and neutral detergent fibre (NDF; i.e. MS and SS) were less selected by sheep. Diet selection was influenced by group feeding, but not removal of dietary ingredient in sheep. Therefore, scarcity of one dietary component cannot force animals to feed on undesired available feeds of the similar or poor quality to absent dietary ingredient.

Key words: cereal straws, grazing, rangelands, ruminants

3.1 Introduction

In Africa, most ruminants graze in rangelands that are composed of a vast number of complex native plant species (Salem and Smith, 2008), which vary in level and type of nutrients and secondary metabolites. Theoretically, ruminants eat to meet their nutritional requirements, and their daily selection of diets depends on the age of the plant and animal, plane of nutrition, environmental conditions and animal physiological state (Provenza *et al*., 2003). The nature of natural grasslands gives a cafeteria style of feeding, which provides an opportunity for herbivores to select plants and plant parts to rectify and compensate discomfort caused by over ingestion of toxins and under-or-over ingestion of nutrients (Kawas *et al*., 2010).

Reduced feed intake in herbivores grazing in rangelands during dry season is driven by limited access to adequate quantities of high-quality herbage to meet nutritional requirements. This is associated with overgrazing, soil erosion, and climate change (Salem and Smith, 2010). Crop residues, such as straws are characterised as feedstuffs that are inherently high in lignin and fibre with low crude protein content (Anderson, 1978; Kebede, 2006), which is associated with reducing feed intake in ruminants (Meyer *et al*., 2010). During the dry season when there is low availability of good quality herbage, most pastoral livestock owners feed their ruminants on straws to sustain animals at least at maintenance level or minimal growth and production (Anderson, 1978). Regrettably, the utilisation of straws as feed sources has not been fully exploited, especially in rural production systems, because there is limited understanding of their potential.

In natural environments, ruminants prefer mixture of different plant species due to a number of factors, which include: optimum nutrient balance (Rutter, 2000), maintaining optimal rumen function (Senft *et al*., 1987) and conditioned taste aversion (Duncan *et al*., 2003; Rutter, 2006). Most feeding trials are conducted under field conditions or controlled environments where there are few numbers of plant species for an animal to choose from, and results from such trials can be misleading when applied to animals feeding in natural grasslands. Therefore, there is a need to simulate a natural grazing environment when conducting choice feeding trials by providing several different feeds at once at different levels of feeding and numbers to animals at different stocking rates.

There are constrains in determining feed intake and subsequent diet selection by ruminants grazing in rangelands. Spatial distribution of feed types temporarily makes feed variation difficult to capture. It is not clear if an animal having the same feed choices or with one feed absent in feed ingredient choices, or animals are fed in a group compared to feeding individually, will consume and select diets of the same quantity or quality. Such diets will have variation in dietary ingredient selected and intake but will not differ in digestibility. It is imperative to determine feed choices, intake and composition of herbage that ruminants consume in their natural environment. This will help improve management of available feed resources for ruminants reared by resource-limited farmers in Sub-Sahara Africa through implementation of grazing management strategies that will exploit alternative feed sources (e.g. straws) to sustain production levels especially during dry seasons. The objective of the study was to determine the effect of group feeding and removal of dietary ingredient (*Sorghum bicolor*) on diet selection, nutrient and total intake, and digestibility in choice-fed sheep. It was hypothesised that group feeding and removal of sorghum stover has an effect on diet selection, nutrient and total intake, and digestibility in choice-fed sheep.

3.2 Materials and methods

3.2.1 Study site and ethics

The protocol (AREC/072/2015M) was approved by the Animal Research Ethics Committee for the trial that was conducted at Ukulinga research farm in the University of KwaZulu-Natal, Pietermaritzburg, located at (29° 39' 49, 930" S 30° 24' 14,630" E) in the sub-tropical hinterland at an altitude of approximately 700 m. The area receives an annual rainfall of 735 mm, which falls between October and April with the minimum and maximum mean temperature levels of 8.9° C and 25.7° C, respectively. Predominant vegetation include native grasses *Themeda triandra* (kangaroo grass) and *Heteropogon contortus* (black spear grass).

3.2.2 Study animals and feeds

Twelve clinically healthy *Ovis aries* (Merino sheep) with mean weight of 29.65 ± 4.63 kg were used in the trial. These sheep were dual purpose breed with a wide spectrum of grazing in these climatic conditions (Ngwa *et al*., 2000; Cloete *et al*., 2001). Sheep were ear tagged

for identification. Four pens (length 7.32 m, width 4.24 m and area 30.98 m^2) with concrete floor were used to house sheep. One pen was used for sheep penned as a group and there other three pens were for sheep penned individually. During the 10-day preliminary period, sheep were allowed to adapt to the experimental feeds and the environment. All twelve sheep were grouped together and fed: *Themeda triandra* (veld hay), *Sorghum bicolor* (sorghum stover), *Zea mays* (maize stover), *Medicago sativa* (Lucerne hay) and *Phaseolus lunatus* (bean straw). During the trial three feeds: maize stover (MS), sorghum stover (SS) and veld hay (VH) were fed *ad libitum*. Lucerne hay (LH) and bean straw (BS) were fed at restriction of 0.15 and 0.350 kg/day per sheep, respectively. Lucerne hay was restricted to avoid overconsumption, which may lead to bloat; together with BS both gave supplementary protein. Each feed was provided on separate feeding troughs (length 0.5 m, width 0.35 m and height 0.2 m). Sheep were fed once a day at 0900 h and feeding troughs with VH, MS and SS refilled at 1500 h. Feeds were placed at the same time and randomly in all pens daily to avoid conditioned learning among position of feeding troughs and dietary ingredients (Alonso-Díaz *et al*., 2009).

3.2.3 Experimental design and treatments

Sheep were randomly allocated to treatments. In treatment one, five feeds were fed to sheep fed as a group of 9 sheep (G). In the second treatment, five feeds were fed to sheep penned in isolation (I) and in the last treatment, four feeds (without sorghum stover) were fed to sheep penned in isolation (R). Sorghum stover was removed in one pen out of the 3 pens where sheep fed individually were penned on the first week and then in the other 2 pens in the following week and this was done twice alternatively. There was no adaptation period given during rotation of animals in treatments because of previous exposure to all 5 feeds prior to commencement of the trial. Three days of adaptation to carrying faecal bags was given to sheep. Intake of each feed and total tract digestibility of feeds in treatment G (3 sheep) and those fed individually (I and R) was done for 7 days. After 10 days, 3 sheep from treatment G were randomly allocated to treatments I and R, those in treatments I and R re-allocated back to treatment G and intake of each feed and total tract digestibility of feeds measured for 7 days. This was repeated until all sheep were allocated to all treatments at least once; making G (n=4), I (n=6) and R (n=6).

3.2.4 Sampling and experimental measurements

Intake of each feed was determined by weighing the feed offered and subtracting feed spillage and feed refusal over seven days. Daily feed intake was calculated by dividing the intake by seven. After the trial, the pen used for treatment G was sectioned to calculate feed spillage in sheep fed as a group. Sectioned portion of the pen had dimensions of: length 4.24 m, width 0.72 m and area 3.05 m^2. This sectioned portion was partitioned into two layers; the top and the bottom layer. Top and the bottom layer contained 60% and 10% of the feed respectively with the remaining percentage being faecal and urinary excretions. Layers of sectioned portion of the pen were weighed (DM g/kg) and proportions of feed in them were determined. Spillage of the sectioned portion was determined as the sum of the two proportions of the layers. Quantity of feed from the sectioned area was extrapolated for the whole pen. Feed spillage was calculated by multiplying the amount of spillage in the sectioned portion by the factor (area of the sectioned portion divided by the area of the whole pen). Feed spillage of different feeds in sheep penned individually was picked up daily by the hand, put on separate labelled plastic bags and weighed. Feed selection ($F_{s)}$ was determined by using the selection index used for feed preference by Ibhaze *et al*. (2016) with slight modification from Ngwa *et al*. (2000).

$$F_s = \frac{individual\ dietary\ component\ intake}{total\ dietary\ component\ \ intake}$$

Percentage digestibility was calculated using the following equation:

$$\% \text{ Digestibility} = 1 - \frac{total\ faecal\ output}{total\ feed\ intake} \times 100$$

3.2.5 Chemical analyses of experimental feeds

Feed and faecal samples were oven dried at 60° C for 96 hours. Chemical analyses of all samples were done at the University of KwaZulu-Natal, Pietermaritzburg in the Animal & Poultry Science laboratory. Samples were ground to pass through a 1mm screen (Wiley Hammer mill) prior to analyses. Nitrogen content was determined using LECO TruSpec nitrogen analyser (LECO FP200, LECO, Pretoria, South Africa) following method by Association of Official Analytical Chemistry (AOAC, 1997). Crude protein (CP) was determined by multiplying nitrogen content of all feeds by the factor 6.25 (protein = nitrogen content × 6.25). Moisture content was determined following AOAC Official Method 934.01 (Nancy and Wendt, 2003). Dry matter (DM) was calculated as 100-moisture content. Acid

detergent fibre (ADF) and neutral detergent fibre (NDF) was determined using ANKOM 220 fibre analyser (ANKOM Technology, USA). Hemicellulose was calculated as the difference between NDF and ADF (hemicellulose = neutral detergent fibre-acid detergent fibre).

Table 3. 1 Chemical analyses of experimental feeds

Feed	Chemical composition (g/kg DM)				
	DM	NDF	ADF	CP	HEM
Bean straw	911	697	485	71	212
Veld hay	921	779	503	41	276
Lucerne hay	901	448	334	182	114
Maize stover	915	824	532	37	292
Sorghum stover	916	766	482	37	284
SEM	2.99	59.82	30.83	24.90	30.00

DM: dry matter; NDF: neutral detergent fibre: ADF; acid detergent fibre: CP; crude protein: HEM; hemicellulose: SEM; standard error of mean.

3.2.6 Statistical analyses

Effect of group feeding and removal of sorghum stover on diet selection, nutrient intake, diet intake, dietary ingredient intake, nutrient quality selected and digestibility data sets were analysed using the general linear model of SAS ® 9.2 procedure (PROC GLM). Student-Newman-Kewls (SNK) was used to compare means that were significantly different at $p < 0.05$. All results were presented as means, fitted to the model.

$Y_{ijkl} = \mu + P_i + PO_j + T_k + \varepsilon_{ijkl}$

Where Y_{ijkl} is the observation, μ overall mean, P_i effect of period in weeks ($_i$ = 1-4), PO_j effect of pen position (j = 1-4), T_k effect of treatment (k = 1-3) and ε_{ijkl} residual error.

3.3 Results

3.3.1 Proportion of dietary ingredients consumed

Dietary proportions of BS, VH, LH and MS did not differ ($P > 0.05$) between sheep fed as group and individually with or without sorghum stover. However, sheep fed individually with sorghum stover selected a diet that had a lower proportion ($P < 0.05$) of sorghum stover compared to diet selected by sheep fed as a group. There was a tendency for sheep fed

individually (with or without sorghum stover) to select a diet that was predominantly dominated by veld hay (≈50%), while the diet selected by grouped sheep was dominated by both bean straw and veld hay. The RMSE was highest for veld hay, followed by bean straw then the rest (maize stover, sorghum stover and Lucerne hay).

Table 3. 2 Effect of group feeding and removal of sorghum stover on diet selection (g/g) of Merino sheep

Dietary ingredient	**Treatment**			**RMSE**	**Significance**
	G	I	R		
Bean straw	0.347	0.298	0.249	0.065	NS
Veld hay	0.314	0.473	0.594	0.114	NS
Lucerne hay	0.148	0.139	0.121	0.027	NS
Maize stover	0.071	0.035	0.039	0.035	NS
Sorghum stover	0.132^{a}	0.057^{b}	0.000^{c}	0.028	*

G: group sheep with all feeds; I: individual sheep with all feeds; R: removed sorghum stover in sheep penned individually; RMSE: root mean square error; ^{abc} Means within a row with different superscripts differ *significantly at P <0.05; NS: not significant.

3.3.2 Dietary ingredient intake, total dry matter intake, diet quality selected, digestibility and nutrient intake

There were no differences (P >0.05) in dietary ingredients and nutrient intakes and diet quality in the diet consumed by sheep fed as a group (G) compared to sheep fed individually with (I) or without sorghum stover (R). Dry matter intake was similar (P >0.05) between sheep fed as a group (G) and sheep fed individually (I) assigned to similar dietary choices. There were no differences (P >0.05) in total dry matter intake levels between sheep fed individually with (I) or without sorghum stover (R). However, sheep fed as a group (G) had noticeably low (P < 0.05) dry matter intake levels compared to sheep fed individually without sorghum stover (I).

Table 3. 3 Effect of group feeding and removal of sorghum stover on total dry matter intake (kg/day), diet quality (g/kg day), in-vivo total tract digestibility (g/kg) and nutrient intake (g/kg day) by Merino sheep.

Parameter	Treatment			RMSE	Significance
Intake (kg/day)	G	I	R		
Bean straw	0.316	0.297	0.294	0.015	NS
Veld hay	0.297	0.492	0.707	0.189	NS
Lucerne hay	0.135	0.14	0.143	0.01	NS
Maize stover	0.067	0.038	0.046	0.031	NS
Sorghum stover	0.121	0.057	0	0.027	NS
Total dry matter intake	0.926^{b}	1.106^{ab}	1.183^{a}	0.109	*
Diet quality selected (g/kg)					
Crude Protein	71.60	69.30	69.67	6.147	NS
NDF	703.00	710.94	746.24	44.509	NS
ADF	471.03	474.98	494.88	30.388	NS
Digestibility	634.80	639.30	608.30	57.80	NS
Nutrient intakes (g/ day)					
CPI	65.81	70.02	77.52	5.206	NS
NDFI	651.86	721.11	857.13	98.229	NS
ADFI	436.47	485.28	570.11	63.899	NS

G: group sheep with all feeds; I: individual sheep with all feeds; R: removed sorghum stover in sheep penned individually; CPI: crude protein intake; NDFI: neutral detergent fibre intake; ADFI: acid detergent fibre intake; RMSE: root mean square error; ^{ab} Values within a row with different superscripts differ *significantly at $P < 0.05$.

3.4 Discussion

Voluntary dry matter intake of different dietary components consumed by ruminants is governed by several interacting aspects which include: group size of animals, level of energy required by the animal (Leng, 1990; Weston and Davis, 1991), rumen fill or distention of the gastrointestinal tract (Baumont *et al*., 2000; Drescher, 2003) and physiological state of the animal (Leng, 1990). Adequate intake levels are important in ruminants to ensure growth, maintenance and productivity (Grant and Albright, 2000). Therefore, understanding of factors that govern intake is imperative to achieve such levels and assists in identifying fundamental ways to improve performance of domesticated grazing animals.

Generally, sheep select feeds with the highest crude protein content (Kyriazakis and Oldham, 1993). However, in this study sheep fed as a group selected high proportions of sorghum and maize stover, which are both of inferior crude protein content in their diets compared to diet selected by sheep fed individually. This may have been due to facilitated-

feeding behaviour where visual cues of one animal feeding stimulates the others to feed on the same feed (Rook and Penning, 1991). Establishment of a dominance hierarchy in group-fed animals may have caused dominant animals to feed on poor quality stovers just to prevent animals lower in the hierarchy from eating resulting in high consumption of the stovers. A decline in availability of better-quality alternatives due to competition, limited space and accessibility to VH, which was the only feed with the highest CP that was offered *ad-libitum* may have forced sheep to consume the stovers. Additionally, Garcia *et al*. (1995) reported that sheep grazing on pasture had a tendency of selecting plants material that contain high content of neutral detergent fibre and MS had the highest NDF content than the rest of the feeds. Feeds with high structural cell components (i.e. cellulose, hemicellulose and lignin) in plant tissue (Briske *et al*., 2008) pass slowly in the reticulorumen than feeds with low cell structural components (Allen, 2000) and the earlier is associated with rumen fill which is the physical constraint of voluntary feed intake (Castells *et al*., 2012). Physical and chemical components of the feed play an important role in digestibility (Provenza *et al*., 2003). High level of cell structural components relative to cell soluble components (i.e. protein, lipids and starch) may be the reason for observed low selection level of SS and MS in all diets consumed by sheep across treatments, since feeds with low digestibility are often associated with low levels of intake. However, digestibility of diets consumed was similar among sheep fed as a group and sheep fed individually with or without sorghum stover. This suggest that the diet selected by sheep in this trial was similar in quality and that may be due to animals selecting predominantly veld hay in their diet, which is high in CP. Protein has been reported to increase intake through improvement of associative digestibility, attributed to intensification of microbial activity and subsequently passage rate (Elliott *et al*., 1967; Kartchner, 1980; Allison, 1985).

The results of this study are in contrast to Phillips (2004), who reported increased dry matter intakes in calves fed as a group of three individuals compared to calves fed in isolation. Even though in the present study there was no significant difference in dry matter intake between sheep fed individually with sorghum stover compared to both sheep fed as a group and sheep fed individually without sorghum stover, a trend of total diet intake increments due to decrease in number of animals in the pen was noticed. This implies that the absence of competition and increase in total space availability improved dry matter intake. Sheep are gregarious animals and generally eat at the same time or follow a precedence, therefore space becomes an important factor as an underlying constraint to feed intake. Odoi and

Owen (1993) reported different results compared to the present study where sheep feeding on straws showed an increase in feed intake as the number of animals in the pen increased from one to two. On the other hand, Chua *et al.* (2002) reported no relationship between group size and amount of hay consumed by calves before and after weaning. However, the results of this study are in agreement with Kondo *et al.* (1989) who reported that an increase in number of animals was associated with reducing feed intake in cattle.

Sheep fed individually with or without sorghum stover consumed diets that are similar in proportion of dietary ingredients (BS, VH, LH and MS). This was achieved by slight alterations in the proportions of feeds selected in each treatment (I and R) and it is likely that this was done to buffer changes in digesta composition (Fedele *et al*., 1993; Baumont *et al*., 2000). The results of the present study are in contradiction to the notion postulated by Ginane *et al*. (2002) that suggested stimulation of improved intake levels in ruminants feeding on more diverse plant communities compared to less complex plant communities. On the other hand, Reid and Jung (1956) previously reported increased intake when several hays were provided in cafeteria as opposed to when any hay was provided alone. Despite that, this may be caused by low acceptability (Allison, 1985) or preference, which in the present study was low for sorghum stover, which then led to partial selection and subsequently insignificantly contributing to consumed diet when included as a feed ingredient choice for sheep. Therefore, its removal had no influence on total feed intake, nutrient intake, dry matter intake and digestibility. Nutrient specifically found in SS that animals required and was missing in other present alternative feed choices for animals to exhibit compensatory feed intake to make up for missing dietary nutrient nullified the influence of the removal of SS on quality of diet selected by sheep. Moreover, Allison (1985) proposed that a similar scenario to that of Reid and Jung (1956) would most likely occur in animals grazing on natural pastures. Nevertheless, the threshold of the number of feeds removed in a selection that will change the quality of diets selected and alter intake is still not known. It seems possible that diet quality (nutritive value and digestibility) selected by sheep may be controlled using two methods that are antagonistic: by either slightly altering or adjusting proportions of feeds in the diet consumed while maintaining similar total intake, or by altering intake of feeds in a selection of feeds. If the proposed mechanisms are true, factors influencing the adoption of any one of these proposed mechanisms by ruminants is still undocumented. Verification of these mechanisms warrants further study, given that diet

quality selected in this study was similar across treatments without varying feed proportions selected.

Owing to exposure to feeds in utero and early stages of life, animals extract nutrients efficiently from consumed feeds regardless of quality compared to feeds they are exposed to in later stages of life given that these feeds are offered together in cafeteria feeding system (Villalba *et al*., 2015). The aforementioned epigenetic factor in conjunction with the presence of poor quality roughages supplied *ad-libitum* (i.e. sorghum stover and maize stover) and positive contextual learning (Villalba *et al*., 2015) may have caused sheep across treatments (I and R) to select diet dominated by veld hay, which is inherently low in CP, high in NDF and ADF compared to Lucerne hay and bean straw owing to the fact that sheep in this trial were reared on *Themeda triandra* (veld hay).

3.6 Conclusions

Consumption of poor-quality feeds in a diet when feeds are offered in cafeteria system can be influenced by the presence of companions in sheep due to limited space and accessibility, and competition amongst animals for better-quality alternatives available when animals are fed in an area where their movement is restricted. Group feeding of sheep reduced feed intake compared to sheep fed individually without sorghum stover and had a tendency of making animals select a diet that is predominantly dominated by a high proportion of veld hay, which is the best alternative in feeds offered *ad-libitum*. Ingredient intake, nutrient intake, diet quality and digestibility were insensitive to the removal of sorghum stover. However, the threshold of ingredient choice removal is still not known, above which ingredient removal will alter diet quality and intake, warranting further research on the subject.

Chapter 4

Effect of animal stocking rate on dry matter intake, botanical composition, digestibility and passage rate of diet selected by sheep

Abstract

The objective of the current study was to determine the effect of animal stocking rate on dry matter and nutrient intake, botanical composition, nutrients selected, total tract digestibility and passage rate of diet consumed by sheep. The possibility of predicting dry matter and nutrient intake, botanical composition, nutrients selected and total tract digestibility using inert markers was also assessed. Fifteen sheep (mean body weight 46.5 ±3.3 kg) were blocked by body weight into 4 groups and each sheep was randomly allocated to 4 stocking rates. Sheep were fed maize stover (MS), sorghum stover (SS) and veld hay (VH) *ad-libitum*, each feed was offered separately at stocking rates of 1, 2, 4 and 8 sheep per pen. To assess the effect of animal stocking rate (SR) on passage rate of digesta, 1 sheep each from stocking rates 1 (SR1) and 2 (SR2) animals per pen and 2 sheep each pair from stocking rates of 4 (SR4) and 8 (SR8) animals per pen were randomly selected and dosed with Ytterbium (particulate) and cobalt-ethylenediamine tetra acetic acid (Co-EDTA; liquid) markers. Fractional passage rate (liquid and particulate) in both the rumen and in the hind gut, mean retention time and total mean retention times of diet selected by sheep across treatments were similar. *In-sacco* degradability was measured in 2 fistulated sheep (mean body weight 58.8±5.0) to evaluate the nutritive value of the experimental feeds. Potential degradability of forages ranged from 634.23 to 716.35 (g/kg DM) and effective degradability (ED_{DM}) from 321.93 to 413.58 (g/kg DM). Effective degradability of dry matter of forages was negatively correlated to ADF and CP, and positively correlated to solubility (*a*). Intake of dietary ingredients, nutrients (crude protein: CP, neutral detergent fibre: NDF and acid detergent fibre: ADF), total dry matter and composition of diets selected by sheep across treatments were similar. Total tract digestibility and mineral intake (Ash) differed across treatments. Sheep in SR2 had the highest digestibility and consequently increased dry matter intake. An optimisation procedure was used predict diet selection by minimising the sum of the squared discrepancies between the proportional concentration of the marker (aid insoluble ash: AIA, modified acid detergent fibre: MADF, and acid detergent lignin: ADL) in faeces (A) and their proportional concentration in dietary components (E) (MS, SS and VH), corrected for

faecal recoveries of markers. These results demonstrate that inert markers can be used with wide-ranging application to predict several components of a diet selected by sheep.

Key words: degradability, nutritive value, roughages, space allowance

4.1 Introduction

The amount and quality of dry matter consumed by ruminants are direct determinants of animal performance. Identifying and measuring mechanisms involved in the regulation of voluntary dry matter intake are vital for prediction of animal performance. Accurate formulation of diets and choice of feedstuffs suitable to meet animal nutritional requirements rely on accurate estimation of intake. Voluntary dry matter intake of forages is regulated by interacting physical constraints and metabolic feedbacks (Huhtanen *et al*., 2016). These range from animal factors to physio-chemical properties of feeds (Magadlela, 2001), which are complex and difficult to measure simultaneously. Animal factors include but not limited to: age and production status/stage of an animal, physiological factors (blood glucose level and body fat reserves), ambient temperature, and rumen ecology (Decruyenaere *et al*., 2009). Whereas plant factors include: development stage of the plant and secondary plant metabolites (Salem and Smith, 2008). The physical distention of the rumen (rumen fill) is implicated as a constraint to feed intake and is governed by the rates of degradation and evacuation of digesta in the rumen (Chilibroste *et al*., 2000). Hence, the amount of poor-quality forages consumed by sheep in natural grasslands cannot support the genetic production potential of animals even if the feed is available *ad libitum* because the quantity of forage consumed is restricted (Magadlela, 2001). The rate at which fibrous roughages are cleared from the rumen to subsequent stomach compartments restricts voluntary dry matter intake through prolonged time spent by the roughages and the filling effect they have in the rumen.

Several studies have been conducted to assess the relationship between animal stocking rate (SR) and gain per animal and determined the optimum stocking rate. However, the threshold above which dry matter intake and composition of a diet selected by sheep is affected by animal stocking rate is unknown. Degradability and rate of passage of feedstuffs become important variables in measuring digestive aspects of consumed diet. These determine the rate at which nutrients are degraded and the extent at which nutrients are absorbed and ultimately influence voluntary dry matter intake. Therefore, it is important to determine and

quantify the relationship between animal stocking rate and composition of diet consumed through evaluation of degradability, passage rate and digestibility parameters of a diet consumed by sheep.

The objective of this study was to determine the effect of animal stocking rate on dry matter and nutrient intake, botanical composition, nutrients selected, total tract digestibility and passage rate of diet consumed by sheep. The possibility of predicting dry matter and nutrient intake, botanical composition, nutrients selected and total tract digestibility using inert markers was also assessed. It was hypothesised that animal stocking rate will have an effect on botanical composition and quality selected, dry matter and nutrient intake, total tract digestibility and passage rate. It was also hypothesised that inert markers can be used to estimate the dry matter and nutrient intake, botanical composition, nutrients selected and total tract digestibility using concentration of inert markers in plant and faecal samples. The hypotheses were tested using a zero-grazing approach for precise prediction of dietary parameters.

4.2 Materials and methods

4.2.1 Study site and ethics

All procedures involving animals used in the study were approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (AREC/030/017M). The reader is referred to **chapter 3** for the study site.

4.2.2 Study animals, housing, adaptation and feeds

Fifteen sheep (mean body weight 46.5 ±3.3 kg) of different sex used in the study were orally dosed with abendezole 1.9 % m/v (Reg no: G2101, Act/ wet 36/ 1947) and levamisole hydrochloride 99% m/m (Reg no: G2166, Act/ 36 1947) against gastrointestinal parasites before the commencement of the trail. Sheep were ear tagged for identification. Four pens (length 7.32 m, width 4.24 m and area 30.98 m^2) with concrete floors were used to house sheep. During the 14-day preliminary period, sheep were allowed to acclimatise to the experimental feeds and the environment. All fifteen sheep were grouped together and fed *ad-libitum*: *Zea mays* (maize stover), *Sorghum bicolor* (sorghum stover) and *Themeda triandra* (veld hay) with *ad libitum* access to clean water. Each feed was provided in separate

feeding troughs (dimensions: 610 × 390 × 210 mm). Feeds were allocated twice a day in the morning at 0900 h and at 1500 h. All feeds were milled using a hammer mill to pass through a 12 mm screen (Scientec hammer mill, serial number 400, Johannesburg, lab world Pty Ltd, South Africa). Feeders filled with feed were placed at the same time and randomly in all pens daily to avoid conditioned learning among position of feeding troughs and dietary ingredients (Alonso-Díaz *et al*., 2009).

4.2.3 Experimental design

Fifteen sheep were blocked by initial body weight into 4 groups; 3 groups of 4 individuals and 1 group of 3 individuals. Each sheep within a group was randomly allocated to animal stocking rates (treatments) of either 1 (SR1), 2 (SR2), 4 (SR4) or 8 (SR8) sheep per pen. In each treatment, sheep were provided with 3 experimental feeds: maize stover (MS), sorghum stover (SS) and veld hay (VH) in separate feeding troughs. To evaluate the effect of animal stocking rate on passage rate of digesta, 1 sheep each from SR1 and SR2 and 2 sheep each pair from SR4 and SR8 were randomly selected and dosed with Ytterbium and cobalt-ethylenediamine tetraacetic acid (Co-EDTA) markers. To evaluate the effect of animal stocking rate on diet intake using double adjacent *n*-alkanes and diet composition (*n*-alkanes and long chain alcohols), 1 sheep from SR1, 2 sheep each pair from SR2 and SR4 and 4 sheep from SR8 were orally dosed with a pellet of dotriacontane (C_{32}); an external marker to calculate digestibility. *In-sacco* degradability of feeds was measured using 2 fistulated sheep (mean body weight 58.8±5.0 kg) to evaluate the nutritive value of the experimental feeds.

The trial lasted for 54 days with 14 days of preliminary period and 40 days of sampling. The 14-day adaptation also help in the peck order. During sampling, each sub-period lasted for 10 days including 3 days of adaptation to the environment and carrying faecal bags. During each sub-sampling period, 7 sheep from SR8 were randomly allocated to SR1, SR2 and SR4, those in SR1, SR2 and SR4 grouped together and allocated to SR8. This was repeated four times until all sheep were allocated to all treatments at least once; making n = 4 for all treatments. The study was replicated 4 times (each run lasted for 10 days) and pens were experimental units. Intake of each feed and *in vivo* total tract digestibility of diet consumed were measured for 7 days.

4.2.4 Digestibility

In vivo total tract digestibility (ITD) of a diet selected by sheep was determined as the difference in total amount of feedstuffs consumed and total faecal output divided by total amount of feed stuffs consumed for 7 days, as:

(ITD $= \frac{total\ amount\ of\ feedstuffs\ consumed\ (DM) - total\ faecal\ output(DM)}{total\ amount\ of\ feedstuffs\ consumed\ (DM)}$).

Where: DM was the dry matter basis of the components. The total amount of feed consumed by sheep in each pen was calculated as the total amount of pre-weighed feed in minus total feed out, orts and spillages. Average daily feed intake of each feed in each pen was calculated by dividing total feed intake by 7 and by the number of animals in each pen. Spillages and faeces on the floor were collected every day and collated each week. A sub-sample of 10% of the total weight of spillage was taken, sun dried for 48 hours, then oven dried for 72 hours and stored in airtight plastic bags pending manual separation into faecal and feed components. Total faecal output was measured using faecal bags held in place by clip6yts to adjustable harness in sheep. Faecal bags were emptied every morning before feeding and faeces were oven dried at 60° C for 72 hours. The weight of faeces (including weight of faeces from rectal grab samples) and feeds were recorded.

4.2.5 Degradability

Nylon bags (ANKOM co. New York, United States of America) with pore size of 40 µm were used to assess the rate and extent of degradability of experimental feeds in the rumen. Feeds were milled to pass through a 2 mm sieve in an ultra-centrifugal mill (ZM 200, Retsch-Alle 1-5.42781, Germany). Approximately 3 g of each experimental feed samples were weighed into pre-weighed nylon bags (dimensions: 90 × 50 mm) in duplicates. These bags were tied onto wire rings attached to a stainless steel rectangular disc (dimensions:50 × 23 mm) with 7 evenly spaced holes (4 mm wide and 12 mm apart) on the edge using rubber bands. Samples in duplicates were attached in each disc which served as an anchor. Incubation times of bags in the rumen of fistulated sheep were: 120, 96, 72, 48, 24, 12, 6, 3 and 0 hours, sequentially. All bags were removed from the rumen at the same time and washed with clean running tap water to aerate the bags and to stop any ruminal-microbial activity until the water ran clean. All bags, including those at zero hour (not incubated in the rumen) were then washed in an automated washing machine for 30 minutes (6 cycles with each cycle lasting for 5 minutes). The weight of each bag was recorded after 72 hours of

drying at 60°C in the oven. Dry matter (DM) disappearance from each sampled bag for this incubation was calculated as:

DM disappearance (g/kg) = (initial weight – final weight/ initial weight) × 1000. Degradation parameters were determined using SAS® 9.4 by fitting a model by McDonald (1981) into the SAS program. $Deg_{DM\,(t)} = a + b\,(1 - e^{-c\,(t-L)})$, where: $Deg_{DM\,(t)}$ – degradability at time (t), a - intercept of the proportion of DM solubilised at the beginning of incubation (t = 0), b - slowly degradable fraction of DM in the rumen, c - rate constant of degradation of b; and L – lag time.

4.2.6 Passage rate of digesta

4.2.6.1 Preparation and administration of liquid maker

Cobalt-ethylenediamine tetraacetic acid (Co-EDTA) was used as a liquid marker to determine the rate of passage of liquid fraction of digesta in the rumen of sheep. The Co-EDTA was prepared following procedures by Udén *et al*. (1980). Accurately weighed 1039.2 g of 57:37:6 (w/w) of a freshly prepared mixture of sodium-EDTA, cobalt (II) chloride hexahydrate ($CoCl_2.6H_2O$) and sodium hydroxide (NaOH), respectively, was added to 3200 ml of distilled water in a 5-litre glass beaker. The solution was left for 20 minutes to cool at room temperature. One thousand and six hundred millilitres (1600 ml) of the solution were transferred to a second 5-litre glass beaker. An additional 320 ml (50:50 v/v) of distilled water was added to the solution in each beaker and allowed to stand at room temperature for an additional 4 hours. Thereafter, 2400 ml (50:50 v/v) of 90% rectified alcohol was added to each beaker. The solution, with final volume of 4160 ml in each beaker, was placed in the refrigerator for 120 hours for crystals to form. The solution was filtered using a sieve to separate liquid and crystal fractions of the mixture. The liquid fraction of the mixture was discarded. One thousand nine hundred and eighty millilitres (1980 ml) of 80% (50:50 v/v) rectified alcohol was used to rinse the crystals in each beaker for 3 times (660ml of 50:50 v/v of alcohol for each cycle). Crystals were oven dried at 80°C for 72 hours and then stored in plastic containers in a cool dry place until administered to sheep. Seven hundred and twenty grams (720 g) of crystals were dissolved in 4320 ml of water and the solution was drenched to sheep at a dose rate of 60 ml per sheep.

4.2.6.2 Preparation and administration of solid marker

Ytterbium-mordanted *Themeda triandra* (veld hay) fibre was used as a marker to assess particulate passage rate of digesta in the rumen. Ytterbium-mordanted veld hay was prepared using a method by Hartfield *et al.* (1990). Three hundred and sixty grams (360 g) of veld hay milled using a hammer mill to pass through a 12 mm screen (Scientec hammer mill, serial number 400, Johannesburg, lab world Pty Ltd, South Africa) was soaked in distilled water overnight to remove soluble materials and dried in an oven pre-conditioned to 80°C for 24 hours. Dried veld hay (50 g) was soaked in a freshly prepared solution containing 50 g of ytterbium ($YbCl_3$. 6 H_2O) per litre of distilled water for 120 hours. Thereafter, labelled veld hay was strained and rinsed off with distilled water until the water ran clean to remove unbound ytterbium. The ytterbium-mordanted veld hay was allowed to stand at room temperature overnight to strain all the dripping water. The residue was then dried in an oven for 48 hours at 60°C. Dried ytterbium-mordanted veld hay fibre was stored in airtight plastic bags pending administration. Sheep were dosed with 20 g of ytterbium labelled fibre thoroughly mixed with 10 g of Lucerne hay (*Medicago sartiva*) to ensure total consumption of labelled fibre. All animals consumed about 98 % of a thoroughly mixed ytterbium and Lucerne mixture.

4.2.6.3 Sample collection and analyses

Faecal rectal grab samples for passage rate were taken directly from the rectum by palpation and stored in airtight plastic bags pending oven drying and analysis. Faecal samples were taken at times: 0, 3, 6, 9, 12, 24, 27, 30, 48, 52, 55, 72, 75, 78, 96, 99, 120, 126, 144, and 168 hours after administration of markers. Blank samples (0 hours) were taken before the administration of markers to determine the baseline of the concentration of ytterbium and Co-EDTA in faeces of sheep. All samples were oven dried at a temperature of 60°C for 96 hours. Samples were ground to pass through a 2mm sieve in an ultra-centrifugal mill (ZM 200, Retsch-Alle 1-5.42781, Germany) prior to chemical analyses. Two grams of oven dried faecal samples were weighed into porcelain crucibles and ashed in a furnace for 12 hours at 550 °C. Ashed samples were transferred into a 250 ml conical flask and 5 ml of 37 % hydrochloric acid added. The solution was heated to dryness using a block heater in a fume hood. Volume of 5 ml of 6 M nitric acid was added to a dried sample and re-heated to boil. Digested contents were filtered with what-man filter paper (size: 110mm) into a 100 ml volumetric flask. Contents left on the edges of the conical flask and the filter paper were

washed down the volumetric flask with warm de-ionised water. The volumetric flask was filled to mark with de-ionised water and mixed well. Concentration of cobalt-EDTA and ytterbium markers in faeces was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

4.2.6.4 Calculations of passage rate parameters

The mathematical model developed by Grovum and Williams (1973) version of Blaxter (1956) was used to describe the passage rate parameters. The model used was:

$Y = Ae^{-kl\,(t-TT)} - Ae^{-K2\,(t-TT)}$ when $t > TT$ and $Y = 0$ when $t < TT$ (Osuji *et al*., 1993). Where: Y and A are adjustable marker concentrations in the faecal dry matter, K_1 and K_2 are rate constants, TT is the transit time which is the calculated time of the first appearance of the marker in the faeces and *t* is the sampling time post marker administration. Graphically, concentrations of these markers against time were plotted to inspect and eliminate outliers. Natural logarithm of concentration of the marker from the descending slope was regressed against time to determine an equation: the gradient and y-intercept of the regression line gave the rate of passage in the rumen (k_1) and A_1, respectively. This equation of the regression line from the descending slope was used to estimate the concentration of the ascending portion of the graph by fitting corresponding sampling times. Estimated values were exponentially (e^x) raised to generate predicted values from natural logarithm transformed data. Residual concentrations were calculated as the difference between the predicted concentration values and the actual concentration values of the marker. Natural logarithm of the residual marker concentrations were regressed against corresponding times of the ascending portion of the graph to determine the equation of the curve. The gradient and the y-intercept of the residual regression line gave the rate of passage in the hind gut (K_2) and A_2, respectively. The two regression lines intersect at point (TT, A). Therefore, transit time (TT) was calculated as $TT = (A_2-A_1)/ (K_2-K_1)$. Mean retention time was calculated as the inverse of the passage rate (1/K). Mean retention time of particulate phase of the passage ($MRT_{particulate}$) divided by the mean retention time of the liquid phase of passage (MRT_{liquid}) gave selectivity factor (SF). Total tract mean retention time (TTMRT) of liquid and particulate passage rate were calculated as the sum of transit time, rumen and hind gut mean retention times (liquid and particulate). $TTMRT = 1/K_1 + 1/K_2 + TT$ (Grovum and Phillips, 1973).

Table 4. 1 Chemical composition and degradability parameters of experimental feeds

Feed	Chemical composition (g/kg DM)									Degradability parameters (DM)				
	DM	NDF	ADF	MADF	AIA	ADL	CP	Ash	HEM	a (g/kg)	b (g/kg)	c (h^{-1})	P_D (g/kg)	ED_{DM} (g/kg)
MS	909.70	892.68	541.76	770.60	16.98	21.12	49.79	41.43	350.92	100.66	533.57	0.0213	634.23	321.93
SS	881.76	876.84	511.84	694.74	26.38	38.98	43.41	110.76	365.00	118.18	573.01	0.0200	691.18	347.43
VH	911.32	766.59	429.73	497.32	20.12	73.32	30.37	74.61	336.85	188.14	528.21	0.0223	716.35	413.58
MS	936.97	858.40	545.30	530.59	25.65	26.08	52.88	34.40	313.10	130.22	583.15	0.0123	713.37	299.59
MSL	920.99	836.61	449.74	577.75	13.27	13.56	52.19	39.53	386.87	100.50	535.76	0.0360	636.26	392.60
SSS	916.70	844.03	463.45	538.20	45.44	28.67	43.81	56.02	380.59	174.61	579.58	0.0177	754.19	389.74
SSL	897.70	850.41	472.40	399.48	16.29	19.31	40.07	66.37	378.01	108.29	512.78	0.0241	621.06	336.77
SEM	6.13	14.07	15.94	43.50	3.79	7.02	2.78	9.33	9.37	12.53	9.93	0.0025	17.81	14.68

DM: dry matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; MADF: modified acid detergent fibre; AIA: acid insoluble ash; ADL: acid detergent lignin; CP: crude protein; HEM: hemicellulose; MS: maize stover; SS: sorghum stover VH: veld hay; MSL: maize stover leaves; MSS: maize stover stem; SSL: sorghum stover leaves; SSS: sorghum stover stems; Based on McDonald (1981), equations: $PD=a+b(1-e^{-ct})]$ and $ED_{DM}= a+bc/(c+k)$, where, a: intercept of the proportion of DM solubilised at the beginning of incubation (t = 0); b: slowly degradable fraction of DM in the rumen; c: rate constant of degradation of b; P_D: potential degradability (a+b); ED_{DM}: effective ruminal degradability; k: ruminal outflow rate for ED_{DM} = 0.03 per hour. SEM: standard error of mean.

4.2.7 Chemical analysis of feed and faecal samples for MADF, ADL and ADL markers

Moisture and ash contents were determined following AOAC Official Method 934.01 (Nancy and Wendt, 2003). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and modified acid detergent fibre (MADF) contents of experimental feeds were determined using ANKOM 220 fibre analyser (ANKOM Technology, USA). Hemicellulose was determined as a difference between NDF and ADF (hemicellulose = neutral detergent fibre – acid detergent fibre). Nitrogen content was determined using LECO TruSpec nitrogen analyser (LECO FP200, LECO, Pretoria, South Africa) following procedures by Association of Official Analytical Chemistry (AOAC, 1997). Crude protein (CP) was determined by multiplying nitrogen content of all feeds by the factor 6.25 (protein = nitrogen content × 6.25).

Modified acid detergent fibre (MADF) was determined following a method by Collier and Foulds (1975), the method was modified to accommodate the use of an ANKOM 220 fibre analyser using ANKOM fibre bags. One gram of plant and faecal samples in duplicates were weighed into pre-weighed ANKOM fibre bags. All bags including those at zero (empty ANKOM fibre bags) were completely sealed using a sealer and they were extracted with 2000 ml of acid detergent solution for 2 hours under gentle heat and flux. The acid detergent solution was prepared by weighing 1 % w/v of cethyltrimethylammonium (CTAB) into 0.5 M sulfuric acid in a 2000 ml beaker.

Acid detergent lignin (ADL) content of samples was determined in beakers using method 8 of ANKOM Technology following procedures by Van Soest and Wine (1968). Dry plant and faecal samples in ANKOM fibre bags previously extracted in an acid detergent solution were submerged into a 3-litre beaker and 250 ml of 72% of sulphuric acid was added. A 2-litre beaker was placed inside the 3-litre beaker with ANKOM fibre bags and the acid to submerge the fibre bags. The bags were agitated at the beginning and at successive 30 minutes interval by gently lifting up and pushing down the 2-litre beaker 30 times. After 3 hours the acid was poured off from the beaker leaving the ANKOM fibre bags behind which were then rinsed with water until litmus paper (red) showed a neutral pH colour (blue).

Acid insoluble ash (AIA) of plant and faecal samples was determined following procedures described by Van Keulen and Young (1977) with slight modifications. Five grams of milled (1 mm sieve) samples in duplicates was accurately weighed into crucibles and oven dried overnight at 100 °C. Dried samples were allowed to cool in a desiccator and re-weighed after 15 minutes to determine the dry weight of samples and ashed in a muffle furnace at 550 °C for 12 hours opposed to 6 hours of the method by Van Keulen and Young (1977) which was found to be insufficient. Ashed samples were transferred into 500 ml conical flasks and boiled for 5 minutes in 100 ml 2 *N* hydrochloric acid. Hot

hydrolysate was filtered through an ash free Whatman filter paper with warm distilled water. The filter paper was transferred back to the crucible and ashed at 550 °C for 12 hours. Crucibles from the furnace were left to cool for 2 hours, transferred to the oven and dried for 12 hours at 100 °C and thereafter transferred to the desiccator to cool and were weighed after 30 minutes.

4.2.8. Preparation and administration of dotriaconatne pellet

Dotriacontane (C_{32}) used as an external marker was prepared following a method by Kanga (2010), with slight modifications. A mixture of 1500 ml of water with 77.04 g of maize meal was prepared and put in a microwave oven at 70 °C for 9 minutes. Accurately weighed 3.6 g of dotriacontane (Sigma Aldrich, Co, St. Louis, Mo 63103, USA) was dissolved in 50 ml of *n*-Heptane (Sigma Aldrich, RSA) which was used as a solvent in a 250 ml beaker with mild heat and mixed with the maize meal porridge. The porridge was mixed well using a spatula and allowed to cool overnight. The maize meal porridge mixture was dried in a microwave oven at 70 °C for 15 minutes on the following day with 5-minute interval of starring to achieve uniform distribution of the solution of *n*-heptane and dotriacontane. The dried mixture was compacted into 1.07 g of pellets using a round shaped pipe (dimensions: 1.7 cm diameter and 1.5 cm height) to get a concentration of 50 mg of dotriacontane in each pellet (Charmley and Dove, 2008). The pellets were kept in the freezer at -5 °C pending oral administration to sheep. Each sheep was orally dosed using a pipe directly inserted into the rumen with the dosing frequency of one pellet per sheep a day in the morning.

4.2.9 Sample collection and preparation for n-alkane and long chain alcohols

Faecal samples were collected by rectal palpation in the morning 72 hours post administration of dotriacontane which was used as an external marker when it has stabilised in the faeces (Ferreira *et al*., 2007). A sub sample of 10% of pellets was selected for quantification of the average concentration of C_{32} in pellets. All samples were oven dried at 60 °C for 72 hours, milled to pass through a 1.0 mm sieve using an ultra-centrifugal mill (ZM 200, Retsch-Alle 1-5.42781, Germany) and stored in airtight plastic bags. Samples were prepared for analysis by gas chromatography-mass spectrometry (GC-MS) following a method by Dove and Mayes (2006). Dry milled herbage (0.2 g) and faecal (0.1) samples were accurately weighed in duplicates and put into 4 ml GC vials with polytetrafluoroethylene (PTFE) lined inserts. Stock solutions of internal standards were prepared for both *n*-alkanes and long chain alcohols. For *n*-alkanes, 45 mg of both *n*-docosane (C_{22}) and *n*-tetratriacontane (C_{34}) were dissolved in 150 g of *n*-decane (C_{10}) to form a stock solution with proportions of 0.3 mg of both *n*-docosane and *n*-tetratriacontane in a gram of *n*-decane. For long chain alcohols, 75 g of both ethanol and *n*-heptane (C_7) were mixed with 195 mg of heptacosanol (C_{27}-OH) to form a stock solution with proportions of

1.3 mg of heptacosanol and 0.5 g of both ethanol and heptane. To accurately weigh 0.11 g and 0.15 g of a solution of internal standards for *n*-alkanes and long chain alcohols, respectively, the volume and the weight of each stock solution was used to calculate the density of each solution which was then used to convert the required weight to the volume. Weighed 102.174 g (from the molecular weight) of ethanolic potassium hydroxide was added to 1000 ml volumetric flask and filled up to the mark with distilled water to make 1 M of the solution. Plant (1.5 ml) and faecal (2 ml) samples were heated with 1 mole L^{-1} of ethanolic KOH at 90 $^{\circ}$C for 16 hours in an oven with GC vials capped. After oven heating samples were allowed to cool at room temperature to 55 $^{\circ}$C, 1.5 and 2 ml of *n*-heptane was added to faecal and plant samples, respectively. Thereafter, water was added to faecal (0.4 ml) and plant (0.6 ml) samples and the vials were shaken vigorously. The top non-aqueous layer was removed and evaporated to dryness using nitrogen, re-dissolved in 0.3 ml of *n*-heptane with mild heat using the sonicator (RS PRO ultrasonic cleaner, RSA) and was carefully transferred to a silica gel with 1 ml bed volume. The extracts in the edges of the GC vials were washed with heptane to the column. *N*-alkanes were eluted from the columns with *n*-heptane to GC-vials and the *n*-heptane was evaporated to dryness using nitrogen; samples were kept at room temperature pending GC-MS analysis.

To obtain crude long chain alcohols from the same column where *n*-alkanes were collected, 3 ml of a mixture of heptane and ethyl acetate (80:20 v/v) was added. Gas chromatography vials were used to collect the eluate and the solvents were evaporated to dryness using nitrogen. To remove sterols from the crude long chain alcohols, dried samples were re-dissolved in 0.5 ml *n*-heptane with mild heat in the sonicator (RS PRO ultrasonic cleaner, RSA) and then 0.05 ml of the solution was transferred to the solid phase extraction (SPE) column preconditioned with 1 ml of *n*-heptane. The derivatisation of alcohols to form ethyl acetate required an addition of 1.5 ml of 20:80 v/v acetic anhydrous/ pyridine and oven heating overnight at 50°C. Water (0.3 ml) and 1.5 ml of *n*-heptane were added to separate the sample into top and the bottom layers. The top layer was collected and evaporated to dryness and stored pending identification and quantification of LC-OHs using GC-MS. Dried samples of *n*-alkanes and long chain alcohols were re-dissolved in dodecane and *n*-heptane solvents, respectively before being subjected to GC-auto sampler.

4.2.9.1 Gas chromatography analyses

4.2.9.2 Verification of the compatibility of the GC-MS with the standard protocol specifications

Internal standards for *n*-alkanes (C_{22} and C_{34}) and for long chain alcohols (C_{27}-OH) were analysed on the GC-MS machine to verify if the specifications and the column (slightly polar) of the machine were

able to detect the standards. Retention times and the peak areas of internal standards obtained were compared to those by Smith and Strickland (2007).

4.2.9.3 Sample analysis

Analysis of *n*-alkanes and long chain alcohols (LC-OH) were carried out by gas chromatography (GCMS-QP2010 SE) with no fitted flame ionisation detector (FID). Analytes were separated using a Zebron ZB 5MA-plus column, 30m × 0.25mm with 0.25µm film thickness. Injection volume was 2 µl in a split-less mode and the injection temperature was 280 ºC. The temperature of the oven column was initially maintained at 170 ºC for 4 minutes, increased at a rate of 30 ºC min^{-1} to 215 ºC, held for 1 minute, and increased at a rate of 6 ºC min^{-1} to 300 ºC where it was held for 13 minutes. Therefore, total running time per sample was 33.60 min. The carrier gas used was helium, Intel pressure was 88.0 kPa and linear velocity flow control mode was 36 cm sec^{-1}.

All samples were chromatographically analysed in duplicates. Retention times and peak area calculations were carried out using the Shimadzu GC-MS computer software which was auto peak-picking and integrating. The internal standards for *n*-alkanes (C_{22} = 0.3 mg and C_{34} = 0.3 mg) and LC-OHs (C_{27}-OH = 1.3 mg) were included in constant known amounts in all samples at the beginning of extraction to relatively quantify LC-OHs and *n*-alkanes in plant and faecal samples using the peak areas in relation to known concentration of the internal standards.

4.2.10 Predicting diet composition, digestibility and intake

The experiment was designed to also test whether it is possible to determine the botanical composition of a diet selected by free-ranging ruminants using inert markers when grazing conditions are simulated by feeding sheep on varying animal stocking rates. Diet composition was obtained by estimating proportions of individual dietary components using an optimisation procedure (Solver routine) of Microsoft Excel program. The procedure minimises the sum of squared discrepancies between the proportional concentration of the marker in faeces (A) and the proportional concentration of the marker in dietary components (E) (Krebs, 1989; Ferreira *et al*., 2007), as follows:

$$\sum_{i=1}^{n}[A-E]^2 = \sum_{i=1}^{n}[\frac{Fi}{Ft} - \frac{a*D1i + b*D2i + c*D3i \ldots\ldots z*Dni}{a*D1t + b*D2t + c*D3t \ldots\ldots z*Dnt}]^2$$

Where: *a*, *b*, *c to z* are the proportions (*p*) of components D1, D2, D3 and D*n* in the diet, F*i*, D1*i*, D2*i*, D3*i* and D*ni* are the concentration of the marker in faeces and dietary components, F*t*, D1*t*, D2*t*, D3*t*

and D*nt* are total concentration of markers in faeces and in dietary components. The number of internal markers and dietary components was the same (n=3).

Constrains and assumptions used in Solver routine program (Microsoft excel 2016) to estimate diet composition were as follows: Each feed was initially assumed to be equally available (33.3%) and the proportions were constrained to be all positive and their sum should add up to 1 ($0 \leq p \leq 1$). The second constraint applied to the optimisation was that all the variable (changing) cells should be within a likely range (minimum and the maximum boundaries) of values which were taken from the observed data sets. Each range was calculated between the mean (minimum) minus the standard error and the mean (maximum) plus the standard error of the observed data set. The predicted marker concentrations in the diet were calculated as the sum of the product of proportions of dietary components and corresponding marker concentrations in dietary components ($a * D1i + b * D2i + c * D3i$). The optimiser ran iterations and stopped when the objective cell (sum of the squared differences) had a minimum value at the point at which concentrations of markers in the diet were the closest to the concentrations of markers in faeces.

Apparent *in vivo* total dry matter digestibility was calculated from concentration of the marker in the diet and faeces, as follows:

$$D = 1 - \frac{C_d}{C_f}$$

Where: D is the apparent *in vivo* total dry matter digestibility, C_d is the concentration of marker in the diet and C_f is the concentration of the marker in faeces.

Dietary intake was calculated from total faecal output and digestibility (Langlands, 1987), as follows:

$$I = \frac{F}{1-D}$$

Where: I is dietary intake, F is the total faecal output and D is the apparent *in vivo* dry matter digestibility calculated from the concentration of the marker in dietary components and faeces.

Faecal recoveries of markers (MADF, AIA and ADL) were calculated as the proportion of concentration of the marker in the consumed diet found in faeces, as follows:

$$FRM_i = (\mathrm{F}\times\mathrm{Fi})/(\mathrm{A}\times\mathrm{Ai} + \mathrm{B}\times\mathrm{Bi} + \mathrm{C}\times\mathrm{Ci})$$

Where: *FRMi* is the faecal recovery of the marker *i*, *F* is the total faecal output (observed), F_i is the concentration of i^{th} marker in faeces, *A, B* and *C* are the amount of dietary component in the consumed diet, A_i, B_i and C_i are the concentrations of marker *i* in dietary components.

Kulczynski similarity index was used to evaluate the accuracy of the composition, dry matter intake and digestibility of a diet predicted using MADF, AIA and ADL markers (Ferreira *et al*., 2015), as follows:

$$KSI = 100 \times \sum 2ci / \sum (ai + bi)$$

Where: KSI is the Kulczynski similarity index, *ci* is the lesser proportion of dietary component *i* between observed and predicted values and $ai + bi$ is the sum of the observed and predicted values of dietary component i.

4.2.11 Statistical analyses

The Pearson correlation coefficients of chemical analysis and degradability parameters of experimental feeds were established using correlation procedure (PROC CORR) of SAS® 9.4 (SAS institute Inc. Carry, NC, USA). The relationship between the observed and the predicted; dry matter intake, dietary ingredient intake, nutrient intake, proportion of feed and nutrients selected, and digestibility data sets were assessed by the regression analysis in SAS® 9.4 (PROC REG). R-squared (R^2) was used to measure the precision of predictions, while root mean square error (RMSE) was used to assess the accuracy of the predicted values of parameters evaluated. The effect of animal stocking rate on dry matter intake, nutrient intake, proportion of feed and nutrients selected, digestibility and passage rate data sets were analysed using the general linear model of SAS ® 9.4 procedure (PROC GLM). Response in dietary parameters in relation to animal stocking rates was evaluated using regression of SAS (SAS institute Inc. Carry, NC, USA version 9.4) PROC RS-REG. Probability difference (PDIFF) was used to compare means that were significantly different from each other at $p < 0.05$. The statistical model was:

$$Y_{ij} = \mu + S_i + BWT_j + \varepsilon_{ij}$$

Where Y_{ijkl} is the observation, μ overall mean, S_i effect of animal stocking rate ($_i$ = 1; 2; 4; 8), BWT_j body weight, and ε_{ijkl} residual error.

4.3 Results

4.3.1 In sacco degradability

Chemical composition and degradation parameters of DM of roughages are presented in **Table 4.1.** Potential degradability of forages ranged from 634.23 to 716.35 (g/kg DM) and effective degradability ranged from 321.93 to 413.58 (g/kg DM). Correlation coefficients (*r*) of the relationship between chemical composition and degradability parameters of roughages are given in **Table 4.2**. The portion of dry matter solubilised at the beginning of incubation (*a*) was negatively correlated ($p < 0.05$) to both NDF and ADF but tended to be positively ($p = 0.053$) correlated to ED_{DM}. Dry matter was negatively correlated ($p < 0.05$) to slowly degraded fraction (*b*) of roughages in the rumen. The hemicellulose content of feedstuffs was negatively correlated to degradation rate (*c*) of roughages in the rumen *(p* <0.05). Effective degradability of dry matter of forages was negatively correlated ($p < 0.01$) to ADF and CP ($p < 0.05$) and positively correlated to the portion of dry matter solubilised at the beginning of incubation.

Table 4. 2 Pearson correlation coefficients of chemical analysis and degradability parameters of experimental feeds

	DM	NDF	ADF	ADL	CP	Ash	HEM	a	B	c	PD	ED_{DM}
DM		-0.4411 (0.709)	-0.3065 (0.802)	-0.5485 (0.630)	-0.2418 (0.845)	-0.8538 (0.349)	-0.8895 (0.302)	0.3727 (0.757)	-0.9981 (0.039)	0.9212 (0.254)	-0.1702 (0.8911)	0.2950 (0.809)
NDF			0.9895 (0.093)	-0.509 (0.660)	0.9775 (0.135)	-0.0907 (0.942)	0.8025 (0.407)	-0.9972 (0.048)	0.4950 (0.670)	-0.7555 (0.455)	-0.8093 (0.400)	-0.9877 (0.100)
ADF				-0.6278 (0.568)	0.9974 (0.043)	-0.2340 (0.850)	0.7076 (0.500)	-0.9975 (0.045)	0.3640 (0.763)	-0.6527 (0.5472)	-0.8858 (0.307)	-0.9999 (0.008)
ADL					-0.6788 (0.525)	0.9036 (0.282)	0.1057 (0.933)	0.5715 (0.613)	0.4965 (0.669)	-0.1780 (0.885)	0.9173 (0.261)	0.6371 (0.560)
CP						-0.29877 (0.8068)	0.6584 (0.542)	-0.9905 (0.088)	0.3005 (0.806)	-0.6002 (0.590)	-0.9150 (0.264)	-0.9985 (0.035)
Ash							0.5214 (0.651)	0.1649 (0.895)	0.8204 (0.388)	-0.5839 (0.603)	0.6584 (0.543)	0.2456 (0.842)
HEM								-0.75561 (0.455)	0.9157 (0.263)	-0.9972 (0.048)	-0.2989 (0.807)	-0.6991 (0.507)
a									-0.4286 (0.718)	0.7044 (0.502)	0.8510 (0.352)	0.9966 (0.053)
b										-0.9432 (0.2156)	0.1098 (0.930)	-0.3527 (0.771)
c											0.22663 (0.8545)	0.6436 (0.555)
PD												0.8913 (0.300)
ED_{DM}												

DM: dry matter; NDF: neutral detergent fibre; ADF: Acid detergent fibre; ADL: acid detergent lignin; CP: crude protein; HEM: hemicellulose; *p*-values are in brackets.

4.3.2 Dry matter intake of dietary ingredients, total dry matter intake and nutrient intake

Table 4. 3 Effect of animal stocking rate on dietary ingredients intake, total dry matter intake (kg/day) and nutrient intake (g/day).

Feeds	**Stocking rate (sheep per pen)**				**General linear model**		**Regression**	
Intake (kg/day)	**1**	**2**	**4**	**8**	**RMSE**	**Significance**	**Linear**	**Quadratic**
Veld hay	0.302	0.318	0.431	0.428	0.12	NS	0.073^{NS}	-0.005^{NS}
Maize stover	0.175	0.421	0.137	0.206	0.13	NS	0.202^{NS}	-0.044^{NS}
Sorghum stover	0.304	0.367	0.186	0.183	0.14	NS	0.028^{NS}	-0.017^{NS}
Total dry matter intake	0.781	1.106	0.754	0.817	0.19	NS	0.303^{NS}	-0.066^{NS}
FR-total dry matter intake	0.874	0.905	0.994	0.973	0.08	NS	0.104^{NS}	-0.013^{NS}
FR	81.03	117.90	76.07	76.93	0.14	NS	0.104^{NS}	-0.01^{NS}
KSI = 90.42%	-	-	-	-	-	-	-	-
Nutrient intake (g /day)								
CP	28.496	45.910	31.151	34.411	8.15	NS	17.991^{NS}	-3.539^{NS}
NDF	590.917	942.829	613.263	671.973	178.41	NS	375.863^{NS}	73.3001^{NS}
ADF	342.446	553.317	353.308	388.969	104.70	NS	212.978^{NS}	-43.804^{NS}
Ash	49.970^{b}	85.416^{a}	55.178^{b}	62.439^{ab}	15.13	*	35.948^{NS}	-7.046^{NS}

1: sheep fed individually per pen; 2: Sheep fed in group of 2 sheep per pen; 4: sheep fed in group of 4 sheep per pen; 8: sheep penned in group of 8 sheep per pen; RMSE: root mean square error: CP: crude protein; NDF: neutral detergent fibre; ADF: Acid detergent fibre; FR: faecal recoveries (%); FR-total dry matter intake: predicted total dry matter intake adjusted for incomplete faecal recoveries of markers; KSI: Kulczynski similarity index between the predicted and the observed total dry matter intake; [a, b] Values within a row with different superscripts differ *significantly at $p < 0.05$; NS: Not significantly different at $p > 0.05$.

Dietary ingredients, nutrients (CP, NDF and ADF) and total dry matter consumed by sheep across treatments were similar (p >0.05). However, there was a trend of total dry matter intake across treatments being the highest for SR2 followed by SR8, SR1 and lastly SR4. Mineral (ash) content intake by sheep at SR2 was higher (p <0.05) than that consumed by sheep at SR8 and SR4 which had similar mineral content intake (p >0.05) and sheep at SR8 consumed mineral content intake similar to all other treatments.

4.3.3 Diet selection, diet quality and total tract digestibility

Table 4. 4 Effect of animal stocking rate on diet selection (g/g), predicted diet selection (g/g), diet quality (g/kg), and apparent total tract digestibility (g/kg) in merino sheep.

Parameter	**Stocking rate (sheep per pen)**				**General linear model**		**Regression**	
Diet selection	**1**	**2**	**4**	**8**	**RMSE**	**Significance**	**Linear**	**Quadratic**
Veld hay	0.362	0.305	0.605	0.550	0.16	NS	0.073^{NS}	-0.005^{NS}
Maize stover	0.248	0.374	0.156	0.236	0.16	NS	0.202^{NS}	-0.044^{NS}
Sorghum stover	0.390	0.320	0.239	0.216	0.16	NS	0.028^{NS}	-0.017^{NS}
KSI = 90.26%	-	-	-	-	-	-	-	-
	Predicted							
Veld hay	0.619	0.387	0.620	0.557	0.12	NS	-0.207^{NS}	0.042^{NS}
Maize stover	0.129	0.264	0.151	0.170	0.06	*	0.146^{NS}	-0.029^{NS}
Sorghum stover	0.252	0.349	0.229	0.273	0.08	NS	0.06^{NS}	-0.013^{NS}
Diet quality (g/kg)								
CP	13.31	13.873	13.762	14.033	9.56	NS	0.585^{NS}	-0.075^{NS}
NDF	280.4	283.034	270.863	273.411	17.72	NS	-3.042^{NS}	-0.048^{NS}
ADF	163.5	168.988	155.604	157.988	97.03	NS	0.346^{NS}	-0.689^{NS}
Ash	23.51	25.836	24.102	25.326	18.03	NS	1.701^{NS}	-0.268^{NS}
Digestibility	369^{b}	590^{a}	428^{b}	451^{b}	114.90	*	225.900^{NS}	-49.500^{NS}
FR-digestibility	432^{b}	514^{ab}	594^{a}	566^{ab}	74.57	*	186.158^{NS}	-27.567^{NS}
FR	81.3	117.90	76.10	76.93	0.41	NS	0.104^{NS}	-0.013^{NS}
KSI = 89.35%	-	-	-	-	-	-	-	-

1: sheep fed individually per pen; 2: Sheep fed in group of 2 sheep per pen; 4: sheep fed in group of 4 sheep per pen; 8: sheep penned in group of 8 sheep per pen; RMSE: root mean square error; FR: faecal recoveries (%); FR-digestibility: predicted digestibility adjusted for incomplete faecal recoveries of markers; KSI: Kulczynski similarity index between the predicted and the observed parameters; $^{a, b}$ Values within a row with different superscripts differ *significantly at $p < 0.05$; NS: not significantly different at $p > 0.05$.

Animal stocking rate had no effect (p >0.05) on diet selection and proportions of nutrients (diet quality) consumed by sheep. However, there was a decreasing trend in proportion of sorghum stover in dietary ingredients as stocking rate increased from 1 to 8 animals per pen (SR1> SR2 >SR4 >SR8). Total tract digestibility across SR1, SR4 and SR8 were similar. However, sheep in SR2 on average selected a diet with higher (p >0.05) total tract digestibility than other treatments in this trial. Apparent total tract digestibility across SR1, SR2 and SR8 were statistically similar amongst the predicted values. Sheep in SR4 had the highest predicted total tract digestibility. The predicted and the observed data sets of apparent total tract digestibility and proportion of feed consumed (diet selection) accounted for 10.65% and 9.74% of variation respectively.

4.3.4 Passage rate of digesta

Table 4. 5 Effect of animal stocking rate on fractional passage rate of liquid and solid particles, mean retention time of digesta in the rumen and hind gut, liquid and solid transit time, total tract mean retention time and selectivity factor in the rumen and hindgut.

	Stocking rate (sheep per pen)				**General linear model**		**regression**	
Parameter	**1**	**2**	**4**	**8**	**RMSE**	**Significance**	**Linear**	**Quadratic**
	Fractional passage rate (h^{-1})							
RR (k_s)	0.036	0.034	0.025	0.027	0.01	NS	-0.009^{NS}	0.001^{NS}
HG (K_s)	0.040	0.036	0.045	0.039	0.01	NS	0.003^{NS}	-0.001^{NS}
RR (K_l)	0.102	0.054	0.062	0.080	0.61	NS	-0.088^{NS}	0.0165^{NS}
HG (k_l)	0.110	0.093	0.093	0.099	0.66	NS	-0.032^{NS}	0.006NS
	Mean retention time (h)							
RR_s	28.970	31.900	39.010	38.690	15.29	NS	7.690^{NS}	-0.813^{NS}
HG_s	22.782	30.731	23.206	24.377	12.72	NS	8.199^{NS}	-1.695^{NS}
RR_l	9.782	20.146	16.311	15.201	6.83	NS	15.584^{NS}	-2.869^{NS}
HG_l	9.160	13.393	11.553	11.294	5.98	NS	6.071^{NS}	-1.123^{NS}
	Total tract mean retention time (h)							
$TTMRT_S$	57.24	59.98	81.94	69.92	22.22	NS	24.450^{NS}	-3.690^{NS}
$TTMRT_l$	41.300	35.589	32.131	21.743	7.87	NS	-0.367^{NS}	-1.169^{NS}
	Selectivity factor							
RR	1.844	1.736	2.344	2.924	1.34	NS	-0.475^{NS}	0.172^{NS}
HG	1.437	3.011	3.106	2.192	1.35	NS	3.346^{NS}	-0.622^{NS}

1: sheep fed individually per pen; 2: Sheep fed in group of 2 sheep per pen; 4: sheep fed in group of 4 sheep per pen; 8: sheep penned in group of 8 sheep per pen; RR: reticulo-rumen; HG: hind gut; K_s: fractional passage rate of solid particles; K_l: fractional passage rate of liquid particles; RRs: reticulo-rumen solid particles; HGs: hindgut solid particles.

RR_l: reticulo-rumen liquid particles; HG_l: hindgut liquid particles; $TTMRT_s$: solid total tract mean retention time; $TTMRT_l$: liquid total tract mean retention time; RMSE: root mean square error; NS: not significantly different at $p > 0.05$.

Fractional passage rate (liquid and particulate) from both the rumen and the hind gut, mean retention time and total mean retention times across treatments were similar ($p > 0.05$). Fractional particulate passage rate in the rumen and hind gut across treatments had a similar trend (SR2>SR8>SR4) with corresponding mean retention times (SR4>SR8>SR2). Total mean retention time had a trend of SR4>SR8>SR2 and lastly SR1. Sheep across treatments had statistically similar ($p > 0.05$) selectivity index factors between ranges of 1.844 - 2.924 and 1.437 - 3.106 in the reticulo-rumen and hindgut, respectively.

4.3.5 Faecal recoveries and predicted components of diets selected by sheep

Table 4. 6 Effect of animal stocking rate on faecal recoveries of internal markers (%)

Marker	**Stocking rate (sheep per pen)**				**General linear model**		**Regression**	
	1	**2**	**4**	**8**	RMSE	Significance	Linear	Quadratic
MADF	35.5	31.3	32.9	34.9	0.07	NS	-7.770^{NS}	1.550^{NS}
ADL	49.1	49.3	53.1	47.4	0.15	NS	39.715^{NS}	-9.025^{NS}
AIA	81.0	117.9	76.1	76.9	0.41	NS	7.245^{NS}	-1.475^{NS}

1: sheep fed individually per pen; 2: Sheep fed in group of 2 sheep per pen; 4: sheep fed in group of 4 sheep per pen; 8: sheep penned in group of 8 sheep per pen; MADF: modified acid detergent fibre; ADL: acid detergent lignin; AIA: acid insoluble ash; RMSE: root mean square error; NS: not significant at $p > 0.05$.

Faecal recoveries (FR) of each of the markers (MADF, AFL and AIA) were similar ($p > 0.05$) across treatments. Acid insoluble ash had the highest FR followed by ADL and lastly MADF; ranges of FR were 31.3 – 34.9 (MADF), 49.1 – 53.1 (ADL) and 76.1 – 117.9 (IAA) between the minimum and the maximum, respectively

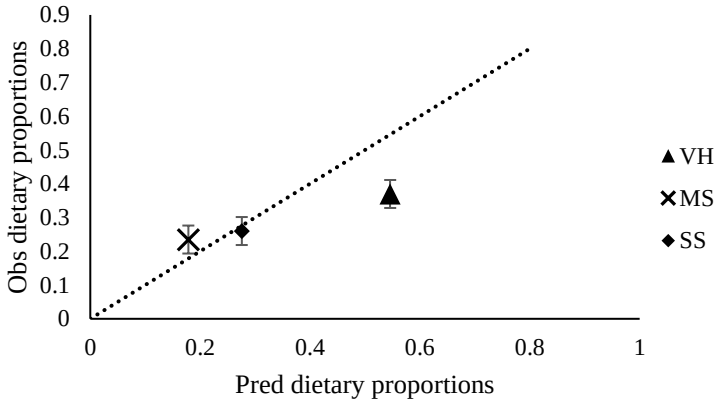

Figure 4. 1 Observed (Obs) versus predicted (Pred) dietary proportions selected by sheep. Regression line y = 1.159 (±0.360) x - 0.0006 (±0.112). RMSE = 0.13089; n =12 and R^2 = 0.503; p = 0.0098; error bars represent standard error.

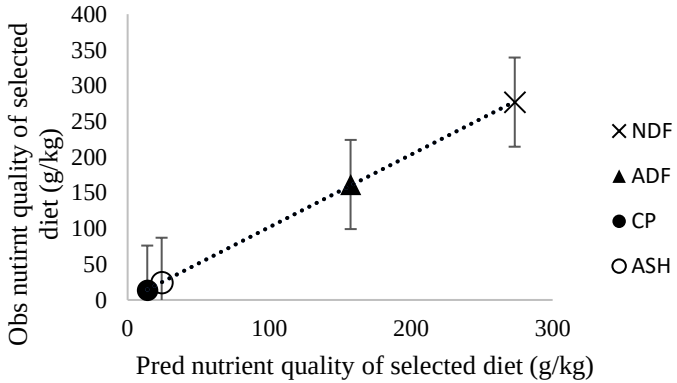

Figure 4. 2 Observed versus predicted (Pred) nutrient quality selected by sheep. Regression line y = 0.984 (±0.007) x - 0.254 (±1.1017). RMSE = 2.9603; n =16 and R^2 = 0.999; p = 0.001); error bars represent standard error.

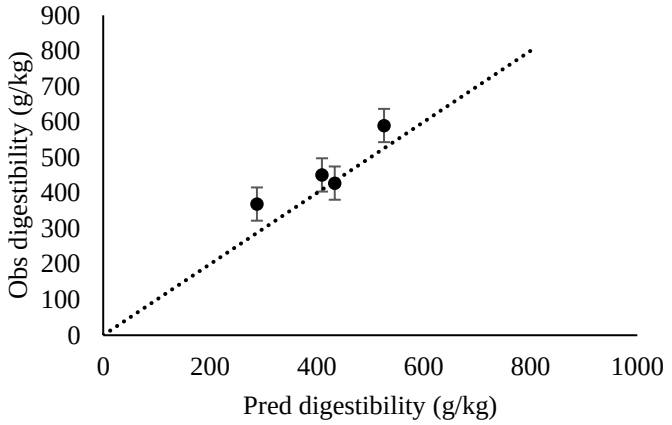

Figure 4. 3 Observed (Obs) versus predicted (Pred) total tract digestibility (g/kg) in sheep. Regression line y = 0.967 (±0.284) x - 29.931 (±132.6014). RMSE = 46.075; n =4 and R^2 = 0.853; p = 0.0766; error bars represent standard error.

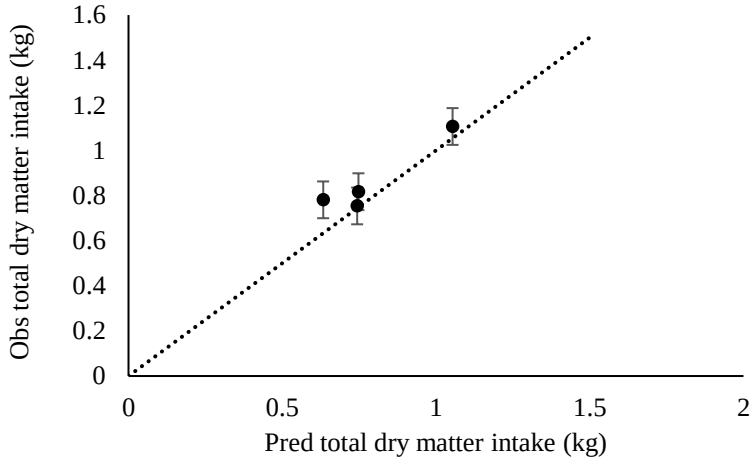

Figure 4. 4 Observed (Obs) versus predicted (Pred) dry matter intake (kg) in sheep. Regression line y = 1.053 (±0.247) x - 0.116 (±0.217). RMSE = 0.0698; n = 4 and R^2 = 0.901; p = 0.0510; error bars represent standard error.

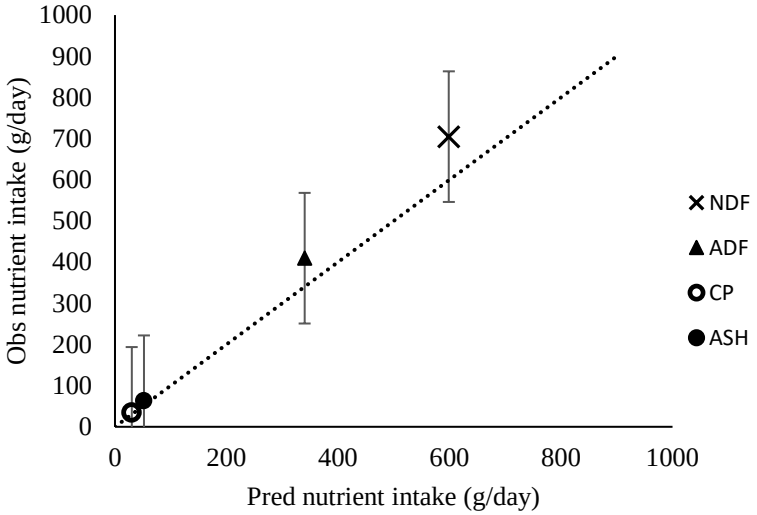

Figure 4. 5 Observed (Obs) versus predicted (Pred) dietary intake by sheep. Regression line y = 0.601 (±0.324) x + 0.0956 (±0.100). RMSE = 0.116; n =12; R^2 = 0.256; p = 0.0931; error bars represent standard error.

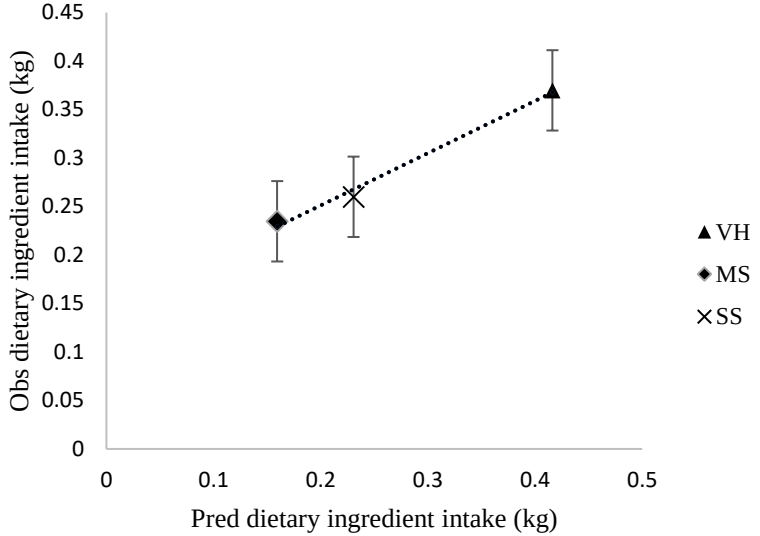

Figure 4. 6 Observed (Obs) versus predicted (Pred) nutrient intake by sheep. Regression line y = 0.797 (±0.05) x + 13.520 (±19.160). RMSE = 52.68; n =16; R^2 = 0.956; p = 0.0001; error bars represent standard error.

Figure 4.1 to **Figure 4.2** shows the linear regression relationship between the predicted and the observed data sets of: dry matter intake, dietary ingredient intake, nutrient intake, proportion of feed and nutrients selected, and digestibility of a diet selected by sheep in this study. There were little discrepancies between predicted and observed components of diets selected by sheep. Dietary intake had the least whereas the nutrient quality of the diets selected by sheep had the highest R-squared values of 0.256 and 0.999, respectively.

4.3.6 N-alkanes and long chain alcohols

The retention times of the internal standards for *n*-alkanes (C_{22} and C_{34}) were 11.216 and 26.106 minutes, respectively, whereas Smith and Strickland (2007) reported 14.500 and 26.000 minutes for C_{22} and C_{34}, respectively. There is scanty literature available on retention times of LC-OHs to refer to for comparison. However, the retention time of LC-OHs (C_{27}-OH) in the present study had the retention time of 20.446 min. *N*-alkanes and long chain alcohols were not detected in the present study. Possibly this could be attributed to low accuracy in analytical determination due to generally decreasing concentration of plant cuticular wax hydrocarbons as plant ages, given that plants used in the present study were harvested at late maturity. Additionally, cereal grains have been reported elsewhere (Charmley and Dove, 2008) to contain minute amounts of plant cuticular wax hydrocarbons. As a result, others (Ali *et al*., 2004; Bugalho *et al*., 2004 and Dove and Oliván, 2006) suggested use of other markers as alternatives to complement particularly long chain fatty acids, long chain alcohols and alkenes. On the other hand, Elwert and Dove (2005) suggested coating cereal-based supplements with beeswax due to low concentration of hydrocarbons found in the wax of epicuticular layer.

4.4 Discussion

Correlation analysis from the current study showed a strong negative (r = 0.999) relationship between ADF content of roughages and ED_{DM}. The portion of dry matter solubilised at the beginning of incubation (a) tended (p = 0.053) to be positively correlated to ED_{DM} and negatively correlated to ADF. Dry matter and hemicellulose content of roughages both had strong negative correlations of r = 0.9981 and r = 0.9972 with slowly degraded fraction (b) and degradation rate (c), respectively. These findings suggest that there are possibilities of

estimating degradability parameters (a, *b*, *c* and ED_{DM}) of a given roughage using chemical composition. Gerber (2014) and Moyo *et al*. (2019) applied these principles in the estimation of degradability of protein and dry matter of feeds, respectively. Nsahlai *et al*. (1995) postulated similar approaches for estimation of nitrogen degradable parameters from DM degradability for fresh leaves from multipurpose trees. However, findings from the current study fall short of being conclusive due to the lack of significant correlations between PD_{DM} and chemical composition of roughages.

Diet selection in ruminants is governed by dynamic interrelated processes, which include reticulo-rumen digestibility, intake (Arnold, 2017), microbial synthesis (Harrison and McAllen, 1980), rumen fermentation and rate of passage of digesta (Bernard and Doreau, 2000). Our findings, even though not statistically different, showed that sheep in SR2 selected a diet mostly dominated by maize stover. The general contention is that cereal straws have low intake levels because of poor digestibility due to inherently high fibre content. However, in the present study all feeds used were roughages. Maize stover had the highest crude protein content than other dietary ingredients (VH and SS), which might be the cause of the observed increase in consumption of maize stover in SR2. These findings agree with the proposed concept of improved intake in relation to high protein content of the selected diet (Kyriazakis and Oldham., 1993). Therefore, high total dry matter intake levels by sheep in SR2 might be attributed to associative digestibility increase because of proliferation of microbial population due to high intake of crude protein (g/day) in the selected diet in SR2. Furthermore, it can be projected that SR2 was the maximal stocking rate, where facilitation of feeding and competition had a little effect on consumption of a diet predominated by a roughage with better protein content (MS). Odoi and Owen (1993) reported results in agreement with the present study where the dry matter intake increased in lambs fed in pairs than lambs fed individually. Generally, availability of companion(s) in group orientated animals increases activity and visual cues, which stimulate eating than when one animal is penned in isolation. Therefore, the decreased dry matter intake by sheep fed in isolation compared to sheep in other treatments was expected due to the lack of social facilitated behaviour. It is possible that, in attempt to compensate for high competition owing to increased number of animals per unit area, sheep in SR8 selected a diet consisting of mainly veld hay. Such compensation is likely motivated by the reticulo-rumen filling effect of veld hay in the diet, owing to its DM content with low CP content compared to maize and sorghum stovers. The adoption of this selection behaviour was likely linked to attainment of satiety. Illius and Gordon (1990) reported a similar response

where large grazing animals selected grasses in large proportions in their diet relative to forbs and shrubs because of the high biomass and filling effect of grasses compared to shrubs and forbs. Although not assessed, sheep preferred leaves more than stalks in both sorghum and maize stovers. This may have caused sheep to select veld hay due to its high biomass and sheep can eat little amounts of veld hay and have their reticulo-rumen filled for longer period rather than leaves which have low biomass.

Unexpectedly, passage rate of diet selected by sheep in treatment SR1 was the highest with short mean retention time. The fast passage rate of digesta of a diet selected by sheep in treatment SR1 is similar to an observation reported by Murphy *et al*. (1989) where rate of passage of digesta was fast in swamp buffaloes and cattle fed diet predominated by rice straw at *ad libitum* access due to prolonged rumination. After re-swallowing the digesta, small particles which have undergone extensive digestion exit the rumen relatively faster than large particles (Murphy *et al*., 1989). Additionally, Poncet and Abd (1984) examined the passage of chromium and ytterbium mordant fibres in sheep feeding on hay-based diet and concluded that ytterbium adheres to small particle fraction of digesta in the rumen. Hence, the controversy of low feed intake and mean retention time with high passage rate of digesta observed in the present study in treatment SR1 can be partly explained by that the rate of passage of digesta is influenced by other physical characteristics of digesta particles (i.e. specific gravity) of the diet selected. Digesta particle size decreases and specific gravity increases with time due to rumination and digestion leading to increased rate of passage of small particle size. Large sized particles have low density and float in the liquid phase of digesta in the rumen and get trapped in the fibre fraction formed in the dorsal part of the rumen (Bernard and Doreau, 2000; Clauss and Lechner-Doll, 2001). After rumination and bacterial digestion, large particles get reduced to below critical size and sink to the bottom of the rumen where they get washed out of the rumen by rumen contractions (Clauss and Lechner-Doll, 2001). Therefore, the concentration and distribution of ytterbium marker recovered in faeces is altered because of the fractionation of digesta into large and small particles given that ytterbium adheres to small-size particle fraction of digesta. Selectivity factors of diets selected across treatments were all within the range of 1.56-3.80 which is similar to earlier reports

(Clauss and Lechner-Doll, 2001); Moyo *et al*., 2018). This reflects that animals were able to retain the diets they selected long enough in the GIT for efficient digestion.

Faecal recovery rates of markers in nutritional studies are the true reflection of the efficacy of the markers and allows the establishment of representative relative comparison of markers in estimating dietary parameters in question (Kanga, 2010). Faecal recoveries of inert markers were similar to findings by Huhtanen *et al*. (1994) where AIA had the highest FR in comparison to ADL which had the least FR. However, in the present study there was an additional inert marker (MADF) not used in the study by Huhtanen *et al*. (1994) which had the least FR. Low faecal recoveries of MADF (FR<50%) and ADL (FR≈50%) may be due to a behaviour manifested by sheep of mainly selecting leaves rather than stems on their diets, given that stems had higher MADF and ADL contents in comparison to leaves. In contrary to low faecal recoveries of other inert markers, AIA had faecal recoveries of more than 75%. In treatments (SR1, SR2 and SR4) incomplete (FR <100%) faecal recovery of AIA may also be partly explained by the aforementioned selective behaviour manifested by sheep in this study. Faecal recoveries of AIA more than 100% in SR2 may be due to sheep to licking areas where urination previously occurred resulting to consumption of soil particles. In agreement with these results, Huhtanen *et al*. (1994) concluded that there are two possible explanation of inflated recoveries (>100%) of AIA which were: soil contaminated the grass and that there might be errors in getting representative sample. The earlier possibility is considered to be the source of disparity in this study. Additionally, sheep may have consumed some of the spillages contaminated with dust which may inflate the results (Van Keulen and Young, 1977). As expected, the FR rates of markers were not affected by animal stocking rate because the total faecal outputs were bulked within treatments to reduce the diurnal variation in concentration of the markers in faeces. Both predicted total dry matter intake (KSI = 90.42%) and apparent total tract digestibility (KSI = 89.35%) adjusted for incomplete faecal recovery rates had fairly good similarity indices compared to respective observed data sets. The predicted and the observed botanical composition of a diet had a high KSI value of 90.26%. Also, the regression relationship between the predicted and the observed (not adjusted for incomplete faecal recovery rates); dry matter intake and digestibility had R-squared values of 0.901 and 0.853, respectively. In agreement with this study, Ferreira *et al*. (2015) when using long chain alcohols (LCOH) alone or in combination with either *n*-alkanes or long chain fatty acids (LCFA) to estimate diet composition, LCOH showed to be less sensitive to faecal recovery correction and provided the most accurate estimates. Hence, these fairly good indices of the accuracy and precision of predicted parameters give an impression that the approach of using indigestible markers in predicting dietary parameters in nutritional studies on free ranging

animals is possible despite the fact that faecal recoveries are difficult to determine in free ranging animals.

4.5 Conclusions

There was no significant difference on rate of passage, intake and diet selected by sheep in relation to animal stocking rate. The total tract digestibility was affected by animal stocking rate. Dry matter intake and total tract digestibility were the highest for sheep fed in stocking rate of two animals relative to others. The rate of passage, rumen digestibility and intake of selected diets by sheep is governed by several factors, which are not fully understood in respect to animal stocking rate necessitating in-depth studies. Predicted dry matter intake and digestibility of a diet selected by sheep were less sensitive to correction of incomplete faecal recovery of the markers and they tended to be similar to observed dietary parameters. Therefore, this study demonstrated that inert markers can be used with wide-ranging application to estimate several components of a diet selected by sheep.

Chapter 5

General discussion, conclusions and recommendations

5.1. General discussion

Grazing is the most common and economical system of feeding for all classes of ruminants. Identifying and improving pre-existing methods of predicting intake, composition of diet selected and factors affecting intake in sheep grazing in rangelands is of importance. Quantifying how much can be supplemented to meet animal requirements depends on the ability to accurately predict composition and quantity of selected diet. These components of diets selected by grazing sheep are difficult to measure in a free-range feeding system. Hence, the proof of the concept of using inert markers is imperative where animals are fed in a simulated natural environment by providing several different feeds from *ad-libitum* to restriction feeding levels at different animal stocking rates. In a controlled environment it is possible to compare the observed and the predicted botanical composition of selected diet. Use of inert markers in predicting diet composition in rangeland would be determined by the result from the proof of such concept.

The hypotheses in the study were all tested in a zero-grazing approach for precise and accurate measurements and predictions of dietary parameters. Chapter 3 tested the hypothesis that group feeding and removal of sorghum stover has an effect on diet selection, nutrient and total intake, and digestibility in choice-fed sheep. Group feeding sheep relative to individuals had an effect on total dry matter intake and diet selection but no effect on digestibility and nutrient intake. Whereas the removal of ingredient intake had no influence on diet selection, nutrient and total intake, and digestibility in choice-fed sheep. This was achieved by slight alterations in the proportions of feeds selected in each treatment (I and R) and it is likely that this was done to buffer changes in digesta composition. The hypothesis is partly accepted based on the effect group feeding had on total dry matter intake and diet selection. It can be concluded that the number of animals per unit area need to be considered as one of the underlying factors when intake and diet selection are predicted in grazing animals.

Chapter 4 tested the hypotheses that: (1) animal stocking rate will have an effect on botanical composition and quality selected, dry matter and nutrient intake, total tract digestibility and passage rate, and (2) inert markers can be used to predict the dry matter and nutrient intake,

botanical composition, nutrients selected and total tract digestibility using concentration of inert markers in plant and faecal samples. The rate of passage, intake and diet selected by sheep in relation to animal stocking rate were all similar and total tract digestibility was different across treatments. The hypotheses were accepted on the view of that total tract digestibility was the highest for sheep fed at a stocking rate of 2 sheep per pen compared to all other stocking rates in the study. The predicted dietary parameters were fairly accurately and precisely close to observed data sets of parameters. Additionally, when total tract digestibility and dry matter intake were predicted without faecal recoveries of markers they gave good predictions with R^2 values of 0.853 and 0.901, respectively. Therefore, incomplete faecal recovery of the markers had negligible effect on predicted components of a diet select by sheep. In conclusion, inert markers can be used with wide-ranging application to estimate several components of a diet selected by sheep.

5.2 Conclusions

Removal of less preferred feed in offered dietary ingredients in sheep fed individually in a cafeteria feeding system cannot force sheep to consume other feeds of the same or poor quality when alternatives of better quality are supplied *ad libitum* or in restriction with the absence of competition. There was no significant difference on rate of passage, intake and diet selected by sheep in relation to animal stocking rate. The total tract digestibility was affected by animal stocking rate. Dry matter intake and total tract digestibility were the highest for sheep fed in stocking rate of two animals relative to others. There is a great possibility of obtaining data with the true reflection of reality from studies conducted in rangelands where inert markers can be used in conjunction with video recording devices. Behavioural data is important for comparison of predicted and observed data to improve the accuracy and effectiveness of the predicted data. Data obtained in this study can serve as a baseline in estimating botanical composition, digestibility and dry matter intake of diets selected by free ranging ruminants.

5.3 Recommendations for future research

Prediction of dietary components selected by sheep when using inert markers may be improved by determination of the stem to leaf ratio of dietary ingredients offered to animals and of the feed refusals left in the feeder. This facilitates the quantification of the amount of marker in

faeces ascending from consumed plant parts, improving prediction of dietary parameters of diets selected by animals using inert markers. There is a need for the development of accurate methods and models for determining diet selection and actual intake in simulated grazing conditions taking into consideration limitations in animal movement and feed choices according to their accessibility in natural pastures in corporation with different seasons. That will allow establishment of an outline for use of alternative feed resources (i.e. straws) to mitigate the shortage of forage with declining quality especially during dry seasons and periods of nutritional stress. Besides, that will enhance achievement of sustenance, optimum production and efficient management of pre-existing feed resources in accordance with dynamic ecosystem and seasons. Given that it is difficult to fully simulate grazing conditions of natural pastures on animals fed in pens because of restricted extent at which spatial distribution and availability of feed can be altered. To increase the credibility and accuracy of data predicted using inert markers, available data needs to be incorporated with data from studies conducted to achieve the following objectives, which are to:

1. Determine how grazing animals alter diet composition and feeding behaviour in relation to a decrease in availability of the preferred plant species or plant parts due to long term or continuous grazing effects.
2. Assess the use of faecal nitrogen excretion as an alternative complementary method to inert markers for estimating intake in free ranging animals.
3. Establish relationship between the predicted components of diets selected by grazing ruminants.

References

Aitchison, E.M., Gill, M., Dhanoa, M.S. and Osbourn, D.F., 1986. The effect of digestibility and forage species on the removal of digesta from the rumen and the voluntary intake of hay by sheep. *British Journal of Nutrition*, *56*(2), pp.463-476.

Ali, H., Khan, E. and Sajad, M.A., 2013. Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, *91*(7), pp.869-881.

Ali, H.A.M., Mayes, R.W., Hector, B.L. and Orskov, E.R., 2005. Assessment of n-alkanes, long-chain fatty alcohols and long-chain fatty acids as diet composition markers: the concentrations of these compounds in rangeland species from Sudan. *Animal Feed Science and Technology*, *121*(3-4), pp.257-271.

Ali, H.A.M., Mayes, R.W., Lamb, C.S., Hector, B.L., Verma, A.K. and Ørskov, E.R., 2004. The potential of long-chain fatty alcohols and long-chain fatty acids as diet composition markers: development of methods for quantitative analysis and faecal recoveries of these compounds in sheep fed mixed diets. *The Journal of Agricultural Science*, *142*(1), pp.71-78.

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *Journal of Dairy Science*, 83, 1598-1624.

Allison, C.D., 1985. Factors affecting forage intake by range ruminants: a review. *Journal of Range Management*, pp.305-311.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., Aguilar-Caballero, A.J. and Capetillo-Leal, C.M., 2009. Sheep preference for different tanniniferous tree fodders and its relationship with in vitro gas production and digestibility. *Animal Feed Science and Technology*.151, 75-85.

Anderson, D.C., 1978. Use of cereal residues in beef cattle production systems. *Journal of Animal Science*, *46*(3), pp.849-861.

Anthony, R.G. and Smith, N.S., 1974. Comparison of rumen and fecal analysis to describe deer diets. *The Journal of Wildlife Management*, pp.535-540.

AOAC, Association of Official Analytical Chemists. (1997). Official methods of analysis, (16th ed., 3rd rev.). Arlington, VA: AOAC.

Arnold, G.W., 2017. Regulation of forage intake. In *Bioenergetics of wild herbivores* (pp. 81-102). CRC Press.

Ashton, C.J., 1998. Evaluation of plant alcohols and sterols as potential faecal markers for estimating diet composition in free-ranging herbivores. MSc thesis, University of Aberdeen, United Kingdom.

Barcia, P., Bugalho, M.N., Campagnolo, M.L. and Cerdeira, J.O., 2007. Using n-alkanes to estimate diet composition of herbivores: a novel mathematical approach. *Animal Consortium, 1*(1), pp.141-149.

Barnes, D.L., 1976. A review of plant-based methods of estimating food consumption, percentage utilisation, species preferences and feeding patterns of grazing and browsing animals. *Proceedings of the Annual Congresses of the Grassland Society of Southern Africa, 11*(1), pp.65-71.

Baumont, R., Prache, S., Meuret, M. and Morand-Fehr, P., 2000. How forage characteristics influence behaviour and intake in small ruminants: a review. *Livestock Production Science*. 64, 15-28.

Bernard, L. and Doreau, M., 2000. Use of rare earth elements as external markers for mean retention time measurements in ruminants. *Reproduction Nutrition Development, 40*(2), pp.89-101.

Blaxter, K.L., Graham, N.M. and Wainman, F.W., 1956. Some observations on the digestibility of food by sheep, and on related problems. *British Journal of Nutrition*, 10(2), pp.69-91.

Bocquier, F. and González-García, E., 2010. Sustainability of ruminant agriculture in the new context: feeding strategies and features of animal adaptability into the necessary holistic approach. *Animal Consortium, 4*(7), pp.1258-1273.

Boling, J.A., Faltin, E.C., Hoekstra, W.G. and Hauser, E.R., 1967. Feed intake of cattle in response to dietary dilution with polyethylene. *Journal of Animal Science, 26*(6), pp.1385-1389.

Breman, H. and De Wit, C.T., 1983. Rangeland productivity and exploitation in the Sahel. *Science, 221*(4618), pp.1341-1347.

Briske, D.D., Derner, J.D., Brown, J.R., Fuhlendorf, S.D., Teague, W.R., Havstad, K.M., Gillen, R.L., Ash, A.J. and Willms, W.D., 2008. Rotational grazing on rangelands: reconciliation of perception and experimental evidence. *Rangeland Ecology Management*, 61, 3-17.

Bugalho, M.N., Dove, H., Kelman, W., Wood, J.T. and Mayes, R.W., 2004. Plant wax alkanes and alcohols as herbivore diet composition markers. *Journal of Range Management*, pp.259-268.

Bugalho, M.N., Mayes, R.W. and Milne, J.A., 2002. The effects of feeding selectivity on the estimation of diet composition using the n-alkane technique. *Grass and Forage Science*, *57*(3), pp.224-231.

Casebeer, R.L. and Koss, G.G., 1970. Food habits of wildebeest, zebra, hartebeest and cattle in Kenya Masailand. *African Journal of Ecology*, *8*(1), pp.25-36.

Chamrad, A.D. and Box, T.W., 1964. A point frame for sampling rumen contents. *The Journal of Wildlife Management*, pp.473-477.

Charmley, E. and Dove, H., 2008. Using plant wax markers to estimate diet composition and intakes of mixed forages in sheep by feeding a known amount of alkane-labelled supplement. *Australian Journal of Agricultural Research*, *58*(12), pp.1215-1225.

Chilibroste, P., Tamminga, S., Boer, H., Gibb, M.J. and Den Dikken, G., 2000. Duration of regrowth of ryegrass (Lolium perenne) effects on grazing behavior, intake, rumen fill, and fermentation of lactating dairy cows. *Journal of Dairy Science*, *83*(5), pp.984-995.

Chua, B., Coenen, E., Van Delen, J. and Weary, D., 2002. Effects of pair versus individual housing on the behavior and performance of dairy calves. *Journal of Dairy Science*, 85, 360-364.

Clauss, M. and Lechner-Doll, M., 2001. Differences in selective reticulo-ruminal particle retention as a key factor in ruminant diversification. *Oecologia*, *129*(3), pp.321-327.

Cloete, S.W.P., Schoeman, S.J., Coetzee, J. and Morris, J.D.V., 2001. Genetic variances for liveweight and fleece traits in Merino, Dohne Merino and South African Meat Merino sheep. *Australian Journal of Experimental Agriculture*, 41, 145-153.

Coates, D.B., Schachenmann, P. and Jones, R.J., 1987. Reliability of extrusa samples collected from steers fistulated at the oesophagus to estimate the diet of resident animals in grazing experiments. *Australian Journal of Experimental Agriculture*, *27*(6), pp.739-745.

Coleman, S.W., Moore, J.E., Wilson, J.R. and Moser, L.E., 2004. Quality and utilization. *Warm-season (C4) grasses. Agronomy Series*, *45*, pp.267-308.

Collier, R.E. and Foulds, S.M., 1975. A measure of the ruggedness of the modified acid detergent fibre method. *Journal of the Science of Food and Agriculture*, *26*(7), pp.949-952.

Cook, C.W. and Stoddart, L.A., 1953. The quandary of utilization and preference. *Journal of Range Management*, *6*, pp.329-335.

Croker, B.H., 1959. A method of estimating the botanical composition of the diet of sheep. *New Zealand Journal of Agricultural Research*, *2*(1), pp.72-85.

Das, M.M. and Kundu, S.S., 1994. The effects of calcium hydroxide, urea and calcium hypochlorite treatments on composition and degradability of wheat straw. *Indian Journal of Dairy Science*, *47*, pp.59-59.

Dawson, L.A., Mayes, R.W., Elston, D.A. and Smart, T.S., 2000. Root hydrocarbons as potential markers for determining species composition. *Plant, Cell & Environment*, *23*(7), pp.743-750.

Decandia, M., Sitzia, M., Cabiddu, A., Kababya, D. and Molle, G., 2000. The use of polyethylene glycol to reduce the anti-nutritional effects of tannins in goats fed woody species. *Small Ruminant Research*, *38*(2), pp.157-164.

Decruyenaere, V., Buldgen, A. and Stilmant, D., 2009. Factors affecting intake by grazing ruminants and related quantification methods: a review. *Biotechnologie, Agronomie, Société et Environnement*, *13*(4), pp.559-573.

Ding, L.M. and Long, R.J., 2009. The use of herbage N-alkanes as markers to estimate the diet composition of yaks on the Qinghai-Tibetan plateau. *Asian-Australasian Journal of Animal Sciences*, *23*(1), pp.61-67.

Doan, T. and Guo, X., 2019. Understanding Bison Carrying Capacity Estimation in Northern Great Plains Using Remote Sensing and GIS. *Canadian Journal of Remote Sensing*, pp.1-24.

Dove, H. and Mayes, R.W., 1996. Plant wax components: a new approach to estimating intake and diet composition in herbivores. *The Journal of Nutrition*, *126*(1), pp.13-26.

Dove, H. and Mayes, R.W., 1996. Plant wax components: a new approach to estimating intake and diet composition in herbivores. *The journal of nutrition*, *126*(1), pp.13-26.

Dove, H. and Mayes, R.W., 2005. Using n-alkanes and other plant wax components to estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. *Small Ruminant Research*, *59*(2-3), pp.123-139.

Dove, H. and Mayes, R.W., 2006. Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. *Nature protocols*, *1*(4), p.1680.

Dove, H. and Moore, A.D., 1995. Using a least-squares optimization procedure to estimate botanical composition based on the alkanes of plant cuticular wax. *Australian Journal of Agricultural Research*, *46*(8), pp.1535-1544.

Dove, H. and Olian, M., 1998. Using synthetic or beeswax alkanes for estimating supplement intake in sheep. *Animal Production in Australia*, *22*, pp.189-192.

Dove, H. and Oliván, M., 2006. The possible use of the alkenes (unsaturated hydrocarbons) of plant cuticular wax as diet composition markers in sheep. *BSAP Occasional Publication*, *34*, pp.1-7.

Dove, H., 1998. Pastures and grazing animals–the interaction continues. *Animal Production in Australia 22*(1): pp.3-13.

Dove, H., 2010, July. Keynote: assessment of intake and diet composition of grazing livestock. In *Proceedings of the fourth Grazing Livestock nutrition conference. Champaign, IL: Western Section of American Society of Animal Science,* (pp. 31-54).

Dove, H., Freer, M. and Foot, J.Z., 2000. The nutrition of grazing ewes during pregnancy and lactation: a comparison of alkane-based and chromium/in vitro-based estimates of herbage intake. *Australian Journal of Agricultural Research*, *51*(6), pp.765-777.

Dove, H., Scharch, C., Oliván, M. and Mayes, R.W., 2002. Using n-alkanes and known supplement intake to estimate roughage intake in sheep. *Animal Production in Australia*, *24*, pp.57-60.

Drescher, M.F., 2003. Grasping complex matter: large herbivore foraging in patches of heterogeneous resources. PhD Thesis, Wageningen University, Netherlands.

Duncan, A.J. and Poppi, D.P., 2008. Nutritional ecology of grazing and browsing ruminants. In *The ecology of browsing and grazing* (pp. 89-116). Springer, Berlin, Heidelberg.

Duncan, A.J., Ginane, C., Gordon, I.J. and Orskov, E.R., 2003. Why do herbivores select mixed diets? In Matching herbivore nutrition to ecosystems biodiversity, VI International Symposium on the nutrition of herbivores. Yucatán, México. pp. 195-212.

Duncan, A.J., Mayes, R.W., Lamb, C.S., Young, S.A. and Castillo, I., 1999. The use of naturally occurring and artificially applied n-alkanes as markers for estimation of short-term diet composition and intake in sheep. *The Journal of Agricultural Science*, *132*(2), pp.233-246.

Dunnet, G.M., Harvie, A.E. and Smit, T.J., 1973. Estimating the proportions of various leaves in the diet of the opossum, Trichosurus vulpecula Kerr, by faecal analysis. *Journal of Applied Ecology*, pp.737-745.

Elliott, R.C., 1967. Voluntary intake of low-protein diets by ruminants: II. Intake of food by sheep. *The Journal of Agricultural Science*, *69*(3), pp.383-390.

Elwert, C. and Dove, H., 2005. Estimation of roughage intake in sheep using a known daily intake of a labelled supplement. *Animal Science*, *81*(1), pp.47-56.

Faichney, G.J., McDonald, I.W. and Warner, A.C.I., 1975. Digestion and Metabolism in the Ruminant, Proceedings of IVth International Symposium on Ruminant Physiology.

Ferreira, L.M.M., Celaya, R., Santos, A.S., Mayes, R.W., Rodrigues, M.A.M. and Osoro, K., 2015. Application of long-chain alcohols as diet-composition markers in sheep fed on grass–white clover and heather–gorse plant species. *Grass and Forage Science*, *70*(1), pp.30-43.

Ferreira, L.M.M., Garcia, U., Rodrigues, M.A.M., Celaya, R., Dias-da-Silva, A. and Osoro, K., 2007. Estimation of feed intake and apparent digestibility of equines and cattle grazing on heathland vegetation communities using the n-alkane markers. *Livestock Science*, *110*(1-2), pp.46-56.

Ferreira, L.M.M., Hervás, G., Belenguer, A., Celaya, R., Rodrigues, M.A.M., García, U., Frutos, P. and Osoro, K., 2017. Comparison of feed intake, digestion and rumen function among domestic ruminant species grazing in upland vegetation communities. *Journal of Animal Physiology and Animal Nutrition*, *101*(5), pp.846-856.

Forbes, J.M., 2007. *Voluntary food intake and diet selection in farm animals*. Cabi.

Fraser, M.D. and Gordon, I.J., 1997. The diet of goats, red deer and South American camelids feeding on three contrasting Scottish upland vegetation communities. *Journal of Applied Ecology*, pp.668-686.

Fraser, M.D., Theobald, V.J. and Moorby, J.M., 2006. Determining diet composition on complex swards using n-alkanes and long-chain fatty alcohols. *Ecological Applications*, *16*(5), pp.1901-1910.

Free, J.C., Sims, P.L. and Hansen, R.M., 1971. Methods of estimating dry-weight composition in diets of steers. *Journal of Animal Science*, *32*(5), pp.1003-1007.

Galyean, M.L. and Gunter, S.A., 2016. Predicting forage intake in extensive grazing systems. *Journal of Animal Science*, *94*(suppl_6), pp.26-43.

Ganskopp, D.C. and Bohnert, D.W., 2009. Landscape nutritional patterns and cattle distribution in rangeland pastures. *Applied Animal Behaviour Science*, *116*(2), pp.110-119.

Gerber, N.D., 2014. The effect of feed type and diet quality on kinetics of digestion: the degradation properties of certain protein supplements, and the effect of concentrate supplementation and basal roughage quality on eating behaviour and particle passage from the rumen of sheep. MSc thesis, University of KwaZulu-Natal, South Africa.

Ginane, C., Baumont, R., Lassalas, J. and Petit, M., 2002. Feeding behaviour and intake of heifers fed on hays of various quality, offered alone or in a choice situation. *Animal Research,* 51, 177-188.

Giráldez, F.J., Lamb, C.S., López, S. and Mayes, R.W., 2004. Effects of carrier matrix and dosing frequency on digestive kinetics of even-chain alkanes and implications on herbage intake and rate of passage studies. *Journal of the Science of Food and Agriculture*, *84*(12), pp.1562-1570.

Goetsch, A.L., Gipson, T.A., Askar, A.R. and Puchala, R., 2010. Invited review: Feeding behavior of goats. *Journal of Animal Science*, *88*(1), pp.361-373.

Gotlib, V.G., Kuzentsova, A.I., Gotlib, G.F., Ushakov, Y.U.A. and Henke, A.N.I., 1977. Digestibility of a diet with ammoniated straw. *Nutrition Abstracts and Reviews*, *47*(4).

Grant, S.A., Torvell, L., Smith, H.K., Suckling, D.E., Forbes, T.D.A. and Hodgson, J., 1987. Comparative studies of diet selection by sheep and cattle: blanket bog and heather moor. *The Journal of Ecology*, pp.947-960.

Grovum, W.L. and Phillips, G.D., 1973. Rate of passage of digesta in sheep: 5. * Theoretical considerations based on a physical model and computer simulation. *British Journal of Nutrition*, *30*(2), pp.377-390.

Grovum, W.L. and Williams, V.J., 1973. Rate of passage of digesta in sheep: 4. * Passage of marker through the alimentary tract and the biological relevance of rate-constants derived from the changes in concentration of marker in faeces. *British journal of Nutrition, 30*(2), pp.313-329.

Haenlein, G.F.W., 2001. Past, present, and future perspectives of small ruminant dairy research. *Journal of Dairy Science*, *84*(9), pp.2097-2115.

Hameleers, A. and Mayes, R.W., 1998. The use of n-alkanes to estimate herbage intake and diet composition by dairy cows offered a perennial ryegrass/white clover mixture. *Grass and Forage Science*, *53*(2), pp.164-169.

Hansen, R.M., 1971. Estimating plant composition of wild sheep diets. In *First Transition, North America. Wild Sheep Conference* (pp. 180-185).

Harrison, D.G. and McAllan, A.B., 1980. Factors affecting microbial growth yields in the reticulo-rumen. In *Digestive physiology and metabolism in ruminants* (pp. 205-226). Springer, Dordrecht.

Hatfield, P.G., Clanton, D.C., Sanson, D.W. and Eskridge, K.M., 1990. Methods of administering ytterbium for estimation of fecal output. *Journal of Range Management*, pp.316-320.

Heublein, C., Südekum, K.H., Gill, F.L., Dohme-Meier, F. and Schori, F., 2017. Using plant wax markers to estimate the diet composition of grazing Holstein dairy cows. *Journal of Dairy Science*, *100*(2), pp.1019-1036.

Hilburger, E.J., 2017. Assessing Plant-Wax Markers as a Tool to Estimate intake and diet Composition in Beef Cattle. MSc thesis, University of Nebreska, Lincon.

Holechek, J.L., Vavra, M. and Pieper, R.D., 1982. Botanical composition determination of range herbivore diets: a review. *Journal of Range Management*, pp.309-315.

Huhtanen, P., Detmann, E. and Krizsan, S.J., 2016. Prediction of rumen fiber pool in cattle from dietary, fecal, and animal variables. *Journal of dairy science*, *99*(7), pp.5345-5357.

Huhtanen, P., Kaustell, K. and Jaakkola, S., 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Animal Feed Science and Technology*, *48*(3-4), pp.211-227.

Ibhaze, G.A., Ekeocha, A.H. and Ojeamiren, B.O., 2016. Free Choice Intake, Growth and Digestibility Responses by West African Dwarf Does Fed Pulverised Maize-Cob Based Diets. *Applied Tropical Agriculture*, 21, pp.128-132.

Illius, A.W. and Gordon, I.J., 1990. Constraints on diet selection and foraging behaviour in mammalian herbivores. In *Behavioural mechanisms of food selection* (pp. 369-393). Springer, Berlin, Heidelberg.

Johnson, M.K. and Pearson, H.A., 1981. Esophageal, fecal and exclosure estimates of cattle diets on a longleaf pine-bluestem range. *Journal of Range Management*, pp.232-234.

Jones, R., Ludlow, M.M., Troughton, J.H. and Blunt, C.G., 1979. Estimation of the proportion of C 3 and C 4 plant species in the diet of animals from the ratio of natural 12 C and 13 C isotopes in the faeces. *The Journal of Agricultural Science*, *92*(1), pp.91-100.

Jung, H.G. and Allen, M.S., 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. *Journal of animal science*, *73*(9), pp.2774-2790.

Kababya, D., Perevolotsky, A., Bruckental, I. and Landau, S., 1998. Selection of diets by dual-purpose Mamber goats in Mediterranean woodland. *The Journal of Agricultural Science*, *131*(2), pp.221-228.

Kanani, J., 2012. Evaluation of Internal Markers for Predicting Digestibility and Fecal Output by Cattle Fed Bermudagrass Hays of Varying Quality. PhD thesis, University of Arkansas, Fayetteville.

Kanga, J.S., 2010. Estimating the voluntary herbage intake and digestibility of growing pigs fed a concentrate supplement on a Kikuyu pasture by the N-alkane and acid-isoluble ash markers. MSc thesis, University of South Africa, South Africa.

Kartchner, R.J., 1980. Effects of protein and energy supplementation of cows grazing native winter range forage on intake and digestibility. *Journal of Animal Science*, *51*(2), pp.432-438.

Kawas, J.R., Andrade-Montemayor, H. and Lu, C.D., 2010. Strategic nutrient supplementation of free-ranging goats. *Small Ruminant Research*, 89, 234-243.

Kebede, G., 2006. Effect of urea-treatment and leucaena (*Leucaena leucocephala*) supplementation on the utilization of wheat straw as feed for sheep. MSc thesis, Haramaya University, Ethiopia.

Kessler, W.B., 1981. Three methods compared for analysis of pronghorn diets. *Journal of Wildlife Management*, *45*, pp.612-619.

Knudsen, K.B., 2001. The nutritional significance of "dietary fibre" analysis. *Animal Feed Science and Technology*, *90*(1-2), pp.3-20.

Kondo, S., Sekine, J., Okubo, M. and Asahida. Y., 1989. The effect of group size and space allowance on the agonistic and spacing behavior of cattle. *Applied Animal Behavioural Science*, 24, 127-135.

Kotb, A.R. and Luckey, T.D., 1972, July. Markers in nutrition. In *Nutrition Abstracts and Reviews* (Vol. 42, No. 3, p. 813).

Krebs, C.J., 1989. Measurement of dietary preferences. *Ecological methodology. New York: HarperCollins*, pp.392-407.

Kyriazakis, I. and Oldham, J.D., 1993. Diet selection in sheep: the ability of growing lambs to select a diet that meets their crude protein (nitrogen× 6.25) requirements. *British Journal of Nutrition*, 69, 617-629.

Kyriazakis, I., 2003. What are ruminant herbivores trying to achieve through their feeding behaviour and food intake. *Matching herbivore nutrition to ecosystems biodiversity'. (Eds L't Mannetje, L Ramirez-Aviles, CA Sandoval-Castro, JC Ku-Vera) pp*, pp.154-173.

Laca, E.A., 2008. Foraging in a heterogeneous environment: intake and diet choice. In *Resource Ecology* (pp. 81-100). Springer, Dordrecht.

Langlands, J.P., 1987. Assessing the nutrient status of herbivores. In 'The nutrition of herbivores. (Eds JB Hacker, JH Ternouth) pp. 363–390.

Laycock, W.A., Buchanan, H. and Krueger, W.C., 1972. Three methods of determining diet, utilization, and trampling damage on sheep ranges. *Rangeland Ecology & Management/Journal of Range Management Archives*, *25*(5), pp.352-356.

Leng, R.A., 1990. Factors affecting the utilization of 'poor-quality' forages by ruminants particularly under tropical conditions. *Nutrition Research Review*, 3, 277-303.

Leury, B.J., Siever-Kelly, C., Simpson, R.J., Gatford, K.L., Ciavarella, T.A. and Dove, H., 1999. Spray-topping annual grass pasture with glyphosate to delay loss of feeding value during summer. I. Effects on pasture yield and nutritive value. *Australian journal of agricultural research*, *50*(4), pp.453-464.

Lewis, R.M., Magadlela, A.M., Jessop, N.S. and Emmans, G.C., 2003. The ability of the n-alkane technique to estimate intake and diet choice of sheep. *Animal Science*, *77*(2), pp.319-327.

Leyequien, E., Verrelst, J., Slot, M., Schaepman-Strub, G., Heitkönig, I.M. and Skidmore, A., 2007. Capturing the fugitive: applying remote sensing to terrestrial animal distribution and diversity. *International Journal of Applied Earth Observation and Geoinformation*, *9*(1), pp.1-20.

Li, C., Xue, S., Tajima, A. and Ishikawa, N., 2015. Estimation of herbage intake and digestibility of grazing sheep in Zhenglan Banner of Inner Mongolia by using n-alkanes. *Animal Nutrition*, *1*(4), pp.324-328.

Lin, L.J., Zhu, X.Y., Jiang, C., Luo, H.L., Wang, H., Zhang, Y.J. and Hong, F.Z., 2012. The potential use of n-alkanes, long-chain alcohols and long-chain fatty acids as diet

composition markers: indoor validation with sheep and herbage species from the rangeland of Inner Mongolia of China. *Animal Consortium*, *6*(3), pp.449-458

López-Bermúdez, F. and García-Gómez, J., 2006. Desertification in the arid and semiarid Mediterranean regions. A food security issue. In *Desertification in the Mediterranean region. A security issue* (pp. 401-428). Springer, Dordrecht.

Magadlela, A.M., 2001. An evaluation of n-alkanes as markers to estimate dry matter intake, diet selection and solid digesta passage rates in ruminants. PhD thesis, University of Edinburgh, United Kingdom.

Malossini, F., Bovolenta, S., Piasentier, E., Piras, C. and Martillotti, F., 1996. Comparison of n-alkanes and chromium oxide methods for estimating herbage intake by grazing dairy cows. *Animal Feed Science and Technology*, *61*(1-4), pp.155-165.

Marais, J.P., 2000. Use of markers. Farm animal metabolism and nutrition: critical reviews. Wallingford: CAB International, pp.255-277.

Mayes, R. and Lamb, C., 1984. The possible use of normal-alkanes in herbage as indigestible fecal markers. In: Proceedings of the Nutrition Society. p A39-A39.

Mayes, R.W. and Dove, H., 2000. Measurement of dietary nutrient intake in free-ranging mammalian herbivores. *Nutrition Research Reviews*, *13*(1), pp.107-138.

Mayes, R.W., Beresford, N.A., Lamb, C.S., Barnett, C.L., Howard, B.J., Jones, B.E., Eriksson, O., Hove, K., Pedersen, Ø. and Staines, B.W., 1994. Novel approaches to the estimation of intake and bioavailability of radiocaesium in ruminants grazing forested areas. *Science of the Total Environment*, *157*, pp.289-300.

Mayes, R.W., Lamb, C.S. and Colgrove, P.M., 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. *The Journal of Agricultural Science*, *107*(1), pp.161-170.

McDonald, I., 1981. A revised model for the estimation of protein degradability in the rumen. *The Journal of Agricultural Science*, *96*(1), pp.251-252.

McDowell, R.E., 1988. Importance of crop residues for feeding livestock in smallholder farming systems. In *Plant breeding and the nutritive value of crop residues. Proceedings of a workshop. ILCA, Addis Ababa (Ethiopia)* (pp. 3-27).

McInnis, M.L., 1976. A comparison of four methods used in determining the diets of large herbivores. MSc thesis, Oregon State University, United States of America.

McInnis, M.L., Vavra, M. and Krueger, W.C., 1983. A comparison of four methods used to determine the diets of large herbivores. *Journal of Range Management*, pp.302-306.

Merchant, M., 1996. The intake of grass and rush (Juncus effusus L.) by goats grazing rush-infested grass pasture. *Grass and Forage Science*, *51*(1), pp.81-87.

Mertens, D.R., 1987. Predicting intake and digestibility using mathematical models of ruminal function. *Journal of animal science*, *64*(5), pp.1548-1558.

Meyer, K., Hummel, J. and Clauss, M., 2010. The relationship between forage cell wall content and voluntary food intake in mammalian herbivores. *Mammal Review*. 40, 221-245.

Michell, A.R., 1977. An inexpensive metabolic harness for female sheep. *British Veterinary Journal*, *133*(5), pp.483-485.

Minson, D.J., 1963. The effect of pelleting and wafering on the feeding value of roughage—a review. *Grass and Forage Science*, *18*(1), pp.39-44.

Minson, D.J., 1967. The voluntary intake and digestibility, in sheep, of chopped and pelleted Digitaria decumbens (pangola grass) following a late application of fertilizer nitrogen. *British Journal of Nutrition*, *21*(3), pp.587-597.

Moyo, M., Bhiya, S.T., Katamzi, M. and Nsahlai, I.V., 2019. Evaluation and Prediction of the Nutritive Value of Underutilised Forages as Potential Feeds for Ruminants. In *Forage Groups*. IntechOpen.

Moyo, M., Kana, E.G. and Nsahlai, I.V., 2018. Prediction of solid digesta passage rate using liquid passage rate as one of the input variables in ruminants. *South African Journal of Animal Science*, *48*(4), pp.758-769.

Mui, N.T. and Ledin, I., 2007. Effect of group size on feed intake, aggressive behaviour and growth rate in goat kids and lambs. *Small Ruminant Research, 72*(2-3), pp.187-196.

Murphy, M.R., Kennedy, P.M. and Welch, J.G., 1989. Passage and rumination of inert particles varying in size and specific gravity as determined from analysis of faecal appearance using multicompartment models. *British Journal of Nutrition*, *62*(2), pp.481-492.

Nancy, J. and Wendt, T., 2003. Association of Official Analytical Chemistry (AOAC), official method 934.01. Revision 2, AOAC International 1.

Nefzaoui, A. and Ben Salem, H., 2001. Opuntia: a strategic fodder and efficient tool to combat desertification in the WANA (West Asia/North Africa) region. *FAO Plant Production and Protection Paper (FAO).*

Newman, J.A., Thompson, W.A., Penning, P.D. and Mayes, R.W., 1995. Least-squares estimation of diet composition from n-alkanes in herbage and faeces using matrix mathematics. *Australian Journal of Agricultural Research*, *46*(4), pp.793-805.

Ngwa, A., Pone, D. and Mafeni, J., 2000. Feed selection and dietary preferences of forage by small ruminants grazing natural pastures in the Sahelian zone of Cameroon. *Animal Feed Science and Technology,* 88, 253-266.

Nsahlai, I.V., Siaw, D.E.K.A. and Umunna, N.N., 1995. Inter-relationships between chemical constituents, rumen dry matter and nitrogen degradability in fresh leaves of multipurpose trees. *Journal of the Science of Food and Agriculture*, *69*(2), pp.235-246.

Odoi, F. and Owen, E., 1993. Encouraging store lambs to eat barley straw at housing: influence on intake of pen to pen visibility and number of lambs per pen. *Animal Production,* 56, 455.

Ordakowski, A.L., Kronfeld, D.S., Holland, J.L., Hargreaves, B.J., Gay, L.S., Harris, P.A., Dove, H. and Sklan, D., 2001. Alkanes as internal markers to estimate digestibility of hay or hay plus concentrate diets in horses. *Journal of Animal Science*, *79*(6), pp.1516-1522.

Osbourn, D.F., Terry, R.A., Outen, G.E. and Cammell, S.B., 1974. Significance of a determination of cell walls as the rational basis for the nutritive evaluation of forages. In *Sectional Papers International Grassland Congress*.

Osuji, P.O., Nsahlai, I.V. and Khalili, H., 1993. Feed evaluation (Vol. 5). ILRI (aka ILCA and ILRAD).

Owens, F.N. and Hanson, C.F., 1992. External and internal markers for appraising site and extent of digestion in ruminants. *Journal of Dairy Science*, *75*(9), pp.2605-2617.

Peiretti, P.G., Meineri, G., Miraglia, N., Mucciarelli, M. and Bergero, D., 2006. Intake and apparent digestibility of hay or hay plus concentrate diets determined in horses by the total collection of feces and n-alkanes as internal markers. *Livestock Science*, *100*(2-3), pp.189-194.

Pellikaan, W.F., Verstegen, M.W.A., Tamminga, S., Dijkstra, J. and Hendriks, W.H., 2013. δ 13 C as a marker to study digesta passage kinetics in ruminants: a combined in vivo and in vitro study. *Animal Consortium*, *7*(5), pp.754-767.

Phillips, C., 2004. The effects of forage provision and group size on the behavior of calves. *J. Dairy Sci.* 87, 1380-1388.

Poncet, C. and Abd, A.A., 1984. Particulate and fluid passage studies in sheep fed a hay-based diet. *Canadian Journal of Animal Science*, *64*(5), pp.77-79.

Provenza, F.D., Meuret, M. and Gregorini, P., 2015. Our landscapes, our livestock, ourselves: restoring broken linkages among plants, herbivores, and humans with diets that nourish and satiate. *Appetite*, *95*, pp.500-519.

Provenza, F.D., Villalba, J.J., Dziba, L., Atwood, S.B. and Banner. R.E., 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. *Small Ruminant Research*, 49, 257-274.

Reid, R.L. and Jung, G.A., 1965. Influence of Fertilizer Treatment on the Intake, Digestibility and Palatability of Tall Fescue Hay 1. *Journal of Animal Science*, 24, 615-625.

Reid, R.L., Jung, G.A. and Thayne, W.V., 1988. Relationships between nutritive quality and fiber components of cool season and warm season forages: a retrospective study. *Journal of Animal Science*, *66*(5), pp.1275-1291.

Rook, A. and Penning, P., 1991. Synchronisation of eating, ruminating and idling activity by grazing sheep. *Applied Animal Behaviour Science*, 32, 157-166.

Roumet, C., Picon-Cochard, C., Dawson, L.A., Joffre, R., Mayes, R., Blanchard, A. and Brewer, M.J., 2006. Quantifying species composition in root mixtures using two methods: near-infrared reflectance spectroscopy and plant wax markers. *New Phytologist*, *170*(3), pp.631-638.

Rutter, S.M., 2000. Graze: a program to analyze recordings of the jaw movements of ruminants. *Behaviour Research Methods, Instruments and Computers*. 32, 86-92.

Salem, H.B. and Smith, T., 2008. Feeding strategies to increase small ruminant production in dry environments. *Small ruminant research*, *77*(2-3), pp.174-194.

Sampaio, C.B., Detmann, E., Valente, T.N.P., Souza, M.A.D., Valadares Filho, S.D.C. and Paulino, M.F., 2011. Evaluation of fecal recovering and long term bias of internal and external markers in a digestion assay with cattle. *Revista Brasileira de Zootecnia*, *40*(1), pp.174-182.

Sandberg, R.E., Adams, D.C., Klopfenstein, T.J. and Grant, R.J., 2000. N-alkane as an internal marker for predicting digestibility of forages. *Journal of Range Management*, pp.159-163.

Sanders, K.D., Dahl, B.E. and Scott, G., 1980. Bite-count vs fecal analysis for range animal diets. *Journal of Range Management*, pp.146-149.

Schiere, J.B., Ibrahim, M.N.M. and Van Keulen, H., 2002. The role of livestock for sustainability in mixed farming: criteria and scenario studies under varying resource allocation. *Agriculture, ecosystems & environment*, *90*(2), pp.139-153.

Schnyder, H., Locher, F. and Auerswald, K., 2010. Nutrient redistribution by grazing cattle drives patterns of topsoil N and P stocks in a low-input pasture ecosystem. *Nutrient Cycling in Agroecosystems*, *88*(2), pp.183-195.

Scotcher, J.S.B., 1979. A review of faecal analysis techniques for determining the diet of wild grazing herbivores. *Proceedings of the Annual Congresses of the Grassland Society of Southern Africa*, *14*(1), pp.131-136.

Sibbald, A.M., Davidson, G.C. and Mayes, R.W., 2000. Effect of dosing regime on intake estimation using the n-alkane technique in sheep fed pelleted grass meal. *Journal of the Science of Food and Agriculture*, *80*(8), pp.1206-1210.

Silanikove, N., Holzer, Z., Cohen, D., Benjamin, R., Gutman, M. and Meltzer, A., 1987. Interrelationship between metabolism of tritiated water, 22sodium and dry matter intake by beef cattle consuming wheat straw and poultry litter in free choice. *Comparative Biochemistry and Physiology Part A: Physiology*, *88*(1), pp.113-118.

Smith, A.D. and Shandruk, L.J., 1979. Comparison of fecal, rumen and utilization methods for ascertaining pronghorn diets. *Rangeland Ecology & Management/Journal of Range Management Archives*, *32*(4), pp.275-279.

Smith, L.L. and Strickland, J.R., 2007. Improved GC/MS method for quantitation of n-alkanes in plant and fecal material. *Journal of agricultural and food chemistry*, 55(18), pp.7301-7307.

Sollenberger, L.E. and Vanzant, E.S., 2011. Interrelationships among forage nutritive value and quantity and individual animal performance. *Crop Science*, *51*(2), pp.420-432.

Sollenberger, L.E., Agouridis, C.T., Vanzant, E.S., Franzluebbers, A.J. and Owens, L.B., 2012. Prescribed grazing on pasturelands. Conservation outcomes from pastureland and hayland practices: assessment, recommendations, and knowledge gaps.

Teferedegne, B., 2000. New perspectives on the use of tropical plants to improve ruminant nutrition. *Proceedings of the Nutrition Society*, *59*(2), pp.209-214.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G. and Slade, N.A., 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ 13 C analysis of diet. *Oecologia*, *57*(1-2), pp.32-37.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. and Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature*, *418*(6898), p.671.

Tulloch, A.P., 1976. Chemistry of waxes of higher plants. In *Chemistry and biochemistry of natural waxes* (pp. 235-287).

Udén, P., Colucci, P.E. and Van Soest, P.J., 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *Journal of the Science of Food and Agriculture*, *31*(7), pp.625-632.

Valiente, O.L., Delgado, P., De Vega, A. and Guada, J.A., 2003. Validation of the n-alkane technique to estimate intake, digestibility, and diet composition in sheep consuming mixed grain: roughage diets. *Australian Journal of Agricultural Research*, *54*(7), pp.693-702.

Van der Merwe, N.J., 1982. Carbon isotopes, photosynthesis, and archaeology: Different pathways of photosynthesis cause characteristic changes in carbon isotope ratios that make possible the study of prehistoric human diets. *American Scientist*, *70*(6), pp.596-606.

Van Keulen, J.Y.B.A. and Young, B.A., 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science*, *44*(2), pp.282-287.

Van Soest, P.J. and Wine, R.H., 1968. Determination of lignin and cellulose in acid-detergent fiber with permanganate. *Journal of the association of official analytical chemists*, *51*, pp.780-785.

Van Soest, P.J., 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. *Journal of Animal Science*, *24*(3), pp.834-843.

Van Soest, P.J., 2018. *Nutritional ecology of the ruminant*. Cornell university press.

Vavra, M. and Holechek, J.L., 1980. Factors influencing microhistological analysis of herbivore diets. *Journal of Range management*, *33*(5), pp.371-374.

Vazquez, O.P. and Smith, T.R., 2000. Factors affecting pasture intake and total dry matter intake in grazing dairy cows. *Journal of Dairy Science*, *83*(10), pp.2301-2309.

Velasquez, A.V., 2017. Evaluation of internal and external markers for estimating dry matter intake and digestibility in cattle. PhD thesis, Universidade de São Paulo, Brazil.

Villalba, J. J., Provenza, F., Catanese, F. and Distel, R.A., 2015. Understanding and manipulating diet choice in grazing animals. *Animal Production Science,* 55, 261-271.

Vulich, S.A., O'riordan, E.G. and Hanrahan, J.P., 1991. Use of n-alkanes for the estimation of herbage intake in sheep: accuracy and precision of the estimates. *The Journal of Agricultural Science*, *116*(2), pp.319-323.

Ward, R.G., 1981. Prediction of range cattle forage intake by infrared reflectance spectroscopy of forage specimens. PhD thesis, New State Mexico University, United State of America.

Westoby, M., 1974. An analysis of diet selection by large generalist herbivores. *The American Naturalist*, *108*(961), pp.290-304.

Weston, R.H. and Davis, P., 1991. The significance of four forage characters as constraints to voluntary intake. Proceedings of the International Symposium on Nutrition of Herbivores. Blacktown, Australia. pp, 33.

Wilson, D.E., Hirst, S.M. and Ellis, R.P., 1977. Determination of feeding preferences in wild ruminants from trocar samples. *The Journal of Wildlife Management*, pp.70-75.

Yadav, B.P.S. and Yadav, I.S., 1989. Comparative study of ammoniated wheat and paddy straws on nutrient utilization and rumen fermentation in cattle. *Indian Journal of Animal Nutrition*, *6*(3), pp.215-222.

Appendix

Ethical approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

18 July 2017

Mr Buliani Nangamso Pepeta (213551846)
School Agricultural, Earth & Environmental Sciences
Pietermaritzburg Campus

Dear Mr Pepeta,

Protocol reference number: AREC/030/017M
Project title: Feed preferences in ruminants

Full Approval – Research Application

With regards to your revised application received on 30 June 2017 and 07 July 2017. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Please note: **Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person.**

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 18 July 2018.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

.....

Prof S Islam, PhD
Chair: Animal Research Ethics Committee

/ms

cc Supervisor: Professor Ignatius Verla Nsahlai
Cc Acting Dean & Head of School: Professor D Mutanga
Cc Registrar: Mr Simon Mokoena
Cc NSPCA: Ms Stephanie Kevlder
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