EFFECTS OF *RHUS LANCEA* AND *CELTIS AFRICANA* ON SHORT-TERM INTAKE RATE, NUTRIENT DIGESTIBILITY, BLOOD METABOLITES AND RUMEN BACTERIA IN NGUNI GOATS AS A MODEL FOR FEEDING CAPTIVE WILDLIFE HERBIVORES IN ZOOLOGICAL GARDENS

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France Phiri

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Supervisor: Prof. K.R. Mbatha
Co-Supervisor: Dr. A.T. Kanengoni
Co-Supervisor: Dr. T.D.E. Mpanza

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DECLARATION OF ORIGINALITY

Name: France Phiri

Degree: Doctor of Philosophy in Agriculture

Title: Effects of *Rhus lancea* and *Celtis africana* on short-term intake rate, nutrient digestibility, blood metabolites and rumen bacteria in Nguni goats as a model for feeding captive wildlife herbivores in zoological gardens

I declare that the above thesis is my work and that all the sources 6I have used or quoted have been indicated and acknowledged using complete references.

I further declare that I submitted the thesis to originality-checking software and tha35t it falls within the accepted requirements for originality.
I further declare that I have not previously submitted this work, or part of it, for examination at Unisa for another qualification or at any other higher education institution.

30 June 2024

Signature Date

DEDICATIONS

This work is dedicated to life and its ways of teaching. To my parents, Mr. James Gawaza Phiri and Mrs. Lethube Moriah Phiri

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SUMMARY

The study assessed *Rhus lancea* and *Celtis africana* as browse species for concentrate selectors in captivity, using indigenous Nguni male goats as a model. The first experiment involved ten indigenous Nguni male goats, each weighing 16 ± 1.7 kg (mean ± SD) in the wet season and 15 ± 1.6 kg (mean ± SD) in the dry season. To assess the short-term intake rate of the browse by the goats, a cafeteria method was employed. *Rhus lancea* (RL) and *Celtis africana* (CA) branches were offered separately and in combination in a 1:1 ratio. The goats' preferences were *R. lancea* > RL:CA > *C. africana* in the wet and dry seasons. In the second experiment, apparent total tract digestibility was estimated using twenty mature male Nguni goats weighing 16 ± 1.7 kg (mean ± SD) in a 2 x 4 (season x diet) randomized factorial experiment. All goats received *Eragrostis curvula* hay as a basal diet and one of four supplementary forages, namely, i) *R. lancea*, ii) *C. africana*, iii) a combination of the two browse species in a ratio 1:1 (RL:CA), and iv) a control diet made up of *Medicago sativa*, butternut squash, apples, spinach, and concentrates. Goats on *R. lancea*, *C. africana*, and RL:CA diets had higher crude protein digestibility ($p < 0.05$) in the wet season than in the dry season. In the third experiment, twenty indigenous male Nguni goats were individually housed and fed either *C. africana* or *R. lancea* browse diets to evaluate the impact on the rumen bacteria composition during both dry and wet seasons in a randomized block design study. The goats had an average weight of 15 ± 1.6 kg (mean ± SD). The results showed an abundance of phyla *Bacteroidetes* over *Firmicutes* (F/B) (0.85:1) ratio ($p < 0.05$) in the dry season than in the wet season. In the fourth experiment, feed intake, live weight changes, urinalysis, and blood metabolites were compared over 30 days in twenty goats weighing 15 ± 1.6 kg (mean ± SD). There were season and diet interactions ($p < 0.05$) on final weight, average daily gain, and dry matter intake. The goats on the control diet had higher serum glucose levels ($p < 0.05$) than those on *R. lancea*, *C. africana,* and RL:CA during the dry season. The results suggested *R. lancea*, *C. africana*, and RL:CA can help meet the nutrient requirements and support the growth of goats, which was supported by serum metabolite profiles and rumen bacterial efficiency during both dry and wet seasons. However, to achieve optimal growth and ensure balanced serum, and rumen bacterial efficiency throughout the year, it is recommended to supplement with other dietary items.

Keywords: Concentrate selectors; Secondary plant metabolites; Browse; Preference; Digestibility; Rumen microorganism; Intake; Body weight; Blood serum.

KAKARETŠO

Dinyakišišo di lekodišišitše *Rhus lancea* le *Celtis africana* bjalo ka mehuta ya diphoofolo tša go fula matlakala a mehlare go tšeo di kgethago phulo di lotilwe, ka go šomiša dipudi tša pholo tša Nguni bjalo ka mohlala. Tekolo ya mathomo e akareditše dipudi tša dipholo tša Nguni tše 10, fao ye nngwe le ye nngwe e bego e na le boima bja 16 ± 1.7 kg (ka bonyane ± SD) ka go sehla sa dipula le tša 15 ± 1.6 kg (ka bonyane ± SD) ka go sehla sa komelelo. Go sekaseka kelo ya phulo ya lebaka le lekopana ya go fula ga matlakala ga dipudi, mokgwa wa khafetheria o šomišitšwe. Dithabe tša *Rhus lancea* (RL) le tša *Celtis africana* (CA) di filwe dipudi ka go arogana le ka go kopanywa ka kelo ya 1:1. Dipudi di ratile *R. lancea* > RL:CA > *C. africana* ka go dihla tša pula le tša komelelo, ka go latelana. Ka go teko ya bobedi, go ile gwa akanywa kgonagalo ya palomoka ya tshepedišo ya tšhilego ka go šomiša dipudi tše 20 tša dipholo tša Nguni tša boima bja 16 ± 1.7 kg (ka bonyane ± SD) ka go tekanyo ya 2 x 4 (sehla x dijo) ya tekolo ya dintlha ya sewelo. Dipudi ka moka di hweditše furu ya *Eragrostis curvula* bjalo ka dijo tša motheo le e tee ya difuru tše nne tša tlaleletšo, e lego, i) *R. lancea*, ii) *C. africana*, iii) motswako wa mehuta ye mebedi ya go fulwa ka kelo ya 1:1 (RL:CA), le iv) dijo tše laotšwego tše di bopilwego ke *Medicago sativa*, sekwaše sa pathanate, diapole, sepeniše, le metswoko. Dipudi tše di bego di eja dijo tša *R. lancea*, *C. africana*, le RL:CA di bile le khurute ya godimo ya tšhilego ya diphrotheine ($p < 0.05$) ka go sehla sa dipula go feta ka go sehla sa komelelo. Ka go teko ya boraro, dipudi tša setlogo tše 20 tša Nguni di ile tša hlahlelwa di nnoši tša fiwa dijo tša go fulwa tša *C. africana* goba *R. lancea* go sekaseka seabe sa sebopego sa dipaktheria tša diphoofolo tša megodu ka nakong ya dihla tša komelelo le tša dipula ka go dinyakišišo tša tlhamo ya sewelo ya lekala. Dipudi di bile le palogare ya boima bja 15 ± 1.6 kg (ka bonyane ± SD). Dipoelo di laeditše bontši bja *phyla Bacteroidetes* godimo ga *Firmicutes* (F/B) (0.85:1) kelo ($p < 0.05$) ka go sehla sa komelelo go feta ka go sehla sa dipula. Ka go teko ya bone, go ja dijo, diphetogo tša boima bja go phela, tshekatsheko ya moroto, le tlhamego ya madi di ile tša bapetšwa mo matšatšing a 30 ka go dipudi tše 20 tša dipholo tša Nguni tša boima bja 15 ± 1.6 kg (ka bonyane ± SD). Go bile le ditsenogare ka go dihla le ka go dijo ($p < 0.05$) go boima bja mafelelo, go nona ga kakaretšo ya letšatši ka letšatši, le go ja dijo tše omilego. Dipudi tše di bego di le go dijo tše di laotšwego di bile le maemo a godimo a tlelukhose ya seramo ($p < 0.05$) go feta dijo tša *R. lancea*, *C. africana,* le tša RL:CA ka nakong ya sehla sa komelelo. Dipoelo di šišintše gore *R. lancea*, *C. africana*, le

RL:CA di ka thuša go fihlelela dinyakwa tša phepo le go thuša ka kgolo ya dipudi, e lego seo se thekgilwego ke diphrofaele tša tlhamego ya seramo le go šoma gabotse dipaketheria tša diphoofolo tša megodu ka go dihla tša komelelo le tša dipula ka bobedi. Le ge go le bjale, go fihlelela kgolo ye kgolo le go netefatša gore go ba le seramo ye e lekanetšego le go šoma gabotse ga dipaktheria tša diphoofolo tša megodu ngwaga ka moka, go šišinywa gore go tlaleletšwe ka dijo tše dingwe.

Mantšu a bohlokwa: Dikgethi tša metswako; ditlhamego tša dimela tša tlaleletšo; Fula; Lerato; Tšhilego; diphedinyana tša diphoofolo tša megodu; Go ja; Boima bja mmele; Seramo ya madi.

ISIFINYEZO

Lolu cwaningo beluhlola ukusebenza koMhlakotshane/iFolishi (i*Rhus lancea and Celtic africana*) njengezimila zokondla izilwane ezivalelwe ndawonye, kusetshenziswa izimbuzi ezingamaduna ezingamaNguni. Ucwaningo lokuqala lwenziwe ngezimbuzi ezingamaduna eziyishumi ezingamaNguni, imbuzi ngayinye inesisindo esingu-16 ± 1.7 kg (okusho ± SD) ngesikhathi semvula kanti zingu-15 ± 1.6 kg (okusho ± SD) ngesikhathi sesomiso. Ukuze kuhlolwe ukudla isikhathi esifushane kwezimbuzi zinqampuna amahlamvu, kusetshenziswe uhlelo lokudla ndawonye. Izimbuzi ziye zaphakelwa amahlamvu e*Rhus lancea* (*RL*) nawe*Celtis africana* (*CA*) ngokuhlukana zaphinda zanikezwa wona esehlanganisiwe esilinganisweni sika-1:1. Lapha kuvele ukuthi imbuzi ithanda i*R. lancea* > *RL:CA* > *C. africana* ngesikhathi sezimvula kanye nangesesomiso. Ocwaningweni lwesibili, bekubhekwa ukugayeka kokudla ngesilinganiso sezimbuzi ezingamaduna esezikhulile ezingamashumi amabili zamaNguni ezinesisindo esingu-16 ± 1.7 kg (okusho ± SD) ngokuka-2 x 4 (isikhathi sonyaka x indlela yokudla) ukuhlola ngokuxuba izimo. Zonke izimbuzi ziphakelwe umsingizane njengokudla okuyisisekelo kanye nefolishi elilodwa, kulawa amane, i) i*R. lancea* , ii) i*C. africana* , iii) inhlanganisela yamafolishi amabili ngokwesilinganiso sika-1:1 (*RL:CA*), kanye iv) nokuphaka ngokulawula i*Medicago sativa*, izintanga zamathanga, ama-aphula, isipinashi, nezingxube. Izimbuzi ezidle i*R . lancea* , i*C. africana*, ne*RL:CA* zinamandla kakhulu okugaya amaphrotheni aluhlaza (*p <0.05*) ngesikhathi sezimvula uma kuqhathaniswa nesikhathi sesomiso. Ucwaningo lwesithathu, kuye kwavalelwa izimbuzi ezingamashumi amabili ezingamaduna ezingamaNguni zahlaliswa ngayinye zase ziphakelwa i*C. africana* noma i*R. lancea* ukuze kuhlolwe umthelela ekwakhekeni kwamagciwane e*rumen* ngesikhathi sezimvula nesesomiso ngokokuhleleka kocwaningo. Izimbuzi bezinesilinganiso sesisindo sika-15 ± 1.6 kg (okusho ± SD). Imiphumela ikhombise inala ye*phyla Bacteroidetes* ngaphezu kwe*Firmicutes (F/B)* (*0.85:1*) isilinganiso (*p <0.05*) ngesikhathi sesomiso uma kuqhathaniswa nesikhathi semvula. Ucwaningo lwesine, kuye kwaqhathaniswa ukudla okudliwayo, ukushintsha kwesisindo, ukuhlaziya umchamo, kanye nokusebenza kwegazi ezinsukwini ezingama-30 ezimbuzini zamaNguni ezingamaduna ezingamashumi amabili ezinesisindo esingu-15 ± 1.6 kg (okusho ± SD). Lapha kube nokusebenzisana phakathi kwendlela yokudla nesikhathi sonyaka (*p <0.05*) esisindweni sokugcina, umthamo odliwa ngosuku, kanye nokudla ngesikhathi sesomiso. Izimbuzi ezidla

ngokulawulwa zinoshukela (i*serum*) ophezulu (*p <0.05*) kunalezo ezidla i*R. lancea*, i*C. africana*, kanye ne*RL:CA* ngesikhathi sesomiso. Imiphumela iphakamisa ukuthi i*R. lancea*, i*C. africana,* kanye ne*RL:CA* kungasiza ukuhlangabezana nezidingo zomsoco futhi kulekelele ekukhuleni kwezimbuzi, okwasekelwa nangamaphrofayili e*serum metabolite* kanye namagciwane e*rumen* ikakhulu ngesikhathi sesomiso nesezimvula. Kodwa-ke, ukuze izimbuzi zikhule kahle futhi kuqinisekiswe ne*serum* elinganayo, kanjalo namagciwane e*rumen* akahle unyaka wonke, kunconywa ukuthi izimbuzi zinikezwe nezinye izinhlobo zokudla.

Amagama amqoka: Izilwane ezikhetha ukudla okugayeka kalula nomsoco ophezulu ama*Secondary plant metabolites*; isihlahla esidliwa izilwane; Ukukhetha; Ukugaya ukudla; i*Rumen microorganism*; Ukudla; Isisindo somzimba; iBlood serum.

SEQUENCE OF CHAPTERS

The thesis consists of six chapters that include a general introduction, a literature review, and three chapters prepared for submission to peer-reviewed journals. These chapters are followed by study conclusions. Chapter 1 is the general introduction, broken into a brief background, a problem statement, the aims and objectives, and the hypotheses. Chapter 2 reviews the literature on: Challenges faced by concentrate selectors kept under different management environments; Foraging behaviour and digestive physiology of wild and domesticated concentrate selectors; Nutritive value of unstructured diets as offered by zoological gardens; Antinutritive factors found in browse; Implications of feeding unstructured and structured feedstuffs on the rumen environment and the effects of diets on blood metabolites in concentrate selectors.

Chapters 3, 4, and 5 are structured as manuscripts prepared for submission to journals. Chapter 3 is entitled “Nutritional composition in *Celtis africana* and *Rhus lancea* during two seasons and their effects on Nguni goats’ short-term intake rate and nutrient digestibility”, Chapter 4 is entitled “Rumen bacterial composition of Nguni goats fed *Rhus lancea, Celtis africana,* and their mixture diet during dry and wet seasons”. Chapter 5 is entitled “A comparison of *Celtis africana* and *Rhus lancea* based diets and concentrate-based diets on feed intake, weight, urinalysis and blood metabolites in Nguni goats”. The last chapter of the study contains the conclusions and recommendations of the research findings and areas for further research as well as a critical evaluation of the study.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADG	Average daily gain
ADL	Acid detergent lignin
ADMI	Actual dry matter intake
ALP	Alkaline phosphatase
ALT	Alanine amino transaminase
AMYL	Amylase
ASC	Ascorbic acid
BIL	Bilirubin
CHOL	Cholesterol
CP	Crude protein
CPD	Crude protein digestibility
CPI	Crude protein intake
CTI	Condensed tannins intake
CT	Condensed tannins
dCP	Digestible crude protein
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
dNDF	Digestible neutral detergent fibre
dOM	Digestible organic matter

GAE	Gallic acid equivalent
GHP	General health profile
GGT	Gamma-glutamyl transferase
GLOB	Globulin
GLU	Glucose
IPHOS	Inorganic phosphate
KET	Ketones
LEU	Leukocytes
NDF	Neutral detergent fibre
NDFI	Neutral detergent fibre intake
NIT	Nitrite
OM	Organic matter
OMI	Organic matter intake
OTUs	Operational taxonomic units
PCR	Polymerase chain reaction
PCoA	Principal coordinate analysis
PDMI	Proportional dry matter intake
RL:CA	*Rhus lancea* and *Celtis africana*
rRNA	Ribosomal ribonucleic acid
RPI	Relative preference index
SALB	Serum albumin
SCAL	Serum calcium
SCR	Serum creatinine

SPMs	Secondary plant metabolites
STE	Sorghum tannin equivalent
STP	Serum total protein
TPCI	Total phenolic compound intake
TPCs	Total phenolic compounds
SUN	Serum urea nitrogen
SBWL	Seasonal body weight loss
UBLD	Urine blood
UGLU	Urine glucose
UMA	Urine microalbumin
UPC	Urine protein to creatinine ratio
URO	Urobilinogen
USG	Urine specific gravity
VFA	Volatile fatty acids

CHAPTER 1: GENERAL INTRODUCTION

1.1. Background

In South Africa, zoological gardens use captive ruminant concentrate selectors like the springbok (*Antidorcus marsupialis*) to attract and educate eco-tourists. However, the seasonal body weight loss (SBWL) that is sometimes experienced by captive, wild, herbivorous concentrate selectors (Animals that prefer plants that are easy to digest and contain high levels of nutrients) that consume grass, herbaceous and browse materials, distracts the visual pleasure experienced by eco-tourists who expect to view sleek, well-groomed animals. One of the ways to mitigate SBWL is to offer these concentrate selectors appropriate browse material. Mbatha and Bakare (2018) suggested that incorporating browse into the diets of concentrate selectors (ruminants such as springbok and goats) could enhance the nutritional value by introducing novel compounds such as tannins, flavonoids, phenols, alkaloids, and terpenoids. These compounds are known for their antioxidant, anti-inflammatory, and medicinal properties. Domestic goats (*Capra hircus*) have been utilized as valuable models for captive concentrate selectors' nutritional research due to their similar foraging behaviour and digestive physiology (Le Meillour et al., 2020). Both springbok and goats exhibit digestive systems well-suited for a mixed forage diet (Gattiker et al., 2014). Concentrate selectors have evolved strategies to manage the spatio-temporal variations in feed resources by consuming both grass and tree forages with differing nutritional values (Kiffner & Lee, 2019; Kerby et al., 2022).

Forage items found in the natural habitats of herbivorous animals consist of various monocotyledonous and dicotyledonous plants (Okada et al., 2015). Within the dicotyledonous group, *Celtis africana* (*C. africana*) and *Rhus lancea* (*R. lancea*) browse species exist in most parts of Southern Africa (Madzinga & Kritzinger, 2020; Arnoldi & Shackleton, 2021). Both *R. lancea* and *C. africana* trees often serve as shade in parks and roadside trees in the urban areas of Johannesburg and Pretoria in South Africa. In addition, the leaves of *R. lancea* and *C. africana* are often collected and offered to captive-managed wild ruminants (Mbatha & Bakare, 2018) and are also consumed by free-ranging domestic ruminants (Yusuf et al., 2020).

Rhus lancea is a drought-tolerant perennial tree that flowers in winter and spring, it thrives in tropical and temperate environments (Gundidza et al., 2008). It bears resin-like fruit, is devoid of thorns and spikes, and uses biological chemical substances such as secondary plant metabolites (SPMs) for defence against predators. The classes of SPMs include phenolics, alkaloids, saponins, and terpenes (Badyal et al., 2020). Gemeda and Hassen (2015) noted that *R. lancea* leaves harvested from Pretoria in the Gauteng province of South Africa during the wet season contained 845.3 g organic matter (OM) g kg^{-1} dry matter (DM), 86.9 g crude protein (CP) g kg^{-1} DM, 390.6 g acid detergent fibre (ADF) g kg^{-1} DM, 424.5 g neutral detergent fibre (NDF) g kg^{-1} DM, 78.7 g acid detergent lignin (ADL) g kg^{-1} DM, 33.0 g condensed tannins (CT) g kg^{-1} DM and 209.1 g total phenolic compounds (TPCs) g kg^{-1} DM.

Celtis africana is a deciduous tree that typically blooms in the wet season, and whose leaves turn yellow in the early dry season before defoliating (Ts'ehlana, 2005). Information about the nutritional values of *C. africana* is scant (Phiri et al., 2022). However, Ryan et al. (2013) reported 27.64 g CP/kg DM, 30.39 g NDF/kg DM, 22.21 g ADF/kg DM, and 13.72 g ADL/kg DM in *C. africana* leaves collected from Kibale National Park, Uganda. Both *R. lancea* and *C. africana* contain SPMs such as condensed tannins (CT), which render them unpalatable and can lead to adverse health effects when consumed in high concentrations (Ts'ehlana, 2005; Costa et al., 2021). Oral consumers of CT experience an astringent taste and decreased nutrient degradation in the rumen (Peng et al., 2021). When consumed above a certain threshold, CT can inhibit weight gain in ruminants (Mkhize, 2008; Dludla, 2010; Phiri et al., 2022). This results from the CT binding dietary protein, reducing the availability of digestible protein and negatively affecting animal CP requirements (Costa et al., 2021).

However, goats and wild concentrate selectors have evolved proline-rich proteins in their saliva, enabling them to counteract the toxicity and adverse effects of SPMs (Ward et al., 2020). When the SPMs in the feed are effectively neutralized, they can enhance nutritional benefits for both goats and concentrate selectors in the wild. On the one hand, this is due

to the positive characteristics of the feed being unlocked once the negative effects caused by SPMs are eliminated (Scogings et al., 2011). The SPMs in *R. lancea* include antioxidants, antibacterial and antifungal substances, and they suppress the activities and growth of pathogens by inhibiting lipid peroxidation (Forsberg et al., 2010; Perveena et al., 2011; Esmaeilnejad et al., 2012; Ekpe et al., 2018). Essential oil and α- pinene in *R. lancea* inhibit *Clostridium perfringens* by reducing cell viability and inhibiting phospholipase activity (Gundidza et al., 2008; Da Silva et al., 2012). Although CT reduces the degradation of CP in the rumen, this process improves the flow of essential amino acids from the rumen to the small intestine (Avila et al., 2020), a process that enhances the absorption in the small intestine of organic compounds such as carbohydrates. Furthermore, CT has been reported to enhance the activity of blood platelets within the vascular system, thereby preventing blood loss and improving blood circulation (Da Camara et al., 2020).

Several *in vivo*, *in vitro,* and *in sacco* techniques have been used to understand the effects of feed on degradability and rumen microorganism composition on livestock (Nozière & Michalet-Doreau, 2000; Mohamed & Chaudhry, 2008; Chebli et al., 2021; El- Nile et al., 2021). In an *in vitro* study, tanniferous browse such as provided by *Acacia saligna (Labill.) H.L.Wendl. s.l.* and *Vachellia nilotica* was shown to reduce the rumen protozoan population by 4.1 and 5.3 times, respectively, consequently decreasing the ammonia nitrogen concentration and improving nutrient digestibility (Amira et al., 2014). However, the extent to which SPMs in *R. lancea* and *C. africana* influence rumen microbial composition and blood serum metabolites has not been fully explored in South African indigenous Nguni goats. Previous results showed that goats exhibit selective behaviour when fed *R. lancea* and *C. africana*, indicating their amenability to confinement (Phiri et al., 2022). The composition of rumen bacteria, whether suppressed or proliferated, in Nguni goats when offered *R. lancea* and *C. africana* leaves, is limited. A reason for this is that culture-dependent techniques used to analyse the rumen bacteria population are scarce because only 1% of the rumen bacteria are culturable (Hugenholtz et al., 1998). Molecular methods are therefore necessary to determine the complexity of the gastrointestinal bacteria population in humans and animals (O'Flaherty & Klaenhammer, 2010; Kau et al., 2011; Gravitz, 2012).

1.2. Problem statement

In goats and other captive concentrate selectors, diet-induced metabolic disorders such as acidosis, bloat, and diarrhoea, as well as mortality, have been observed (Clauss & Dierenfeld, 2008; Mbatha et al., 2012; Gattiker et al., 2014). These issues arise when the animals are primarily fed diets composed of pelleted feed lacking sufficient fibre and browse material, to mitigate seasonal body weight losses. In urban areas, it is logical to harvest browse to feed captive concentrate selectors. However, *C. africana* and *R. lancea* have been identified as potential browse species to address this issue due to their widespread availability in Southern Africa. They are included in dietary enrichment programs for captive concentrate selectors in zoological gardens. They are, however, offered to the animals in small amounts and at irregular intervals. The concentrations of SPMs in these browse species are largely unknown, posing a challenge due to potential impacts on palatability, digestibility, and overall health in animals, especially if fed to naïve animals or in large quantities. In captive environments, the eating behaviour of concentrate selectors is limited to the offered specific types of browse species and available feed, which may have implications for the biological effects of SPMs, a subject that is not fully understood. Whereas when consumed by free-roaming animals, they may lessen the impacts of SPMs by opting for other more palatable browse containing fewer SPMs, but they do not have this luxury in captivity. Despite the potential benefits of the browse species, their effects on rumen microorganisms and overall health have not been extensively researched. Additionally, under natural conditions, herbivores of the same species may perceive SPMs in browse differently depending on harvesting periods and physiological stages of both plants and animals. Notably, most browsing activity occurs from morning to afternoon, indicating that free-ranging goats consume more plant material in the morning and relatively less as the day progresses (Raats, 1998; Meyer et al., 2010). Not following this pattern of feeding behaviour in captive environments may contribute to rumen disorders. However, our understanding is limited due to a lack of knowledge regarding rumen microbial populations, their abundance, richness, and role in feed degradation.

1.3. Justification

The need to address SBWL, sometimes experienced by captive, wild, herbivorous concentrate selectors, should be approached sustainably. The improvement of the feeding management of concentrate selectors using *C. africana* and *R. lancea* forages would lead to better animal health and welfare and better experiences for eco-tourists in the zoological gardens. It would also mean exploiting the browses' antioxidant, anti-inflammatory, and medicinal properties for the benefit of the animals. In addition, it would lead to a more sustainable utilization of resources as the pruning from *C. africana* and *R. lancea* shoots would be used sustainably to feed the animals. Currently, browse material is offered in zoological gardens as a dietary source to elicit the natural wild instincts of wild herbivores. Therefore, this research attempted to investigate the nutritional contribution of *C. africana* and *R. lancea* on wild concentrate selectors through the understanding of the nutrition of domestic indigenous goats (Nguni goats). This knowledge may be used to formulate diets to promote the optimal health of concentrate selectors in captivity. The profiling of the rumen microbial populations in the wet and dry seasons will provide essential information on the rumen bacterial shift due to the interaction of diet and season. This information might be used as a guideline on sustaining the ruminal bacterial population while achieving the nutrient requirements of captive concentrate selectors during the wet and dry seasons.

1.4. Study aim

To determine the value of *Rhus lancea* and *Celtis africana* as browse species for concentrate selectors in captivity using male Nguni goats as a model for concentrate selectors.

1.5. Specific objectives

To determine the following in *C. africana* and *R. lancea* browse material for feeding to male Nguni goats as a model concentrate selector.

- Nutrient composition and secondary plant metabolites and their effects on palatability and nutrient digestibility of male Nguni goats in the dry and wet seasons.
- Effects of feeding diets providing *C. africana* and *R. lancea* browse *ad libitum* on the ruminal bacterial composition of male Nguni goats in the dry and wet seasons.
- Effects of feeding diets providing *C. africana* and *R. lancea* browse *ad libitum* on feed intake, weight, urine, and serum metabolites in male Nguni goats in the dry and wet seasons.

1.6. Hypothesis

The study tested the following null hypotheses:

- The dietary preferences and digestibility of nutrients in Nguni goats are not influenced by nutrient composition and secondary plant metabolites in diets containing *R. lancea*, *C. africana* leaves or their mixture in the dry and wet seasons.
- The ruminal bacterial composition in indigenous Nguni goats is not influenced by diets containing *R. lancea*, *C. africana,* or their mixture in the dry and wet seasons.
- Feeding indigenous Nguni goats *R. lancea*, *C. africana,* or their mixture has no effect on feed intake, weight gain, or urine and serum metabolites during dry and wet seasons.

1.7. Delimitation of the study

Domestic goats were chosen as a model for captive springboks due to several factors: a) Limited availability of captive springboks for research purposes. b) Ethical considerations regarding the use of non-habituated wildlife in experimental setups. c) Similarities in digestive physiology and foraging behaviour between goats and springboks, both being concentrate selectors (Le Meilleur et al., 2020; Gattiker et al., 2014).

The study recognizes that the structured environment of this research experiment may not fully replicate natural foraging conditions. To mitigate this: a) the study designed the experimental setup to simulate aspects of natural foraging as closely as possible. b) the study accounted for potential behavioural differences in analysis and discussion.

Additional Delimitations: a) Species-specific differences: While goats serve as a useful model, this study acknowledges that there may be species-specific differences in foraging strategies and nutritional requirements. b) Environmental factors: This study was conducted in a controlled environment, which may not account for all variables present in natural or diverse captive settings. c) Individual variation: the study recognizes that individual animals may exhibit different foraging behaviours, which could affect the generalizability of our results.

Generalizability of findings: this study discusses the limitations of generalizing the findings:
a) To other concentrate selector species in captivity.
b) To springboks in wild environments.
c) Across different captive management systems.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

The study of wildlife transitioning from wild habitats to zoological gardens and game ranch environments is an important strategy for understanding wildlife nutrition and the effects of dietary ingredients on the average health of the captured animals (Dierenfeld, 1997). A challenge faced by zoo animal nutritionists is to mimic as closely as possible the foraging behaviour of wild concentrate selectors (animals that prefer easily digested plant material that contains high levels of nutrients like protein, fat, and carbohydrates) and to understand their nutrient requirements. Concerted efforts for improved captive wildlife husbandry have been applied through the incorporation of different feedstuffs, including browse materials (Dierenfeld, 1997). However, the browse quality and quantity might compromise conservation efforts, because the nutrition of mammalian herbivores differs concerning the evolution of herbivore species (Mbatha et al., 2012).

2.2. Challenges faced by wild concentrate selectors.

2.2.1. National parks and game ranches

National parks and game ranches share common wildlife conservation objectives, which are to sustain wildlife genotypes and ecotourism (Lindsey et al., 2009). These conservation environments apply management strategies to protect wildlife and provide them with quality feed (Miao et al., 2019). The conservation management strategies include protecting endangered species, habitat conservation and rehabilitation, breeding, and wildlife research (Lindsey et al., 2009). However, these conservation management strategies are affected by the diversity of browse species, which is influenced by geographical location and habitat sizes/area (Miao et al., 2019; Mwangi et al., 2021). This makes it difficult to constantly supply specific animals with the required fresh browse in the conservation institutions. During the dry season, protein is much lower in the grass (e.g., 4.4% DM), thus making it the first limiting nutrient (Mbatha & Ward, 2010; Mwangi et al., 2021).

Depending on the species, browse is high in either condensed tannins (CT) or hydrolysable tannins as chemical deterrents against herbivores (Naumann et al., 2013). Monteith et al. (2019) stated that forages treated with quebracho tannins at 20% concentration inhibited voluntary intake in two to seven-year-old white-tailed deer. Forages high in CT concentrations limit dietary intake and thus influence forage preference by animals during the wet and dry seasons (Makkar, 2003; Naumann et al., 2017; Mokoboki et al., 2019). Moreover, extreme abiotic factors (drought, floods heat, and cold temperatures) compromise forage quality by increasing the level of SPMs, as a defense against external elements (Ahmed et al., 2019). Therefore, as the levels of SPMs increase, the use of such forage as feed to animals decreases. Hence, it is important to treat the browse to attenuate SPMs toxicity to improve their intake by animals.

During the dry season, supplementary feeds (such as concentrates) are often offered to improve good health, sustain body conditions, and lower the incidence of starvation in zoological gardens and game ranches (Mellado et al., 2020). However, in uncontrolled environments such as game ranches, some animal species are adversely affected by supplements that are formulated for other different species (Provenza et al., 2003; Robbins, 2012). For instance, certain non-ruminant animals may accidentally consume a diet containing urea, which is formulated for ruminants. This can result in fatalities due to urea poisoning, as noted by Patra and Aschenbach (2018). Urea is a non-protein nitrogen source that is hydrolyzed into ammonia by urease enzymes in the rumen of ruminants. If urea is not carefully managed, it can be toxic to animals. For example, if a diet containing urea gets wet, it should not be fed to animals (Gimelli et al., 2023).

Overgrazing is a major challenge faced by national parks and ranches where animals have limited grazing space, and this can be linked to uncontrolled animal populations. Population increase in the national parks and ranches is not optimally controlled as in the livestock sector (Niamir-Fuller et al., 2012) where manipulation of reproductive processes and culling is practised to meet management objectives. Grazing areas can support a specific number of animals for a specific period based on forage production potential and may not be able to accommodate an increase in animal populations (Tyrrell et al., 2017). This leads to a decline

in forage availability, especially during the dry season, and implementing grazing systems can enable the pastures to recover efficiently (Bilotta et al., 2007). Unfortunately, grazing management systems, including mob, switchback, and rotational grazing systems are implemented mainly in livestock production (KidWell, 2010; Zhou et al., 2019). In national parks, the continuous grazing system is an obvious preferred system, however, the expansion in numbers of wild ruminants in the continuous grazing system reduces forage availability (Longland, 2013).

Global warming and increasing recurrent droughts lead to water shortages, which negatively affect the growth of different grass and forage species (Hart et al., 2022). This causes herbivorous animals to depend more on supplements (concentrates) than on exploiting their natural forage resources (Mota-Rojas et al., 2021; Moyo & Nsahlai, 2021). Excessive rainfall and floods are major contributors to soil erosions and the washing away of natural and cultivated pastures (Paulik et al., 2021). In addition, while fences are erected around the parks and ranches to reduce potential wildlife-human conflicts (Díaz, 2022), they invariably limit the migration of animals to access quality forages. Naturally, most herbivorous animals migrate when forage quality declines because of the change in season, therefore restriction of movement reduces access to quality forage. The disruptions of ecological balances related to contamination (air, water, and soil) and degradation of ecological resources (soil erosion and deforestation) in national parks and ranches may increase diseases, mortality, and eventually extinction of wildlife (Glicksman, 2008; Chatterjee et al., 2015).

2.2.2. Zoological gardens

The national parks and game ranches face different challenges than zoological gardens. In the latter, providing herbivores with a specific quality and quantity of fodder directly from their natural environments is a challenge. (Mbatha et al., 2012; Gates, 2021). Harvesting browse species to feed zoo herbivores might face challenges such as plant age at harvest, the season, and the plant's location (Codron et al., 2019). These challenges are severe for urban zoological gardens where browse species are scant. Hence, zoo animals' diets are based on what is available and provided (Taylor et al., 2013). Normally, varieties of commercial feedstuffs such as concentrates are offered, and some of the commercial feedstuffs are

rich in protein and energy, which leads to the accumulation of lipids in hepatic tissue (Drackley et al., 2014) and the accumulation of adipose tissue under the skin leads to overweight (Razali et al., 2019).

The maintenance of captive wild animals is achieved by offering balanced protein and energy diets (Dierenfeld, 1997). In a range of feedstuffs offered, animals can achieve the targeted nutritional balance and consume more or opt for another type of feedstuffs whenever that balance is not achieved (Felton et al., 2016). Therefore, to enhance good health and disease-free animals in captivity a balanced diet is often accompanied by a periodic and effective preventative health program. Vaccines or prophylactic substances improve the responses and effectiveness of leukocytes to release pathogen-lysing agents, such as antimicrobial agents, enzymes, nitrogen oxides, and other proteins, which resist attack from pathogens or infections (Attia et al., 2021). Herbivorous animals self-medicate when consuming plant material comprising SPMs due to its antiviral and antiparasitic properties and this self-medicating behaviour might be a learned experience by goats consuming a variety of plant materials (Bricarello et al., 2023).

Rhus lancea contains 8% and 12% CP in the seeds and leaves, respectively, at different growing stages (Aganga & Mosase, 2001; Dludla, 2010), whereas the concentration of CP in the grass is approximately 5% CP such as in *Eragrostis superba* (Ravhuhali et al., 2019). Therefore, *R. lancea* leaf meal can potentially be used to enhance the CP content of grasses to meet animal requirements. Zoological gardens attempt to maintain the daily dietary protein supply of browse for various herbivores by establishing plant nurseries within their premises. Sometimes these establishments do not supply sufficient forage for the captive herbivorous population daily (Van Herk et al., 2019). Guidelines for offering browse materials in satiable quantities to herbivores as are consumed by their free-ranging counterparts are lacking in zoo feeding programs (Nijboer & Dierenfeld, 1996). Furthermore, there is also a lack of information about the optimal amount of SPMs as a daily intake requirement to benefit the health of animals based on their age and body condition.

2.3. Foraging behaviour and digestive physiology of wild and domesticated concentrate selectors

Goats, as well as wild concentrate selectors such as springbok in wild natural habitats, consume a variety of forage material for satiety and to self-medicate by utilizing the plants' CT to inhibit the development of pathogens, including *Escherichia coli* (*E. coli*) from multiplying in the rumen (Štumpf et al., 2020). Domestic goats and wild domesticated animals have been categorized based on similarities in foraging behaviour and digestive physiology (Le Meillour et al., 2020). The advantage of categorizing animals is to improve scientific knowledge to make sound inferences when developing optimal diets. The objective of categorizing animals based on their foraging and rumen physiology is to standardize feeding practices and ease the management of wildlife in captivity.

Wild concentrate selectors are mixed feeders which consume both plants and grass, they prefer easily digestible plant material that contains high levels of nutrients like protein, fat, and carbohydrates (Gattiker et al., 2014). Wild concentrate selectors include animals such as springbok (*Antidorcas marsupialis*), steenbok (*Raphicerus campestris*), eland antelope (*Taurotragus oryx*), kudu (*Tragelaphus strepsiceros*), nyala (*Tragelaphus angasii*), gazelles (Gazella) and impala (*Aepyceros melampus*), and domesticated concentrate selectors including domestic goats (*Capra hircus*) (Claus et al., 2010). Both springbok and goat digestive systems are well adapted to a diet of mixed forage (Gattiker et al., 2014). Concentrate selectors are limited in their ability to degrade fibre in the plant material (Agolisi et al., 2021). The volumes of the four digestive chambers in mature goats can be up to 22. 7 L for the rumen, 15.1 L each for the reticulum and the omasum, and 3.8 L for the abomasum. These animals can balance their body's metabolic requirements against dietary seasonal changes by adjusting their voluntary feed intake rate, digestive capacity, diet composition, and foraging time (Turner et al., 2012).

The different herbivore nutritional strategies, including all relevant animal adaptations (anatomic, gut microbial symbiosis, and host physiological, and metabolic responses should be interrogated to justify why the goat is an appropriate nutritional model for the wild species in question. According to Kiffner and Lee (2019), and Venter et al. (2019), concentrate

selectors are ungulates. They can be classified into ruminants and hindgut fermenters due to the structure of their gut and biological adaptations (Kiffner & Lee, 2019). In ruminants, fermentation of forage, and nutrient absorption occur before the passage of the feed in the lower gut, although in hindgut fermenters, energy is sourced as feed passes through the stomach, and more energy is freed through fermentation (Clauss & Dierenfeld, 2008; Venter et al., 2019).

Concentrate selectors have evolved tactics to deal with the spatio-temporal changes in feed resources by consuming both grass and tree forages varying in nutritional values (Kiffner & Lee, 2019; Kerby et al., 2022). During the wet season, when grass nutrients are high, they graze, and during the dry season, when nutrients in the grass are depleted, they browse on tree leaves, succulents, and forbs (Chebli et al., 2022). They spend over 60% of their time selecting and foraging on a variety of tree leaves, therefore, they excel in the selection of forage comprising SPMs for their survival and reproduction (Gurung, 2020). They can optimise a plant's nutrients by rejecting forage with concentrated SPMs and plant fibre and opt for forage with lower SPMs and plant fibre. Thus, they forage on multiple dietary sources low in SPMs and plant cell wall concentrations (Mkhize, 2008; Dludla, 2010; Müller et al., 2019) and avoid plants with concentrated SPMs or plant cell walls for other foraging specialists, such as browsers. Exposure to diverse types of forages in natural habitats helps elicit natural foraging behaviour and improves the foraging experience in counteracting or averting SPMs (Distel & Villalba, 2018).

In captivity, foraging behaviour, particularly by herbivores born in captivity, might be limited to certain types of browse species and available feedstuffs. Hence, it is difficult to fully understand the biological effects of SPMs on captive wild herbivores (Monteith et al., 2019) because the foraging behaviour in captivity is based on what is offered, rather than what might be selected by the animal in its natural wild environment. Herbivores of the same species might perceive SPMs in browse species differently due to harvesting periods and physiological stages. Browse intake is preferable by goat in the morning period than in the afternoon. During the morning period, the leaves are less exposed to ultraviolet light which is responsible for the increase of SPMs levels in the leaves of the plant (Raats, 1998; Meyer

et al., 2010; Gourlay & Constabel, 2019). As the day progresses, ultraviolet light increases the production of SPMs thus affecting the foraging behaviour of herbivores (Ubi et al., 2006). Thus, goats associate their foraging behaviour with the concentration of SPMs and so browse material that will be fed to wild animals in captivity must be harvested to mimic the wildlife foraging behaviour for captive feeding (Mbatha et al., 2012). It is, however, advisable that goats depending on caretakers for feed resources be fed in line with their natural foraging behaviour though their saliva contains biological compounds that counteract SPMs.

2.4. Unstructured and structured diets

Unstructured or extrapolated diets are developed by mixing different ingredients comprising vitamins, minerals, carbohydrates, crude protein, and other nutrients (Ely & Fike, 2022). They have been developed from the understanding of the nutrient requirements of livestock and other domesticated animals to achieve specific nutritional requirements of those animals under human care (Mbatha et al., 2012; Eshar et al., 2019). Unlike unstructured diets, structured diets are not synthesized, modified, or cultivated (Milton, 1999). Animal nutritionists often use nutrients in structured diets as references when formulating diets for particular animals in captivity (Caravaggi et al., 2018). This was done through observation of different herbivores browsing in their natural habitats to develop methods of preserving forage in the form of browse silage which maintains similar nutritional quality for feeding animals in captivity (Mbatha & Bakare, 2018).

2.4.1. Unstructured diets as feed sources in zoological gardens

Unstructured diets are accepted for feeding wild herbivores in zoological gardens to enhance the quality of available forages. Domesticated sheep have been used as a model to produce concentrates for feeding red deer, reindeer, and white deer in zoological gardens (Dierenfeld, 1997). The major advantage of concentrates is that they can be formulated as pellets, crumbs, and or mash to meet the animal's nutritional requirements (Clauss et al., 2010). Some of the most common unstructured diets offered to herbivores in zoological gardens include grass hay, a variety of concentrates, silage (maize, legume, grasses, and browse silage), and available fresh produce (fruits and vegetables) from the retail markets.

Unstructured diets may not always provide positive nutritional benefits for wild herbivores in captivity (Chharang et al., 2020; James et al., 2021) compared to domestic herbivores.

This is because the goals for keeping wild ruminants in zoological gardens might not be the same as those for livestock production. Some of the primary goals of zoological gardens are to improve wildlife husbandry through scientific research and, to educate the public and protect wildlife against diseases (Scaglione et al., 2019). Experimental models to justify the worth of unstructured diets in wild herbivorous husbandry are limited to the understanding of domesticated livestock nutrition (Dierenfeld, 1997; Clauss et al., 2010). Thus, experimental models to test dietary ingredients consumed in wild habitats with the view of developing optimal diets for the captive wild herbivorous population are limited (Ullrey, 1996; Clauss et al., 2010). Allen (1996) reported that when commercial kibbles developed for domestic dogs are offered to captive iguanas, they get stuck between the tongue and palate scratching the surface area and causing oral lesions.

The digestive morphology and physiology of elephants are similar to that of domestic horses; therefore, they are often offered diets extrapolated from horses to achieve their nutritional needs (Oftedal et al., 1996; Greene et al., 2019; Chharang et al., 2020; Sach et al., 2020). Sach et al. (2020) observed zinc deficiency in captive African savanna elephants (*Loxodonta africana*) offered commercial pellets that were extrapolated from the diet of horses (*Equus caballus*). Mbatha et al. (2012), however, proposed that feedstuffs must be offered to wild captive herbivores according to a greater understanding of their specific nutritional needs and foraging behaviour. Dietary feedstuffs such as apples, hay, concentrates, and pumpkins are offered to captive herbivores because they are palatable and mostly preferred over other dietary items (Allen, 1996). Therefore, forage items (grass hay, legume, and browse material) should be offered before palatable fresh produce. What the animal selects, or rejects should be scrutinized by determining its nutrient composition to improve future feeding decisions.

2.4.2. Structured diets as feed in the zoological gardens

Different types of browse species form part of the diet for a variety of concentrate selectors. The tree leaves mostly found in South Africa and commonly edible by cattle and goats include *Vachellia nilotica, Bauhinia galpinii, Euclea natalensis*, *Combretum erythrophyllum*, and *Tecoma stans* (Chepape et al., 2014). These tree species and many others differ in nutrient composition, and they are seldom fed to wild herbivores in South African zoos such as the Johannesburg City Parks Zoo and the National Zoological Gardens of South Africa, locally known as the Pretoria Zoo. *Rhus lancea* and *C. africana* trees are found within the urban areas of Gauteng Province and have the potential to provide the needed nutrients to concentrate selectors in captivity. Their leaves can be offered fresh during the wet seasons or ensiled and offered as browse silage for concentrate selectors during the dry season. *Rhus lancea* contains 100.0 g kg^{-1} DM CP, 632 g kg^{-1} DM NDF, 550 g kg^{-1} DM ADF, and 178 g kg^{-1} DM ADL in the early dry season (Phiri et al., 2022). *Celtis africana* contains 97.0 g kg^{-1} DM CP, 554 g kg^{-1} DM NDF, 532 g kg^{-1} DM ADF, and 765 g kg^{-1} DM OM (Phiri et al., 2022). Thus, *R. lancea* and C. *africana* might provide the animals with optimal CP for metabolic function.

It has been suggested that a variety of forages found in different wild habitats have sufficient NDF, ADF, and ADL for herbivorous animals (Codron et al., 2007) (as shown in Table 2.1). Studies indicate that feedstuffs with ADF levels greater than 18% and 6.19 MJ/kg ME reduce dry matter intake in goats (Lu et al., 2005; Nastir, 2012). If this value is applied to the nutrient intake and digestibility of captive concentrate selectors, the results may be similar to those observed in domestic goats. Ruminants derive energy from dietary fibre, and feedstuffs low in fibre reduce chewing time and cause an increase in pH levels in the rumen (van Soest., 1991; Galyean & Goetsch, 1993; Nastir, 2012).

Wild fruits are often scarce due to deforestation, wildfires, industrialization, and urbanization; hence they may not be available for zoo feeding (Dikeman, 2006). Therefore, cultivated fruits and vegetables from fresh produce markets replace wild fruits and the vegetable diet for herbivores and wild concentrate selectors. However, some cultivated fruits and vegetables

have more soluble carbohydrates and high moisture content, which might reduce the fibre concentration and increase digestibility in the rumen (Leschine, 1995; Dikeman, 2006; Clauss and Hatt, 2006; Schwitzer et al., 2009; Nastir, 2012; Schilcher et al., 2013). Nijboer and Dierenfeld (1996) reported that cultivated fresh produce has a lower fibre value (12.5% DM NDF and 6.3% DM ADF) and a higher moisture level (75.4% DM) than their wild counterpart (Table 2.1). Cultivated fresh produce is palatable and is bred for human nutrition (Milton, 2000).

Table 2.1: Comparison of nutritional concentration (% DM) of the zoological and wild diets (mean ±SD)

Parameters	**Zoo diets (n=7)**	**Wild diets (n=154)**	**References**
Moisture	75.4±2.4	78.3±31.7	Nijboer & Dierenfeld (1996)
Crude protein	15.0 ± 3.0	21.1±5.2	Dierenfeld et al. (2000)
NDF	12.5±4.6	66.7±43.7	Dikeman (2006)
ADF	6.3±2.8	52.3±26.1	Dierenfeld et al. (2000)
ADL	1.0±1.1	-	Dierenfeld et al. (2000)

NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin.

2.5. Dietary protein and energy requirements of concentrate selectors

Information on the nutritional requirements of concentrate selectors is gradually improving because of enhanced communal farming systems and the nutritional importance of goat meat in developing countries (Monau et al., 2020). Knowledge of the protein and energy requirements of goats is key to improving production efficiency to meet food security demands. A crude protein concentration of 70 - 80 g kg^{-1} DM in forages is essential for optimal rumen activity and feed intake in ruminants (Akinsola, 2019). These levels in forages suggest that goats weighing 45 kg require 7.4 MJ of energy per day for maintenance (NRC, 1987). It has been suggested that the dietary energy of 12.85 MJ per day can meet the metabolic energy (ME) requirements of goats during late pregnancy, considering that the diet contained 7% to 11% CP DM (NRC, 1987; Castro et al., 2021).

The dietary CP requirements of herbivores whose diet comprises tree leaves are faced with setbacks due to the presence of CT, which binds and precipitates dietary CP, thereby reducing its availability for rumen and physiological needs (Dludla, 2010). Furthermore, Lata and Mondal (2021) observed a low growth rate of 37.3 g/day in Mozambiquan indigenous goats offered *Albizia lebbock* leaves comprising 5.9% DM of CT and 18.3% DM of CP. Weight loss was recorded in Nguni goats offered *R. lancea* and *C. africana* as the only feed source (Phiri et al., 2022). The *R. lancea* leaves offered to Nguni goats contained 9.6 g kg^{-1} DM CT and 100 g kg^{-1} DM CP while *C. africana* leaves contained 140 g kg^{-1} DM CT and 111 g kg^{-1} DM CP (Phiri et al., 2022). Therefore, it was proposed that browse forages comprising high CT levels and low CP should be supplemented with CP to enhance the effectiveness of CT utilization in ruminants (Oyeyinka & Afolayan, 2019; Phiri et al., 2022).

Dietary mineral imbalances affect all aspects of animal productivity and health. Wild concentrate selectors and domestic goats managed in various production systems (i.e. free-ranging, semi-intensive, and intensive environments) should be provided with forages comprising minerals that are equivalent to their nutritional needs (Thornton, 2002; Barboza et al., 2009). The forages containing 0.3 - 2.5% calcium (Ca) DM, 0.4 - 0.8% phosphorus (P) DM, are adequate to satisfy the dietary mineral requirements of goats (Hart, 2015). Forages such as tree leaves can meet the mineral requirements of goats (Kour et al., 2021). However, dietary mineral contents of forages are affected by geographical location, age of the tree, soil pH, climatic temperatures, and season which can either increase or limit the mineral composition (Vázquez-Hernández et al., 2019). Stadlmayr et al. (2020) reported variations in the calcium composition in baobab fruits harvested from Kibwezi inland (213 - 538 mg) and Malindi coast (303 - 422 mg) in Kenya. This suggests that goats foraging on different plants are unlikely to experience dietary mineral imbalances compared to those foraging predominantly on one plant species (Gurung, 2020).

2.6. Antinutritional factors in browse

2.6.1. Total phenolic compounds and related substances

Total phenolic compounds (TPCs) are chemical substances formed by plants' secondary metabolism. They are deterrents against herbivory because they comprise other toxic

chemical substances including condensed tannins, flavonoids, and alkaloids (Karantzi et al., 2021). Nonetheless, they are non-nutritious in the plants' metabolism protect the plant from diseases, and mainly attract pollinators to distribute seeds (Karantzi et al., 2021). The TPCs also protect the plants against abiotic stress (ultraviolet light, parasites, and insects) by increasing the synthesis of flavonols and salicylates (Lattanzio et al., 2009; Albuquerque et al., 2021).

Goats as concentrate selectors select browse with fewer TPCs to increase digestibility compared to those with high phenolic content (Nyamukanza & Sabata, 2020) which are often rejected. The TPCs are non-nutritious to herbivores, because of their antioxidants, antibacterial and anti-inflammatory character they help to advance the physiological processes by increasing organ and immune response against internal parasites in goats (Jaiswal et al., 2020). Chávez-Servín et al. (2018) reported high TPCs (34.7% DM) in the milk of free-ranging Alpine goats foraging on *Rosopis laevigata*, *Acacias* spp., *Celtis* spp., and *Opuntia* spp compared to low TPCs (30.1% DM) in the milk of those offered alfalfa hay and corn silage. This implies that since TPCs are antioxidants, antibacterial, and anti-inflammatory, they may benefit the immunity of suckling kids (Ebadi et al., 2019).

2.6.2. Condensed tannins

The CTs are synthesised by the condensation of flavan-3-ols and produces coloured anthocyanidins (Gourlay & Constabel, 2019; Zeller, 2019). The CT differ from other SPMs because of their biological makeup and chemical activities. They have an aromatic ring bearing one or more hydroxyl substituents and are soluble in water (Hagerman, 1995) and some chemical solvents such as ethanol (Lattanzio et al., 2006). They do not possess nutritional effects on the plant's metabolism (Hagerman, 1995) but they are toxic at certain concentrations, and they bind with crude protein (Tian et al., 2021) which deters herbivory (Tontini et al., 2019). They differ in their biological roles based on plant type, and structural differences, and this makes it difficult to establish specific models to accurately determine and predict their effects when consumed by animals (Hagerman, 1995). The reason for this is that concentrations of CT differ with the location and developmental stages of the plant (Rufino-Moya et al., 2019; Kelln et al., 2020). Consequently, an animal browsing on a plant

that is in the vegetative stage might experience negative effects compared to when the plant is matured.

2.7. Implications of feeding unstructured and structured feedstuffs on the gut environment

2.7.1. Rumen microbes of concentrate selectors

The rumen microbial population is composed of diverse types of bacteria, fungi, and protozoa of distinct species linked to each other by their functional relationships (Qi et al., 2010). The rumen microbes assist in the fermentation of forage, degradation, and extraction of nutrients for absorption in the gut (Firkins, 2021). Feed fermentation takes place in an anaerobic environment at pH 6 to 7 (Pech-Cervantes et al., 2021). The SPMs binding complexes enable them to bind to nutrients (binding to carbohydrates, protein, and plant cell wall) and they dissociate in acid (pH 3.5) in the abomasum or alkaline (pH 8) in the duodenum (Mezzomo et al., 2011). Therefore, the acidic pH level in the abomasum enables microbes to extract nutrients from the feed to ease absorption in the small intestine.

2.7.2. Rumen bacteria

The composition of the rumen bacterial community and its role in the degradation of forages and concentrates was well demonstrated in dairy cattle, beef steers, and sheep (Gagen et al., 2015; Rahchamani et al., 2019; Ramos et al., 2021). Concentrate selectors depend on rumen bacteria to break down forages into basic carbohydrates and volatile fatty acids (VFAs), and to synthesize microbial protein for protein and energy sources (Ramos et al., 2021). The rumen bacterial community accounts for 10^{10} - 10^{11} cells/gram in the rumen and their role differs according to the bacteria (Choudhury et al., 2015a). Plant cell wall material is hydrolysed to oligomers and monomers in the anaerobic condition in the rumen and degraded to form VFA (Kong et al., 2010). The rumen genus *Lactobacillu*s is responsible for producing the VFA in the rumen. In addition, this genus helps to improve feed conversion and production of milk in animals (Chen et al., 2017). A balanced diet comprising all essential nutrients and minerals helps to enhance the activity of the rumen bacteria community including the genus *Lactobacillus*. Thus, the health of concentrate selectors is affected by the diet that subsequently affects the ruminal bacterial community (Russell, 2002). A nutrient-

deficient diet alters the efficiency of some rumen bacteria resulting in rumen microbial imbalance (Clauss & Dierenfeld, 2008).

A shift in rumen phyla *Firmicutes* and *Bacteroidetes* has been observed in lactating Saanen goats offered a highly concentrated:forage ratio (70:30) diet compared low to concentrate:forage ratio (30:70) (Ma et al., 2021). The efficiency and perhaps the effectiveness and abundance of these two phyla are affected by the nutrients in the diet (Russell, 2002). In addition, Li et al. (2019) identified genes encoding carbohydrate enzymes in *Bacteroidetes* genomes. A decline in the rumen phylum *Bacteroidetes* population was reported in the rumen content of mature fistulated Brahman cross steers fed *Panicum maximum*, *Cenchrus ciliaris*, and *Chloris gayana* (Gagen et al., 2015). It is, however, unclear if the decline in rumen *Bacteroidetes* indicates insufficient organic matter in the diet, which the bacteria degrade in the rumen. The decline in the rumen 2-hydroxypropanoic acid was reported 8 hours after feeding *Glycyrrhiza glabra* as opposed to *Urtica dioica* to Dallagh sheep (Rahchamani et al., 2019). Thus, a change in diet quality may alter the rumen biome (Gharechahi et al., 2021; Ramos et al., 2021), suggesting that a single forage type may not meet the nutrient requirements of an animal. This suggests that multiple forages varying in nutrient quality, quantity, and composition are important for the efficiency and activity of the rumen bacterial community. In this regard, evidence as to the effect of *R. lancea* and *C. africana,* used singly or in combination on the rumen bacterial composition of Nguni goats is scant.

2.8. Importance of the diet on microbial efficiency

Rumen microbes are exposed to different types of forage diets, and are required to degrade the forages to enhance animal performance (Qi et al., 2010; Wadhwa et al., 2016). The nutrient in the forage causes the rumen bacteria to proliferate or decrease in number by only allowing an increase of specific bacterial populations to degrade specific constituents in the forage (Karasov & Carey, 2009; Fernando et al., 2010; Wadhwa et al., 2016). Furthermore, Kamra (2005) found that tannin-resistant rumen bacteria such as *Streptococcus caprines* and *Selenomonas ruminantium* are relatively increased in feral goats fed *Calliandra calothyrsus* that contain CT. The effects of CT on rumen microbes determine the extent of

rumen microbial activity. The dietary oxalates increase rumen bacteria such as *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Eubacterium* at the genus level in herbivores (Miller & Dearing, 2013). These oxalate-degrading bacteria are important because they help to lower the bioavailability of oxalates in the blood thus assisting in balancing the Ca and P in the blood (Miller & Dearing, 2013; Naik et al., 2014).

2.9. Challenges of identifying and quantifying rumen microbes

It is a challenge to determine rumen microbes using culture methods because of the difficulty in determining a suitable medium to maintain the growth of most rumen microbial communities (Suenaga, 2012). This may explain the reason why only 1% of the rumen microbial population is culturable. Knowledge about the rumen microbe community has advanced along with methods of separating bacteria and determining their function. Recently, metagenomic techniques have been employed, especially in the animal sciences (Deusch et al., 2015), to analyse the 16S rRNA genes (Olsen et al., 1986; Suenaga, 2012). The metagenomic techniques are categorized into three groups: (i) analysis using mass genome sequencing, (ii) activity-driven approach investigating specific microbial activity, and (iii) sequence-driven investigation of phylogenetic or functional gene expression (Riesenfeld et al., 2004; Neelakanta & Sultana, 2013). Metagenomic techniques are employed to determine the entire microbial community without creating a metagenomic library (Shendure & Ji, 2008; Harismendy et al., 2009).

2.10. Urinalysis of goats

Urinalysis tests are important in goats because they provide a reference for the animals' metabolic health status (Parrah et al., 2013; Batan, 2021). Urinalysis tests measure leukocytes, protein, bilirubin, urobilinogen, ketones, nitrites, pH, specific gravity, red blood cells, and glucose levels (Batan, 2021). Concentrations of urinary glucose (>1000 mg/dl) and ketone (>30 mg/dl) suggest diabetes mellitus or diabetic ketoacidosis in ruminants (Parrah et al., 2013; Sarker et al., 2020). Nevertheless, the use of urinalysis tests in goats is limited and there is a need to implement urine collection methods and instruments to measure urine sediments for small ruminants such as goats (Parrah et al., 2013). In addition, the collection of urine samples such as free catch, cystocentesis, and catheterisation were proposed.

Catheterisation is one of the most reliable collection methods and provides accurate results, however, it may lead to stress due to handling and can result in biased readings (Bianch-Bosisio et al., 1997). A study by Ban et al. (2022) using high-performance liquid chromatography to analyse urine from Thai-native × Anglo-Nubian male goats fed mangosteen peel powder, concluded that this diet does not reduce urine nitrogen. Batan (2021) used a dipstick as a measuring tool and found that Boer goats fed local *Indigofera* have higher bilirubinuria levels.

The presence of bilirubinuria is an indication of a blocked bile duct, leptospirosis, inflammation of the liver, hepatic necrosis, acute cholestasis, and/or erythroblastosis fetalis (Jacob, 2020; Batan, 2021; Choudhary et al., 2023). According to the literature, conjugated bilirubin is excreted in the bile and degraded by intestinal bacteria to urobilinogen, therefore some of the urobilinogen is oxidized to urobilin and some is reabsorbed and excreted in the urine (Jacob, 2020; Batan, 2021). The urinalysis study by Batan (2021) also found high concentrations of leukocytes (25 leu/L) in the urine of Boer goats fed local forage and *Indigofera* in the Bali Province of Indonesia. The presence of leukocytes is a biological indication of inflammation of the urinary tract and can be linked with urolith-induced injury to the urinary tract (Costanzo et al., 2017; Batan, 2021). Therefore, the analysis of urine and blood metabolite samples can be significant in making sound references to the health status of goats.

2.11. Effects of diets on blood metabolites in concentrate selectors

When ruminants consume diets lacking in dietary energy, their blood sugar levels become imbalanced. Glycogenolysis then converts glycogen to glucose in hepatocytes and myocytes (Paredes-Flores & Mohiuddin, 2020). Feed consumed by ruminants may have adverse effects on or benefit the blood's biochemical composition, which can significantly contribute to glucose regulation by the liver assisting in producing glucose via glycogenolysis and gluconeogenesis to maintain balanced blood glucose levels when ruminants were fasting (Tadaishi et al., 2018). Fadiyimu et al. (2010) found that red Sokoto goats that were fed *Ficus polita* fodder with CT composition of 68.0 g/kg for 12 weeks had serum liver enzyme composition within healthy threshold levels - alanine amino transaminase (ALT) were

between 7 - 24 IU/L, aspartate transaminase (AST) were between 43 - 132 IU/L, and alkaline phosphatase (ALP) were between 7 - 30 IU/L (Olafadehan et al., 2014). These results suggested that the liver and kidney function were not negatively impacted nor had decreased adverse effects. However, the availability of CT in the forage is related to hypoglycemia (Olafadehan et al., 2014). Changes in liver enzymes AST, albumin (ALB), ALP, ALT, and serum α- amylase (AMYL) are mostly used to measure metabolic health status, while urea, creatinine, albumin, glucose, total protein are used to determine the quality of diet offered (Fadiyimu et al., 2010). Wang et al. (2022) observed increased concentration of AST in the caecal chyme of male goats offered high concentrate diet (concentrate: forage = 90:10), suggesting liver disease, while Pambu-Gollah et al. (2000) observed a high urea concentration suggesting low energy level in diets of free-ranging indigenous goats.

2.12. Conclusions

Browse produces SPMs that influence the composition of rumen microbes, the blood intermediate metabolite profile, urine composition, and growth performance of mammalian herbivores in complex ways. It is therefore crucial to conduct a comprehensive investigation into the potential effects of different structural diets provided to herbivores in captivity on their health and well-being. There is a need for a concerted investigation of the impact of *R. lancea* and *C. africana* on concentrate selectors herbivores being kept in captivity, especially regarding their rumen bacterial profile and urine composition. This is necessary to understand how *R. lancea* and *C. africana* influence the rumen bacterial makeup, renal health, and blood metabolites in concentrate selectors. The purpose of this research was:

- Nutrient composition and secondary plant metabolites and their effects on palatability and nutrient digestibility of male Nguni goats in the dry and wet seasons.
- Effects of feeding diets providing *C. africana* and *R. lancea* browse *ad libitum* on the ruminal bacterial composition of male Nguni goats in the dry and wet seasons.
- Effects of feeding diets providing *C. africana* and *R. lancea* browse *ad libitum* on feed intake, weight, urine, and serum metabolites in male Nguni goats in the dry and wet seasons.

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CHAPTER 3: NUTRITIONAL COMPOSITION IN *CELTIS AFRICANA* AND *RHUS LANCEA* DURING TWO SEASONS AND THEIR EFFECTS ON NGUNI GOATS' SHORT-TERM INTAKE RATE AND NUTRIENT DIGESTIBILITY

Abstract

This chapter reports on the analysis and concentration of selected nutrients and secondary plant metabolites in *Celtis africana* (*C. africana*) and *Rhus lancea* (*R. lancea*), and their impact on short-term intake rate and nutrient digestibility determined in two separate, repeat experiments in the dry and wet seasons. In the first study, ten male Nguni goats weighing 16 ± 1.7 kg (mean ± SD) in the wet season and 15 ± 1.6 kg (mean ± SD) in the dry season were individually housed in a completely randomized design to determine preference using a cafeteria system. The *R. lancea, C. africana* or their mixture (RL:CA) were mounted on separate forage boards and each goat was allocated a 30-minute time frame. The goats' preferences were *R. lancea* > RL:CA > *C. africana* during the dry and wet seasons. There were season x diet interactions on dry matter intake, relative palatability index (RPI), and time spent eating ($p < 0.05$). The objective of the second study was to investigate how the individual or combination of *Rhus lancea* and *Celtis africana* browse affected nutrient digestibility in goats consuming *Eragrostis curvula* hay. Nutrient digestibility was evaluated using 20 individually housed male Nguni goats weighing 16 ± 1.7 kg (mean ± SD) in the wet season and 15 ± 1.6 kg (mean ± SD) in the dry season. The goats were fed an *Eragrostis* curvula hay basal diet, supplemented with i) *R. lancea*, ii) *C. africana*, iii) RL:CA, and iv) a control diet made up of *Medicago sativa*, green apples, butternut squash, spinach, and commercial pellets. Crude protein (CP) digestibility was higher in the wet season ($p < 0.05$) for the *R. lancea, C. africana,* and RL: CA-based diets. Goats consuming the control diet had higher dry matter digestibility ($p < 0.05$) than those on *C. africana* during the dry season. It was concluded that goats can use *C. africana* and *R. lancea* as substitute browsing forage in both the wet and dry seasons, but they require additional CP supplementation to improve overall digestion and satisfy CP requirements across seasons.

Keywords: Forage intake, preference, digestibility, ruminants

3.1. Introduction

Feeding choice in ruminants is a complex phenomenon influenced by multiple factors, including adaptability, foraging experience, secondary plant metabolite (SPM) concentrations, and geographical location (Odo et al., 2001; Yayneshet et al., 2008). Shipley (1999) emphasized that forage preference is closely tied to an animal's rumen morphology and physiology, affecting its ability to select, chew, digest, absorb nutrients, and defecate efficiently. Ruminants, particularly goats and wild concentrate selectors, thrive on dietary diversity. They typically consume a variety of forages, including grasses, succulents, shrubs, and tree leaves, while avoiding those with high concentrations of SPMs (Shipley, 1999; Naumann et al., 2017). Prolonged management on a single forage type often results in suboptimal performance and failure to meet nutrient requirements (Odo et al., 2001).

Secondary plant metabolites, found in many plant species, can take the form of chemical compounds (e.g., tannins) or mechanical components (e.g., hooks and thorns) (Naumann et al., 2017). These compounds often reduce dietary intake and digestibility in ruminants (Hagerman, 1995). However, goats and wild concentrate selectors have evolved mechanisms to either avoid SPMs when foraging or consume them at low levels.

Rhus lancea and *C africana* are common browse species frequently offered to wild concentrate selectors in captive settings, such as the Johannesburg City Park Zoo and the National Zoological Gardens of South Africa. This feeding practice is based on observations of free-ranging goats (Naumann et al., 2017) and wild concentrate selectors (Selebatso et al., 2018; Cunningham & Cunningham, 2024) consistently foraging on these species in Southern Africa. However, the extent of preference for *R. lancea*, *C. africana*, or their mixture has not been thoroughly investigated using goats as a model for wild concentrate selectors in a captive cafeteria system. This study aims to address this knowledge gap through two experiments conducted during both wet and dry seasons:

The first experiment investigates the concentration of selected nutrients and secondary plant metabolites in *R. lancea* and *C. africana* during dry and wet seasons. It also examines the extent to which goats prefer either browse species when supplied separately or in

combination. The second experiment explores how individual and combined *R.lancea* and *C. africana* browse affect nutrient digestibility in goats consuming *Eragrostis curvula* hay.

By conducting these experiments, we aim to provide valuable insights into the feeding preferences and digestibility patterns of goats as a model for wild concentrate selectors. This information can inform more effective feeding strategies for captive wild ruminants, potentially improving their nutrition and overall well-being.

3.2. Materials and methods

3.2.1. Study area

The study was conducted at the Agricultural Research Council-Animal Production (ARC- AP) located in Irene. (25°89.9′23″ S, 28°21.5′23″ E). The average annual rainfall is 646 mm, with the wet season spanning from September to March, and the dry season from April to August. The temperatures range from 17°C to 28°C in January and 5°C to 21°C in July, respectively (Adeyemi et al., 2015; Simpson & Dyson, 2018). Prior to starting, the study received ethical permission from the UNISA Animal Ethics Committee (Ref no. 2019/CAES/072) and the ARC-AP Ethics Committee (Ref no. APIEC18/14).

3.2.2. Animals and their management

Twenty (n=20) yearling male Nguni goats with an average body weight of 16 ± 1.7 kg (mean ±SD) were bought from smallholder farmers in the Sekhukhune region, Limpopo province, South Africa. The animals were humanely transported in a truck designed for livestock transportation, ensuring compliance with animal welfare standards. The goats were free-ranging and obtained from farmers who were at least 5 km apart to minimize potential biases and confounding factors that could arise from sourcing animals from a single location or closely situated farms. On arrival at the ARC–AP, the goats were quarantined and treated for internal and external parasites using injectable Ivermax 1% (Aspen Veterinary Resources Ltd, Liberty, MO 64068, USA), and oral Ovi dose 4 (Ascendis Health, Monument Park 0105, Pretoria, RSA). Goats were adapted for seven days on commercial pellets (400 g DM) (Epol

Lamb and Ewe 13®) and *Eragrostis curvula* hay (100 g DM) before the onset of the study. The individual pens were disinfected with a product containing quaternary ammonium compounds and chlorine bleach (DynaChem, Honeydew, Johannesburg, South Africa) and allowed to dry a week before the arrival of the goats. The pens were cleaned daily in the morning (07:00 to 08:00) before feeding. The goats were observed daily for morbidity and a health recording sheet was used to capture breathing rate, behavioural, eating, and drinking observations of each goat.

The sample sizes for animals used in this study were not determined using power tests. Instead, they were based on a combination of factors:

1. Previous studies: referred to similar research in the field to guide our sample size selection. Specifically, this study looked at studies by Higgins et al. (1996), Charan and Kantharia (2013), and Onzima et al. (2018), which used comparable methodologies and had a sample size range.
2. Practical constraints: sample sizes in this study were also influenced by logistical limitations, particularly: a) The number of available pens for housing the animals and b) The total number of animals in this study could feasibly be managed within resource constraints
3. Ethical considerations: This study aimed to use the minimum number of animals necessary to achieve the research objectives, in line with the principles of the 3Rs (Replacement, Reduction, Refinement) in animal research.

While a power analysis would have been ideal for determining the optimal sample size, the study's approach ensured a balanced statistical consideration with practical limitations and ethical responsibilities. This study acknowledges this as a potential limitation of our study and suggests that future research in this area could benefit from a priori power analyses to determine sample sizes, provided the necessary resources are available.

3.2.3. Preparation of feedstuffs

Celtis africana and *R. lancea* browse species were collected from the ARC Vegetable, Industrial, and Medicinal Plants (ARC-VIMP) at Roodeplaat, South Africa (25°61.5′47″ S, 28°36.4′35″ E). The browse was collected every week during the wet (October to February) and dry (May to July) seasons. The collection period was limited to two hours each morning, from 08:00 to 10:00. This approach was implemented to ensure consistency in the data collection process. A tree lopper was used to randomly cut small branches (approximately 50 cm to 100 cm in length) with leaves of *R. lancea* and *C. africana* on trees, which were at least 5 m apart. Browse samples were collected from young trees at vegetative and mature stages to represent the total population of species in each season. As described by Gundidza et al. (2008), during the dry season, some *C. africana* trees defoliate and the leaves fall to the ground. In this research study, and for the preference and palatability experiments, *C. africana* trees that kept their leaf material during the dry season were chosen.

3.2.4. Assessment of preference and palatability

For the first experiment involving preference and palatability testing per season, ten indigenous male goats weighing 16 ± 1.7 kg in the wet season and 15 ± 1.6 kg in the dry season were housed individually in pens (1.5 m length x 2.5 m in wide). The goats were offered fresh *R. lancea* and *C. africana* browse separately, as well as in combination, using a cafeteria technique (Mpanza et al., 2013). The browse material was weighed, mounted on three separate foraging boards, tightened with strings, and offered to one animal at a time for 30 minutes. After that, the foraging board with the remaining forage was removed and the forage was weighed again after browsing to estimate the intake. A separate representative branch of each browse species was put aside every time the browse was offered to the goat, and they were weighed to estimate the weight lost due to wilting. Browse samples were dried from each of the representative branches at 60°C in an oven to determine the dry matter of each browse. The position of the browse was altered on the foraging board daily to prevent the development of a ‘habit reflex’ (Kaitho et al., 1996). The data collection took place for four consecutive days. The basal diet was withdrawn at 18:00 and each goat was offered *Eragrostis curvula* hay and pellets parallel from the forage treatments.

The RPI was calculated by dividing all forage intake values by that of the highest intake value and multiplying the result by 100. The eating rate was calculated as the amount of browse consumed divided by the time spent eating. A video camera was used to record goats foraging behaviour. The browse dry matter and intake were used to estimate the actual dry matter intake (ADMI) and proportional dry matter intake (PDMI) across seasons. The ADMI and PDMI were calculated according to NRC (2016), Lardy and Goesch, (2017), and Seles and De Oliviera (2019).

$$ADMI\ g = Forage\ consumed\ (as\ fed)\ g/day\ X\ Forage\ DM\%$$

The PDMI was calculated as;

$$PDMI\ \% = \frac{ADMI}{Body\ weight}\ X\ 100$$

The refusals were collected, weighed, and recorded after the preference and palatability trials; goats were offered commercial pellets and *E. curvula* hay and kept with the rest of the group. The preference study was carried out in two respective seasons (wet and dry seasons).

3.2.5. Apparent total digestibility study

In the second experiment, apparent total tract digestibility was investigated using the total faecal collection method repeated over two seasons. Twenty mature male Nguni goats weighing 6 ± 1.7 kg in the wet season and 15 ± 1.6 kg in the dry season were included in this experiment and were placed in individual pens (1.5 m in length x 2.5 m wide). Each goat constituted an experimental unit with five replicates per treatment to ensure the reliability and validity of the results. All goats received 100 g DM of *E. curvula* hay as a baseline feed to encourage the intake of other dietary items and one of four supplements, for four different diets, namely, i) *Rhus lancea* (*R. lancea* + *E. curvula* hay), ii) *Celtis africana* (*C. africana* + *E. curvula* hay), iii) a combination of the two browse species in a ratio 1:1 (RL:CA + *E. curvula*

hay), and iv) a control diet (*Medicago sativa* + concentrates + fruits + vegetables as shown in Table 3.1) representing an unstructured diet. The concentrates (Epol lamb and ewe 13® pellets were purchased from Epol Animal Feeds (Epol Animal Feeds®, Westville, South Africa), *M. sativa* (Lucerne) was purchased from ARC-VIMP, and the green apples, butternut squash, and spinach were procured weekly from the Tshwane fresh produce market.

Each goat received approximately 3% feed DM of their body weight in the form of *R. lancea*, *C. africana*, or their mixture (RL:CA), and control diets. The forages were restricted based on the body weight of the animal, however, for goats that finished their feed, an additional 5% was offered in the next feeding. *Eragrostis* and *M. sativa* hay were milled (3 - 5 mm) separately with a hammer mill crusher (Drotsky Hammer Mills and Feed Mixers (Pty) Ltd, Alberton, South Africa), and 100 g of each was weighed and offered to the goats each day. The fruit and vegetables were cut to 5 - 10 mm using a sharp table knife. Dietary treatments were weighed and placed in separate feeding troughs (each pen comprised seven troughs for the control and two for the experiment). Forage intake was estimated by weighing forage before and after being consumed by each animal. Fresh drinking water was offered *ad libitum* daily. The total faecal collection was achieved by mounting a harness bag on each goat. The straps of the harness bags were adjustable to ensure comfort for the goats and were adapted to harness bags for seven days before data collection. The harness bags of each goat were emptied daily. The faecal collection was conducted (08:00 to 9:00) before feeding for five successive days during data collection in each season. Apparent digestibility was calculated as defined by Ban et al. (2022).

$$Apparent\ nutrient\ digestibility\ (\%) = \frac{Nutrient\ intake\ \left(\frac{g}{day}\right) - Faecal\ excretion\ \left(\frac{g}{day}\right)}{Nutrient\ intake\ \left(\frac{g}{day}\right)}\ x\ 100$$

The fresh faeces were weighed immediately after collection and were dried per goat at 60°C in an oven for dry matter analysis. The dried faecal samples were grouped for each goat and milled to pass through a 1 mm sieve using a Wiley Mill grinding machine (Wiley Mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA, USA) and stored awaiting chemical analysis. The digestibility trial was conducted in two seasons (wet and dry seasons).

Table 3.1: Ingredient and chemical composition (%DM) of the control diet in the wet and dry seasons.

	Wet season						**Dry season**					
Parameters	*Medicago sativa*	Apples	Butternut squash	Spinach	Pellets*	*Eragrostis* hay	*Medicago sativa*	Apples	Butternut squash	Spinach	Pellets*	*Eragrostis* hay
DM	95.6	14.2	12.6	12.4	94.8	95.3	95.5	14.4	12.9	11.4	87.1	95.1
OM	84.5	11.8	7.0	2.1	83.7	91.9	85.7	12.1	8.1	5.8	76.7	90.7
CP	20.3	2.1	11.5	31.7	17.5	4.8	20.9	2.8	11.7	32.2	17.7	3.6
NDF	36.9	6.8	9.5	18.7	29.8	74.9	30.7	8.1	9.5	21.5	28.9	68.9
ADF	27.9	4.5	7.9	13.2	17.8	40.0	22.2	5.5	7.8	11.8	12.3	34.2
ADL	4.2	1.3	1.6	2.1	1.5	2.1	3.2	1.4	1.3	2.2	2.1	1.5

*Epol Lamb and Ewe 13, Epol Animal Feeds®, 10, The Boulevard, Westway Office Park, Westville 3629, South Africa. DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin, Wet season ranged from October to February, Dry season ranged from May to July.

3.2.6. Chemical analyses

Forage and faecal samples were grouped according to treatment and season and dried and processed to meet the study's objectives.

3.2.7. Proximate analyses

Three fresh samples (approximately 50 g) of the four dietary treatments were dried in an oven at 60°C until constant weight, to determine the dry matter (DM). The dried samples were ground through a 1 mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA, USA). Approximately 0.5 g of each sample in duplicate was ashed by incinerating it in a muffle furnace at 550°C (Method No. 930.05; AOAC, 2010) for 3 hours. The organic matter (OM) was calculated by subtracting ash from DM. The acid detergent fibre (ADF), neutral detergent fibre (NDF), and acid detergent lignin (ADL) were analysed according to the recommendations of Van Soest et al. (1991). The crude protein (CP) was determined by following the standard methods of the procedures prescribed by AOAC (2010).

3.2.8. Total phenolic compounds

Total phenolic compounds (TPCs) were measured following the techniques described by Hattas et al. (2005). Briefly, 3 ml of 70% acetone was added to samples (measuring 2 g) and sonicated in an ice water bath for 30 minutes and centrifuged at 2000 rpm for 10 minutes (Hagerman, 1995; Hattas & Julkunen-Tiitto, 2012). The gallic acid was expressed as GAE (Gallic Acid Equivalent) (mg GAE/g DM) and was used as a standard for TPCs at an absorbance of 720 nm on visible-light spectrophotometry (Hagerman, 1995; Hattas et al., 2005). The mg GAE/g DM was converted to percentage dry matter and expressed as % DM.

3.2.9. Condensed tannins

Condensed tannins (CT) were also analysed in *C. africana*, *R. lancea*, RL:CA, and *M. sativa* using the method described by Hattas and Julkunen-Tiitto (2012). A 70% aliquot of aqueous acetone was used to extract samples by sonicating in an ice-water bath for 30 minutes and centrifuging at 2000 g for 10 minutes. The supernatants were transferred into a screwcap tube containing acid butanol and 2% $FeNH_3SO_4$ reagent as outlined by Porter et al. (1986), and Hattas and Julkunen-Tiitto (2012). The purified sorghum tannin (mg STE/g DM) was extracted and used as a standard on a visible-light spectrophotometer at 550 nm absorbance (Hagerman, 1995; Hattas & Julkunen-Tiitto, 2012). The mg STE/g DM was converted to a percentage dry matter and expressed as % DM.

3.2.10. Statistical analyses

The seasonal effects on DM, OM, CP, ADF, NDF, ADL, CT, and TPCs levels in the forages were analyzed using analysis of variance in Stata (version 17, 2019). The following model was used:

$Yijk = \mu + \alpha i + \beta j + (\alpha\beta)ij + \varepsilon ijk$

Where:

Yijk = The observed value of the dependent variable (DM, OM, CP, ADF, NDF, ADL, CT, or TPCs) for the kth replicate of the ith season and jth forage type

μ = The overall mean

αi = The fixed effect of the ith season (i = 1, 2; where 1 = dry season, 2 = wet season)

βj = The fixed effect of the jth forage type (j = 1, 2, 3, 4; where 1 = *R. lancea*, 2 = *C. africana*, 3 = RL:CA, 4 = *M. sativa*)

(αβ)ij = The interaction effect between the ith season and jth forage type

εijk = The random error term

k = The number of replicates within each season-forage type combination

The RPI, DM intake, time spent eating, eating rate, and time spent sniffing the browse were subjected to analysis of variance in Stata: (version 17, 2019) with *R. lancea*, *C. africana,* and RL:CA as treatments and individual goats as replicates in a completely randomized design. The intake of DM, OM, CP, NDF, and apparent digestibility of DM, OM, CP, ADF,

and NDF in the *R. lancea*, *C. africana,* and RL:CA per season were analysed using the GLM procedures in Stata (version 17, 2019). The following model was used:

$Yijk = \mu + \alpha i + \beta j + (\alpha\beta)ij + \varepsilon ijk$

Where:

Yijk = The observed value of the dependent variable (RPI, DM intake, time spent eating, eating rate, and time spent sniffing, intake of DM, OM, CP, NDF, or apparent digestibility of DM, OM, CP, ADF, NDF) for the kth replicate of the ith diet and jth season.

μ = The overall mean.

αi = The fixed effect of the ith diet (i = 1, 2, 3, 4; where 1 = R. lancea, 2 = *C. africana*, 3 = RL:CA mixture, 4 = Control).

βj = The fixed effect of the jth season (j = 1, 2; where 1 = wet season, 2 = dry season)

(αβ)ij = The interaction effect between the ith diet and jth season.

εijk = The random error term.

k = The number of replicates within each diet-season combination.

The relationships of the observations, goats consuming *R. lancea*, *C. africana,* and RL:CA during wet and dry seasons and the variables (DM; DMI; ADF; NDF; CP; CT; TPCs; time spent eating, eating rate and time spent sniffing; preference) were evaluated through Spearman's correlation coefficient in Stata, and analysed using Principal Component Analysis and the Biplot analysis command in Stata (version 17, 2019).

3.3. Results

The chemical composition of DM, OM, ash, CP, NDF, ADF, ADL, CT, and TPCs levels in *R. lancea*, *C. africana,* and RL:CA are indicated in Table 3.2. There were significant season x diet interactions ($p < 0.05$) on DM, OM, ash, CP, NDF, ADF, ADL, CT, and TPCs levels. The DM of *R. lancea* was higher ($p < 0.05$) in the dry season than the wet season whereas the DM of *C. africana*, RL:CA, and *M. sativa* were similar ($p > 0.05$) across seasons. The DM of *M. sativa* was higher than that of *R. lancea*, *C. africana,* and RL:CA ($p < 0.05$) in the wet season. *Celtis africana* had lower OM ($p < 0.05$) than *R. lancea* and *M. sativa* across seasons. The ash in *C. africana* was higher than in *R. lancea* ($p < 0.05$) in the dry season. The CP levels were *M. sativa* > *R. lancea* > *C. africana* ($p < 0.05$) during the dry season

and in the wet season *M. sativa* > *C. africana* > *R. lancea* ($p < 0.05$). The NDF levels were similar ($p > 0.05$) for *C. africana*, *R. lancea*, and *M. sativa* in the dry season. *Medicago sativa* had the highest NDF levels ($p < 0.05$) in the wet season and higher ADF levels than *C. africana* and *R. lancea,* and *R. lancea* had higher levels than *C. africana* in both seasons ($p < 0.05$). During the dry season, the CT levels for *R. lancea* and RL:CA were higher ($p < 0.05$) in the wet season. The TPCs levels for *R. lancea* and RL:CA were higher ($p > 0.05$) than *C. africana* and *M. sativa* during both the wet and dry seasons.

Table 3.2: Chemical composition (%DM) of Medicago sativa, *C. africana*, *R. lancea* or RL:CA forage during the wet and dry seasons.

		Parameters								
Season	**Diet**	DM	OM	Ash	CP	NDF	ADF	ADL	CT	TPCs
Dry	*C. Africana*	88.3^{ab}	66.4^{a}	21.9^{b}	7.8^{a}	24.0^{a}	13.2^{a}	3.1^{a}	4.3^{b}	2.0^{ab}
	R. lancea	93.9^{c}	87.9^{c}	6.0^{a}	11.7^{b}	25.4^{ab}	16.9^{b}	2.8^{a}	15.1^{e}	8.0^{de}
	RL:CA	92.5^{bc}	78.9^{bc}	13.6^{ab}	9.4^{ab}	24.3^{ab}	15.5^{ab}	3.3^{ab}	11.0^{d}	6.2^{cd}
	Medicago sativa	95.5^{c}	85.7^{c}	9.8^{ab}	20.9^{c}	30.7^{bc}	22.2^{d}	3.2^{ab}	0.2^{a}	0.3^{a}
Wet	*C. Africana*	86.4^{a}	73.6^{ab}	12.8^{ab}	14.5^{c}	22.7^{a}	12.8^{a}	2.9^{a}	4.1^{bc}	2.6^{b}
	R. lancea	87.8^{a}	82.4^{c}	5.4^{a}	10.4^{b}	25.9^{ab}	16.9^{b}	2.8^{a}	11.0^{d}	8.1^{e}
	RL:CA	87.6^{ab}	76.7^{abc}	11.0^{a}	10.9^{b}	23.9^{a}	14.6^{ab}	2.8^{a}	6.0^{c}	5.0^{c}
	Medicago sativa	95.6^{c}	84.5^{c}	11.2^{ab}	20.3^{c}	36.9^{c}	27.9^{e}	4.2^{b}	0.3^{a}	0.3^{a}
	SEM	0.72	1.66	1.3	0.60	0.58	0.57	0.08	7.78	4.56
	CV (%)	4	10	58	35	17	26	19	69	65
	p-values									
	Season	0.093	0.013	0.0001	0.0001	0.345	0.704	0.311	0.538	0.309
	Diet	0.0001	0.0001	0.0001	0.0001	0.001	0.0001	0.204	0.0001	0.0001
	Season*Diet	0.009	0.002	0.021	0.0001	0.008	0.004	0.008	0.0001	0.005

Diet; *C. africana* = *Celtis africana*, *R. lancea* = *Rhus lancea*, RL:CA = *Rhus lancea* and *Celtis africana* mixture**. Parameters**; DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin, CT = Condensed tannins, TPCs = Total phenolic compounds, Superscripts that differ in the same column denote values that are statistically different at the $p < 0.05$ level. SEM-standard error of the mean. *The wet season ranged from October to February, and the Dry season ranged from May to July.

The results on DM intake, relative preference index (RPI), preference ranking, time spent eating, eating rate, and time spent sniffing by goats on *R. lancea*, *C. africana*, or RL:CA across the two seasons on a dry matter basis are indicated in Table 3.3. There were significant ($p < 0.05$) interactions between season x diet on DMI, RPI, and time spent eating. During the dry season, the DMI of *C. africana* was significantly lower ($p < 0.05$) than RL:CA and *R. lancea*. The DMI during the wet season was similar for all dietary treatments but lower than in the dry season ($p < 0.05$). The goats' preference rankings were *R. lancea* > RL:CA > *C. africana* during the dry and wet seasons. The goats spent less time eating ($p < 0.05$) *C. africana* than *R. lancea* or RL:CA during both seasons. The goats spent more time eating *R. lancea* than *C. africana* ($p < 0.05$) during the wet season.

Table 3.3: Relative preference index, dry matter intake and time spent eating and sniffing (mean SEM) of *C. africana*, *R. lancea*, or RL:CA forage by Nguni goats during the dry and wet seasons.

		Animal behaviour					
Season	**Diet**	DMI (g)	RPI %	RP	Time spent eating (sec)	Eating rate (g/sec)	Time spent sniffing (sec)
Dry	*C. africana*	40.9^{bc} ±5.61	88.7^{a}	3	141^{c} ±59.5	0.24±0.078	13±3.3
	RL:CA	62.8^{a} ±4.55	92.2^{a}	2	449^{a} ±48.3	0.15±0.063	16±2.7
	R. lancea	60.4^{ab} ±4.66	96.5^{a}	1	383^{ab} ±49.4	0.18±0.065	19±2.7
Wet	*C. africana*	29.7^{c} ±4.58	60.1^{b}	3	211^{bc} ±48.6	0.28±0.064	16±2.7
	RL:CA	30.2^{c} ±5.70	67.5^{b}	2	503^{a} ±60.4	0.19±0.079	20±0.1
	R. lancea	39.4^{c} ±4.59	87.4^{a}	1	402^{ab} ±48.7	0.15±0.064	14±2.7
SEM%		5.91	4.75		57.74	0.02	0.92
CV%		33	30		41	26	14
p-values							
Season		0.1382	0.0001		0.361	0.735	0.971
Diet		0.0029	0.247		0.0001	0.390	1.000
Season* Diet		0.0001	0.040		0.001	1.000	1.000

Diet; *C. africana* = Celtis africana, *R. lancea* = *Rhus lancea*, RL:CA = *Rhus lancea* and *Celtis africana* mixture. Superscripts that differ in the same column denote values that are statistically different at the $p < 0.05$ level. **Animal behaviour;** DMI = dry matter intake, RPI = relative preference index, RP = Relative Preference. SEM = standard error of means, CV = coefficient of variation, Wet season ranged from October to February, Dry season ranged from May to July.

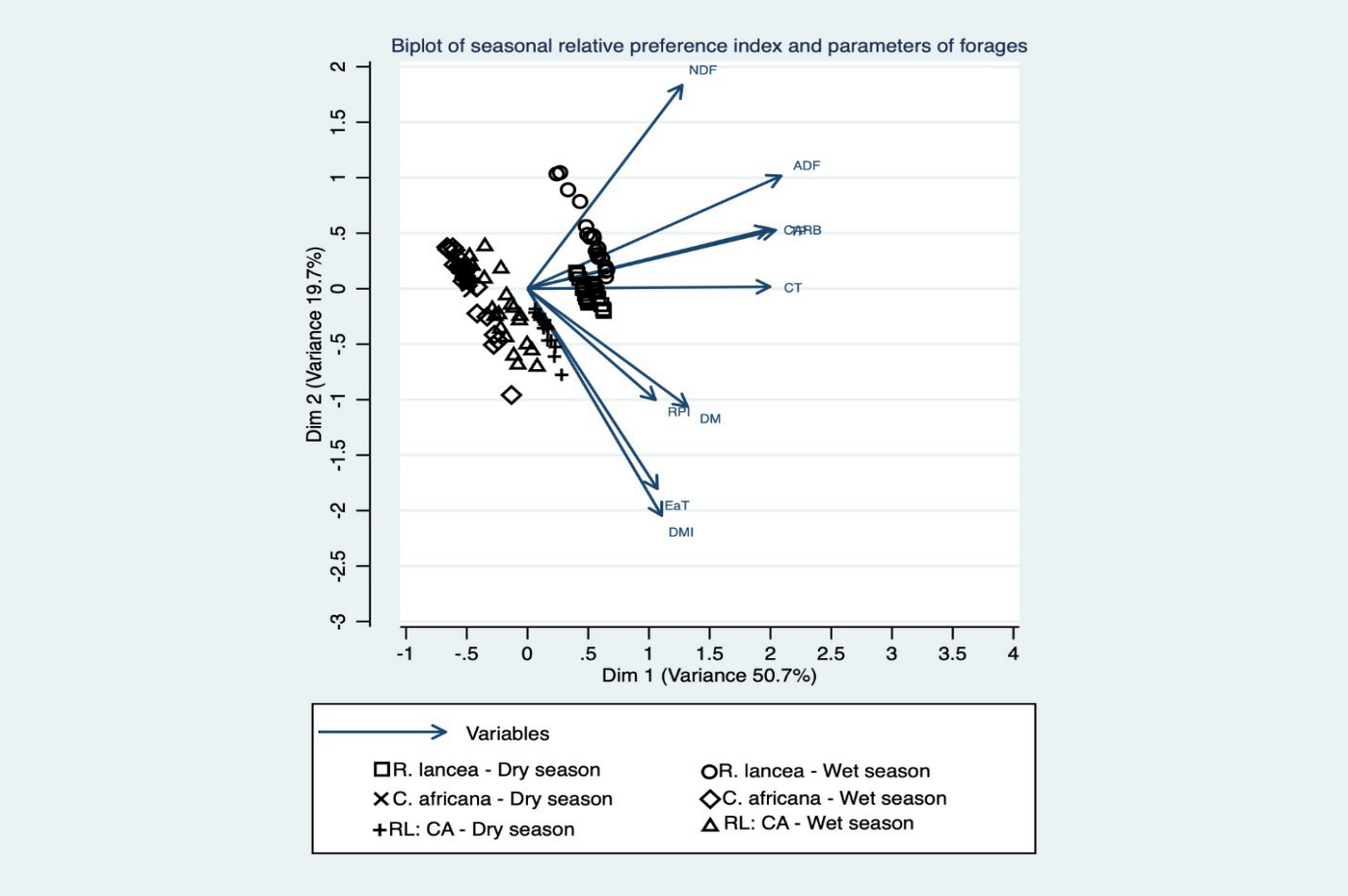

Figure 3.1: The relationships between the chemical composition, dry matter intake, preference, and eating time of *R. lancea*, *C. africana* or RL:CA when consumed by Nguni goats during wet and dry seasons.

Note: DM – dry matter, DMI – dry matter intake; ADF – acid detergent fibre; NDF – neutral detergent fibre; CT – Condensed tannins; TPCs – Total phenolic compounds; EaT- time spent eating; RPI – relative preference index; Wet season ranged from October to February; Dry season ranged from May to July.

The biplot in Figure 3.1 displays the relationships between the chemical composition, dry matter intake, preference, and eating time of *R. lancea*, *C. africana,* and RL:CA when consumed by Nguni goats during wet and dry seasons. The model accounted for a total of 70.4% of the variance in the first and second dimensions. The *C. africana* and RL:CA during the dry and wet seasons clustered together while *R. lancea* in the wet and dry seasons are

clustered together slightly to the right. The variances between the clusters were influenced mostly by NDF, ADF, CT, and TPC which were positive in both dimensions. The EaT, RPI, DM, and DMI had positive variance contribution in dimension 1 but negative variance in dimension 2.

Correlation coefficients of diet parameters (DM, TPCs, CT, CP, NDF, ADF, ADL) and goat-based parameters (dry matter intake, relative preference index, eating time, eating rate, and sniffing time) irrespective of treatment and season are shown in Table 3.4. Dry matter intake was significant and positively correlated ($p < 0.05$) to DM, RPI, eating time, sniffing time, TPCs, CT, ADF, and ADL. Relative preference index was positively correlated and significant ($p < 0.05$) with DM, DMI, time spent eating, TPCs, CT, NDF, ADF, and ADL. Eating time was negatively correlated to eating rate ($p < 0.05$) and positively correlated to TPCs, CT, ADF, and ADL ($p < 0.05$). The eating rate was significant and positively correlated to sniffing time ($p < 0.05$). Sniffing time was positively correlated to CT and CP ($p < 0.05$).

Table 3.4: Spearman's correlation coefficients of diet and goat-based parameters.

	Parameters											
	DM	DMI	RPI	Eating time	Eating rate	Sniff time	TPCs	CT	CP	NDF	ADF	ADL
DM	1.00											
DMI	0.52***	1.00										
RPI	0.33**	0.38*	1.00									
Eating time	0.43**	0.75***	0.31*	1.00								
Eating rate	-0.07	-0.08	-0.08	-0.62***	1.00							
Sniff time	0.11	0.23	-0.08	0.05	0.20	1.00						
TPCs	0.61***	0.44***	0.25	0.43**	-0.13	0.21	1.00					
CT	0.74***	0.40	0.40***	0.30	-0.03	0.21	0.71***	1.00				
CP	-0.20	0.10	0.02	-0.01	0.10	0.20	0.30	0.34**	1.00			
NDF	0.37*	0.03	0.50***	-0.02	-0.01	-0.10	0.50***	0.60***	0.03	1.00		
ADF	0.77***	0.40*	0.42*	0.40*	-0.11	0.10	0.80***	0.74***	0.03	0.70***	1.00	
ADL	0.83***	0.29	0.34**	0.20	-0.01	0.00	0.20	0.54***	0.49***	0.43**	0.50***	1.00

Parameters; DM = Dry matter, TPCs = Total phenolic compounds, CT = Condensed tannins, CP = crude protein, NDF = neutral detergent fibre, ADL= acid detergent lignin, ADF = acid detergent fibre, DMI = dry matter intake, RPI = relative preference index. * = significant at p <0.05. **= significant at p = 0.01. *** = significant at p = 0.001.

The proportional dry matter intake (PDMI), actual dry matter intake (ADMI), organic matter intake (OMI), crude protein intake (CPI), neutral detergent fibre intake (NDFI), the digestibility of dry matter (DMD), organic matter (OMD), crude protein (CPD) and neutral detergent fibre digestibility (NDFD) in goats fed *C. africana*, *R. lancea*, RL:CA or control diet across two seasons are presented in Table 3.5. There were significant season x diet interactions ($p < 0.05$) on PDMI, ADMI, OMI, CPI, and NDFI. Goats fed the control diet had higher PDMI ($p < 0.05$) than those on *R. lancea* during the dry season. Goats fed the *C. africana* diet had higher PDMI ($p < 0.05$) than those on the *R. lancea* diet during the wet season. The ADMI was the highest on the control followed by *C. africana* ($p < 0.05$) and then *R. lancea* and RL:CA diets across all seasons. Goats fed the *R. lancea* diet had the highest OMI ($p < 0.05$) during the wet season compared to those that were fed *C. africana*, RL:CA, and control diets. Goats fed the control diet had the highest OMI ($p < 0.05$) during the dry season compared to goats on the other diets. The CPI was the highest ($p < 0.05$) for the control diet during the dry season and the highest ($p < 0.05$) for the *C. africana* diet during the wet season. The NDFI was the highest on the control diet during the dry season and *C. africana* NDFI was more than *R. lancea* NDFI ($p < 0.05$). In the wet season, the NDFI in *R. lancea* was significantly ($p < 0.05$) higher than in *C. africana* and the control, while NDFI was similar to *R. lancea* and RL:CA.

The goats consuming the control diet had higher DMD ($p < 0.05$) than those on *C. africana* during the dry season. There were significant season x diet interactions ($p < 0.05$) on CPD. The CPD was higher during the wet season than during the dry season ($p < 0.05$) for the *R. lancea*, *C. africana*, and RL:CA based diets and there were no seasonal differences for the control diet ($p > 0.05$).

Table 3.5: Nutrient intake and digestibility coefficients (dry matter basis) of nutrients in *C. africana*, *R. lancea* and or RL:CA diets and control diets offered to Nguni goats during the wet and dry seasons.

		Nutrient intake feed DM/day					Digestibility coefficients (%)			
Season	**Diet**	PDMI %	ADMI	OMI	CPI	NDFI	DMD	OMD	CPD	NDFD
Dry	*C. Africana*	3.1^{bcd}	510^{b}	401^{cd}	35.5^{d}	169^{b}	49^{b}	62	30^{b}	33
	R. lancea	2.1^{b}	384^{c}	362^{de}	39.2^{d}	147^{cd}	56^{ab}	60	42^{b}	33
	Control	4.1^{ac}	827^{a}	722^{a}	155.3^{a}	253^{a}	72^{a}	74	79^{a}	46
	RL:CA	3.0^{bd}	388^{c}	339^{e}	31.6^{d}	143^{d}	52^{ab}	62	40^{b}	28
Wet	*C. Africana*	4.1^{a}	493^{b}	414^{c}	103.3^{b}	151^{cd}	51^{b}	59	71^{a}	34
	R. lancea	3.0^{bcd}	485^{b}	458^{b}	86.1^{c}	170^{b}	60^{ab}	63	69^{a}	29
	Control	3.3^{acd}	472^{b}	411^{c}	94.2^{bc}	161^{bc}	63^{ab}	65	77^{a}	41
	RL:CA	3.2^{acd}	462^{b}	411^{cb}	87.3^{c}	159^{bc}	55^{ab}	64	65^{a}	24
SEM		0.18	18.3	15.9	3.80	5.4	8.9	9.8	6.9	13.3
CV (%)		3	31	30	55	24	8	7	6	24
p-values										
Season		0.0001	0.511	0.572	0.0001	0.020	0.98	0.71	0.001	0.55
Diet		0.0001	0.0001	0.0001	0.0001	0.0001	0.001	0.19	0.001	0.14
Season*Diet		0.0001	0.0001	0.0001	0.0001	0.0001	0.35	0.54	0.001	0.97

[abcd] Means within a row that do not share a common superscript differ significantly at the p <0.05. **Diet;** *C. africana*= *Celtis africana*, *R. lancea* = *Rhus lancea*, RL:CA = *Rhus lancea* and *Celtis africana* mixture. **Nutrient intake**; PDMI = proportional dry matter intake (intake as % of body weight), ADMI = actual dry matter intake, OMI = organic matter intake, CPI = crude protein intake, NDFI = neutral detergent fibre intake. **Digestibility coefficients**; DMD = dry matter digestibility, OMD = Organic matter digestibility, CPD = crude protein digestibility on a dry matter basis, NDF= neutral detergent fibre digestibility dry matter. SEM = standard error of means, CV = coefficient of variation. *Wet season ranged from October to February, and Dry season ranged from May to July

Digestible organic matter (dOM), crude protein (dCP), and neutral detergent fibre (dNDF) in *C. africana*, *R. lancea,* or RL:CA based diets and control fed to goats during the wet and dry seasons are indicated in Table 3.6. There were significant season x diet interactions ($p < 0.05$) on dCP. There were higher dCP ($p < 0.05$) in goats on the control diet than in goats on the *C. africana*, *R. lancea,* and RL:CA based diets during the dry season. The dCP levels in *C. africana*, *R. lancea,* and RL:CA based diets were higher ($p < 0.05$) during the wet season than during the dry season but there were no seasonal differences for the control diet ($p > 0.05$).

Table 3.6: Digestible organic matter (dOM), crude protein (dCP), and neutral detergent fibre (dNDF) in *C. africana*, *R. lancea,* RL:CA diets or control diet fed to goats during the wet and dry season.

		Parameter (g/ kg DM)		
Season	**Diet**	dOM	dCP	dNDF
Dry	*C. africana*	48.8	2.1^{a}	10.9
	R. lancea	56.1	4.2^{a}	13.0
	Control	64.4	14.8^{c}	14.0
	RL:CA	53.8	2.7^{a}	10.2
Wet	*C. africana*	49.9	14.7^{bc}	16.6
	R. lancea	59.9	12.1^{b}	13.6
	Control	57.0	15.3^{c}	15.5
	RL:CA	56.8	12.5^{bc}	15.4
SEM		8.41	1.16	4.86
CV (%)		7	1	20
p-values				
Season		0.96	0.001	0.096
Diet		0.04	0.001	0.90
Season* Diet		0.45	0.001	0.69

^{abcd} Means within a row that do not share a common superscript differ significantly at the $p < 0.05$. **Diet**; *C. africana* = *Celtis africana*, *R. lancea* = *Rhus lancea*, RL:CA = *Rhus lancea* and *Celtis africana* mixture. **Parameters;** dOM = digestible organic matter, dCP = digestible crude protein, dNDF = digestible neutral detergent fibre. SEM = standard error means, CV = coefficient of variation. *Wet season ranged from October to February, and Dry season ranged from May to July.

3.4. Discussion

3.4.1. Nutritional composition

The study determined the CP, DM, OM, NDF, ADF, TPCs, and CT levels in *R. lancea*, *C. africana,* and RL:CA over the wet and dry seasons. The large coefficient of variation, which is above 20% (Table 3.2), indicates significant fluctuations in the average concentrations of ash, crude protein (CP), acid detergent fibre (ADF), crude fibre (CT), and total phenolic compounds (TPCs) across different types of browse and throughout different seasons. These fluctuations resulted in inconsistent nutrient and antinutrient intake by goats. *Celtis africana* had lower OM and higher ash content than the *R. lancea*, during the dry season. The lower OM content implies that goats will not achieve a high nutrient intake when fed the species alone. The ash component may comprise minerals or silicates that may be critical for growth and metabolic functions or have adverse health effects on the goats. Several studies have identified the negative effects of dietary mineral sulphur (0.2%) on dietary preference, intake, and growth in wild concentrate selectors and livestock (Zinn et al., 1997; Spears et al., 2011; Ceacero et al., 2015). Studies on the mineral content of *C. africana* are scant, and more studies would be invaluable in investigating the specific minerals affecting short-term intake in goats.

The CP levels in *C. africana* during the dry season were less compared to the wet season while *R. lancea* maintained similar CP levels during the dry and wet seasons. However, the CP levels in *R. lancea* and RL:CA mixture during the dry season and *C. africana*, *R. lancea*, and RL:CA mixture during the wet season were slightly above the normal CP requirements for maintenance in ruminants (7% to 8% DM) (Thamina et al., 2020). The findings of this current research agree with reports on elevated CP levels in fresh mature leaves in *R. lancea*, during the wet and dry seasons in the periphery of Pretoria, South Africa (Gemeda & Hassan, 2015). Crude protein of 15.1% DM has also been observed in young fresh leaves of *C. africana* during the wet season in the mid-Rift Valley, Ethiopia (Shenkute et al., 2012). The current study observed CP values for *C. africana*, *R. lancea,* and RL:CA mixture, which were above the minimum of 8% DM, except for *C. africana* during the dry season. According to Naumann et al. (2017), the values are suitable for meeting the maintenance requirements of ruminants. This suggests that browse can be a

protein source for Nguni goats to fulfil their CP maintenance requirements. However, this study showed that the digestible crude protein (dCP) level during the dry season is insufficient to support the concentrate selectors. According to these authors (Nsahlai et al., 2011; Tshabalala et al., 2013; Naumann et al., 2017), the nutritional value of forage in Southern Africa varies between wet and dry seasons, posing a challenge for domestic ruminants and wild animals. However, the current study suggests that a mixture of *C. africana*, *R. lancea*, and RL:CA can help overcome feed shortages and lower NDF content in forages during dry seasons. The CP levels in the *R. lancea* and RL:CA mixture remain consistent with slight fluctuations of 9.4 - 11.7 and 10.4 - 10.9 % DM respectively across seasons, making it a viable option to supplement low-quality forages and enhance dietary CP levels across seasons. The CP of *M. sativa* was significantly higher (20.3 - 20.9 % DM) across seasons and can be essential to meet the animal's protein requirements for physiological needs.

The NDF levels in *C. Africana*, *R. lancea*, and *M. sativa* were similar during the dry season, suggesting that both browse and *M. sativa* are potential sources of slowly available energy for rumen microbes. While CP is important, so are NDF and ADF, as the NDF interacts with CP and influences the increase of microbial protein in the rumen (Song et al., 2018). However, increased concentrations of NDF in the diet reduce DM, ADF, CP, metabolizable energy intake, and digestibility in ruminants (Cuong et al., 2009; Truong & Thu, 2019). Neutral detergent fibre and ADF levels in *R. lancea* were inferior compared to the finding of Naumann et al. (2017) on the tender leaves of *R. lancea* in the Limpopo province, South Africa. This indicates that differences in maturity among species influence nutrient composition, probably due to differences in geography and environment (Davidson et al., 2011).

The significantly lower ADF levels in the *R. lancea, C. africana*, and RL:CA browse forages, compared to *M. sativa*, indicates that there is less of the relatively undigestible fibre component in the browse species used in this research study (Lu et al., 2008). *Medicago sativa*, however, had higher NDF levels during the wet season. The NDF levels (25 – 30% DM), contributed by the browse and *Medicago sativa* are much lower than those

contributed by *Eragrostis curvula* hay of more than 70%. The higher fibre content in *Eragrostis curvula* hay was attributed to the matureness of the hay (Lu et al., 2008). *Medicago sativa* had higher ADF levels than *C. africana* and *R. lancea*, while *R. lancea* had higher levels than *C. africana* in both seasons. The ADF is a less digestible plant component and higher levels impact negatively on the availability of digestible energy (Lu et al., 2008) resulting in poor nutrient absorption. The NDF and ADF levels reported in *C. africana, R. lancea*, and RL:CA mixture can enhance nutrient feed intake, aid in achieving the NDF and ADF requirements, and positively influence digestibility in goats (Norton, 1994; Osuga et al., 2020).

The TPCs and CT levels in *R. lancea* were higher than in *C. africana* in both the wet and dry seasons. The seasonal effects on the TPCs and CT values of evergreen and semi-deciduous trees have been demonstrated in research studies (Rana et al., 2006; Ahmed et al., 2017; Marais, 2019). Differences have been attributed to the environment and ecology of the browse species (Pant et al., 2021). In addition, as the day length and light intensity are low, the biogenesis of TPCs and CT decreases and increases as day length and light intensity increase (Ahmed et al., 2017). The evolutionary and ecological basis for the observed patterns in TPCs and CT production likely stems from the complex interplay between plant defense strategies, resource allocation, adaptation to seasonal changes, and responses to environmental stressors. This flexibility in secondary metabolite production allows plants to optimize their fitness in varying environmental conditions and in the face of changing herbivore pressures. The TPCs levels in the two-browse species (*R. lancea* and *C. africana*) were unaffected by the season. The *R. lancea*'s CT levels were higher during the dry than during the wet season, showing variations in the two-browse species' TPCs and CT metabolism. Higher levels of CT (>5% CT DM) forages are known to depress forage intake, nutrient digestibility, and nitrogen retention (Mergeduš et al., 2018). The negative results of TPCs and CT on ruminant performance have been well documented (Nsahlai et al., 2011; Tshabalala et al., 2013). It was outlined that intake of high CT forages decreased protein digestibility and weight gain in Boer goats (Dludla, 2010). The CT levels observed in *R. lancea* (11.3% DM) during the wet season were comparable to those described by Naumann et al. (2013) on *R. lancea* (10.8% DM) in the

Limpopo province of South Africa. Several factors including the environment, soil nutrients and pH level, affect the browse nutrient content and their secondary metabolites (Naumann et al., 2017) and could explain this difference. The composition of TPCs and CT levels is also affected by the genotype profile of the forages used in the research (Dykes et al., 2014).

3.4.2. Relative preference and palatability

The high coefficient of variations for DMI, RPI, time spent eating, and eating rate is a testament to the individual variation in response among the goats for these parameters. The goats' preferences for the browse were *R. lancea* > RL:CA > *C. africana* during the dry and wet seasons respectively. The RPI was positively correlated to DMI and eating time for TPCs, CT, NDF, ADF, and ADL. Nonetheless, the results suggest that the high CT and TPCs in *R. lancea* (11.0 - 15.1% CT and 8.0 - 8.1% TPCs) and RL:CA (6.2 - 11.0 % CT and 5.0 - 6.0 % TPCs) were either not at sufficient levels to deter the goats from consuming more than the other offered choices, or the goats sought and desired these forages. The goats' preference for *R. lancea* despite high CT and TPC levels likely results from a complex interplay of physiological adaptations, nutritional strategies, and potential benefits related to thirst and thermal regulation. This behaviour underscores the sophisticated nature of goat foraging strategies and their ability to balance nutritional gains against the challenges posed by plant defense compounds. It also highlights the importance of considering multiple factors, including seasonal variations, when studying animal foraging behaviour and dietary preferences. This preference was also demonstrated in the higher *R. lancea* DMI and the fact that the goats spent less time eating *C. africana* than *R. lancea* during the dry season. In addition, there were no diet effects on the eating rate and time spent sniffing.

The high DM intake of *R. lancea* can be attributed to its succulence during the dry season compared to *C. africana*. Succulent forages reduce water intake thus increasing nutrient intake (Silva et al., 2021). In a different study, Ekwe et al. (2020) observed a higher preference for succulent forages by red Sekoto and western African dwarf goats using a

quadrant method to determine forage availability. Dry matter intake was related to RPI, time spent eating, time spent sniffing, nutrients (ADF and ADL), and SPMs (TPCs and CT). Thus, higher DM intake in this research study was attributed to higher time spent eating *R. lancea*, RL:CA and *C. africana*. This is in accord with the report of Osolo et al. (1996), and Kalio et al. (2006).

The CP levels of *C. africana* were higher than *R. lancea* and RL:CA during the wet season. The findings showed that the forage preference by goats was not influenced by the crude protein value, but rather by their feeding behaviour. Pulina et al. (2013) proposed that hormones, including dopamine, opioids, and ghrelin, that promote the behaviour of "wanting" and "liking," both of which are components of hunger in goats, might be responsible for the short-term modulation of feeding behaviour. Goats can recognize and recall forages they have tasted before (Scherer et al., 2019). Seemingly, they discriminated between *R. lancea*, *C. africana,* and RL:CA mixture by sniffing and tasting. The *R. lancea* has an aromatic smell (Irish, 2000; Beinart, 2011) and its pleasant smell can override the smell of other browse material. Furthermore, the narrow leaf size of *R. lancea* to *C. africana* may have contributed to its high relative preference. It was reported that browse with large leaf sizes decreased intake rate, due to the required high bite rate to finish the leaves (Heuermann et al., 2011; Ortíz-Domínguez et al., 2022).

During the dry season, the goats spent the most time eating RL:CA, while spending the least amount of time eating the triangular-shaped leaves of *C. africana*. This is because the narrow lanceolate (narrow tapering to the pointed apex) leaves of *R. lancea* and triangular leaves of *C. africana* have different shapes, causing goats to spend more time selecting and unplugging the narrow-shaped *R. lancea* leaves in the RL:CA mixture. The observation during data collection showed that goats unplugged the leaves and stem of *R. lancea* until it was completely consumed from the foraging board before moving on to RL:CA mixture. These findings are consistent with previous research by Freund (2005), and Swanepoel (2016). The higher eating rate is a result of chewing which assisted in increasing the surface area for enzymes to digest and ingest the forages. This agrees with a previous report by Mkhize et al. (2018), that browsing forages rich in CT necessitates

longer chewing than forages with low or no CT. A previous study stated that Nguni goats have a higher preference for *R. lancea* compared to *Dichrostachys cinerea* (Werekeh, 2012). The findings of the current research indicate that, owing to similarities in eating rate, the palatability of *R. lancea* and RL:CA mixture might, however, not be a problem for goats.

3.4.3. Nutrient intake and digestibility

There were season x dietary interactions in the intake of DM, OM, CP, and NDF. While there was a relatively similar intake of nutrients during the wet season, there were differences in intake during the dry season across all diets. Goats on the control diet consumed 827 g/DM feed/day, while the goats on the *C. africana* consumed 40% less feed at 510 g/DM feed/day, and those on *R. lancea* consumed 54% less feed at 384 g/DM of feed during the dry season. Similarly, goats on the control diet consumed 155 g CP/kg feed DM/day while goats on *R. lancea* consumed 39 g CP/kg feed/day, a fourfold difference in intake, and animals on *C. africana* 36 g CP/kg feed/day. The digestibility coefficients of DM of the control diet (72%) were also 16% higher than that of *R. lancea* (56%) and 23% higher than those of *C. africana* (49%). The dCP was low in *C. africana*, *R. lancea*, and RL:CA compared to the control diet. This effectively means that, even though the CP content in *R. lancea*, *C. africana*, and RL:CA may be sufficient to meet the goats' requirements, the available digestible protein for their physiological needs was quite low, and this may be owing to the negative consequences of TPCs and CT in the rumen.

The differences in nutrient intake, suggest that goats consuming less forage would not get sufficient nutrients for the physiological stage, or meet maintenance requirements. The PDMI showed that goats on the control diet consumed more forage per kg of body weight than goats on the *R. lancea*, *C. africana,* and RL:CA browses during the dry season. The high PDMI in the control diet enhanced digestive efficiency resulting in higher digestibility and this can be positively related to high body weight (Silanikove, 2000). The low PDMI in goats fed *C. africana*, *R. lancea,* and RL:CA showed that their digestive efficiency was low followed by low digestibility, body weight, and high nutrient requirements. Goats consume 3 - 5% of their body weight to meet physiological needs depending on their age and growth

status (Zemmelink & Meinderts, 1985; Lu, 1988). The findings of the current research contradict the results of Zemmelink and Meinderts (1985), and Lu (1988). The study found that goats fed *R. lancea*, *C. africana*, and RL:CA consumed less than 3% DM. This low consumption can be attributed to the unspecific feeding patterns of goats (Papachristou et al., 1999) and low digestibility (Aregheore, 2002). During the wet season, goats on the *C. africana* diet showed a higher PDMI than those on the control, *R. lancea*, and RL:CA. This is informed by enhanced CP values in *C. africana* browse during the wet season, supporting the findings that *C. africana* is a more nutritious browse during the wet season (Shenkute et al., 2012). The *C. africana* browse component in RL:CA can be the reason why there was no variation in the concentrations of PDMI between RL:CA and control throughout the wet season.

The high content of indigestible fibre in roughages depresses the digestibility of dietary components (Lu et al., 2008). Dietary protein influences fibre (ADF, NDF, and ADL) degradation in the rumen (Da Cruz et al., 2021). The dietary CP provides adequate amounts of ammonia nitrogen to the rumen (Da Cruz et al., 2021), which supports the growth of fibrinolytic bacteria that enhance fibre degradation (Souza et al., 2010; Franco et al., 2017; Rufuno et al., 2016; Da Cruz et al., 2021). The availability of CP in the browse enhanced the NDFD in the rumen. The higher NDFD resulted in higher DMI (Xu et al., 2023) and can improve the digestible energy in the rumen.

During both the wet and dry seasons, *C. africana* had the lowest TPCs and CT and the highest ash content. The ash content in *C. africana* resulted in low CPD, based on the observations that as the CP levels declined, the ash content rose. Shirley and Parsons (2001) also observed enhanced ash levels which reduces the protein quality. The high ash content in *C. africana* forage is, however, an indication of potentially available mineral content (Wambui et al., 2006) and needs further investigation. The CT bind to CP resulting in tannin-protein complexes which decreased the degradability of the CP in the rumen and negatively affected the degradability of NDF (Barry & McNabb, 1999; Muir 2011; Thurau et al., 2021). Chebli et al. (2021), recorded CT levels of 20 - 45 g kg^{-1} DM that depressed dietary protein degradability and proteolytic bacteria in ruminants. On the one hand, the

results of this research demonstrated that the CT levels in *R. lancea*, *C. africana,* and RL:CA were higher than 3.5% DM (35 g/kg DM) and this resulted in depressed dCP and CPD due to tannin binding complexes. However, on the other hand, the CT binding to CP can slow down microbial degradation and prevent nitrogen loss in the rumen (Makkar, 2003).

3.5. Conclusions

Celtis africana had a higher ash content and lower TPCs and CT than *R. lancea* across seasons. The CP levels in *R. lancea*, *C. africana* during the wet season, and *R. lancea* during the dry season were sufficient to meet the ruminant protein requirements. However, CP intake from diets based on these forages was lower than the control diet. Even though the crude protein values during the dry season are adequate for meeting the maintenance needs of ruminants and imply that browse can serve as a protein source for Nguni goats, this study indicates that the digestible crude protein (dCP) levels are insufficient to support the concentrate selectors during the same period. In the first experiment, the study investigated the extent to which goats prefer either browse species when supplied separately or in combination, it was concluded that goats preferred *R. lancea*>RL:CA>*C. africana* across seasons. The choice of *R. lancea* by the goats despite high CT and TPCs levels may be attributed to their physiological adaptations and nutritional advantages. This behaviour demonstrates the advanced nature of goat foraging techniques and their capacity to balance nutritional benefits in the presence of plant defense compounds, highlighting the importance of considering seasonal variations when analyzing animal foraging patterns. In the second experiment, apparent total tract digestibility was investigated in goats offered *R. lancea*, *C. africana,* and RL:CA mixture across seasons. It was concluded that the high levels of TPCs and CT in *R. lancea*, *C. africana,* and RL:CA mix posed a challenge to the digestibility of nutrients resulting in low digestibility in Nguni goats. However, *C. africana* and *R. lancea* can be potential browse for goats during the wet season but require protein supplementation to enhance the protein supply to improve digestibility in goats in the dry season.

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CHAPTER 4: RUMEN BACTERIAL COMPOSITION OF INDIGENOUS NGUNI GOATS FED *RHUS LANCEA*, *CELTIS AFRICANA* DURING DRY AND WET SEASONS

Abstract

The study determined the effects of diets based on *Rhus lancea (R. lancea, RL)*, *Celtis africana (C. africana, CA),* and a combination (RL:CA) of the two plants on the rumen bacterial composition in Nguni goats. This was evaluated in twenty male Nguni goats with an average weight of 16 ± 1.7 kg (mean ±SD) in the wet season and 15 ± 1.6 kg (mean ±SD) in the dry season in a randomised block design during the wet and dry seasons. Rumen fluid samples collected during the wet and dry seasons were processed and subjected to metagenomic analysis of prokaryotic 16S ribosomal RNA (16S rRNA). Out of the 12 identified phyla, *Bacteroidetes* and *Firmicutes* phyla were the most dominant with the combined contribution to the total comprising more than 85% across the seasons. The mean contribution for all treatments during the wet season (92.5%) was higher (p <0.05) than in the dry season (87.4%). The *Firmicutes* to *Bacteroidetes* (F/B) ratio of the four diets during the dry season (0.85:1) was higher (p <0.05) than in the wet season (0.58:1). There was a nearly 12.7% decrease for *Firmicutes* in goats fed *R. lancea* during the dry season than the wet season. In contrast, goats fed *C. africana* had a more stable *Firmicutes* population in either season, with a difference of 6.1% while goats on the RL:CA forage only had a 4.5% difference. The *Bacteroidia* class and *Bacteroidales* order were expressed more abundantly (p <0.05) in goats on *R. lancea* than those on RL:CA diets during the wet season. The abundances of *Clostridia* class, *Clostridiales* order and *Ruminococcaceae* family were greater (p <0.05) in goats fed RL:CA than those fed *R. lancea* diet during the wet season. The abundances of *Clostridia* class and *Clostridiales* order in goats fed *R. lancea* diet were higher than (p <0.05) those in goats fed *C. africana* during the dry season. It can be concluded that *R. lancea, C. africana* and RL:CA diet positively affects rumen microorganism composition in Nguni goats across seasons. Further studies on the aforementioned forages are required to determine their role in the long-term effects, stability and health of rumen microbiota in Nguni goats across seasons.

Keywords: browse, rumen microorganisms, ruminants.

4.1. Introduction

The ruminant gastrointestinal tract includes a diverse community of microbes belonging to the Archaea, Bacteria, and Eukarya (Gharechahi et al., 2020). The captive herbivore husbandry, small ruminant mammals including goats (*Capra hircus*) and springbok (*Antidorcas marsupialis*) are provided with a variety of unconventional feedstuffs in captivity (Mbatha et al., 2012). Thus, the goat rumen microbes adjust to a variety of forages from which they extract nutrients and respond to anti-nutrients with antimicrobial properties such as secondary plant metabolites (SPMs). When the type and amount of forage and the resident gut bacteria are in balance, this contributes to an optimally functioning gut and healthy ruminants. Rumen microbes obtain approximately 70% of their energy using fermentation processes (Bergaman, 1990) involving the biological conversion of plant fibre into mono- and disaccharides that may then be absorbed from the gut (Jami and Mizrahi, 2012; Liu et al., 2016). However, depending on the type of forage, the intake of SPMs such as condensed tannins (CT) can adversely affect microbial function and inhibit the digestion of nutrients. Thus, a moderate daily intake of CT (3-4% DM) benefits rumen microbes by improving nitrogen supply and enhancing nutrient efficiency (Min et al., 2014).

Intensive research has been conducted to understand how forage affects the rumen environment (Kumar, 2003; Kamra, 2005; Karasov and Carey, 2009; Qi et al., 2010; Wang et al., 2011; Steuer et al., 2012), and blood markers with the view to sustaining good health (Dziba et al., 2003; Ikhimioya and Imaseun, 2007; Olafadehan *et al.,* 2014). For example, Ban (2019) reported on the use of fresh *Garcinia mangostana* leaves in feeding crossbred Thai native × Anglo-Nubian male goats even though they contain oligomeric flavonoids. These leaves were reported to sustain gut bacteria such as *Fibrobacter succinogenes, Ruminococcus albus,* and *Butyrivibrio fibrisolvens* that produce volatile fatty acids (VFA) (Ma et al., 2017; Purba et al., 2020). However, limited studies have been conducted to understand the rumen microbial diversity in goats (Liu et al., 2016) and how the homeostasis of this microbial community is maintained in goats fed various types of forage. Plant species such as *Rhus lancea* (*R. lancea*) and *Celtis africana* (*C. africana*) are indigenous to southern Africa (Ndou et al., 2020; Tapfuma et al., 2020) and these plants may be used to feed herbivorous animals. Studies have reported on the use of *R. lancea*

as browse forage (Dludla, 2010) and to understand the growth performance in Boer goats (Mkhize, 2008). In addition, *C. africana* was used as a potential fodder to feed ruminants during the wet season in the mid-Rift Valley of Ethiopia (Shenkute et al., 2012). Furthermore, a *C. africana* extract was used to determine the biological effects of endophytic microflora and mycoflora and their significance in human medicine (Magobo et al., 2017).

However, little information is available on the effect of feeding *R. lancea* and *C. africana* on the composition of gut microbes in South African indigenous Nguni goats. Thus, the purpose of this study was to determine the effects of *R. lancea*, *C. africana,* and a combination of the two plants (RL:CA) on the composition of gut bacteria in Nguni goats. This study hypothesized that feeding goats *R. lancea, C. africana,* or RL:CA plant species during the dry and wet seasons have distinct significant variable forage effects on gut microbe profiles.

4.2. Materials and methods

The study was conducted at the Agricultural Research Council-Animal Production (ARC-AP) located in Irene and received ethical permission from the UNISA Animal Ethics Committee and the ARC-AP Ethics Committee.

4.2.1. Animals and their management

Twenty (n=20) indigenous male Nguni goats aged around 12 months with an average body weight of 16 ± 1.7 kg (mean ±SD) in the wet season and 15 ± 1.6 kg (mean ±SD) in the dry season were used in this study. The details on animal husbandry for this study are in Chapter 3, section 3.1.2.

4.2.2. Preparation of feedstuffs and study design

Each goat constituted an experimental unit and there were five replicates for each of the four diets. All goats received 100 g DM of *Eragrostis curvula* hay and one of

four supplements for four different diets, namely, i) *Rhus lancea* (*R. lancea* + *Eragrostis curvula* hay), ii) *Celtis africana* (*C. africana* + *Eragrostis curvula* hay), iii) a mixture of the two browse species in a ratio of 1:1 (RL:CA + *Eragrostis curvula* hay) and iv) a control diet (*Medicago sativa*+ concentrates + fruits + vegetables, the same diet used in see Chapter 3) representing the unstructured diet. Goats were offered fresh water and the quantity of forage offered to each goat was illustrated in Chapter 3, section 3.1.5. The study was conducted over 30 days in the wet and dry seasons.

4.2.3. Data collection

Rumen fluid samples were collected during the wet and dry seasons before an initial seven-day adaptation period and at the end of the 30-day trial, to provide two samples per goat per season. To ensure comfort and ease of handling, the rumen fluid was collected using a custom-made stomach tube (approximately 0.3 cm - 1 cm internal diameter and 1 metre in length). Before inserting the stomach tube, it was warmed in water for two minutes to make it pliable. A co-worker comfortably restrained the goat in a standing position between the legs and gently held it steady by its front hooves. Then the veterinarian inserted a mouth gag to prevent chewing on the tube, before inserting the stomach tube through the opening of the mouth gag, over the tongue, and down the oesophagus of the goat into the rumen. When a fermented odour and rumbling or gurgling (borborygmus) sounds were detected through the tube this was taken as confirmation that the tube was in the rumen. A syringe attached to the outer opening of the tube was used to aspirate the rumen contents. Each rumen sample (approximately 100 ml) was transferred into separate, sealable, marked plastic sterile containers and stored in a cooler box with ice packs. The stomach tube was rinsed twice with clean warm water after collecting from each animal to prevent cross-contamination of rumen contents. The rumen fluid pH was recorded directly after collection before the samples were sent to Inqaba Biotec™ (Inqaba Biotechnical Industries (Pty) Ltd., (Pretoria, South Africa), for microbial composition analysis.

4.2.4. Metagenomic analyses

The study used Microbiome Analyst and followed the recommendations of Inqaba Biotechnical Industries (Pty) Ltd., (Pretoria, South Africa). The rumen fluid samples from the goats were subjected to DNA extraction, PCR, and metagenomic analysis of prokaryotic 16S ribosomal RNA (16S rRNA). This gene segment consists of almost 1 500 bp and contains nine variable regions interspersed between conserved regions (Brayton et al., 2007). The variable regions of 16S rRNA were used to phylogenetically identify and order the bacterial species in the diverse microbial population of the rumen.

The sequences of the full-length 16S PCR forward and reverse primer sequences recommended by Jiang et al. (2017) and Inqaba Biotechnical Industries (Pty) Ltd., (Pretoria, South Africa) to track this region are:

F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG – 3'

R: 5'–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC –3'

Briefly, each 25 µl PCR reaction consisted of microbiome DNA (5 ng/µl) (2.5 µl), forward primer 1 µM (5 µl), reverse primer 1 µM (5 µl) and 2x KAPA HiFi HotStart ready-mix (12.5 µl). The plate was sealed, and the PCR was performed in a thermal cycler using the following cycling and temperature conditions: Denaturation at 95°C for 3 minutes followed by 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and extension for 5 minutes at 72°C before the reaction was held at 4°C. Each PCR reaction was cleaned using AMPure XP beads at room temperature to separate the 16S V3 and V4 amplicons from free primers and primer dimers (Bourlat et al., 2016). Initially, the PCR plate

was centrifuged at 1,000 x g at 20°C for 1 minute to collect condensate, and the seal was removed. Then the AMPure XP beads were vortexed for 30 seconds to disperse beads evenly before a multichannel pipette was used to add 50% of AMPure XP beads into each well of the PCR plate. The pipette tips were changed between each addition of beads. Each PCR well-containing beads were pipetted up and down and then shaken at 1800 rpm for 2 minutes. The beads were washed twice with 80% ethanol before amplicons were eluted from the beads using a volume of 50 µl of elution buffer. The sample amplicons were then transferred to the index PCR for further processing to attach dual indices and Illumina sequencing adapters using the Nextera XT Index Kit. The PCR was cleaned again using the AMPure XP beads to prepare the final library before quantification.

For the library quantification, normalization, and pooling, the Illumina quantifying libraries used a fluorometric quantification method that uses dsDNA binding dyes to accurately determine the next generation sequencing library (Nakayama et al., 2016). The DNA concentration was calculated in nM, based on the size of DNA amplicons as determined by an Agilent Technologies 2100 Bioanalyzer trace as follows:

$$DNA\ concentration\ in\ nM = (Concentration\ in\ ng\ /\ (\mu l)\ x\ \text{〖10〗}\ \text{^6})/((660\ g)\ /\ mol\ x\ average\ library)$$

The samples were then subjected to library denaturation and loading onto the MiSeq apparatus. The illumina uses the MiSeq v3 reagent kits to improve run metrics in formation for cluster generation and sequencing. The pooled libraries were denatured with 0.2 NaOH (10 µl), diluted with hybridization buffer (1540 µl), and then heat denatured before MiSeq sequencing (1 cartridge). Each run involved a minimum of 5% PhiX to serve as an internal control for these low-diversity libraries. The DNA was denatured and diluted at 10 nM PhiX to the same loading concentration as the Amplicon library to contain 5% PhiX. Heat denaturation was conducted prior to loading the library into the MiSeq reagent cartridge to warrant

effective template loading on the MiSeq Reporter software (MSR) flow cell. After the samples were loaded on the MSR the Metagenomics workflow was selected.

The metagenomics workflow was selected to classify organisms from the V3 and V4 amplicons using a database of 16S rRNA data. The classification was based on the Greengenes database. The output of this workflow was a grouping of reads at several taxonomic levels: kingdom, phylum, class, order, family, genus, and species.

4.2.5. Data analyses

Statistical analysis was performed using Microbiome Analyst, a web-based pipeline for the analysis of microbiome data (Dhariwal et al., 2017; Chong et al., 2020). A total of 40 ruminal samples were collected across the two seasons for all the diets at the end of each study. Out of these, 31 rumen fluid samples (7 for *C. africana* and 8 for each of *R. lancea*, RL:CA and control) and 368 operational taxonomic units or features were used. Those features or operational taxonomic units (OTUs) with identical values (i.e. zeros) across all samples were excluded as OTUs that appeared in only one sample and were considered artefacts. Data were filtered to eliminate poor-quality or unproductive OTUs to enhance the downstream statistical analysis. A total of 183 low abundance OTUs were eliminated, while a total of 6 low variations in OTUs were deleted because of the inter-quantile ranges. After the data filtering steps, 46 OTUs remained. To permit more biologically expressive comparisons, the data were normalized by rarefying or drawing without substitution from each sample such that all samples have a similar quantity of total counts to address the difference in sampling depth and the sparseness of the data.

Various methods in the Microbiome Analyst pipeline were used to analyse the data as follows;

- Visual exploration using stacked bar/area plots, rarefaction curves, and heat trees.

- Community profiling included alpha and beta diversity analyses.
- Clustering analysis included heatmaps, dendrograms, and correlation analysis.
- Differential abundance analysis comprised univariate analysis.

4.2.5.1. Visual exploration

Rarefaction curve analysis presented the association related to the number of OTUs and the number of sequences using the modified function *ggrare* that emanated from the *ranacapa* package (Kandlikar et al., 2018). Heat tree analysis compared the abundance of diverse taxonomic levels for each pair of factors in a metadata variable using the hierarchical structure of taxonomic groupings to quantitatively (median abundance) and statistically (non-parameter Wilcoxon Rank Sum test) illustrate taxon variations among populations utilizing the R package metacoder package (Foster, 2017).

Community profiling alpha diversity analysis measured the total number of species (richness) and the abundance of the species (evenness) present in the samples and was done using the Phyloseq package (McMurdie, 2013). The following indices were used:

- The observed index to estimate the number of unique OTUs in each sample (richness).
- The Chao1 index considered the observed OTUs to account for unobserved species based on low-abundance OTUs (richness).
- The Shannon and Simpson indices accounted for both richness and evenness.

The OTU level results were plotted across samples and reviewed as box plots for each diet per season. The beta diversity method compared the diversity or composition between microbial communities across diets using the phyloseq

package (McMurdie, 2013). Each sample was associated with every other sample generating a distance or dissimilarity matrix. Similarity or distance between samples was measured using the non-phylogenetic Bray-Curtis distance. Visualization of the dissimilarity matrix in lower dimensions was performed using Principal Coordinate analysis (PCoA).

The statistical significance of the clustering pattern in ordination plots was evaluated using permutational ANOVA (PERMANOVA) and analysis of similarities (ANOSIM). The homogeneity of group dispersions (PERMDISP) analysed the multivariate homogeneity of group dispersions (variances) and differs from the other two in that it specifically tests for differences in the spread (dispersion, variability).

4.2.5.2. Clustering analysis

Hierarchical clustering was performed with the *hclust* function in the package *stat* (R Core Team, 2023). Each sample began as a distinct cluster and the algorithm proceeded to associate them until all samples belonged to one cluster. The two parameters that were regarded when performing hierarchical clustering were resemblance or distance between samples as measured using the Bray-Curtis distance. The other parameter involved the use of clustering algorithms, comprising regular linkage (using the centroids of the observations), complete linkage (using the farthest pair of observations between the two groups), single linkage (using the closest pair of observations), and Ward's linkage (minimizing the sum of squares of any two clusters). In Microbiome analysis, the clustering analysis is supported using a Heatmap and dendrograms (Chong et al., 2020).

4.2.5.3. Differential abundance analysis

Univariate analysis methods identified differentially abundant features using ANOVA across the treatments (Microbiome Analyst R, 1.0, 2020). Features were considered to be significant based on their p-value of below 5%.

4.3. Results

4.3.1. Visual exploration

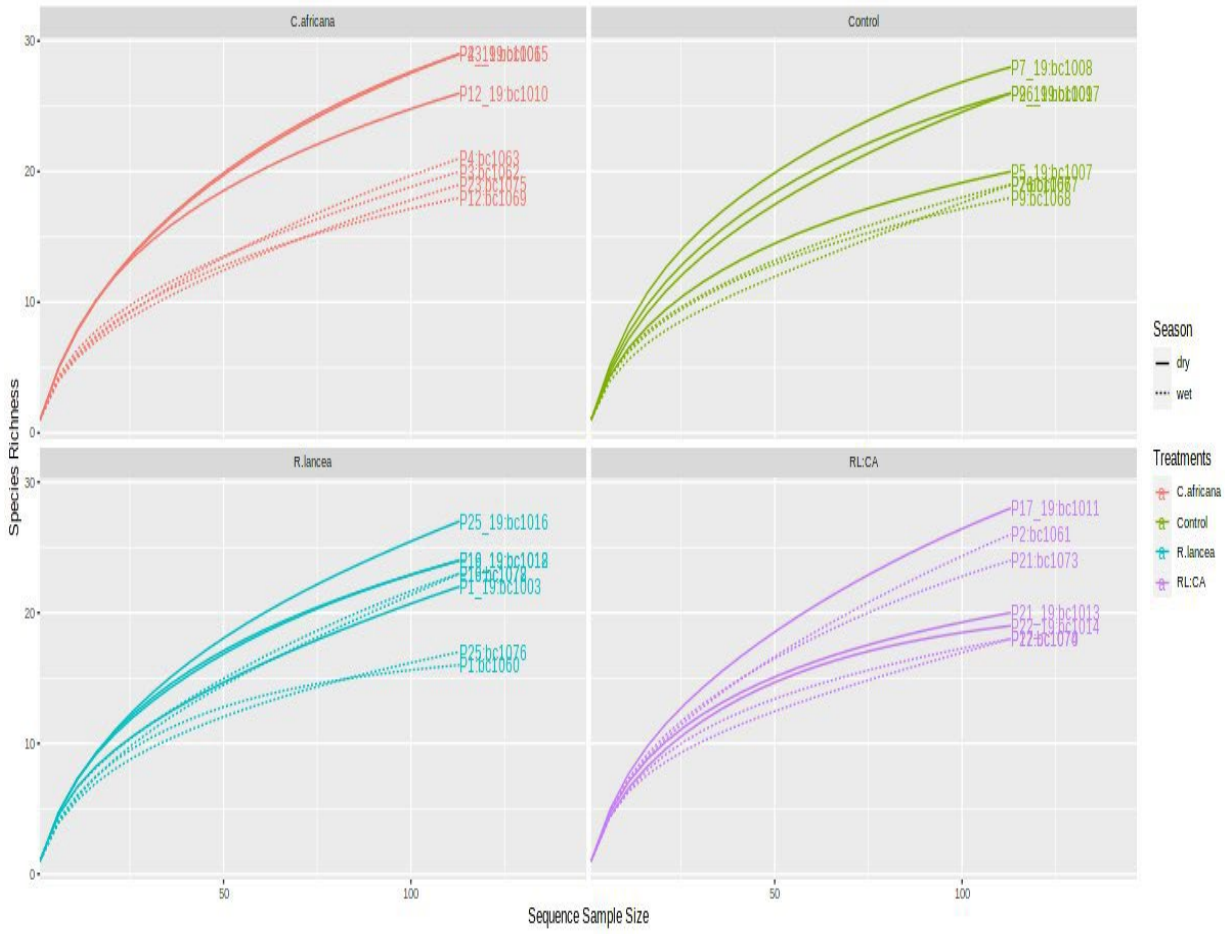


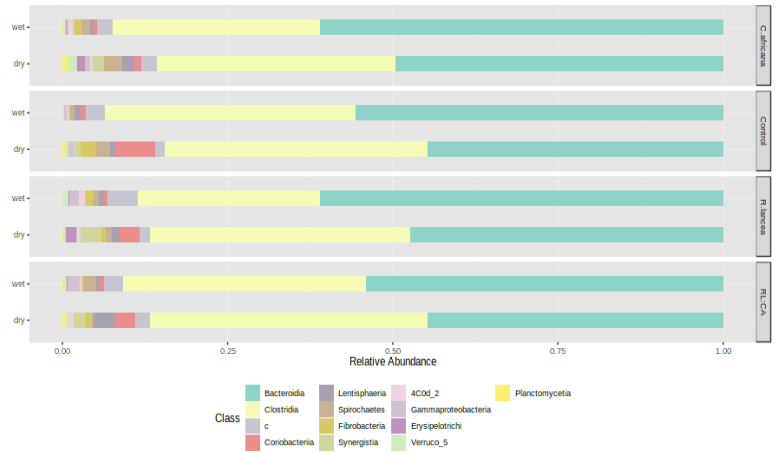
Figure 4.1: Rarefaction curves of rumen bacteria from goats fed *C. africana*, Control, *R. lancea*, or RL:CA diets during wet and dry seasons.

Figure 4.1 displays rarefaction curves showing the connection between the number of OTUs and the number of sequences. Good's coverage values showed that on average 92% of the total species richness was described across the treatments (see Appendix 1). The rarefaction curves indicated that there was little variance between the diversity of the bacteria across the treatments, but there was a seasonal effect on bacterial diversity for goats fed the *C. africana*, *R. lancea*, RL:CA forage, or the control diets. Both data sets rarefaction curves approached but did not reach a plateau, indicating that the bacterial communities' diversity was not fully captured.

The stacked bar plots of the phylum, class, order, and family levels showed that diet and season influenced the goats' rumen bacteria (Figure 4.2). Phyla showed that *Bacteroidetes* and *Firmicutes* phyla contributed more than 80% to the relative abundance with a clear seasonal influence. The relative abundances of *Actinobacteria, Lentisphaerae, Spirochaetes, Fibrobacteres,* and *Synergistetes* contributed less than 10% in varied proportions and the influence of the diets and seasons was not clear. The class showed that *Bacteroidia* and *Clostridia* classes contributed more than 60% to the relative abundance and this was influenced more by season than by diet. The other classes of note contributing less than 10% relative abundance were *Coriobacteria*, *Lentisphaeria*, *Spirochaetes, Fibrobacteria, and Synergestia.* Similarly, season influenced the order *Bacteriodales* and *Clostridiales* which contributed more than 50% to the relative abundance, and the family *Prevotellaceae* which contributed more than 15% to the relative abundance (Figure 4.2 Family).

Phyla

Class



Family

Figure 4.2: Relative abundance of bacteria on goats *C. africana*, *R. lancea* or RL:CA mixture and control diets during wet and dry season.

Table 4.1 shows the actual abundances of *Bacteroidetes* and *Firmicutes* in goats fed *C. africana*, control, *R. lancea* or RL:CA diets during the wet and dry seasons. In the dry season and the wet season, *Bacteroidetes* was quantitatively more abundant in goats fed *C. africana* and *R. lancea* diets followed by goats on the control diet and the RL:CA (Table 4.1). However, *Firmicutes* showed different patterns, where goats on the *C. africana* diet showed a lower percentage compared to those on the control diet during the dry season. There was a nearly 12.7% decrease (from 41.1% to 28.8%) for *Firmicutes* in goats fed *R. lancea* when comparing the dry season to the wet season. In contrast, goats fed *C. africana* had a more stable *Firmicutes* population in either season, (38.1% in the dry season and 32.0% during the wet season (i.e., a difference of 6.1%) and this is reflected in goats fed RL:CA forage with only a 4.5% difference in the *Firmicutes* when comparing the dry and the wet seasons. The *Firmicutes* to *Bacteroidetes* (F/B) ratio of the four diets in the dry season (0.85:1) was higher when compared to the wet season (0.58:1).

Table 4.1: Abundance (%) of Bacteroidetes and Firmicutes in goats fed *C. africana*, control, *R. lancea* or RL:CA diets during the wet and dry seasons.

	Dry season taxon abundance (%)				**Wet season taxon abundance (%)**			
Phylum	*C. africana*	Control	*R. lancea*	RL:CA	*C. africana*	Control	*R. lancea*	RL:CA
Bacteroidetes	50.5	44.9	48	45.2	61.5	56.4	61.6	54.7
Firmicutes	38.1	40.1	41.1	41.8	32	38.2	28.4	37.3
F/B ratio	0.75	0.89	0.86	0.92	0.52	0.68	0.46	0.68
F+B	88.6	85	89.1	87	93.5	94.6	90	92

Note: F/B ratio– *Firmicutes*/*Bacteroidetes* ratio; F + B – *Firmicutes* plus *Bacteroidetes* abundances.

The heat trees for pair-wise comparisons of rumen bacteria from the goats fed *C. africana*, control, *R. lancea,* and RL:CA diets during the wet and dry seasons revealed a strong seasonal influence on the abundance of rumen bacteria at the genus and species levels (Figure 4.3). The *Prevotella* genus and *Ruminicola* species were more abundant in the wet season ($p < 0.05$) in goats on the control, *C. africana,* and *R. lancea* diets compared to the dry season.

Tax_rank	Tax_name	Treatment_1	Treatment_2	Mean_diff	Wilcox_p_value
Genus	Prevotella	COD	COW	-0,13	0,05
Genus	Prevotella	RLD	RLW	-0,33	0,03
Genus	Prevotella	CAD	CAW	-0,27	0,05
Species	ruminicola	COD	COW	-0,06	0,05
Species	ruminicola	RLD	RLW	-0,11	0,03
Species	ruminicola	CAD	CAW	-0,08	0,06

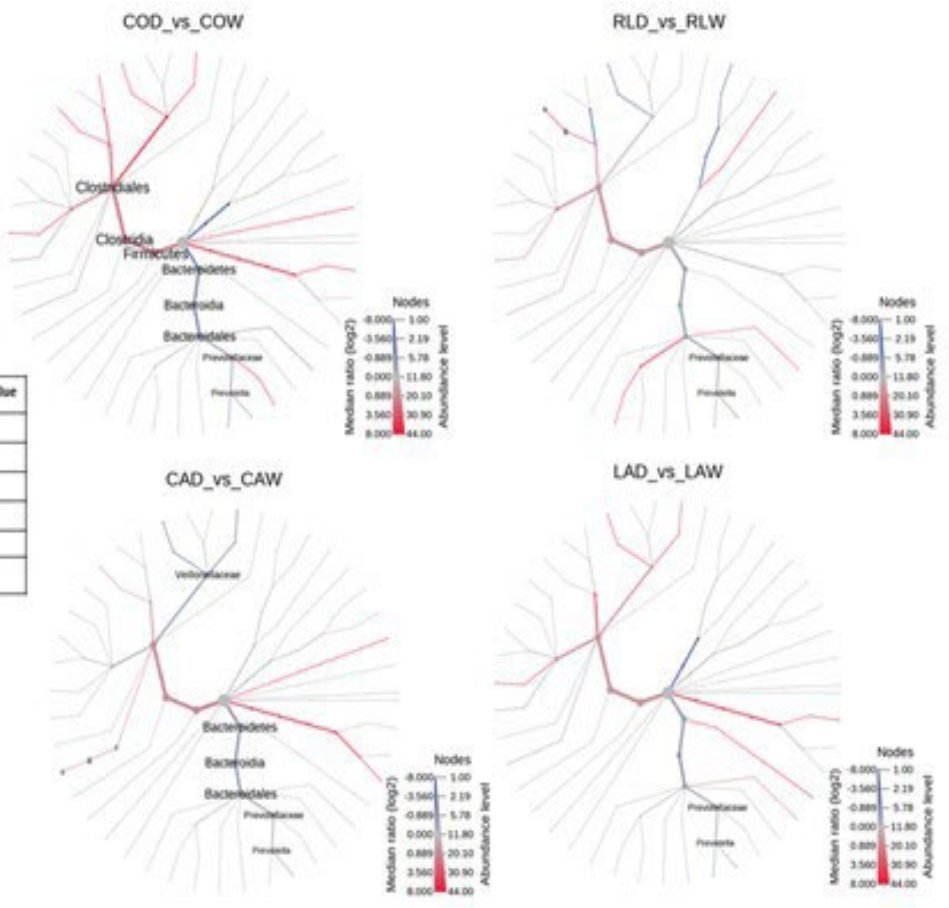


Figure 4.3: Heat trees for seasonal comparisons for rumen bacteria from goats fed *C. africana*, *R. lancea*, RL: CA or control diets in the dry and wet seasons.

Figure 4.4 shows the heat trees for dietary comparisons for rumen bacteria from goats fed *C. africana, R. lancea,* and RL:CA and control diets in the dry and wet seasons. The *Bacteroidia* class and *Bacteroidales* order were significantly ($p < 0.05$) abundant in goats fed on *R. lancea* compared to goats on RL:CA diets during the wet season. Within the *Firmicutes* phylum, the *Clostridia* class, *Clostridiales* order, and *Ruminococcaceae* family were significantly ($p < 0.05$) abundant in goats reared on RL:CA compared to goats on *R. lancea* diet during the wet season. The abundance of *Clostridia* class and *Clostridiales* order in goats fed *R. lancea* diet were significantly ($p < 0.05$) higher in goats fed *C. africana* during the dry season. The abundance of the *Clostridiaceae* family and *Clostridium* genus was significantly ($p < 0.05$) increased in goats fed *C. africana* diets when compared with goats fed a *R. lancea* diet in the dry season.

Tax_rank	Tax_name	Treatment_1	Treatment_2	Mean_diff	Wilcox_p_value
Class	*Bacteroidia*	RLW	LAW	0,12	0,04
Class	*Clostridia*	RLW	LAW	-0,12	0,03
Class	*Clostridia*	RLD	CAD	0,08	0,05
Order	*Bacteroidales*	RLW	LAW	0,12	0,04
Order	*Clostridiales*	RLW	LAW	-0,11	0,03
Order	*Clostridiales*	RLD	CAD	0,08	0,05
Family	*Clostridiaceae*	RLD	CAD	-0,02	0,04
Family	*Ruminococcaceae*	RLW	LAW	-0,02	0,05
Genus	*Atopobium*	RLD	COD	-0,04	0,05
Genus	*Butyrivibrio*	RLD	LAD	-0,03	0,05
Genus	*Clostridium*	RLD	CAD	-0,02	0,04

Note: COD – Control dry season; COW- Control wet season; RLD– Rhus lancea dry season; RLW– Rhus lancea wet season; CAD – Celtis africana dry season; CAW– Celtis africana wet season; LAD –RL:CA dry season; LAW– RL:CA wet season;

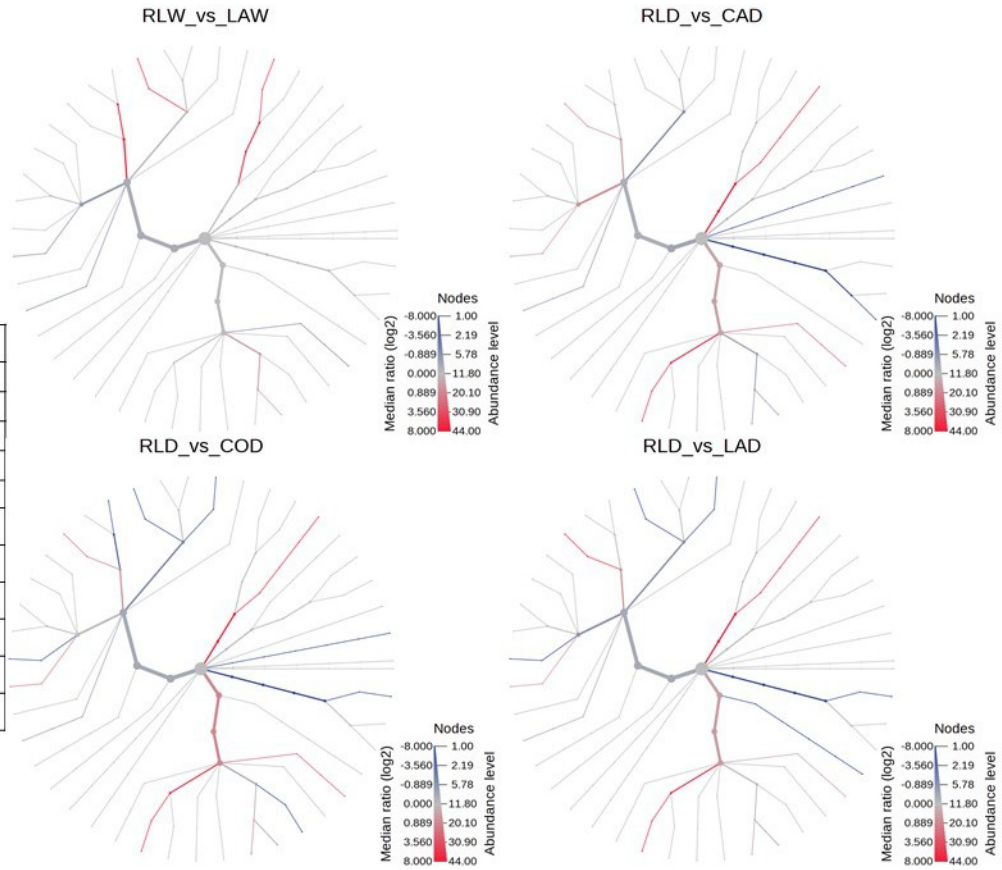


Figure 4.4: Heat trees for dietary comparisons for rumen bacteria from goats fed *C. africana*, *R. lancea*, RL:CA or control diets in the dry and wet seasons.

4.3.2. Community profiling using alpha and beta diversity analyses.

Table 4.2 shows Alpha diversity indexes comparing the ruminal OTUs of goats fed different diets in the wet and dry seasons. The Observed and Chao1 alpha diversity indexes did not display any variation in the richness of rumen bacteria from goats subjected to various diets during wet and dry seasons ($p > 0.05$). Nevertheless, the Shannon and Simpson indexes showed significant richness and evenness variations ($p < 0.05$).

Table 4.2: Alpha diversity indexes comparing the ruminal taxa of goats fed different diets during the wet and dry seasons.

Index	Phylum	Class	Order	Family	Genus	Species
Observed	0.169	0.285	0.290	0.493	0.081	0.076
Chao1	0.638	0.562	0.712	0.741	0.550	0.574
Shannon	0.008*	0.021*	0.041*	0.005**	0.001**	0.004**
Simpson	0.045*	0.064*	0.075	0.002**	0.001**	0.035*

Indexes within a row with an asterisk * are significant at $p < 0.05$, ** at $p < 0.01$.

Figure 4.5 shows the Shannon and Simpson indexes at OTU level across all the samples. The richness and evenness of the treatments across the seasons at the feature level were significantly different ($p < 0.05$) as shown by the Shannon and Simpson indexes (Figure 4.5 a, b).

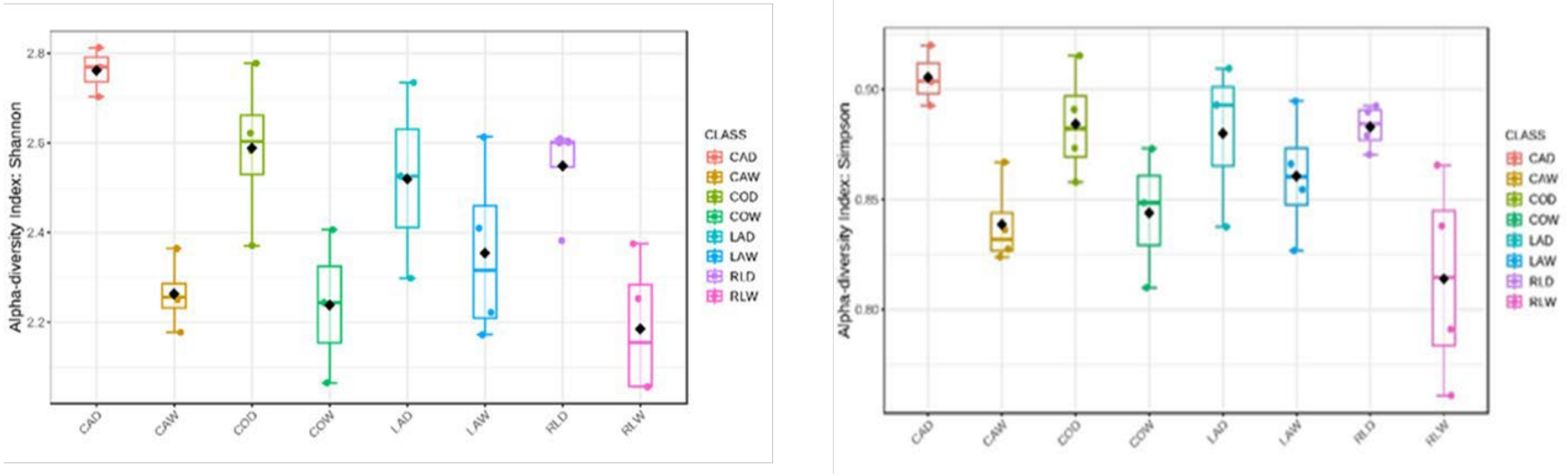


Figure 4.5: Alpha-diversity indexes a) Shannon, b) Simpson showing richness and evenness of rumen microbiota in goats fed *C. africana*, *R. lancea*, control or RL:CA diets over wet and dry seasons.

Note: CAD – Celtis africana dry season; CAW – Celtis africana wet season; RLD – Rhus lancea dry season; RLW – Rhus lancea wet season; LAD –RL:CA dry season; LAW– RL:CA wet season; COD – Control dry season; COW- Control wet season.

Table 4.3 shows the results of assessing the significance of the clustering pattern in ordination plots of ruminal OTUs from goats fed the various diets in the wet and dry seasons using Permutational ANOVA (PERMANOVA), Analysis of Similarities (ANOSIM), and Multivariate Homogeneity of Dispersions (PERMDISP). This indicates that a significant difference was noted in the clustering pattern in ordination plots of ruminal taxa from goats fed the various diets during wet and dry seasons using permutational ANOVA (PERMANOVA) and Analysis of Similarities (ANOSIM) ($p < 0.05$) but not from using Multivariate Homogeneity of Dispersions (PERMDISP) ($p > 0.05$).

Table 4.3: Use of PERMANOVA, ANOSIM and PERMDISP to determine significant differences in bacterial clustering patterns.

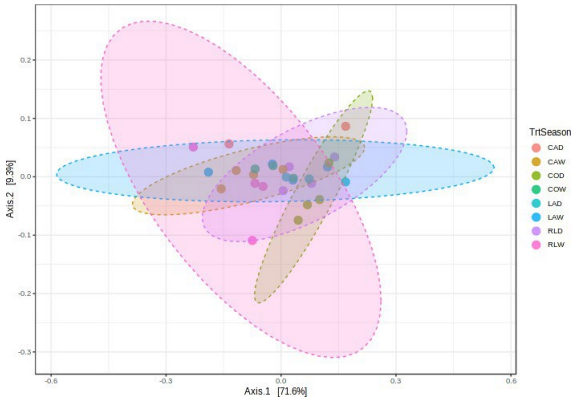
Analysis Method	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
PERMANOVA	0,036*	0,031*	0,029*	0,001**	0,002**	0,001**
ANOSIM	0,029*	0,027*	0,021*	0,001**	0,002**	0,002**
PERMDISP	0,712	0,770	0,816	0,333	0,561	0,802

p-values within a row with an asterisk * are significant at $p < 0.05$, ** at $p < 0.01$.

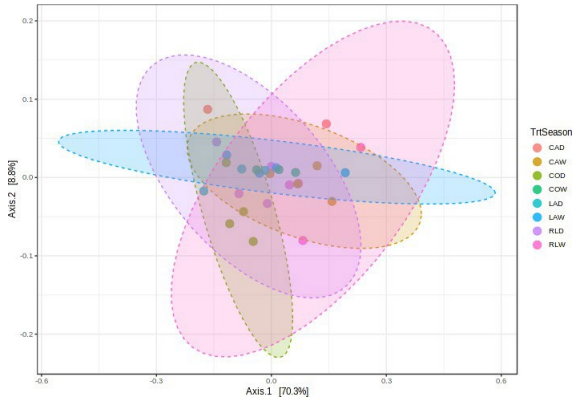
Figure 4.6 shows the analysis of differences amongst samples or groups using beta diversity and expressed as Principal Coordinate Analysis (PCoA) plots. Each axis reflects the percent of the difference between the samples with the x-axis demonstrating the highest dimension of dissimilarity and the y-axis representing the second highest dimension of change. Furthermore, each point or sample showed on PCoA plots is coloured because of the sample group. The group clusters were represented by five ellipses, viewed at various angles and with varying degrees of overlap (or separation) and indicated the ordination plots. Similarities or variances between samples were determined using the non-phylogenetic Bray-Curtis distance. The ‘Bray-Curtis dissimilarity’ uses abundance data and calculates variations in feature abundance. The PERMANOVA results indicated that the clusters of the four diets per season were significantly different ($p < 0.05$) across the phylum, class, order, and family

taxonomic levels. However, results showed that the rumen microbiota in relation to the treatment and season groups clustered together as an indication of a high level of microbial community similarity. Phylum-level analysis accounted for 71.6% of the variation on axis 1 and 9.3% on axis 2, respectively. Similar to the class level, where there were five ellipses (70.3% and 18.8% variance along axes 1 and 2), the order level had five ellipses (69.9% and 10% variance along axes 1 and 2), and the family level had five ellipses (63.8% and 10.4% variance along axes 1 and 2).

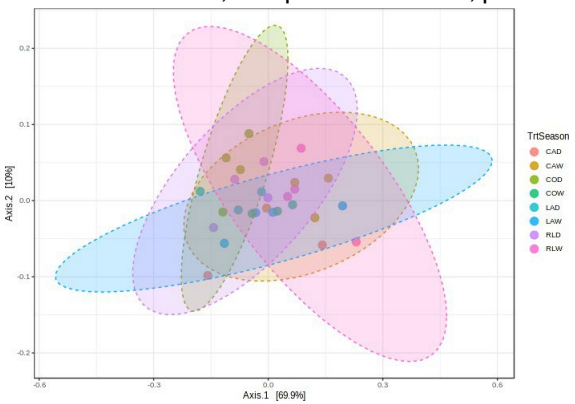
Phylum
F-value: 2.2482; R-squared: 0.44036; p-value: 0.036



Class
F-value: 2.2799; R-squared: 0.44382; p-value: 0.031



Order
F-value: 2.3495; R-squared: 0.45125; p-value: 0.029



Family
F-value: 4.3078; R-squared: 0.60123; p-value: 0.001

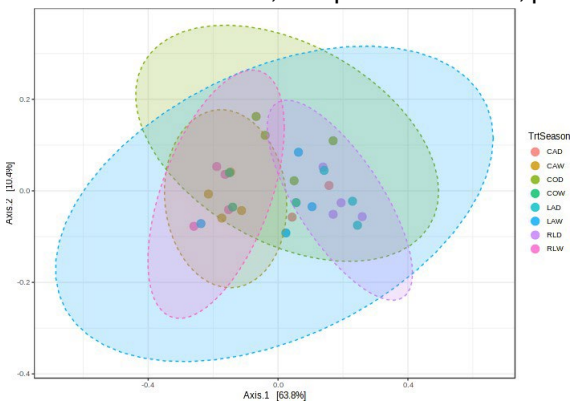


Figure 4.6: Beta diversity analysis expressed as Principal Coordinate Analysis (PCoA) plots of rumen microbiota in goats fed experimental or control diets over wet and dry seasons.

Note: CAD – Celtis africana dry season; CAW– Celtis africana wet season; RLD– Rhus lancea dry season; RLW– Rhus lancea wet season; LAD –RL:CA dry season; LAW– RL:CA wet season; COD – Control dry season; COW- Control wet season.

4.3.3. Clustering analysis using dendrograms, heatmaps and correlation analysis.

The dendrogram in Figure 4.7 displays the clustering relationships of gut microbes in goats that were fed different diets (*C. africana*, control, *R. lancea*, and RL:CA) during wet and dry seasons. There were no visible clustering patterns detected at the phylum, class, order, and family taxonomic levels. Heatmap analyses of rumen microbiota changes from goats offered the various forage diets across all seasons are in Figures 4.8 and 4.9. The expression patterns of OTUs were grouped using a clustering method combined with heatmaps based on their similarity. The heatmap scale on the right of each figure shows the abundance scores. The positive abundancy scores indicate values above the mean, while negative abundancy scores showed values below the mean in standard deviation units. The scaled data were then converted into colours. Hierarchical clustering is based on the Euclidian similarity index.

The phylum taxa that expressed significantly across most treatments were *Firmicutes* and *Bacteroidetes* (All four diets), *Actinobacteria* (*C. africana,* control*,* RL:CA), and *Lentisphaerae* (RL:CA, *R. lancea*). The Phyla clustered into three main groups comprising i) *Spirochaetes*, *Fibrobacteres,* and *Verrucomicrobia*; ii) *Synergistetes*, *Firmicutes,* and *Lentisphaerae* and iii) *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria* and Unclassified. The class taxa expressed differentially abundantly across most diets as follows: *Bacteroidia* and *Clostridia* (all four diets), *Coriobacteriia* (*C. africana,* control*,* RL:CA), and *Lentisphaeria* (RL:CA, *R. lancea*). The following classes clustered together i) *Spirochaetes*, *Fibrobacteria*, *Verruco_5* and *Coriobacteria*; ii) *Gammaproteobacteria*, 4C0d_2 and *Bacteroidia*, and iii) *Synergistia*, *Clostridia,* and *Lentisphaeria*. The order taxa expressed differentially abundantly across most diets as follows: *Clostridiales* and *Bacteroidales* (All four diets) and *Coriobacteriales* (*C. africana,* control*,* RL:CA). Three clusters identified comprised i) *Spirochaetales*, *Fibrobacterales,* and *Coriobacteriales*; ii) *Synergistales*, *Clostridiales,* and alpha proteobacterium *Z20 spp*, and iii) *Bacteroidales*, *Aeromonadales*, *WCHB1_41* and *Victivallales*. The family taxa expressed differentially abundantly across most diets as follows: *Prevotellaceae* and *Ruminococcaceae* (All four diets), *Lachnospiraceae* (control*,* RL:CA, *R. lancea*), *Coriobacteriaceae* (*C. africana,* control*,*

RL:CA), and *Veillonellaceae* (*C. africana,* control). The following families clustered together i) *Spirochaetaceae*, *Lachnospiraceae*, *RFP12* and *Mogibacteriaceae*; ii) R4_45B and *Paraprevotellaceae*; iii) *Coriobacteriaceae, Barnesiellaceae, p_2534_18B5* and *Fibrobacteraceae; iv) Succinivibrionaceae, S24_7* and *Prevotellaceae; v) Ruminococcaceae, Dethiosulfovibrionaceae, Veillonellaceae; and vi) Victivallaceae* and *Clostridiaceae*

Figure 4.7: Dendrograms based on rumen microbiota of goats fed *C. africana*, *R. lancea* or RL:CA diets over the wet and dry seasons.

Note: CAD – Celtis africana dry season; CAW– Celtis africana wet season; RLD– Rhus lancea dry season; RLW– Rhus lancea wet season; LAD – RL:CA dry season; LAW– RL:CA wet season; COD – Control dry season; COW–Control wet season.

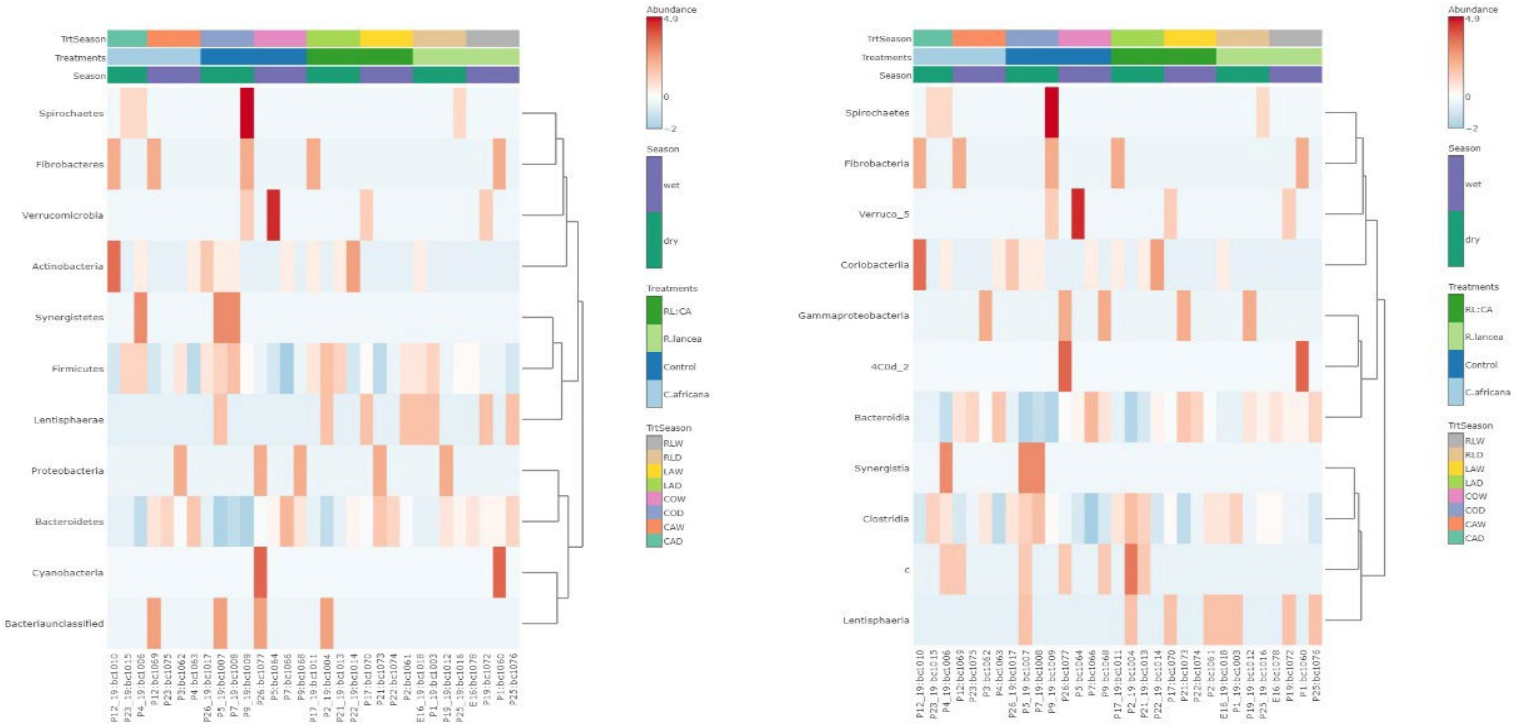


Figure 4.8: Heatmaps showing the phylum (left) and class (right) relative abundances from goats fed various diets over the wet and dry seasons.

Order **Family**

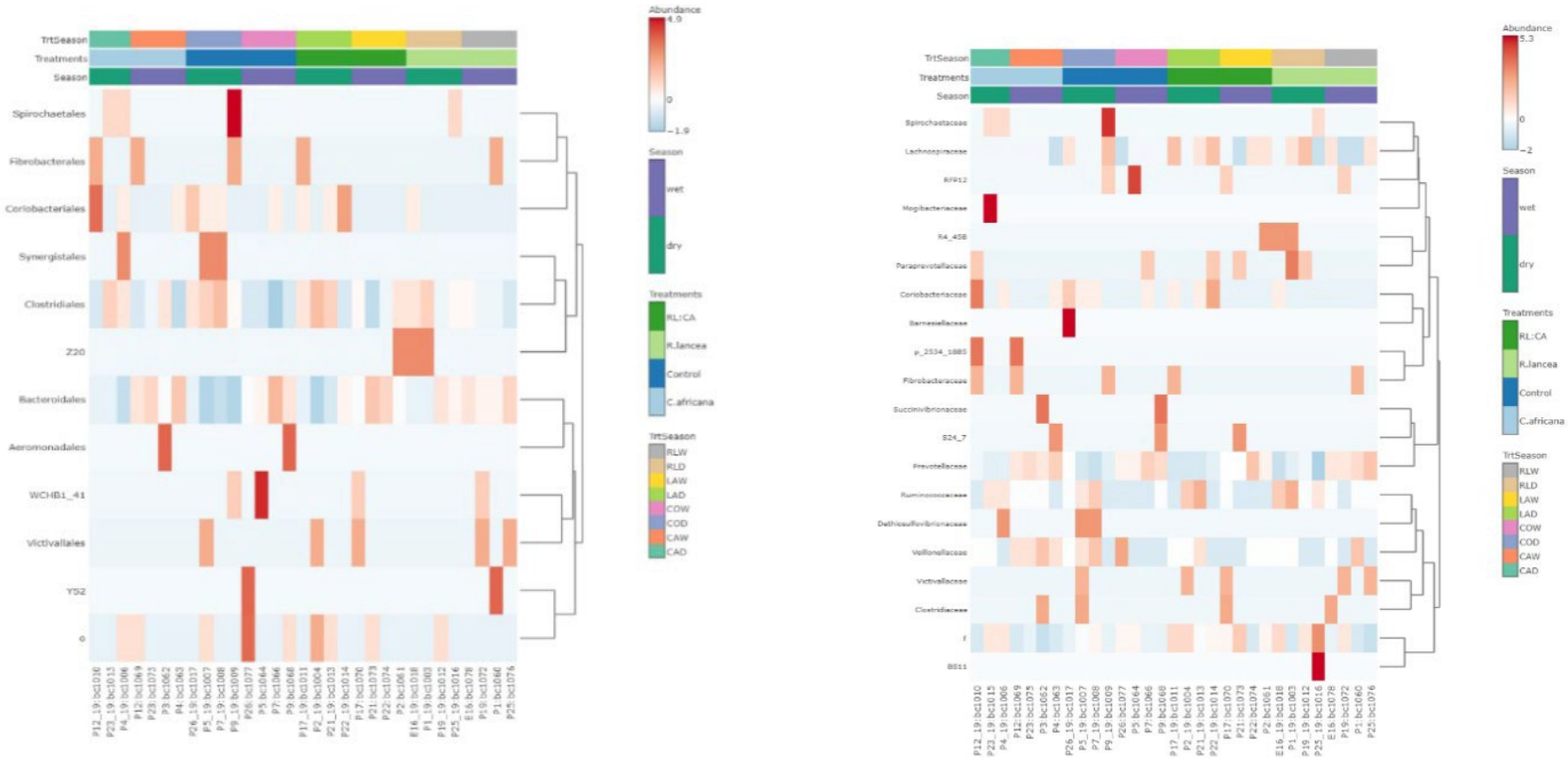


Figure 4.9: Heatmaps showing the order (left) and family (right) relative abundances from goats fed the various diets over the wet and dry seasons.

4.3.4. Differential abundance analysis

Figures 4.10 and 4.11 show the abundance of the rumen bacteria at the genus level in goats fed *R. lancea*, *C. africana*, RL:CA, and control diets during the dry and wet seasons. During the wet season, the phylum *Bacteroidetes* and class *Bacteroidia* were significantly ($p < 0.05$) abundant in goats fed *C. africana*, *R. lancea*, and control diets than in goats fed corresponding diets during the dry season (see Appendices 2 and 3). Family *Prevotellaceae* and genus *Prevotella* were significantly ($p < 0.05$) abundant in goats fed *C. africana* and *R. lancea* during the wet season rather than in goats fed corresponding diets during the dry season. Moreover, the family *Victivallaceae* were significantly ($p < 0.05$) abundant in goats fed RL:CA and *R. lancea* diets than in goats fed *C. africana* and control diets. The family *Mogibacteriaceae* was significantly ($p < 0.05$) abundant in goats fed *C. africana* diets compared to goats fed all the other diets across seasons. In the dry season, the family *Clostridiaceae* and genus *Clostridium* were significantly ($p < 0.05$) abundant in goats fed *C. africana* diets rather than in goats fed the control, RL:CA, and *R. lancea* diets. Additionally, genus *Victivallis* was significantly ($p < 0.05$) abundant in goats fed RL:CA in the dry season than in goats fed *C. africana* (wet and dry seasons), control (dry season), and RL:CA (wet season). Finally, genus *YRC22* was significantly ($p < 0.05$) abundant in goats fed RL:CA in the dry season than in goats fed control (in all seasons), *R. lancea* (wet season), and RL:CA (wet season).

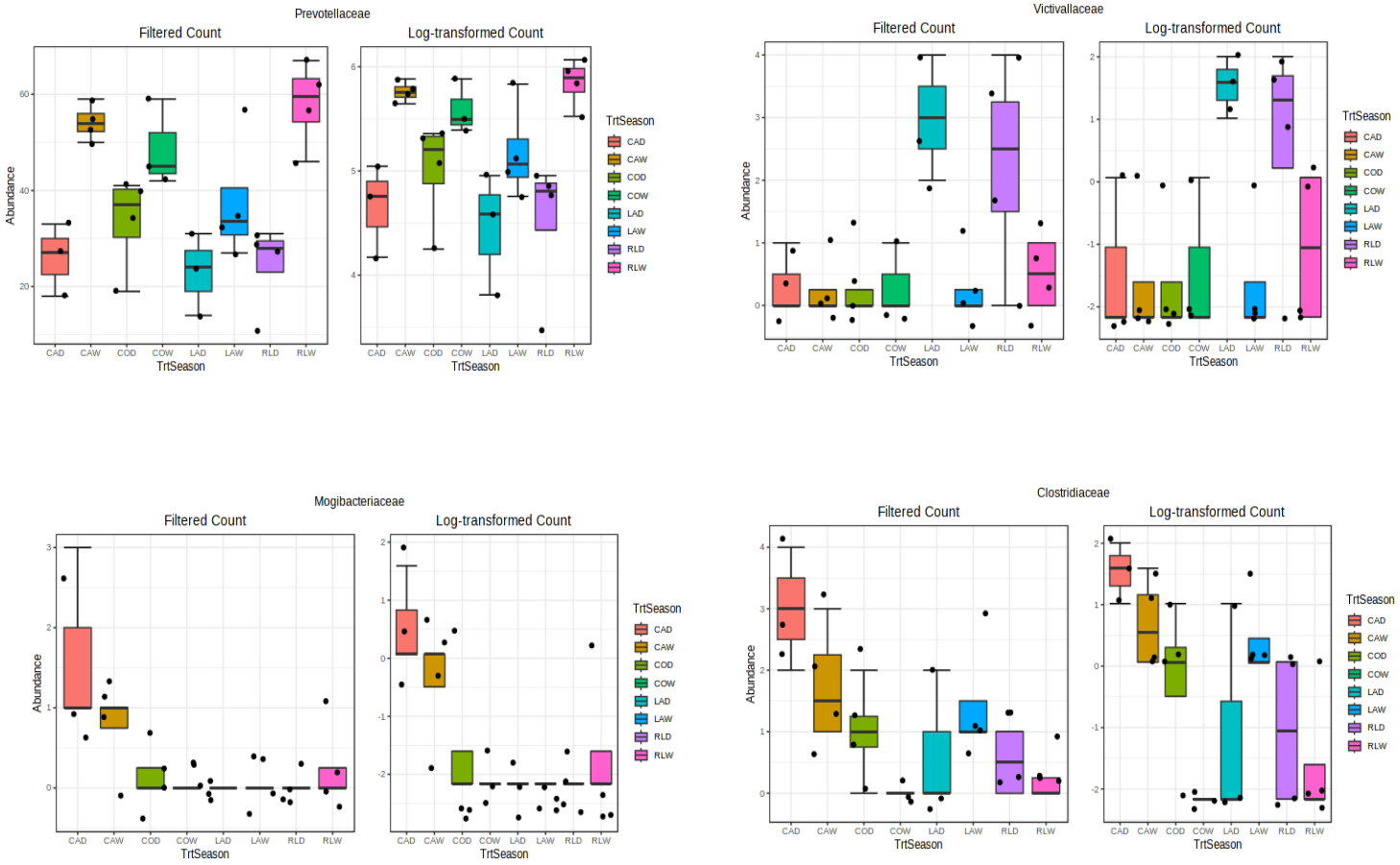


Figure 4.10: Boxplots of univariate analysis of significant rumen bacteria at family level from goats offered *R. lancea, C. africana*, RL:CA or control diets in the dry and wet seasons.

Note: CAD – Celtis africana dry season; CAW– Celtis africana wet season; RLD– Rhus lancea dry season; RLW– Rhus lancea wet season; LAD –RL:CA dry season; LAW– RL:CA wet season; COD – Control dry season; COW– Control wet season.

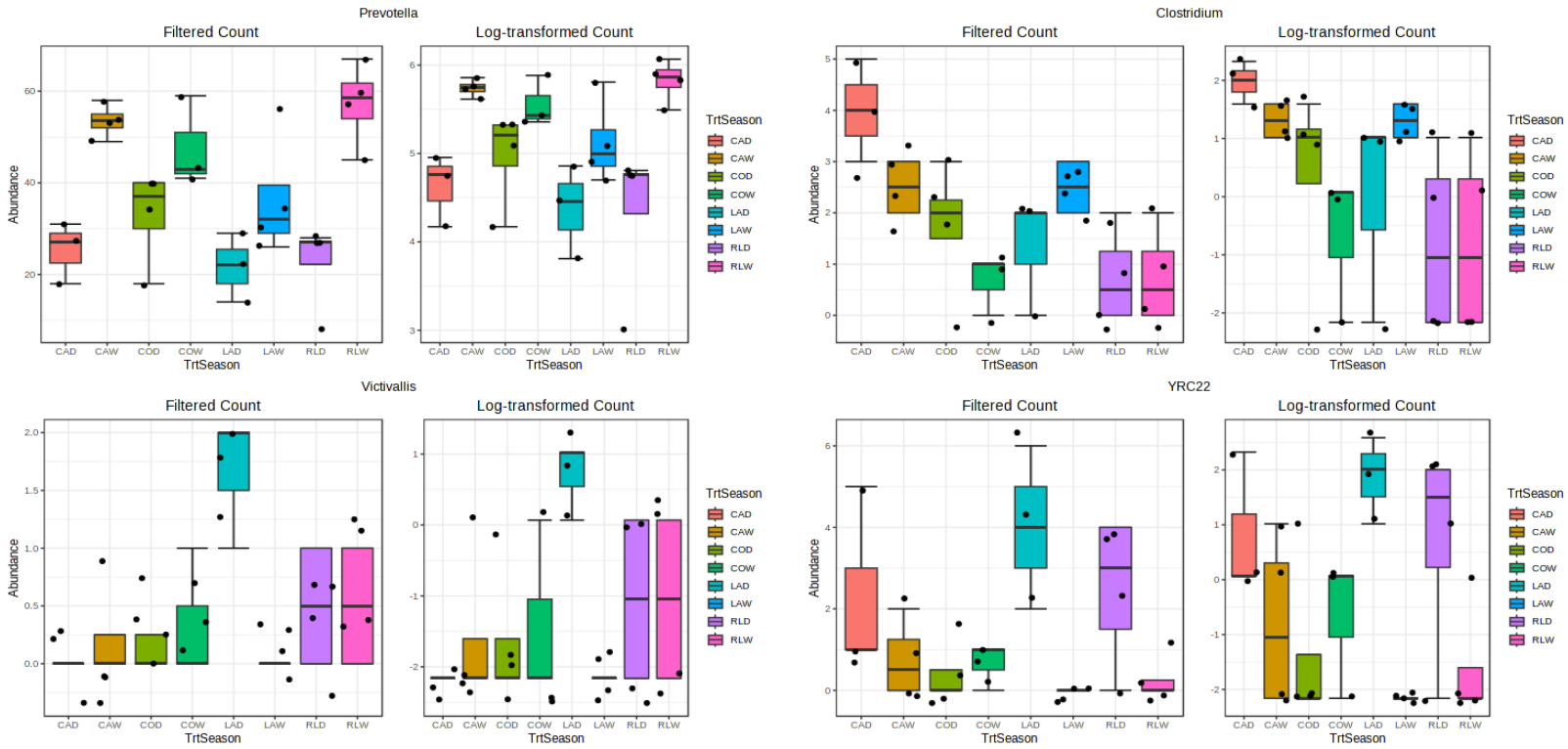


Figure 4.11: Boxplots of univariate analysis of significant rumen bacteria at genus level from goats offered *R. lancea, C. africana*, RL:CA or control diets in the dry and wet seasons.

Note: CAD – Celtis africana dry season; CAW– Celtis africana wet season; RLD– Rhus lancea dry season; RLW– Rhus lancea wet season; LAD –RL:CA dry season; LAW– RL:CA wet season; COD – Control dry season; COW– Control wet season.

4.4. Discussion

4.4.1. Visual exploration

Rumen microorganisms are crucial for feed fermentation, and absorption of various nutrients. They also help to mitigate viral and other pathogenic agents by reacting as antimicrobial reservoirs (Auffret et al., 2017). Therefore, it is important to identify and discuss the factors that determine the rumen bacteria for the goats' overall health (Umanets, 2019). The rarefaction curves showed minimal difference in bacterial diversity across the diets. The minimal difference in bacterial diversity across diets suggests a stable, adaptable, and functionally redundant rumen microbiome in the goats. This observation highlights the complex relationship between diet, host physiology, and microbial ecology in the rumen. Further studies (Nathani et al., 2015; Guo et al., 2020; Zhi et al., 2022) focusing on specific bacterial taxa, functional genes, or metabolic outputs could provide additional insights into how the rumen microbiome adapts to different dietary inputs while maintaining overall community stability.

However, there is a seasonal impact on bacterial diversity for goats fed the *C. africana*, *R. lancea*, RL:CA browse, or control diets. This showed that changes in season influence nutrient composition, subsequently influencing goats' bacterial diversity. It can be deduced from these findings that the bacterial diversity in goats enables them to adapt to a variety of diets in different seasons. It should be noted that the information on rarefaction curves approached but did not reach a plateau, indicating that the sampling depth was not sufficient to capture the full diversity. A deeper sequencing might reveal more subtle differences between diets. An earlier study by Warner (1962) reported that the character of the forage and interval influences the number and kinds of microorganisms within the rumen. On the one hand, Wu et al. (2023) found that forage treatments had no substantial effect on goats' rumen microbial abundance. On the other hand, Umanets (2019) supported the idea that geographical factors and seasonality can affect rumen microorganisms in wild species.

Several studies (Nathani et al., 2015; Umanets 2019; Guo et al., 2020) described the predominance of the *Bacteroidetes* and *Firmicutes* phyla in weaned goat kids subjected to

different forage diets. In the current research, the Ngun' goats' rumen displayed a greater abundance of *Bacteroidetes* compared to *Firmicutes* across all forage diets. However, Min et al. (2019) reported a high abundance of *Firmicutes* compared to *Bacteroidetes* when meat goats grazed on Bermuda grass (*Cynodon dactylon*), Sunn hemp (*Crotalaria juncea*) forages, or combined. Similarly, Domínguez et al. (2022) observed an increased abundance of *Firmicutes* over *Bacteroidetes* in Creole goats fed cacti and *Salicornia*.

Firmicutes and *Bacteroidetes* respond differently even if they are both affected by similar internal and external factors. According to Paster et al. (1994), the *Bacteroidetes* are Gram-negative, chemo-organotrophic rods that don't develop endospores. They are also composed of both motile and non-motile genera that glide. *Firmicutes*, are either Gram-positive or Gram-variable stains, while other phylum classes, such as *Negativicutes*, have both spore-forming and asporogenous individuals depending on the environment (Galperin, 2013). The proportion of *Bacteroidetes* linked to organic decomposition was reported to decline with age, while *Firmicutes* was related to high fibre degradation (Li et al., 2019; Domínguez et al., 2022).

The current research showed that the *Firmicutes* to *Bacteroidetes* (F/B) ratio was higher in the dry season diets (0.85:1) compared to the wet season diets (0.58:1). This was similar to the findings by Guo et al. (2020). These results suggest that the diets during the dry season may have had a higher fibre content. The F/B ratio has been linked to goat physiological performance parameters such as the average daily weight gain (ADG) (Min et al., 2019) and milk fat (Jami et al., 2014). A high F/B ratio was also associated with a decline in the acetate/propionate ratio and enhanced energy extraction from high-fibre diets (Min et al., 2019). The increased proportion of *Bacteroidetes* is negatively related to ADG in meat goats (Zhi et al., 2022). Nonetheless, the results of the current research show that phylum *Bacteroidetes* was abundant in goats during both the wet and dry seasons. Phylum *Bacteroidetes* contain a large number of genes encoding carbohydrate-active enzymes and therefore enhance the catabolism of cellulose, chitin, and ferment amino acids into acetate in the rumen of sheep and goats (Wei et al., 2018; Qiu et al., 2019; Zhang et al., 2021; Islam

et al., 2021; Fan et al., 2023). Phylum *Bacteroidetes* with its dominating non-cellulolytic bacteria *Prevotella* have saccharolytic activities enabling it to bind, and degrade glycans and release oligosaccharides (Naas et al., 2014; Grondin et al., 2017). Their relative abundance in this current research suggests that *R. lancea* and *C. africana* diets provided significant proportions of protein, carbohydrates, and fibre for the goats in accordance with the proposal by Aganga and Mosase (2001), and Naumann et al. (2017).

4.4.2. Community profiles

The alpha-diversity indexes of Shannon and Simpson revealed that the dry season diets exhibited greater bacterial diversity than in the wet season, with the control and *C. africana* treatments showing the highest diversity and *R. lancea* diet exhibiting the lowest diversity. Alpha diversity demonstrates the diversity within a sample, including richness and evenness measurements (Li et al., 2019). The current research study showed that *Firmicutes* and *Bacteroidetes* formed the bulk of rumen phyla in the goats in line with what was reported by Pinnell et al. (2022). They indicate a criterion for the diversity and population composition of the rumen bacteria linked to a type of phylum, class, order, and family. Their abundance could be used to contextualize the impact of season and diet in this current research. The study conducted on Zulu sheep (Xulu et al., 2020) detected the seasonal effect on changes in the rumen microbiome. Similarly, research studies by Shabana et al. (2021), Tian et al. (2021), and Wolf et al. (2021) demonstrated that the richness and evenness of goats' rumen microbiome were influenced by seasonal changes. These findings align with the current study's results on the impact of seasonal changes on the richness and evenness of the rumen microbiome in goats.

The findings from ANOSIM and PERMANOVA analyses indicated that there were significant variations in the diversity and composition of microbial communities in goats across different diets (*C. africana*, *R. lancea*, control, and RL:CA) and across seasons. The results from PERMDISP indicate that the group dispersions (variances) of bacterial communities in goats on the four diets during the two seasons were not significant. In this research study, the non-significant result from PERMDISP implies that the differences observed in PERMANOVA

are solely due to diet and season and not so much by factors directly associated with the goats. Several factors likely contributed to the differences in diversity between diets and seasons. This can include improved nutritional composition such as DM, OM, CP, and NDF of *R. lancea*, *C. africana,* and RL:CA and control diets during the wet season than in the dry season. Forage high in nutrient composition (DM, OM, CP, and NDF) level can influence the results of PERMANOVA. Dry matter and OM are significant in providing substrates with energy for bacterial fermentation in the rumen, this leads to increased bacterial diversity and improved r digestion efficiency in goats and wild concentrate selectors (Mao et al., 2013). In a study by Mao et al (2013) investigating the changes in rumen bacterial community composition in goats fed forage-to-concentrate ratios found that diets with different forage-to-concentrate ratios led to distinct rumen bacterial shifts, highlighting the significance of diet in shaping the bacterial community in the rumen of goats.

Dietary interventions can impact rumen microbial communities in goats. This study demonstrated the effect, as shown by Zhang et al. (2014), Deusch et al. (2017), and Pinnell et al. (2022). Deusch et al. (2017) reported a high abundance of OTU at phylum (*Proteobacteria*) and family (*Succinivibrionaceae*) levels in lactating cannulated Jersey cows offered corn silage-based diet high in starch and sugars. In the context of the season, the temperature change from cold to warm slightly contributed to the difference in the observed diversity. Islam et al. (2021) observed a high Shannon diversity index in spring and summer rather than in winter in Holstein and Jersy steers fed a total mixed ration.

4.4.3. Clustering analyses

Based on the dendrograms, it was not possible to cluster the goats into discernible diet or season-based patterns at phylum, class, order, and family taxonomic levels. This indicates that the rumen bacteria populations within the gut were not consistently affected by either diet or season. One possible explanation for this could be the limited number of experimental goats per diet used in the research study, which may have led to significant variations in rumen bacteria among individual animals, thereby concealing any differences caused by diets.

The Heatmap analyses described the differential abundance of various taxa across different diets. The phylum taxa that were commonly expressed across most treatments were *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Lentisphaerae*. The phyla clustered into three main groups, while the class and order taxa also formed clusters based on their abundance across the diets. At the phylum level, *Actinobacteria* includes gram-positive rumen bacteria which produce α-amylase and along with the *Bifidobacteriaceae* family, they assist in the degradation of polysaccharides in the forage (Deusch et al., 2017; Ratnakomala & Perwitasari, 2020). There was a higher abundance of *Bacteroidales* at the genus level in goats fed *R. lancea* during the dry season. The genus *Bacteroidales* is responsible for the activation of acetate production (Li et al., 2019). According to the literature, acetate is related to proportions of total volatile fatty acids, and therefore high acetate content subsequently indicated high volatile fatty acid levels (Li et al., 2019). The low richness of genus *Clostridiales* in goats fed *C. africana* during the dry season suggests low acetate production and volatile fatty acids. During the wet season, goats fed diets containing *R. lancea* or RL:CA had a high abundance of the phylum *Firmicutes*. This phylum includes various species, orders, and families that possess the potential to catabolize protein and carbohydrates present in the feed (Zhang et al., 2020*).*

Prevotellaceae family was expressed abundantly in goats fed *R. lancea* diet during the wet season. The families that expressed abundantly across most diets were *Prevotellaceae*, *Ruminococcaceae*, *Lachnospiraceae*, *Coriobacteriaceae*, and *Veillonellaceae*, and they also clustered together based on their abundance. The *Clostridiales, Prevotellaceae*, *Ruminococcaceae*, *Lachnospiraceae*, *Coriobacteriaceae,* and *Veillonellaceae* show that they have a common function. They have the potential to degrade specific substrates and are thus classified into different functional groups: cellulolytic, proteolytics, amylolytic, lipolytics, methanogens, anxiolytic, saccharolytic, pectinolytic, acetogenes, ureolytic and acid utilizers in degrading nutrients in the diet (Luo et al., 2004; Choudhury et al., 2015b; Vasta et al., 2019; Yildirim et al., 2021). *Ruminococcaceae* and *Lachnospiraceae*, both belonging to the *Firmicutes* phylum, convert cellulose or xylan into volatile fatty acids that ruminants can utilize (Cammack et al., 2018; Hu et al., 2018). Moreover, research has

demonstrated that specific families and genera within the *Firmicutes* phylum, such as *Lachnospiraceae*, *Veillonellaceae*, *Anaerovibrio*, *Dialister,* and *Acidaminococcus*, are related to increased average daily gain (ADG) in ruminants (Myer et al., 2015; Min et al., 2019).

A decline in CP due to binding with tannins can restrict the CP available for microbial action, providing an advantage to *Proteobacteria* over *Firmicutes* (Cui et al., 2019). In contrast, Zhang et al. (2020) reported a decline in *Proteobacteria* caused by increased CP in Dazu black goats. Cui et al. (2019) found that a diet primarily composed of protein and energy led to a significant increase in *Firmicutes* and *Proteobacteria* in early-weaned Hu lambs. The intricate binding of CP and plant cell wall in the rumen has been discussed (Mkhize, 2008; Dludla, 2010; Barry et al., 2021). The phylum *Proteobacteria* expressed low abundance in goats offered the control diet compared to those on *R. lancea*, *C. africana,* and RL:CA based diets during the dry season. This trend showed that as the CP and energy levels of diet fluctuate, the abundance of *Proteobacteria* also fluctuates during fermentation processes in the rumen (Zhang et al., 2022). As demonstrated by Zhang et al. (2022), enhanced CP concentrations in forage cause a decline in *Proteobacteria* abundance in the rumen. However, this was not observed in goats that were fed *C. africana* and control diets during the wet season. Instead, the current research observed an increase in *Proteobacteria* in goats that were fed *C. africana* and control diets during the wet season, along with an improvement in CP levels. More studies are proposed to investigate the connection between CP levels and *Proteobacteria* abundance in the rumen.

4.4.4. Differential abundance analyses

Research has demonstrated that shifts in ruminal microorganism composition and abundance are caused by SPMs such as TPCs and CT (Vasta & Luciano, 2011; Vasta et al., 2019). Tannins have the potential to inhibit the enzyme activity of ruminal microorganisms, however, the toxic effect depends on the rumen bacterial species and the amount of SPMs consumed by ruminants (Jones et al., 1994). The current research study

showed that the *Clostridia* class and *Clostridiales* order in goats fed *R. lancea* diet were higher when compared to goats fed *C. africana* in the dry season.

The abundance of *Clostridia* class and *Clostridiales* order in goats can be due to the nature and chemical structure of CT and TPCs in *R. lancea* thereby not showing toxic and inhibitory activities of *Clostridia* and *Clostridiales* (Jakhesara et al., 2010). *In vitro* methods demonstrated that the activity of CT against *Clostridium aminophilum*, *Clostridium proteoclasticum,* and *B. fibrisolvens* depended on their chemistry (Sivakumaran et al., 2004; Vasta et al., 2019). However, CT and TPCs may have a specific influence against hemicellulases, endoglucanases, and proteolytic enzymes of numerous rumen microbes including *Fibrobacteres* phylum and *Ruminococcaceae* family (Jones et al., 1994; Bhat et al., 1998; Vasta et al., 2019). This may be the reason they were at low abundances in goats reared on RL:CA and *R. lancea* during the dry season (Giger-Reverdin et al., 2020). Nonetheless, the *Prevotella* genus and *Ruminicola* species expressed genes more abundantly in goats fed the control, *C. africana,* and *R. lancea* diets, respectively, during the wet season compared to the dry season. Genus *Prevotella* is involved in the breakdown of fibre and *Ruminicola* species are involved in the degradation of plant cell wall along with cellulolytic bacteria *Ruminococcus* and *Fibrobacter* (Bekele et al., 2010). Therefore, the abundance of *Prevotella* genus and *Ruminicola* species in goats is a result of the concentrations of fibre in control, *C. africana,* and *R. lancea*-based diet during the wet season. However, the *Ruminococcaceae* family were significantly higher in goats fed RL:CA than those fed the *R. lancea* diet, suggesting that the morphological and mechanical properties of fibre in *R. lancea* were different from that of *C. africana* as a diet in RL:CA mixture during the wet season (Sorieul et al., 2016).

Research conducted by Qiu et al. (2019) assessed the effects of season and diet on rumen bacterial communities. The study found that the diet had a greater impact on bacterial communities than seasonal changes. Specifically, the consumption of *C. africana* browse forage by goats had an adverse consequence on the availability and composition of *Clostridiales* gram-positive anaerobic spores that form bacillus and are commonly found in

animal faeces. Earlier research by Bibbò et al. (2014), and Golić et al. (2017), showed that *Clostridiales* can cause diarrhoea.

The abundance of *Prevotella* and *Clostridiales* is, on the one hand, linked with body weight gain and enhanced performance (De Freitas et al., 2020; Betancur-Murillo et al., 2023). On the other hand, an increased abundance of *Prevotella* might also be a sign of rumen acidosis (Pitta et al., 2010). Cremonesi et al. (2018) observed an abundance of *Bacteroidetes* and *Firmicutes* in female Alpine goats fed *Linum usitatissimum, Cannabis sativa,* and control diet during a 7-day rumen fluid sampling period. The change in nutrient quality of the browse forages (*R. lancea*, *C. africana,* and RL:CA) from the dry season to the wet season resulted in an abundance of *Bacteroidetes* which may have produced more energy and transport of amino acids to maintain metabolism (Wolf et al., 2020). Concerning the forage composition, the results of the current research indicate that TPCs and CT in *R. lancea, C. africana,* and RL:CA browse have the potential to alter rumen bacterial composition, causing a change in the dominant bacteria towards CT-resilient *Enterobacteriaceae* and *Bacteroidetes* communities across seasons (Smith et al., 2003). Findings from this research study and others (Aganga & Monyatsiwa, 1999; Dludla, 2010; Shenkute et al., 2012; Naumann et al., 2017; Mokoboki et al., 2019) reported on the composition of CT and TPCs in *R. lancea* and *C. africana* browse species and found contrasting results as to their concentration and effects on goats' intake and digestibility. As there is limited knowledge about rumen microbial species implicated in the degradation of CT and TPCs in goats (Min et al., 2014), future studies could work on the effect of diet on the microbiome population.

The results also showed that at the class level*, Clostridia,* order *Bacteroidales,* and *Clostridiales* showed an abundance in goats fed *R. lancea* or RL:CA during the wet season, and *C. africana* during the dry season. Whereas with the family *Clostridiaceae,* and *Ruminococcaceae,* at genus level *Atopobium, Butyrivibrio,* and *Clostridium,* were less abundant in goats fed the corresponding forages on the pair-wise taxon comparison. The abundance of phylum *Synergistetes* in goats offered the control diet correlates with its

characteristics because its existence is associated with the environment and host within the rumen (Guo et al., 2020).

4.5. Conclusions

This research was conducted to gain an understanding of the rumen bacterial composition of indigenous Nguni goats fed various types of forages, *Rhus lancea*, *Celtis africana,* and RL:CA mixture during the dry and wet seasons. The research showed that there are limited variations between the diversity of the rumen bacteria in goats fed *C. africana*, *R. lancea*, and RL:CA diets compared to the control diets. The control diets comprised a variety of dietary items influencing bacterial diversity in goats, this shows the importance of diet on bacterial diversity. *Firmicutes* and *Bacteroidetes* were the most abundant rumen phylum, which were attributed to the need to degrade complex nutrients in *Rhus lancea*, *Celtis africana,* and RL:CA diets during both the wet and dry seasons. This research also demonstrated that intake of *R. lancea*, *C. africana,* and RL:CA by goats showed a shift in *Bacteroidetes* and *Firmicutes* (F/B) ratio with higher abundance in the dry season (0.85:1) than in the wet (0.58:1) season. The shift was minimal and related to dietary intake and season, therefore notably assisted in maintaining rumen microbial community homeostasis in goats. The findings suggest that Nguni goats have a resilient and adaptable rumen microbiome, allowing for efficient digestion across varied diets and seasons. This has implications for sustainable goat husbandry, particularly in variable environments where local browse species are available.

Based on our findings, we recommend the following:

1. Given that the control diets with various dietary items influenced bacterial diversity, it's recommended to maintain a diverse diet for goats, even when incorporating specific browse species like *R. lancea* and *C. africana*. This could help ensure a robust and diverse rumen microbiome.
2. The observed shift in the *Firmicutes to Bacteroidetes* (F/B) ratio between seasons suggests that feeding strategies should be adjusted seasonally. Farmers and researchers

can consider increasing the proportion of *R. lancea* and *C. africana* in the dry season diet to support the natural shift in rumen microbial composition.

3. The study demonstrates that *R. lancea* and *C. africana* can be successfully integrated into goat diets without drastically altering the core rumen microbiome. These browse species should be considered valuable feed resources, especially in areas where they are readily available.

4. The F/B ratio can be used as an indicator of rumen health and adaptation to different diets and seasons and to monitor the rumen microbial community composition regularly

5. Farmers and researchers should utilize locally available browse species like *R. lancea* and *C. africana* as sustainable feed resources, particularly during dry seasons.

6. Researchers should focus further research on the functional aspects of the rumen microbiome under different diets and seasons, possibly using metatranscriptomics or metabolomics approaches.

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CHAPTER 5: EFFECTS OF *CELTIS AFRICANA*, *RHUS LANCEA* OR CONCENTRATE SUPPLEMENTATION ON FEED INTAKE, WEIGHT CHANGES, BLOOD METABOLITES AND URINALYSIS IN NGUNI GOATS

Abstract

The study evaluated the feed intake, live-weight changes, urinalysis, and blood metabolite composition in Nguni goats offered *R. lancea* and *C. africana*, or their combination compared to those fed on concentrates. Twenty indigenous male Nguni goats aged around 12 months with an average body weight of 16 ± 1.7 kg (mean ±SD) in the wet season and 15 ± 1.6 kg (mean ±SD) in the dry season were used in this study. The goats received a basal diet of *Eragrostis* hay, supplemented with one of the following: i) *R. lancea*, ii) *C. africana*, iii) a 1:1 combination of *R. lancea* and *C. africana* (RL:CA), or iv) a control diet. Urobilinogen and UBIL in goats fed the control diet were higher than those on *C. africana*, *R. lancea,* and RL:CA during the two seasons (p <0.05). There were season x diet interactions on urobilinogen (URO), urine bilirubin (UBIL), urine protein (UPRO), and urine calcium (UCAL). The goats reared on *R. lancea* had higher (p <0.05) UPRO than those on the RL:CA diet in the wet season. Goats on *C. africana* had higher (p <0.05) serum gamma-glutamyltransferase (GGT) than those on the control or RL:CA diet across seasons. There were significant (p <0.05) diet effects on goats' serum GGT, albumin, alkaline phosphatase, urea nitrogen, creatinine, cholesterol, glucose, and inorganic phosphate across seasons. It was concluded that *C. africana* and *R. lancea* supplementation balance some blood serum parameters, however, it should be investigated further on the renal and hepatic health of the goats.

Keywords: Browse, secondary plant metabolites, urinalysis, ruminants, health.

5.1. Introduction

Goats and wild concentrate selectors (ungulates that consume grass, herbaceous and browse materials) in captivity are at risk of developing diet-related metabolic disorders and are susceptible to potential mortality when they are provided with concentrated-based diets lacking sufficient browse material (Amina et al., 2020). Browse contains secondary plant metabolites (SPM), which could potentially reduce intake and negatively harm goats' health, particularly when consumed inappropriately or in higher concentrations (Makkar, 2003). For example, free-ranging wild concentrate selectors can select browse with low SPM, while those in captivity do not have that privilege. However, the specific extent of this risk is not currently well-defined. Goats have often been modelled for wild concentrate selectors kept in captive environments whose nutrition is a challenge. The current surge in interest in researching alternative animal feed sources, specifically for goats (Ay et al., 2023; Ayaşan et al., 2018; Ekizoğlu et al., 2020), is anticipated to have positive implications for captive wild concentrate selectors. In the Gauteng province of South Africa, goats often feed on *Rhus lancea* (*R. lancea*) and *Celtis africana* (*C. africana*), both of which have been identified as suitable browse options because of their availability (Phiri et al., 2022). The study hypothesized that even small amounts of SPM consumption may cause subclinical pathology in wild concentrate selectors. However, monitoring the subclinical effects of SPM on these animals presents a challenge.

Blood metabolites are important indicators of the physiological state of animals, as well as determinants of the accepted differential responses of these animals to specific conditions such as those presented by seasonal body weight loss (SBWL) (Lérias et al., 2015). There is little information available on the physiological responses of South African Nguni goats on supplemented forage (Dludla, 2010; Phiri et al., 2022). Creatinine, urea, non-esterified fatty acids (NEFAs) and cholesterol showed significant differences in goats due to feed restriction; there was urea and cholesterol reduction after a lower amount of protein and fat ingestion, and a creatinine and NEFAs increment owing to higher metabolization of muscle proteins and fatty acids (Lérias et al., 2015). Urinalysis can also detect metabolic diseases

by measuring glucose and ketone concentrations, and liver diseases based on bilirubin measurement and urolithiasis (Parrah et al., 2013). While urinalysis is crucial for diagnostic purposes, it has not been well investigated in monitoring goats' metabolic health. Therefore, this study was conducted to investigate the physiological responses of Nguni goats when fed *R. lancea*, *C. africana*, or a 1:1 mixture of both browse species and control diet all offered hay grass. The objective was to compare the urine and blood metabolite composition of Nguni goats offered *R. lancea* and *C. africana*, to those fed on the control diet. The objective is to determine the effects of *R. lancea, C. africana*, 1:1 mixture of both browse species and zoological diet on urine and blood metabolite composition.

5.2. Materials and methods

The study was conducted at the Agricultural Research Council-Animal Production (ARC-AP) located in Irene and received ethical permission from the UNISA Animal Ethics Committee and the ARC-AP Ethics Committee. The details of where the animals were sourced and their health status are provided in Chapter 3 section 3.1.

5.2.1. Animals and experimental design

Twenty (n=20) indigenous male Nguni goats aged around 12 months with an average body weight of 16 ± 1.7 kg (mean ±SD) in the wet season and 15 ± 1.6 kg (mean ±SD) in the dry season were adapted for seven days. Data were collected for 30 days during wet and dry seasons respectively. All the goats received 100g DM of *Eragrostis curvula* hay per goat and were allotted to one of four dietary treatments and each treatment was repeated five times. Each of the four dietary treatments offered *ad libitum*, was: i) *R. lancea*, ii) *C*. *africana*, iii) a mixture of the two browse species in a ratio 1:1 (RL:CA), or iv) a control diet comprising *Medicago sativa* (lucerne), green apples, butternut squash, spinach, and commercial pellets representing an unstructured diet. The diets and quantity of forage offered to each goat were outlined in Chapter 3 section 3.1.5. The acquisition and management of the goats were demonstrated in Chapter 3 section 3.1.2. The sample sizes for animals used in this study were not determined using power tests. Instead, they were based on a combination of factors as in chapter 3.

5.3. Data collection

5.3.1. Body weights and feed intake measurements

The weights of goats were recorded three times in each season; at the onset of data collection (day 1), at the intermediate (day 15), and at the end of the trial (day 30) to have a robust evaluation of the responses. The average daily gain (ADG) was determined by dividing the difference between the final weight and the initial weight by the number of days the goats were part of the study. The weights of goats during both the wet and dry seasons differed significantly, metabolic body weight ($BW^{0.75}$) was therefore used for nutritional comparisons across the seasons. This represents the amount of biological tissue in the body using energy and the energy needed by the goat for maintenance when resting (Marzocchi et al., 2020). Feed offered and its refusal from each goat were recorded daily, to evaluate the average daily feed intake (ADFI).

5.3.2. Urine sample collection

Urine samples were collected at the end of each trial during the wet and dry seasons. The samples were collected in the morning (08:00 - 11:00) by attaching a clean transparent plastic bag to the ventral abdomen and incorporating the preputial orifice of the goat (Mui et al., 2002; Jia et al., 2009; Raghu, 2015). A transparent plastic bag was used for ease of observation, and to minimise the handling of the goats. The plastic bag was gently held in position with masking tape, to ensure comfort and minimise stress. The urine was transferred into sealable tubes of 50 mL and kept frozen (-20°C) in the freezer, until urinalysis.

5.3.3. Blood sample collection

Blood samples were collected (08:00 to 09:00) at the onset and at the end of the trial before feeding, this was achieved by using an electrical clipper to shave the hair around the jugular vein area, for clear visualisation and hygiene. Thereafter, alcohol wipes were used to sterilize the site, and a needle (21 G x 38 mm) with a green hub (triple bevelled point for smooth venous puncture) was used to puncture the skin into the vein. Approximately 5 ml

blood sample was drawn into 4 ml SST Gel and glucose vacutainer tubes (IDEXX Laboratories Pty Ltd, Kyalami, Johannesburg, South Africa). The blood samples were stored in a cooler box with an icepack and transported to IDEXX laboratories for analysis (IDEXX Laboratories Pty Ltd, Kyalami, Johannesburg, South Africa).

5.3.4. Chemical analyses

The chemical analysis of feed for dry matter (DM), ash and crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), and acid detergent lignin (ADL) was done as outlined in Chapter 3 section 3.1.6.1. The total phenolic compounds (TPCs) and condensed tannins (CT) were determined, as outlined in Chapter 3 sections 3.1.6.2 and 3.1.6.3, respectively. The serum metabolite levels were determined using IDEXX Vettest® Chemistry Analyzer (IDEXX Laboratories, Inc., Westbrook, ME. USA). The General Health Profile (GHP) evaluated, comprised of serum albumin (SALB), alkaline phosphatase (ALP), alanine amino transaminase (ALT), gamma-glutamyltransferase (GGT), amylase (AMYL), serum urea nitrogen (SUN), serum calcium (SCAL), cholesterol (CHOL), serum creatinine (SCR), globulin (GLOB), glucose (GLU), inorganic phosphate (IPHOS) and serum total protein (STP). Urine samples were analysed for Leukocytes (LEU), ketones (KET), nitrite (NIT), urobilinogen (URO), urine bilirubin (UBIL), urine glucose (UGLU), urine protein (UPRO), urine specific gravity (USG), pH, urine blood (UBLD), ascorbic acid (ASC), urine microalbumin (UMA), urine calcium (UCAL), urine creatinine (UCR), and a calculated urine protein creatinine ratio (UPC) using a portable urine VetScan UA® analyzer (Zoetis, Parsippany, New Jersey, USA).

5.3.5. Statistical analyses

The DM intake per metabolic weight, final metabolic weight, ADG, FCR and the blood metabolites (SALB, ALP, ALT, GGT, AMYL, SCR, BUN, SCAL, CHOL, SCR, GLOB, GLU, PHOS, and STP) per season and urine analytes (URO, UBIL, UGLU, UPRO, USG, pH, UMA, UCAL and UCR) were analyzed using the GLM procedures in Stata (version 17, 2019). The following model was used:

$y_{ijk} = \mu + T_i + \beta_j + (T\beta)_{ij} + \epsilon_{ijk}$

Where:

μ = the baseline mean.

T_i = the seasonal effects (dry and wet seasons).

β_j = the dietary effects (*R. lancea*, *C. africana*, RL:CA and control).

$(T\beta)_{ij}$ = the ijth season x diet interaction effects.

ϵ_{ijk} = the random error of the kth observation from the ij cell.

Significance was set at $p < 0.05$. Means were compared via the adjusted Bonferroni test. The daily mean intake of DM, CP, CT and TPC expressed per metabolic weight, were plotted on graphs using the marginsplot command of Stata (version 17, 2019).

5.4. Results

Table 5.1 shows the nutrient intake (g/kg $BW^{0.75}$/d) comprising organic matter intake (OMI), crude protein intake (CPI), neutral detergent fibre intake (NDFI), acid detergent fibre intake (ADFI), acid detergent lignin intake (ADLI), and condensed tannin intake (CTI) and total phenolic compounds intake (TPCI) in diets based on *C. africana*, *R. lancea*, RL:CA and Control measured across the two seasons. There were significant season x diet interactions ($p < 0.05$) on OMI, CPI, NDFI, ADFI, ADLI, CTI, and TPCI ($p < 0.05$). The goats on *C. africana*, *R. lancea* or RL:CA diets had higher OMI, CPI, NDFI, ADFI and ADLI ($p < 0.05$) during the wet season compared to the dry season. Goats on the control diet had higher ($p < 0.05$) OMI, CPI, NDFI and ADFI than goats on *R. lancea, C. africana* or RL:CA diets across seasons. The goats fed *R. lancea* had higher OMI and CPI ($p < 0.05$) than goats fed *C. africana* during the dry season. There were higher OMI and CPI ($p < 0.05$) in goats fed *C. africana* than in goats on *R. lancea* or RL:CA diets during the wet season. During the dry season, the goats fed the control diet had higher NDFI ($p < 0.05$) than those on *C. africana* and RL:CA diets. There were higher ADFI and ADLI ($p < 0.05$) in goats on *C. africana* than goats on the *R. lancea* or RL:CA diets in the dry season. Goats on the control diet had similar ($p > 0.05$) CTI and TPCI across seasons. Goats on the *C. africana* diet had higher CTI and TPCI ($p < 0.05$) during the wet season compared to the dry season, and those on the RL:CA diet had higher CTI and TPCI ($p < 0.05$) during the dry than wet season. Goats on *R. lancea* diet had higher TPCI ($p < 0.05$) during the wet season compared to the dry season.

Table 5.1: Nutrient intake (g/ kg $BW^{0.75}$/d) in Nguni goats offered *Celtis africana*, *Rhus lancea,* RL:CA, or control diets during the wet and dry seasons.

		Parameter (g/ kg $BW^{0.75}$/d)						
Season	**Browse**	OMI	CPI	NDFI	ADFI	ADLI	CTI	TPCI
Dry	*C. africana*	39.3^{a}	3.4^{a}	16.9^{a}	9.9^{a}	1.8^{b}	1.2^{b}	0.6^{b}
	Control	73.0^{f}	15.3^{f}	25.7^{d}	16.3^{d}	1.8^{b}	0.1^{a}	0.1^{a}
	R. lancea	44.5^{b}	4.9^{b}	17.9^{a}	10.7^{ab}	1.4^{a}	5.5^{f}	3.1^{f}
	RL:CA	39.7^{a}	3.7^{a}	16.6^{a}	9.7^{a}	1.4^{a}	4.0^{e}	2.3^{e}
Wet	*C. africana*	64.6^{e}	10.2^{d}	23.0^{bc}	39.5^{e}	2.4^{d}	2.0^{c}	1.1^{c}
	Control	59.9^{cd}	11.6^{e}	23.3^{bc}	13.1^{c}	1.8^{b}	0.1^{a}	0.1^{a}
	R. lancea	62.5^{d}	7.0^{c}	23.8^{c}	14.1^{c}	1.7^{b}	5.7^{f}	4.5^{g}
	RL:CA	56.6^{c}	7.4^{c}	22.1^{b}	12.5^{bc}	2.1^{c}	3.3^{d}	2.1^{d}
SEM		1.07	0.17	0.37	0.41	0.04	0.08	0.05
CV (%)		31	55	26	63	32	76	85
p-values								
Season		0.001	0.001	0.001	0.001	0.001	0.001	0.001
Browse		0.001	0.001	0.001	0.001	0.001	0.001	0.001
Season*Browse		0.001	0.001	0.001	0.001	0.001	0.001	0.001

^{abcd} Means within a column that do not share a common superscript differ significantly at the $p < 0.05$. **Browse**; *C. africana* = *Celtis africana*, *R. lancea* = *Rhus lancea*, RL:CA = *Rhus lancea* and *Celtis africana* 1:1 mixture. **Parameter;** OMI = organic matter intake, CPI = crude protein intake, NDFI = neutral detergent fibre intake, ADLI = acid detergent lignin, CTI = condensed tannins intake, TPCI = total phenolic compounds intake. SEM = standard error of the mean, CV = coefficient of variation.

Figure 5.1 indicates the daily dry matter and crude protein intake per kg metabolic body weight of goats fed *C. africana*, *R. lancea*, RL:CA or control diets. Goats on the control diet had a higher daily DMI and CPI than goats on the other diets during the dry season. During the wet season, there was no variation in DMI and CPI across the diets. Goats consuming *C. africana* had a numerically higher CPI than those consuming *R. lancea* or RL:CA. The

daily total phenolic compounds and condensed tannins intake per kg metabolic body weight of goats fed *C. africana*, *R. lancea*, RL:CA and control diets are shown in Figure 5.2. Goats on the *R. lancea* diet had higher CTI and TPCI than those on the *C. africana*-based diet.

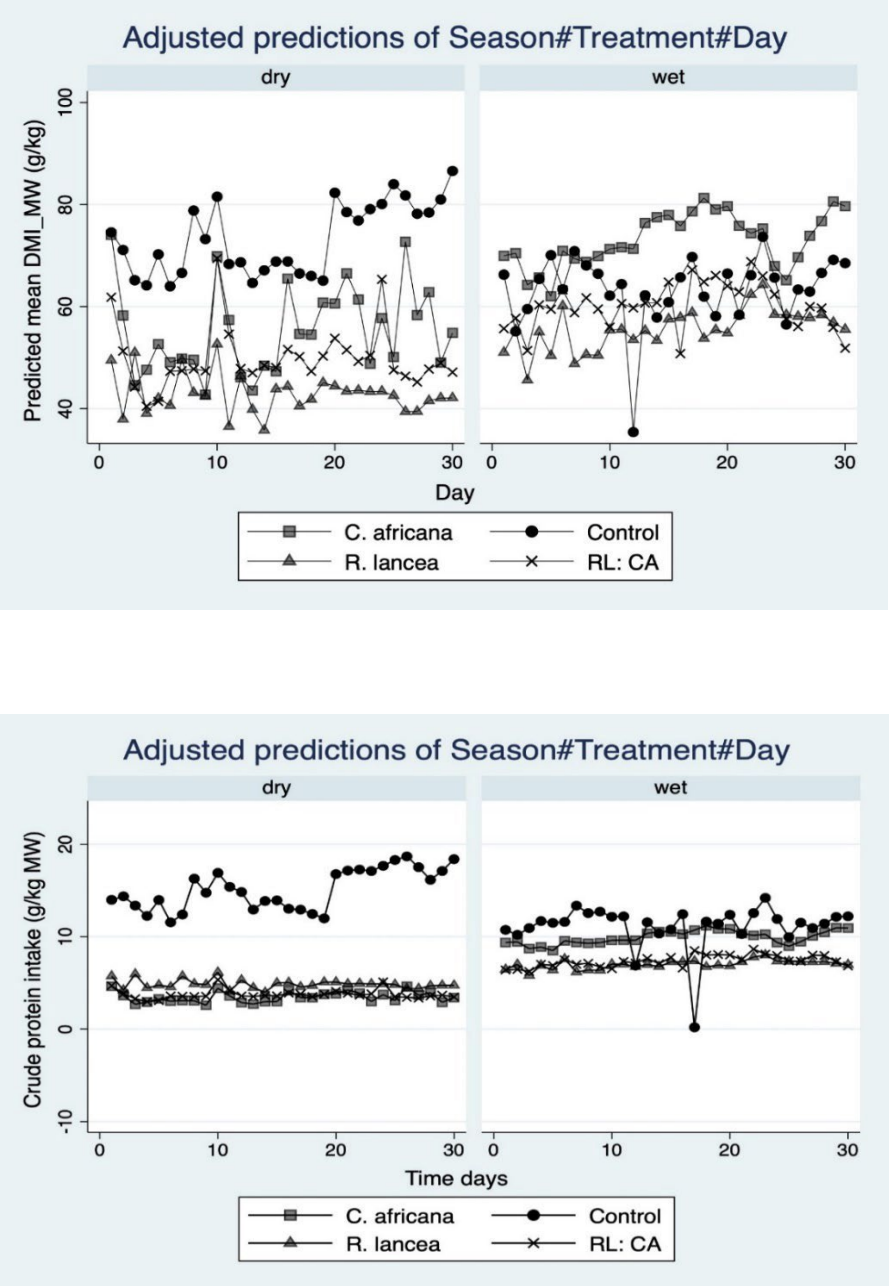
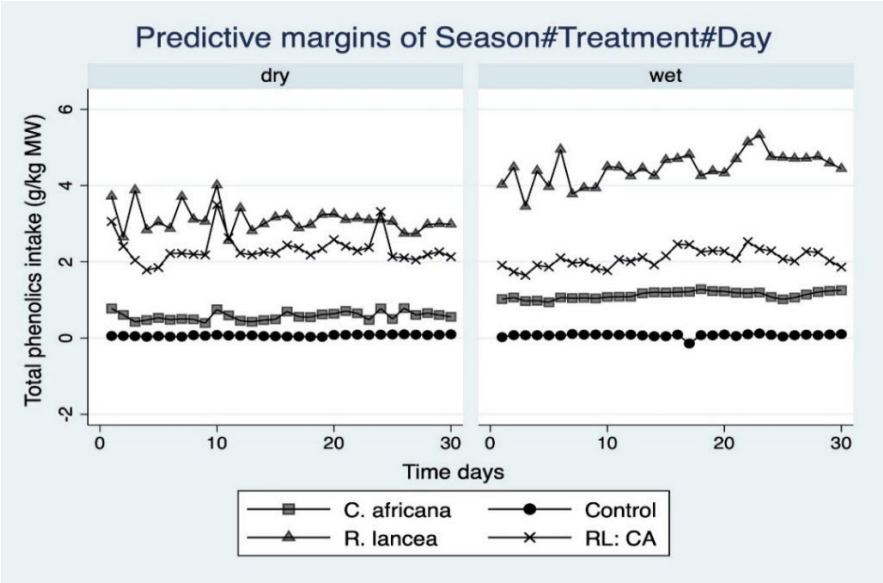


Figure 5.1: Daily dry matter and crude protein intake per kg metabolic weight of goats fed *C. africana*, *R. lancea*, RL:CA or control diets.



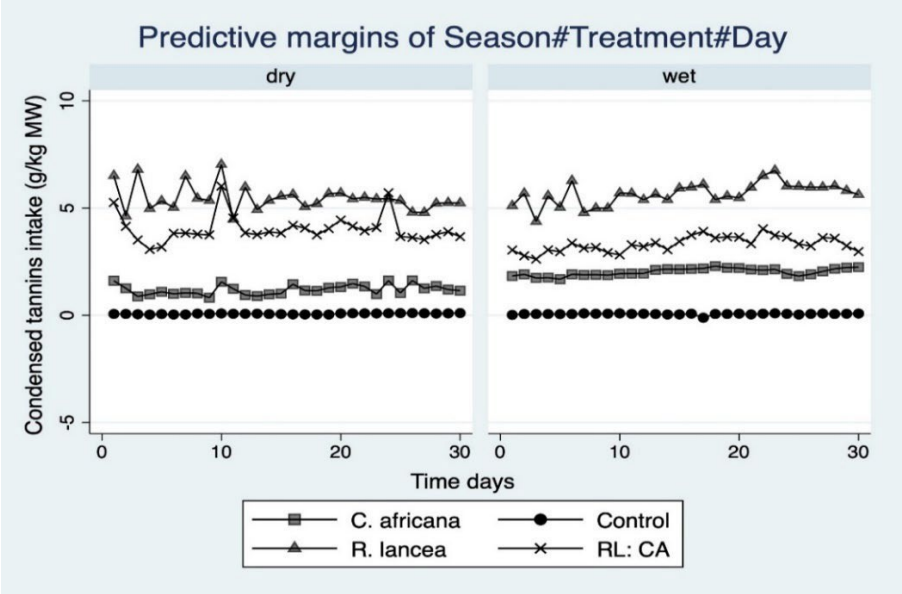


Figure 5.2: Daily total phenolics and condensed tannin intake per kg metabolic weight of goats fed C. *africana*, *R. lancea*, RL:CA or control diets.

The metabolic body weight parameters of Nguni goats offered *C. africana*, *R. lancea,* and RL:CA forage and a control diet during the wet and dry seasons are in Table 5.2. The initial body weight of goats fed *R. lancea* was high ($p < 0.05$) compared to goats fed with *C.*

africana and RL:CA during both the dry and wet seasons. Goats on the control diet had higher final metabolic body weight ($p < 0.05$) than goats on *R. lancea, C. africana* and RL:CA diets during the dry season, while there were no differences ($p > 0.05$) in the final weights of the goats across the diets during the wet season. During the dry season, goats on *R. lancea* had higher final metabolic body weight ($p < 0.05$) than goats on *C. africana* and RL:CA during the dry season.

During the dry season, goats on the control diet had higher ($p < 0.05$) and positive weight gain while the goats on *C. africana, R. lancea* or RL:CA lost weight and had lower ADG. There were significant ($p < 0.05$) interactions between season and browse on final metabolic body weight, ADG, DMI and FCR ($p < 0.05$). Goats on *C. africana*, RL:CA and control diets had similar ($p > 0.05$) ADG during the wet season. Goats on the *C. africana* and RL:CA diets had higher DMI ($p < 0.05$) during the wet season than the dry season while goats on the control and *R. lancea* diets had similar DMI ($p > 0.05$) during both the wet and dry seasons. Goats fed with *C. africana* had a higher DMI ($p < 0.05$) than goats on the *R. lancea* diet during the wet season. Goats on the control diet had higher DMI ($p<0.05$) than goats on the other three diets during the dry season. Only the goats on the control diet had a positive FCR during the dry season. During the wet season, goats on the control diet had a lower FCR ($p < 0.05$) than goats on the *R. lancea* diet.

Table 5.2: Dry matter intake and body weight parameters (kg $BW^{0.75}$), unless otherwise stated) of Nguni goats offered control diet, *C. africana, R. lancea* or RL:CA forage during the wet and dry seasons**.**

Season	Browse	Parameters				
		Initial weight kg $BW^{0.75}$	Final weight kg $BW^{0.75}$	ADG g/day	DMI g/kg $BW^{0.75}$/d	FCR
Dry	*C. africana*	8.1^{cd}	7.5^{b}	-20.0^{ab}	52.8^{ab}	-2.6^{a}
	Control	8.5^{de}	9.8^{d}	43.3^{d}	71.3^{cd}	1.6^{bc}
	R. lancea	9.4^{e}	8.6^{c}	-26.6^{a}	42.7^{a}	-1.6^{ab}
	RL:CA	7.9^{cd}	7.1^{ab}	-26.6^{a}	48.6^{ab}	-1.8^{ab}
Wet	*C. africana*	6.5^{ab}	7.0^{ab}	16.7^{c}	78.9^{d}	4.7^{cd}
	Control	6.3^{a}	6.6^{ab}	10.0^{c}	62.3^{bc}	6.2^{bc}
	R. lancea	7.3^{bc}	7.3^{ab}	0^{bc}	56.9^{abc}	0^{d}
	RL:CA	6.1^{a}	6.4^{a}	10.0^{c}	67.9^{cd}	6.8^{bc}
SEM		0.23	0.24	15.78	3.27	3.58
CV (%)		15.8	15.3	-	21.8	-
p-values						
Season		0.0001	0.184	0.0001	0.0001	0.0001
Browse		0.0001	0.0001	0.0001	0.0001	0.0001
Season* Browse		0.156	0.0001	0.0001	0.0001	0.001

abcd Means within a column that do not share a common superscript differ significantly at the $p < 0.05$. **Browse**; *C. africana* = *Celtis africana*, *R. lancea* = *Rhus lancea*, RL:CA = *Rhus lancea* and *Celtis africana* 1:1 mixture. **Parameters**; $BW^{0.75}$ = metabolic body weight, ADG = average daily gain, DMI = dry matter intake, FCR = feed conversion ratio. SEM = standard error of the mean, CV = coefficient of variation.

The concentrations of URO (urobilinogen), UBIL (urine bilirubin), urine protein (UPRO), urine specific gravity (USG), pH, urine microalbumin (UMA), urine calcium (UCAL), urine creatinine (UCR) and Urine Protein to Creatinine ratio (UPC) in Nguni goats offered *C. africana*, *R. lancea* or RL:CA mixture across the two seasons are shown in Table 5.3. The urine samples from all the goats across the treatments did not show any trace of leucocytes, glucose, or red blood cells. As a result, these parameters were not reported. There were significant ($p < 0.05$) interactions between season and browse on URO, UBIL, UPRO, USG, pH, UCAL and UPC. Goats had similar URO, UBIL, UPRO, USG, pH, UCAL and UPC values ($p > 0.05$) in all diets during the dry season. During the wet season, goats on the control diet had significant ($p < 0.05$) higher URO and UBIL concentrations than goats on the other three diets. Goats on the control diet had higher UPRO concentrations ($p < 0.05$) than goats on the *R. lancea* diet, and goats on the *R. lancea* diet had higher USG values ($p < 0.05$) than goats on the control diet. Similarly, during the wet seasons goats on the control diet had a higher pH and UPC ratio ($p < 0.05$) than those on the *R. lancea* or RL:CA diets.

Table 5.3: Urinalysis of Nguni goats offered control diet, *C. africana*, *R. lancea* or RL:CA mixture during the wet and dry seasons.

		Parameters								
Season	**Browse**	**URO mg/dL**	**UBIL mg/dL**	**UPRO mg/dL**	**USG**	**pH**	**UMA mg/L**	**UCAL mg/dL**	**UCR mg/dL**	**UPC**
Dry	*C. africana*	0.3[a]	0.2[a]	143[ab]	1.013[ab]	8.7[b]	1.0	23.3[b]	100	1.6[ab]
	Control	1.0[a]	0.3[a]	92ab	1.010[ab]	8.9[b]	0.8	21. 7[b]	125	1.4[ab]
	R. lancea	1.0[a]	0.3[a]	186[ab]	1.010[ab]	8.5[ab]	0.5	20.0[b]	88	2.0[ab]
	RL:CA	0a	0.0[a]	158[ab]	1.010[ab]	8.6[b]	0.6	22.0[b]	100	0.9[a]
Wet	*C. africana*	0.4[a]	0.1[a]	220[ab]	1.016[abc]	8.0[ab]	1.0	12.0[a]	110	2.3[ab]
	Control	3.5[b]	1.6[b]	108[ab]	1.003[a]	9.0[b]	1.0	12.5[a]	113	3.4[b]
	R. lancea	0a	0.0[a]	300[b]	1.029[c]	6.9[a]	0.6	20.0[b]	150	0.2[a]
	RL:CA	0.5[a]	0.0[a]	24[a]	1.023[bc]	6.8[ab]	0.8	22.5[b]	150	0.5[a]
SEM		0.468	0.20	52.2	0.0035	0.36	0.18	2.99	30.7	0.24
CV (%)		172	218	57	0.34	4.5	25	16.6	27.13	101
Reference values					1.020-1.040	7.5-8.5	Neg to 0	8.82-12.22	5.0-13.6	
p-values										
Season		0.913	0.799	0.268	0.560	0.167	1.00	0.004	0.806	0.295
Browse		0.252	0.505	0.458	0.445	0.587	0.053	0.430	0.519	0.347
Season* Browse		0.006	0.0001	0.042	0.017	0.002	0.170	0.045	0.386	0.012

[abcd] Means within a column that do not share a common superscript differ significantly at the p <0.05. **Browse;** *C. africana* = Celtis africana, *R. lancea* = *Rhus lancea*, RL:CA = *Rhus lancea* and *Celtis africana* 1:1 mixture. **Parameters**; URO = urobilinogen, UBIL = urine bilirubin, USG = urine specific gravity, pH = acidity and alkaline, UMA = urine microalbumin, UCAL = urine calcium, UCR = urine creatinine, UPC = Urine protein to creatinine ratio. SEM = standard error of the mean, CV = coefficient of variation. Neg = negative. Reference values = Parrah et al. (2013), David et al. (2015).

Table 5.4 shows the concentrations of gamma-glutamyl transferase (GGT), serum total protein (STP), albumin (ALB), globulin (GLOB), alanine transaminase (ALT), alkaline phosphatase (ALP), serum urea nitrogen (SUN), serum creatinine (SCR), serum calcium (SCAL), cholesterol (CHOL), serum glucose (SGLU), inorganic phosphate (IPHOS), total bilirubin (TotalBil), and Amylase (AMYL), in Nguni goats offered *C. africana*, *R. lancea* or RL:CA mixture across the two seasons. There were significant ($p < 0.05$) interactions between season and browse on GGT, CHOL and SGLU concentrations. During the wet season, goats had similar GGT, CHOL and SGLU concentrations ($p > 0.05$) across all the diets. During the dry season, goats on the *C. africana* diet had lower serum GGT concentrations than those on the other three diets ($p < 0.05$). During the dry season, goats on the *C. africana* and RL:CA diets had higher serum CHOL concentrations than goats on the control diet ($p < 0.05$). During the dry season, goats on the control diet had higher SGLU serum concentrations ($p < 0.05$) than goats on the other three diets. There were seasonal and browse effects ($p < 0.05$) on ALB, SUN and SCR concentrations. During the dry season, goats on the RL:CA diet had higher serum ALB concentrations ($p < 0.05$) than those on the control and *R. lancea* diets during the wet season.

The goats' SUN concentrations during the wet season were higher ($p < 0.05$) than during the dry season, across all the diets. Goats on the control diet had higher SUN concentrations ($p < 0.05$) than goats on the other three diets in each season. During the wet season, goats had similar SCR concentrations across all the diets. Goats on *C. africana*, *R. lancea* or RL:CA during the dry season had higher SCR concentrations than those on the same diets during the wet season ($p < 0.05$). There were seasonal effects ($p < 0.05$) on SCAL and TotalBil concentrations. Goats on the control and *R. lancea* diets had higher SCAL concentrations during the dry season than during the wet season ($p < 0.05$). Goats on *C. africana* and *R. lancea* diets had higher serum TotalBil concentrations during the wet season than during the dry season ($p < 0.05$). There were browse effects ($p < 0.05$) on ALP and IPHOS. Goats on the control diet had higher serum ALP and IPHOS concentrations ($p < 0.05$) than those on the *C. africana* and *R. lancea* diets.

Table 5.4: Blood metabolite composition of Nguni goats offered control diet, *C. africana*, *R. lancea* or RL:CA forages during the dry and wet seasons.

		Parameters													
Season	**Browse**	GGT U/l	STP g/l	ALB g/l	GLOB g/l	ALT U/l	ALP U/l	SUN mmol/l	SCR umol/l	SCAL mmol/l	CHOL mmol/l	SGLU mmol/l	IPHOS mmol/l	TotalBil umol/l	AMYL U/l
	C. africana	17^{b}	68	36^{ab}	32	22	129	1.5^{a}	65^{c}	2.5^{ab}	2.8^{cd}	3.0^{a}	1.3^{a}	1.0^{a}	15.3
Dry	Control	44^{a}	65	33^{ab}	32	17	848	6.5^{c}	45^{ab}	2.6^{b}	1.5^{ab}	4.0^{b}	2.8^{b}	1.5^{ad}	15.5
	R. lancea	25^{ab}	66	34^{ab}	32	20	201	1.4^{a}	68^{c}	2.5^{ab}	2.1^{bc}	2.9^{a}	1.9^{ab}	1.3^{a}	10.8
	RL:CA	28^{ab}	69	37^{b}	32	20	371	2.1^{a}	59^{bc}	2.6^{ab}	3.0^{d}	2.7^{a}	1.5^{a}	1.6^{abd}	14.0
	C. africana	43^{a}	70	35^{ab}	35	25	130	6.1^{bc}	43^{ab}	2.4^{abc}	1.4^{ab}	2.8^{a}	1.6^{a}	3.0^{bc}	13.5
Wet	Control	40^{a}	66	32^{a}	34	16	564	9.1^{d}	30^{a}	2.3^{c}	1.2^{a}	2.9^{a}	2.8^{b}	2.8^{bcd}	11.3
	R. lancea	36^{ab}	64	31^{a}	33	23	157	4.3^{b}	41^{a}	2.3^{ac}	1.2^{a}	3.1^{a}	1.6^{a}	3.3^{c}	11.5
	RL:CA	43^{a}	66	34^{ab}	32	20	534	5.1^{bc}	36^{a}	2.4^{abc}	1.6^{ab}	2.8^{a}	1.5^{a}	3.0^{bc}	11.5
SEM		4.1	2.4	1.0	2.1	4.1	154.1	0.41	3.4	0.05	0.15	0.13	0.23	0.30	3.25
CV (%)		13.14	3.57	3.00	6.39	20.46	66.17	13.91	7.55	1.92	9.23	4.50	13.32	16.38	25.65
*Reference values		39-137	55-72	20-30	24-52	29-53	0-552	4.6-9.6	63-111	1.9-2.5	3.4-8.9	3-5	1.4-3.3	8.4-14.8	-
p-values															
Season		0.003	0.741	0.007	0.301	0.622	0.710	0.001	0.001	0.001	0.0001	0.013	0.976	0.0001	0.407
Browse		0.024	0.359	0.003	0.888	0.374	0.003	0.001	0.001	0.404	0.0001	0.0003	0.0001	0.729	0.788
Season*Browse		0.012	0.624	0.587	0.861	0.951	0.551	0.094	0.423	0.205	0.004	0.0002	0.670	0.450	0.893

abcd Means within a column that do not share a common superscript differ significantly at the p <0.05. **Browse;** *C. africana* = Celtis africana, *R. lancea* = *Rhus lancea*, RL:CA = *Rhus lancea* and *Celtis africana* 1:1 mixture. **Parameters;** GGT= gamma-glutamyl transferase, STP = serum total protein, ALB = albumin, GLOB = globulin, ALT = alanine transaminase, ALP = alkaline phosphatase, SUN = serum urea nitrogen, SCR = serum creatinine, SCAL = serum calcium, CHOL = cholesterol; SGLU = serum glucose, IPHOS = inorganic phosphate, TotalBil = total bilirubin, AMYL = Amylase. SEM = standard error of means, CV = coefficient of variation. Normal ranges from IDEXX Laboratories (IDEXX laboratories Pty, Kyalami, Johannesburg, South Africa).

5.5. Discussion

5.5.1. Nutrient intake

Forage dry matter intake (DMI) is an important dietary factor, which correlates with nutrient supply and absorption in ruminants (Magalhães et al., 2021). The DMI of goats offered the control diet was higher than those offered *C. africana*, *R. lancea* or RL:CA during the dry season, which is commensurate with the higher CPI. This is in line with the findings of Nasrullah (2015), that high CP in *M. sativa* enhanced CPI in female Beetal breed goats and Lohi sheep. Forages with a high CP content enhance dry matter intake, thereby aiding in achieving nutrient requirements in ruminants (Thamina et al., 2020). The DMI of goats fed *R. lancea* and *C. africana* was not within the same pattern even though the intake of the two forages was comparable, the CPI was higher from the *C. africana* diet during the wet season. Observations of defoliating *C. africana* leaves, while *R. lancea* leaves were more succulent, could explain the lower CPI during the dry season. It was observed that goats consuming *C. africana* had lower OMI as compared to those on *R. lancea*. This indicates that the concentration of ash in *C. africana* was higher.

The results of the current research indicated that the higher DMI and OMI observed in goats fed the diet containing *C. africana* during the wet season positively influenced the goats' growth. This positive influence was noticeable in all goats during the wet season. The study found that lower crude protein intake levels in *C. africana* did not affect the dry season DMI of goats. This is consistent with previous reports that goats can adapt their metabolic nutrient requirements and consume low crude protein forages during the dry seasons (Silanikove, 2000; Allegretti et al., 2012). Goats offered *R. lancea* had higher CPI levels despite higher TPC and CT levels than those on *C. africana* or RL:CA diets during the dry season. The CTI and TPCI were measured to evaluate if the level of their intake influenced DMI, OMI, CPI, NDF, ADF and ADL in goats. During the wet season, goats on a *C. africana* diet had a high CPI and low CTI and TPCI, however, goats needed more CPI to achieve their protein needs (Mendez-Ortiz et al., 2018). The CTI levels of goats offered *C. africana*, *R. lancea* or RL:CA were below the threshold that causes negative effects in ruminants, regardless of the season (Mkhize et al., 2018a; Da Silva et al., 2021). A CTI above 5% DM inhibits nutrient utilization and voluntary feed intake in ruminants (Pamar

et al., 2022). The results of the current research indicate that CTI wasn't a problem with goats' nutrient intake and can benefit nitrogen utilization in the rumen (Kelln et al., 2020). This agrees with earlier reports that goats can consume TPC and CT and exhibit few or no toxic effects because of the proline-rich protein substances they secrete in their saliva, which act as detoxifying agents (Njidda & Nasiru, 2010).

Condensed tannins have higher molecular weights compared to other phenolic compounds and this affects their solubility, bioavailability, and interactions with proteins and fibre in the rumen (Soldado et al., 2021). They have the potential to contribute significantly to the total phenolic content, but they may not be completely represented in some TPCs assays that target simpler phenolic compounds (Dhull et al., 2016; Granato et al., 2016). Methods using aqueous acetone or methanol to extract CT versus phenolic compounds can influence the results (Schofield et al., 2001). In addition, analysis methods including the Folin-Ciocalteu, Butanol-HCl and vanillin-HCl may be more effective at isolating CT than other phenolic compounds, leading to increased levels of CT in the analysis (Food, 2000; Schofield et al., 2001; Khadambi, 2007; Formagio et al., 2014; Dhull et al., 2016; Granato et al., 2016). Some assays or analytical methods may specifically target CT, resulting in higher concentrations being measured compared to methods that detect a variety of phenolic compounds (Schofield et al., 2001). This results in a biased reading of CT relative to the TPCs (Del Pino et al., 2005). The higher concentrations of CT compared to TPCs in *R. lancea, C. africana* or RL:CA in this research can be described by the structural complexity of tannins, differences in extraction methods, and analytical specificity of the assay used. Literature on CTI and TPI levels is scant, and this warrants further investigation. However, since CT levels are higher than TP (Food, 2000; Schofield et al., 2001; Khadambi, 2007; Formagio et al., 2014), it is therefore, expected that CTI will be higher than the TPI supporting the findings of the current study.

The NDFI, which comprises cellulose and hemicellulose, was below 35% and did not inhibit DMI in goats fed the control diet during the dry season. A higher NDF content (above 35%) e.g. in total mixed ratio would have retarded fermentation and prolonged forage retention time in the rumen (Macedo et al., 2007; Vargas et al., 2021). High NDFI

concentrations were reported in male crossbred Dorper lambs offered hay and concentrates (Herzog et al., 2021). Neutral detergent fibre intake reported in this current research can be beneficial to goats by adjusting their intestinal immunity through the regulation of rumen bacteria composition (Clauss & Dierenfeld, 2008). The ADL is negatively associated with forage value by decreasing forage consumption and digestibility (Jiwuba et al., 2021).

5.5.2. Weight changes

There were no variations in the final metabolic body weights of goats offered *C. africana*, *R. lancea* or RL:CA during the wet season. Therefore, there was no advantage in feeding the choice of *R. lancea* and *C. africana* on DMI, as hypothesized. The results of the study showed that goats experienced weight loss during the dry season when they were offered *R. lancea*, *C. africana* and RL:CA diets. However, it is worth noting that all goats received *Eragrostis curvula* hay, but in a limited amount (100 g/kg DM per day) to encourage intake of studied forages, contrary to previous research by Phiri et al. (2022). A possible explanation is the inability of the goats to consume sufficient browse dry matter during the dry season compared to the wet season. A study by Okoruwa and Ikhimioya (2020) demonstrated that West African dwarf male goats gained body weight when offered fresh mature leaves of *Paraserianthes falcataria*, *Moringa oleifera* and *Calliandra calothyrsus* and mixed hay grass (guinea grass and elephant grass). Indeed, DMI and CPI are important for growth and reproductive performance in ruminants (Ayyat et al., 2021).

In the current research, the CPI remained lower in the browse-based diets coupled with the higher CTI and TPCI, especially during the dry season. The CTs are supposed to bind the CP and carbohydrates inhibiting their fermentation in the rumen (Bele et al., 2010) for digestion and absorption in the gut. The fact that this yielded contrasting outcomes on growth in the current research warrants further investigation. Feeding *R. lancea* to goats as a sole ingredient was reported to lower body weight gain in goats (Dludla, 2010; Phiri et al., 2022). However, during the wet season, the *C. africana* and *R. lancea* diets showed that they have the potential to meet goats' maintenance requirements and moderate growth. The results presented in this research study agree with those of Beigh et al. (2020), that the fresh leaves of *Ulmus* foliage were adequate for achieving maintenance

requirements and moderate body weight gain in Bakerwal goats. However, the results of the current research suggest that goats offered *C. africana*, *R. lancea* or RL:CA diets need supplementation with other forages high in CP and energy to supersede the binding effects of CT and achieve CP demand for weight gain.

5.5.3. Urinalysis

Urinalysis is routinely used in assessing the well-being of the urinary system in companion animals and has the potential to determine subclinical disease and organ health but is comparatively underutilized in ruminants (Ihedioha et al., 2019). The negative urinalysis results for erythrocytes, leucocytes, and glucose for all goats across the treatments were indicative of a lack of overt inflammation and/or infection in the distal urinary or genital tracts (Cork et al., 2019). Urine bilirubin is a product of the breakdown of erythrocytes and should not be present in healthy goats' urine (Parrah et al., 2013; Tang et al., 2019). The goats in the current research had urine bilirubin (>0.1 mg/dL) indicating either hepatic insufficiency or bile duct obstruction (Cork et al., 2019). This conclusion is informed by the observation of the USG being also lower than the reference range (1.02 - 1.04 USG) for the species, as was the case in the current research study (Finley, 2013; Wuillemin et al., 2023).

Urobilinogen, a byproduct of intestinal microflora's bilirubin metabolism, enters the portal circulation and is eliminated by the kidneys (Finley, 2013). A small amount of urinary urobilinogen is expected to be normal, but increased levels occur when the liver produces excessive amounts of bilirubin because the body is destroying red blood cells faster than it can produce them, or because of a liver disease that prevents the liver from recycling urobilinogen into bile (Ihedioha et al., 2019; Hedayanti & Wahyudi, 2023). High levels of serum bilirubin should also be associated with urine bilirubin and urobilinogen, but this was not seen in the goats used in this investigation. However, during the wet season, the urine bilirubin levels in goats given the control diet were higher than those in goats given *R. lancea*, *C. africana* or RL:CA diets, suggesting that the livers of the goats on the control diet may have been more compromised than those of goats receiving the other treatments.

Goats' urine is supposed to be negative for protein (Parrah et al., 2013), contrary to the findings in the current research study for all the goats across the treatments. Non-clinical sources of proteinuria comprise of concentrated protein diet, physical movement and distress; while the clinical source of proteinuria comprises nephropathy, resulting from glomerulonephropathy, tubular transport fault, swelling, or contamination within the renal system (Finley, 2013; Parrah et al., 2013; Aboagye et al., 2019). Given that other findings in goats in this research study reduce the likelihood of inflammation and infection, contributory factors to the goats' proteinuria are likely to be stress associated with keeping the goats in individual pens and handling them while collecting samples at the end of each period, glomerulonephropathy, and tubular transport defects.

Ihedioha et al. (2019) demonstrated that the abnormal urine protein concentrations in cattle in their study may have been attributed to the impotence of kidney tubules to reassimilate protein. Fernandez et al. (2003) posited that renal amyloidosis should be a differential diagnosis in sheep with any level of proteinuria and this premise is extended to goats in this research study. Renal disease was confirmed by an elevated UPC ratio, a protein analysis technique that standardizes the urine protein concentrations, altering the differences occurring from variations in urine volume and USG. Katsoulos et al. (2020) reported that a UPC of ≥0.19 assumed an ideal cut-off point for the diversity among the cattle and those with nephropathy. The UPC findings indicate that goats fed the control or *C. africana* diets stood a higher risk of developing a diet-induced renal disease than those on the *R. lancea* or RL:CA diets during the wet season. The USG is used to measure the solutes concerning hydration. The USG levels in the urine of goats offered *C. africana*, *R. lancea*, RL:CA or control diets across all seasons were lower than the normal range in ruminants (Finley, 2013; Parrah et al., 2013; Sauvé et al., 2015). Jones et al. (2012) noted that USG must be elucidated prudently and not centered on a single sample, as values from as low as 1.003 were recorded in clinically normal goats.

The urine pH levels in goats offered *C. africana*, *R. lancea* or RL:CA diets were alkaline and within the normal range (Finley, 2013). The UCR levels in goats offered *C. africana*, *R. lancea*, RL:CA or control diets were higher than the reference values across all seasons (Makkar, 2004; David et al., 2015). There is no clear reason for this but high levels of

creatinine in urine can indicate kidney damage or disease, and goats have also been reported to secrete creatinine into urine (Udeh et al., 2021). The goats in the current research study experienced various degrees of weight loss and were handled physically during the collection of samples. This would have contributed to rhabdomyolysis and myoglobin release which is known to cause myoglobinuric nephrosis, which in turn causes kidney damage and renal azotemia and could explain the elevated proteinuria and UCR levels (Udeh et al., 2021).

Hypercalciuria is a condition that occurs when there is an excessive amount of calcium in the urine as demonstrated in goats in the current research study. There were seasonal and browse interactions with goats on the control or C. africana diets, which had normal UCAL concentration in the wet season compared to the dry season. Hypercalciuria can be caused by various factors such as metabolic acidosis, primary hyperparathyroidism, excessive ingestion of calcium (Ca) or vitamin D, disparity in the Ca to phosphorus (P) ratio in the forage and low protein intake (Muscher et al., 2011). The incidence of hypercalciuria in goats is not well documented but it has been reported that it is a common cause of urolithiasis in goats (Muscher et al., 2011). This suggests that all the goats on the various treatments except for those on the control or *C. africana* diets during the wet season were at a higher risk of developing urolithiasis.

5.5.4. Blood metabolites

Blood metabolites can give an objective indication of the physiological state as well as responses of animals to conditions such as SBWL and dietary changes (Lérias et al., 2015). Sequential measurements of GGT, Aspartate transaminase, sorbitol dehydrogenase, serum bilirubin, and bile acids are normally applied to evaluate liver dysfunction and illness in ruminants (Foreman, 2014). Goats in the current study had lower GGT, ALT and total bilirubin levels than the reference ranges (IDEXX laboratories Pty, Kyalami, Johannesburg, South Africa; Kaneko et al., 2008). This could be attributed to the fact that serum levels of liver enzymes differ in goats according to age, breed, and sex (Karaşahin et al., 2022) and these have not been established for indigenous goats in Southern Africa (Foreman, 2014).

Although low GGT, ALT, and total bilirubin levels are typically thought to be of negligible clinical consequence, they have been linked to an imbalanced diet, vitamin or mineral deficiencies, or some underlying pathology in goats (Al-Bulushi et al., 2017; Zaher et al., 2020). They may also signify a low-functioning or non-functioning liver, implying that such animals' livers would not release a large amount of GGT or ALT into the blood as needed. For example, low GGT has been shown as a biomarker for poor prognosis in children with biliary atresia and indicative of severe hepatic disease (Sun et al., 2022). Yusuf et al. (2017) observed elevated levels of bilirubin and uric acid in West African dwarf goats which may have served as a potential antioxidant. Further investigations are needed to determine the effects of diet on GGT or ALT concentrations during the dry season. This could be invaluable in understanding liver enzymes and their value when interpreting physiological changes in goats.

In the current research study, Nguni goats on the *C. africana* or *R. lancea* diets showed no clinical signs of toxicosis due to CTI and TPCI, probably because of their ability to efficiently neutralize CT and TPCs (Silanikove et al., 1996). This is supported by the normal globulin levels across all seasons in the current research study. When there is liver injury or disease in goats, globulin levels normally increase, whereas albumin, a negative acute phase protein, is usually lowered (Chovanová et al., 2021) and that was not experienced by goats in the current research study. Instead, albumin levels were slightly higher than reference values, which is frequently caused by dehydration or volume contraction due to fluid loss (Idamokoro et al., 2019).

Intake of insufficient dietary CP negatively affects STP and ALB metabolism, leading to hypoproteinemia (Nnadi et al., 2007). The findings of the current research showed STP levels in goats offered *C. africana*, *R. lancea* or RL:CA diets being within the reference values. The STP and ALB are indicators of goats' protein nutritional status (Bobbo et al., 2017) demonstrating that there were no adverse protein effects physiologically, even though goats on the *C. africana*, *R. lancea* or RL:CA diets ingested considerably low protein content diets when compared with goats on the control diet, and proteinuria was observed in goats on all treatments. The probable explanation is that the proteinuria was transient, and stress-induced and a demonstration of goats’ unique potential to reserve

nitrogen by endogenic reprocessing method, particularly during periods of dietary protein shortage (Harmeyer & Martens, 1980). In accordance with the results of the current research study, Muscher et al. (2011) reported that when nitrogen (N) consumption was reduced in young goats, plasma urea levels were also reduced and there was an inverse relationship between creatinine in plasma and urine, which improved during a dietary N drop because of a low renal task to reserve urea during N shortage.

The results of the current research showed that goats offered *C. africana*, *R. lancea* or RL:CA diets had lower SCR levels during the wet season. The lower SCR levels are a consequence of lower CPI rather than CTI and can be associated with the metabolic energy imbalance relating to reduction in muscle mass, attributed to the use of muscle protein as energy (Njidda et al., 2021). The SCR levels in goats eating *C. africana* and *R. lancea* were at normal range during the dry season, indicating that the energy balance was satisfactorily attained. The SCAL levels in goats offered *C. africana*, *R. lancea*, RL:CA or control diets were consistent with the reference values reported for serum SCAL in goats (IDEXX laboratories Pty, Kyalami, Johannesburg, South Africa; Turner et al., 2005; Kaneko et al., 2008; Radin et al., 2017). The IPHOS levels were higher in goats on the control diet than in goats on *C. africana*, *R. lancea* or RL:CA diets and correlated with ALP levels. This is consistent with the fact that ALP is associated with the breakdown of phosphate esters in the body. It occurs in many tissues such as the liver, bone, and gut (Radin et al., 2017).

The CHOL levels in Nguni goats offered *C. africana*, *R. lancea*, RL:CA or control diets were lower than the reference values recommended by IDEXX laboratories Pty (IDEXX laboratories Pty, Kyalami, Johannesburg, South Africa) across all seasons. Measurement of CHOL levels can indicate hepatic function, gastrointestinal disease, and metabolic disorders (Turner et al., 2005; Kaneko et al., 2008). Usually, the reasons for low cholesterol include protein-losing enteropathy, decreased production and decreased intake. The CT and TPC have contributed to the decline of CHOL levels in earlier research (Farooq et al., 2007; Saxena et al., 2013). The SGLU levels in goats offered the *C. africana*, *R. lancea* or RL:CA diets during both the dry and wet seasons were at normal

range as observed by Radin et al. (2017) except in goats fed *C*. *africana*, *R. lancea* or control diets which were somewhat lower during the wet season.

5.6. Conclusions

The study demonstrates that while evaluating the value of feeding *R. lancea* and *C. africana* to goats, it is essential to consider multiple dietary criteria to ensure that goats' physiological needs are adequately met. The findings showed that inadequate DMI and CPI during the dry season led to body weight loss and potential underlying renal and hepatic dysfunctions affecting all the goats. However, during the wet season, goats on *R. lancea* or *C. africana* or RL:CA diet gained weight, this was supported by a slight improvement in DMI and CPI. The study recommends supplementing goats and wild concentrate selectors with other feedstuffs to meet their growth requirements during the dry season, but it should be done with caution, ensuring a balanced diet.

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CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Wild concentrate selectors at the National Zoological Gardens in Pretoria and the Johannesburg Zoo are being fed *R. lancea* and *C. africana* as part of dietary enrichment. However, the impact of these browse species on their growth weight, physiology, and health has not been fully explored. The current study evaluated the effects of *R. lancea* and *C. africana*, on feed intake, weight, urinary, and blood serum in indigenous Nguni male goats as a model animal for wild concentrate selector in captivity. Experimental diets were based on anecdotal reports of browse consumed in the wild and their availability in urban areas. The control diet was based on what is being fed in zoos to achieve the nutritional needs of wild concentrate selectors.

6.1.1. Conclusion 1

Study objective 1 was to determine the nutritional composition and concentrations of secondary plant metabolites in *C. africana* and *R. lancea* browse material and their effects on palatability and nutrient digestibility of male Nguni goats in the dry and wet seasons. In addressing this study objective, the findings relating to conclusion 1 may be stated as follows:

The current study found that Nguni goats preferred *R. lancea* over *C. africana* in both dry and wet seasons, indicating differences in nutritional quality and utility of these browse forages. This led to the rejection of the null hypothesis. The preference for the RL:CA mixture suggested the presence of *R. lancea* improved the intake of *C. africana* within the mixture. This indicates an associative effect in the mixture. High intake of mixed forages in goats may suggest an associative, goats are known to selectively choose their diet based on nutrient composition and palatability. This indicates that they can consume certain forages in higher quantities. However, the exact reason for the high intake of mixed forage requires further investigation to determine if indeed an associative effect has a role. The higher RPI was due to foraging patterns confirming that the concentrations of TPCs and CT in *R. lancea* and *C. africana* do not negatively affect

intake. Despite *R. lancea* having a variety of nutrients and being associated with TPCs and CT that potentially act as chemical deterrents at an intra-species level, it was the most preferred browse forage for goats, followed by RL:CA or *C. africana*, across seasons. The low preference for *C. africana* and high ash content indicates the presence of inorganic minerals in the browse, which has the potential to reduce feed intake. Abiotic factors such as geographical location and habitat size influence the diversity of browse species, which could be the main factors impacting the nutritional composition of browse used in captive feeding management. The fluctuation of crude protein levels of *C. africana* and *R. lancea* across seasons provides goats with alternative feed resources with other dietary items, which may have lower crude protein in particular seasons.

The current research study showed that dCP and CPD were lower in goats fed the *R. lancea*, *C. africana* or RL:CA than in goats fed the control diet across seasons. This research inferred that the low levels of dCP and CPD in goats fed *R. lancea*, *C. africana* or RL:CA can be attributed to the presence of CT in them. Therefore, goats must constantly adjust their diet to make up for the undigested CP to meet their physiological needs. This is particularly challenging as they must deal with varying seasonal levels of TPCs and CT. One way for goats to cope with this challenge is by consuming other types of forage that have lower TPCs and CT levels. These include dietary items that have different nutritional value and quality, such as forbs, succulents, fruits, and grasses.

6.1.2. Conclusion 2

Study objective 2 was to ascertain the effects of feeding diets containing *C. africana* and *R. lancea* browse on the ruminal bacterial composition of male Nguni goats in the dry and wet seasons. In tackling this study objective, the findings relating to conclusion 2 may be addressed as follows:

As for the rumen bacteria, the study identified 12 phyla and among the phyla, the *Bacteroidetes* and *Firmicutes* were the most dominant, at a combined abundance of 85% across the seasons. This supports the rejection of the null hypothesis. The *Firmicutes* to *Bacteroidetes* (F/B) ratio was higher (0.85:1) in the dry season compared to the wet season (0.58:1) and this can be linked to nutrient composition particularly fibre because of the seasonal effects on the forages used. This study demonstrated that an increase in

forage fibre content increases the F/B ratio, and it is, however, imperative to state that seasonal change and plant cell wall makeup (pectin, cellulose, hemicellulose and lignin) of the forages played a role in their composition.

Genus *Prevotella* is responsible for the breakdown of protein, and it was noted to be abundant in various levels in goats reared on *C. africana*, *R. lancea* or RL:CA across seasons. The abundance of *Prevotella* can be linked to its efficiency to degrade CP. The family *Prevotellaceae* aid in safeguarding the goats from negative or toxic effect of TPCs and CT. There was a high abundance of taxa *Fibrobacteres* in goats fed *C. africana* or *R. lancea* in the wet season and *C. africana*, control or RL:CA in the dry season. This indicated that the fibre composition in the leaves of *C. africana*, *R. lancea*, RL:CA or control differed across seasons thereby variously affecting fibre degradation. The abundance of the family taxa belonging to *Lachnospiraceae* and *Ruminococcaceae* in goats differed with the high abundance observed in treatments during the dry season. It is, however, worth noting that CT binds to fibre and the abundance of proteolytic bacteria is an attempt to access and degrade fibre.

6.1.3. Conclusion 3

Study objective 3 evaluated the effects of feeding diets containing *C. africana* and *R. lancea* browse on feed intake, weight, urine, and serum metabolites in male Nguni goats in the dry and wet seasons. In addressing this study objective, the results relating to conclusion 3 of chapter 5 can be addressed as follows;

On voluntary feed intake, goats on the control diet consumed 827 gDM/kg feed/day, 155 g CP/kg feed and on either *C. africana* and *R. lancea* diets they consumed less than 60% of this amount, and 36% CP, and 39% CP, respectively, across seasons. This suggests that goats on *C. africana, R. lancea* or RL:CA diets during the dry season needed to consume more browse forage, considering that the nutrient quality improved to enhance weight gain during the dry season. On the one hand, the weight gain in goats reared on the control diet is supported by higher CP content than those on *C. africana*, *R. lancea* or RL:CA across seasons. On the other hand, goats fed *C. africana*, *R. lancea* or RL:CA proved their utility, and this was informed by improved weight gain during the wet season thereby indicating that maintenance requirements were achieved.

Under urinalysis, while it is normal for goats to have little URO, the levels of URO in goats offered the control diet were higher compared to those on forage-based diets across seasons. Urobilinogen is derived from the breakdown of bilirubin by intestinal bacteria and its higher concentration is informed by concentrated levels of bilirubin in goats fed the control diet during the wet season. The URO is reabsorbed via the portal vein to the liver, and a portion of it is excreted by the kidney. It can be concluded that intake of *R. lancea, C. africana* or RL:CA by goats results in lower positive concentrations of URO and UBIL suggesting that there were no health disorders associated with hepatobiliary or jaundice in goats, supporting the rejection of the null hypothesis. The goats on *R. lancea* diet had higher concentrations of UPRO during the wet seasons, the reason for this is that UPRO leaked into the urine. However, based on the low concentration of UPRO in the dry season, it can be concluded that the higher UPRO concentrations during the wet season were a result of dehydration in goats. This is informed by the high USG concentration which indicates a demand for more water intake during the wet season. It can be deduced from the results of the current research study that the pH levels of forage-based diets were unstable and alkaline across seasons. However, goats offered the control diet showed high alkaline concentrations, this can be caused by intake of fruit and vegetables reacting as pH precursors across seasons.

The study showed that the treatments and seasons did not affect the composition of STP, GLOB, ALT, and AMYL across seasons. The resistance of serum to short-term changes due to diet and season in the current research study can be due to homeostatic balance. Based on the low GGT and ALT levels, the liver of the goats was not negatively impacted by their consumption of *C. africana*, *R. lancea,* or RL:CA. This conclusion is further supported by the normal GLOB levels, indicating that the goats remained healthy with no signs of liver damage or disease. It can therefore be inferred that the intake of these browse supports the maintenance of GLOB, which is closely linked to liver enzymes. Additionally, based on the findings regarding serum STP, GLOB, SGLU, ALP, SCAL, and IPHOS in goats, it is evident that they were able to resist any negative effects of high TPI and CTI levels, maintaining normal levels regardless of the season. These results have led to the rejection of the null hypothesis regarding blood metabolites.

6.2. Recommendations

- The study investigated the effects of structured vs unstructured diets for wild concentrate selectors in captivity using goats as a model animal. Goats are regarded as domestic concentrate selectors and are suitable model animals for the simulation of feeding wild concentrate selectors in captivity. The acceptance of structured and unstructured diets by goats suggests that wild concentrate selectors in captivity will exhibit similar foraging behaviour and there they should be fed a combination of structured and unstructured diets.
- A study of goat behaviour and their ability to choose forages high in these nutrients and anti-nutrients can be done using *Rhus lancea* as a model, as it is available throughout the year. Goats' behaviour demonstrates their sophisticated foraging strategies, ability to balance nutritional gains and importance of considering multiple factors when studying dietary preferences."
- There is a need to investigate the mineral composition including silicon, magnesium, sulphur and copper in *R. lancea, C. africana* and how they affect feed intake and digestion in goats. These browse species are known to contain various phenolic compounds, including tannins, which can affect mineral availability and potentially impact browse intake by goats.
- More studies are required to investigate the role of phyla *Firmicutes* and *Bacteroidetes* in the degradation of feed. Their abundance is complex however their specific role in the presence of TPCs and CT is unclear.
- There is a need to identify the correct supplementation to improve weight gain in goats being fed *R. lancea* and *C. africana* in the dry seasons. This is imperative because most SBWL occurs in the dry season and the use of goats as a model animal losing weight in the dry season suggests the importance of supplementary diet intervention in captivity.
- There is a need to study the causes of proteinuria in goats foraging on *C. africana, R. lancea* or RL:CA-based diets.

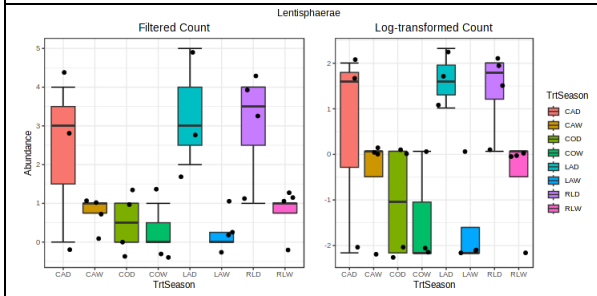
APPENDICES

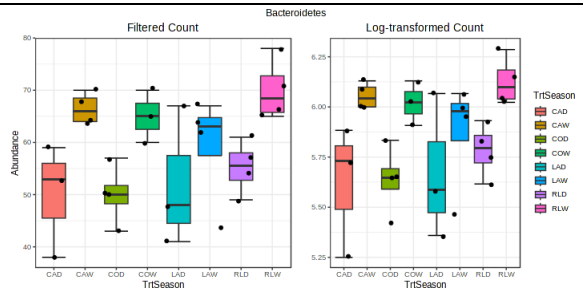
Appendix 1: Rumen microorganism profile

Sample	No.singleton	no.seqs	Goods coverage index
E16_19:bc1018	9	113	92.0353982300885
E16:bc1078	11	113	90.2654867256637
P1_19:bc1003	11	113	90.2654867256637
P12_19:bc1010	10	113	91.1504424778761
P12:bc1069	7	113	93.8053097345133
P17_19:bc1011	13	113	88.4955752212389
P17:bc1070	9	113	92.0353982300885
P19_19:bc1012	9	113	92.0353982300885
P19:bc1072	14	113	87.6106194690265
P1:bc1060	3	113	97.3451327433628
P21_19:bc1013	6	113	94.6902654867257
P21:bc1073	10	113	91.1504424778761
P22_19:bc1014	4	113	96.4601769911504
P22:bc1074	6	113	94.6902654867257
P23_19:bc1015	11	113	90.2654867256637
P23:bc1075	10	113	91.1504424778761
P25_19:bc1016	13	113	88.4955752212389
P25:bc1076	7	113	93.8053097345133
P26_19:bc1017	12	113	89.3805309734513
P26:bc1077	12	113	89.3805309734513
P2:bc1061	14	113	87.6106194690265
P3:bc1062	10	113	91.1504424778761
P4_19:bc1006	12	113	89.3805309734513
P4:bc1063	11	113	90.2654867256637
P5_19:bc1007	7	113	93.8053097345133
P7_19:bc1008	9	113	92.0353982300885
P7:bc1066	8	113	92.9203539823009
P9_19:bc1009	9	113	92.0353982300885
P9:bc1068	7	113	93.8053097345133

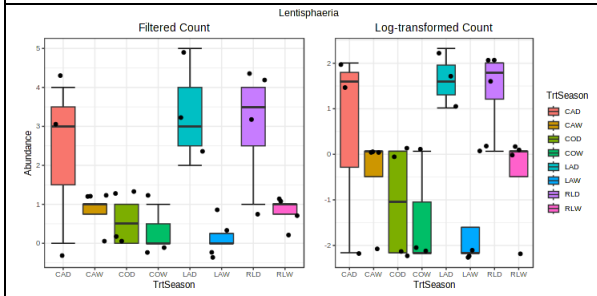
Appendix 2: Differential abundance analysis

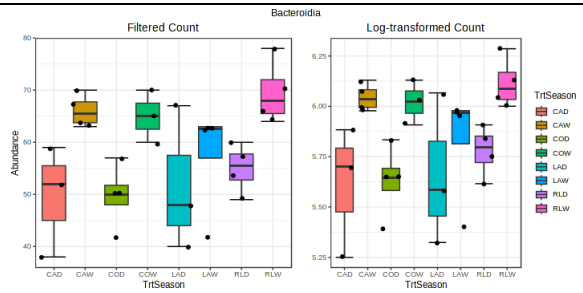
Phylum



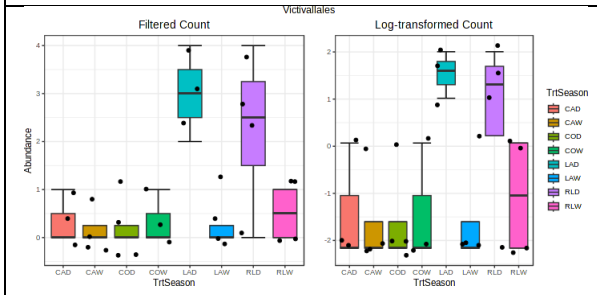


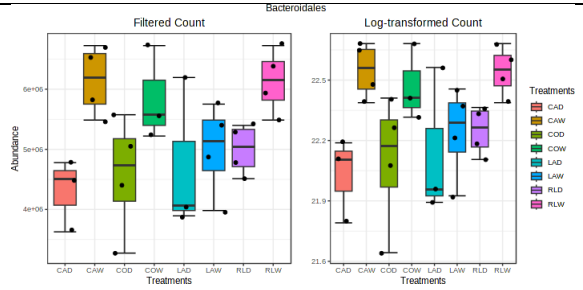
Class



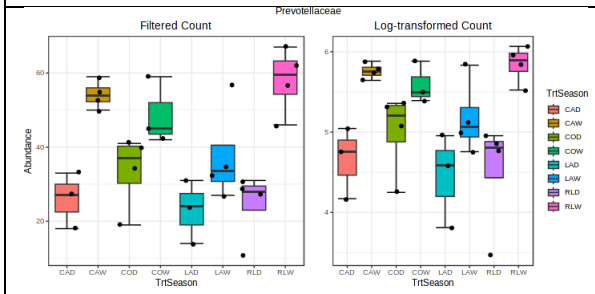


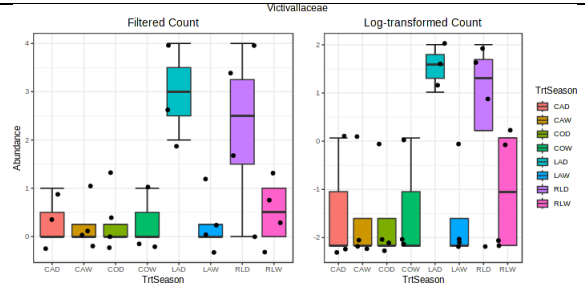
Order





Family


Prevotellaceae
Filtered Count
Log-transformed Count
Abundance
TrtSeason
CAD
CAW
COD
COW
LAD
LAW
RLD
RLW


Victivallaceae
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COD
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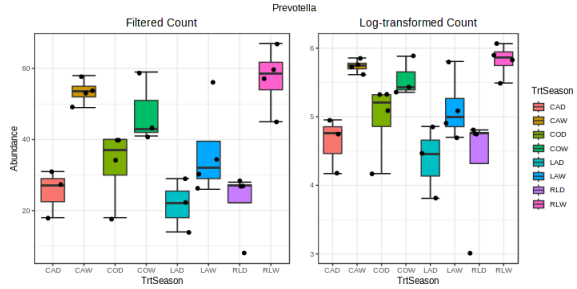
RLD
RLW


Prevotella
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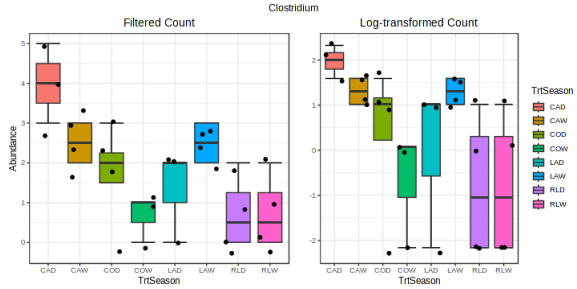
Clostridium
Filtered Count
Log-transformed Count
Abundance
TrtSeason
CAD
CAW
COD
COW
LAD
LAW
RLD
RLW

Appendix 3: Univariate analysis of significant features from goat's rumen microbiota offered *R. lancea*, *C. africana*, RL:CA and control diets

Features	P- Values	FDR
Phylum		
Lentisphaerae	0,0017	0,021
Bacteroidetes	0,0089	0,053
Class		
Lentisphaeria	0,0017	0,022
Bacteroidia	0,0106	0,069
Order		
Victivallales	0,0011	0,015
Bacteroidales	0,0106	0,074
Family		
Prevotellaceae	0,0001	0,002
Victivallaceae	0,0011	0,012
Mogibacteriaceae	0,0028	0,017
Clostridiaceae	0,003	0,017
Paraprevotellaceae	0,0172	0,076
Genus		
Prevotella	0,0001	0,001
Clostridium	0,0012	0,012
Victivallis	0,0076	0,041
YRC22	0,0078	0,041

FDR = False discovery ratio is the rate which features called significant are truly null.