Nutritive value, *in vitro* dry matter degradability and ensiling characteristics of citrus pulp in sorghum silage

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Declaration

I, Atharlia Bontle Ditabo declare that this dissertation is my work. All the utilized literature information has been rightfully and correctly acknowledged in the form of in-text and full references in each chapter. This dissertation has not been submitted to any other higher education institution but exclusively to North-West University with the consent of my supervisors.

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General Abstract

The forage nutritive quality and quantity decline rapidly during dry seasons, putting the livestock animals under feed insecurity. For these reasons, other ways to combat feed insecurity should be employed and exercised. Thus, agro-industrial products that are highly nutritious and under-utilized should be used to improve the nutrition of livestock animals. The present study centred around the nutritive value and fermentation profile of the graded inclusion levels of citrus species pulp in sorghum (*Sorghum bicolor* L. Moench) silage. The study was carried out at the North-West University research farm (25°49′22″S and 25°36′54″E). The first objective aimed to determine the nutritional status and physical characteristics of citrus species pulps. The citrus fruits comprising oranges, grapefruits, lemons and tangerines were purchased from a local store and the juice was extracted using Taurus Liquafruits Pro Juice Extractor to obtain the pulps. They were chopped to obtain the 10 mm theoretical chop length. The pulps were dried, ground and analyzed for chemical (proximate, fiber fractions and mineral) composition, chemical estimates and physical characteristics. The one-way completely randomized design (CRD) by ANOVA using the GLM procedure of SAS (2010) was adopted. Study results showed that LP had high ($P<0.05$) DM, ash, EE, and NDF with low ($P<0.05$) DM, ash, and high ($P<0.05$) CP content in OP. The LP had the highest ($P<0.05$) concentration of all minerals except for aluminium when in comparison with other citrus species pulps. The WR and PV were both high ($P<0.05$) in OP. All the chemical estimates (DMD, DE, ME and TDN) were higher ($P<0.05$) on TP than in other pulps. The citrus species pulps had better chemical composition, chemical estimates and physical characteristics. But the LP exhibited better chemical composition, with tangerine pulp demonstrated improved chemical estimates. The pulps require consideration based on the limited nutrients such as DM, CP and micro-minerals to meet the ruminants' requirements. Additionally, their DM is too low, which might cause quick spoilage in storage, thus requiring absorbent for improved shelf life and preservation. Regarding the CP and micro-minerals, the pulps require

protein supplementation. There was mainly a negative correlation among the parameters (chemical composition, chemical estimates and physical characteristics) in citrus species pulps.

The second objective aimed to determine the effect of citrus species pulp inclusion levels on the chemical composition and fermentation profile of sorghum (*Sorghum bicolor* L. Moench) silage. The citrus species pulp at 5, 10, 15, 20 and 25% levels were homogenously mixed with sorghum and ensiled for 6 weeks. The pre-ensiled treatments were analyzed for chemical composition whilst the silage treatments were analyzed for chemical composition, *in vitro* dry matter digestibility (IVDMD), nitrogen solubility and ensiling characteristics. The data were analyzed in a 5 x 4 factorial arrangement in a completely randomized design (CRD). The DM, NDF and ADL decreased ($P<0.05$) with increasing inclusions. While ash, EE and CP increased ($P<0.05$) with increasing inclusion levels. The SG had an acceptable DM at all levels and had high ($P<0.05$) ADL whilst ST had high ($P<0.05$) CP. The Cel of the silages were decreasing ($P<0.05$) with increasing inclusions whilst, DMD, DMI, DE, ME and TDN were increasing ($P<0.05$) with increasing inclusions. The SG had high ($P<0.05$) K and Mg. The SL had high ($P<0.05$) Ca. The ST had high ($P<0.05$) Mn. The SO had high ($P<0.05$) Na, Fe, Cu, Zn, B and Al at most of the inclusion levels. The IVDMD was increasing ($P<0.05$) with increasing inclusion levels and withdrawal hours; with SO having the highest ($P<0.05$) IVDMD than other silages. High ($P<0.05$) total N content was observed on the ST and low ($P<0.05$) content on the SG and was increasing ($P<0.05$) with the increasing levels for all the citrus species pulp in sorghum silage.

The silages had better nutritive value in different nutrients (sections) and most of the parameters increased with the increasing inclusion levels. In detail, regarding chemical composition and chemical estimates, the SG silage had acceptable DM and the ST silage had better CP and ADL. Generally, except K, Mg and Fe, all the silages had the mineral concentration level which was below the threshold. The macro- and micro-minerals were better at 15 and 10% of citrus species pulp inclusion. The SO and ST silages had superior IVDMD (greater than 50% DM) in

comparison with the other silages. The 25% inclusion level produced better results on chemical composition and IVDMD of citrus species pulp in sorghum silages. The ST silage had a higher total N, and 25% produced a better nitrogen solubility index. The silage pH, WSC, and YM counts were optimized at an average of 23.2, 22.18 and 21.86% citrus pulps inclusion levels on sorghum silage. Citrus species pulp, inclusion levels, and level with species interaction affected all the micro-minerals, IVDMD, and silage fermentation of sorghum silage. These raw materials produced good quality silage, there is a need for supplementation, due to lower CP and minerals (except K, Mg and Fe) to improve the nutritional quality of the silage and thus, overall growth, metabolism and productivity of ruminants. There is a need to assess the aerobic stability across all the silages, and also run metabolic trials to assess the feeding value of the silages.

Keywords: Ensiling, citrus species pulp, sorghum, inclusion, nutrition, ruminants

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DEDICATION

This MSc degree is dedicated to my parents (Mr Nicodemus and Mrs Norah Ditabo), sisters (Lerato and Phinah Ditabo) and brothers (Lethabo and Morgan Ditabo).

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LIST OF ABBREVIATIONS

AA:	Acetic Acid
ADF:	Acid Detergent Fibre
ADL:	Acid Detergent Lignin
ARC:	Agricultural Research Council
CO_2:	Carbon Dioxide
CP:	Crude Protein
CRD:	Completely Randomized Design
DM:	Dry Matter
EE:	Ether Extract
GDP:	Gross Domestic Product
GLM:	General Linear Model
GP:	Grapefruit pulp
ICP:	Inductively Coupled Plasma
IVDMD:	*In vitro* Dry Matter Digestibility
LA:	Lactic Acid
LAB:	Lactic Acid Bacteria
LP:	Lemon pulp
NDF:	Neutral Detergent Fibre
NRC:	National Research Council
OM:	Organic Matter

OP:	Orange pulp
pH:	Potential of Hydrogen
RPM:	Revolutions Per Minute
SA:	South Africa
SG:	Sorghum: Grapefruit Pulp
SL:	Sorghum: Lemon Pulp
ST:	Sorghum: Tangerine Pulp
SO:	Sorghum: Orange Pulp
TDN:	Total digestible nutrients
TP:	Tangerine pulp
WS:	Whole Sorghum
WSC:	Water Soluble Carbohydrates
YM:	Yeast and Mould

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

In South African ruminant production, forages perform a significant role as they are easily available as a primary feed resource. Ruminant production forms a large part of the southern African agricultural sector, comprising 40% of the sub-Saharan African agricultural Gross Domestic Product (Enahoro *et al*., 2019). In semi-arid regions of southern Africa, ruminant production is limited by low nutritive value and unavailability of forage during dry seasons (Balehegn *et al*., 2022; Tulu *et al*., 2023). Dryland livestock production plays an important part in increasing the role of feeding the ever-growing global human population, as it contributes to 40% of the world and 70% of the South African land map (Boote *et al*., 2022). Recent studies highlighted that the fermentation of ensiled forages from rangelands is an important process that does not affect climatic change and enhances fermentation characteristics (Hawu *et al*., 2022; Usman *et al.*, 2022). The study done by Hariprasanna and Rakshit (2016) stated that the production of sorghum (*Sorghum bicolor* L. Moench) as a forage is a fast-growing crop for human and animal consumption. The growth and utilization of sorghum rely on its ability to grow in different environmental and climatic conditions (McCary *et al*., 2020; Bakari *et al*., 2022). Additionally, the rapid increase in the human population is expected to reach 9.6 billion by 2050 (FAOSTAT, 2018). In sub-Saharan regions, the main feed sources of energy are expensive as climate plays a role in their availability. Studies have shown that sorghum is the second-largest-grown cereal crop in Africa, and it is the fifth-produced cereal crop globally (Hariprasanna & Rakshit, 2016; Hadebe *et al*., 2017). Sorghum originates in Africa and has been observed to acclimatize and grow well in both tropical and temperate climates (Bakari *et al*., 2022). Nunes *et al*. (2022) stated that the preservation of forage crops should be an important part of ruminant production given the growth of small-scale intensive systems and the increasing concentrate feedstuffs prices.

Economically, the utilization of by-products as feed supplements is cost-effective (Roda & Lambri, 2019). Citrus fruits are grown globally, and their production amounted to 143 755.6 metric tons in 2019 (FAOSTAT, 2021). Africa produced about 15 579.3 metric tons of citrus fruits while South Africa is estimated to have produced 2 771.7 metric tons (FAOSTAT, 2021). Citrus fruits are grown in the autumn season in South Africa which makes them a seasonal fruit. Although citrus pulp is known as the hazardous by-product or agricultural waste that affect the environment, it has been shown that it could be recycled in the manufacture of animal feed while preventing environmental pollution and contributing to the reduction of the cost of feed (Ülger *et al*., 2020). The major citrus species examples are lemon, orange, lime, pomelo, mandarin and grapefruit (Sharma *et al*., 2017). The citrus pulp is rich in flavonoids, ascorbic acid, sugar and vitamin C, making it a valuable feed supplement for animal production. However, during dry periods the nutrional value in citrus pulp decreases, necessitating preservation methods to conserve its nutrients for later use.

Preservation methods of agro-industrial by products or forages includes haymaking and silage, thus securing feed throughout the year, conserving and enhancing livestock health and production (Balehegn *et al*., 2022). Silage is crucial due to its ability to retain nutrients in the fresh matter basis and can be harvested and made in different climate conditions. During dry periods, silage is an important feed for ruminant animals and preserves nutrient availability. Sorghum (*Sorghum bicolor* L. Moench) is more likely the same in terms of nutritional quality as maize (Alix *et al*., 2019), making it a good source of silage. The citrus pulp addition in the sorghum silage is expected to improve the nutritional status and the fermentation process due to its low fibre, pH, high pectin, antioxidants and anti-inflammatory unique characteristics amongst others (Czech *et al*., 2020). The current study aimed to assess the effects of varying graded inclusion levels of citrus species pulp on the nutritive value and fermentation profile sorghum silage.

1.2 Problem statement

With the rising human population and increasing demand for animal products, there is a need to explore alternative and supplementary feed for animal production to meet the demand for animal products. Southern African rural communal farmers face significant challenges due to inadequate livestock feed availability, particularly during dry seasons. This feed scarcity leads to nutritional deficiencies in livestock, ultimately compromising animal productivity and production (Mapiye *et al*., 2020). Additionally, the use of commercial supplements is economically unviable for communal farmers for improved animal production.

Ensiling sorghum as a preservation method, yield silages of low protein content and high energy value (Borba *et al*., 2012). In contrast, citrus pulps on their own do does not ferment properly due to their high level of moisture and sugar content leading to unstable silage, high effluent losses and quick deterioration (Bakr, 2020; Ülger *et al*., 2020). Citrus pulps are further characterized by high levels of water-soluble carbohydrates (WSC) and low buffering capacity, which can lead to rapid pH drop and secondary fermentation, inhibiting proper fermentation (Lee *et al*., 2019). Sorghum is less digestible than maize due to its higher lignin content and tannins, which bind protein that occurs in slightly higher concentrations in sorghum (Sabertanha *et al*., 2021). Therefore, there is a need to explore alternative means of feed that will sustain the production of animals throughout the year (Premarathne & Samarasinghe, 2020).

1.3 Justification

Feed preservation methods such as ensiling, haymaking, and utilization of agro-industrial by-products can improve livestock nutrition (Asar *et al.*, 2010) at low costs while enhancing the quantity of animal products with high biological value (Fernández-Herrero *et al.,* 2013). Sorghum (*Sorghum bicolor* L. Moench) grows well in varying climate conditions such as drought areas or high rainfall areas and has a high yield (Wagaw, 2019; Sabertanha *et al*., 2021). Getachew *et al*. (2016) reported that sorghum silage is a good alternative feed to maize silage,

as it is well fermented, and is a good source of nutrients for livestock. Sorghum silage should not be fed alone (Chen *et al*., 2019) as it is a high-energy source. Sorghum is the tropical cereal crop most used for silage because of its high yields of dry matter (DM) (Rooney *et al*., 2007), water-soluble carbohydrates content and lower buffering capacity (de Matos Teixeira *et al*., 2014). It is also known to improve soil fertility, has low production costs, better drought and high rainfall and high-temperature resistance (Prasad *et al*., 2021). Citrus pulp has low pH which influences the fermentation process of silage positively. The low pH of citrus pulp results from the high concentration of tartaric, ascorbic, malic and citric acid (Ülger *et al*., 2020). Citrus pulp also has low dry matter (DM), low lignin, moderate crude protein (CP) and low cellulose (Ülger *et al*., 2020), which makes it a good complement to the sorghum silage. Citrus pulp is affordable, palatable and has highly digestible nutrients (Bakr, 2020). Additionally, these fruits have high concentrations of vitamin C and antioxidants, which positively impact the health and improve the performance of ruminants. The citrus pulp increases feed intake by ruminants due to its pleasant odour (Bakr, 2020).

1.4 Aim and objectives of the study

The study aimed to evaluate the effects of varying inclusion levels of citrus species pulp on the nutritive value and fermentation profile of sorghum (*Sorghum bicolor* L. Moench) silage.

Objectives:

1. To determine the chemical composition, chemical estimates and physical characteristics of citrus species pulp.
2. To evaluate the effect of graded inclusion levels of citrus species pulp on chemical composition and fermentation profile of sorghum (*Sorghum bicolor* L. Moench) silage.

1.5 Hypotheses

1. Citrus species pulp would have varying chemical composition, chemical estimates and

physical characteristics.

2. Graded inclusion levels of citrus species pulp would affect chemical composition and fermentation profile of sorghum (*Sorghum bicolor* L. Moench) silage.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Nutrition is a significant component of livestock production, constituting ±75% of the total cost of production (Mudzeng *et al*., 2020). Such a high cost must be reduced without limiting the quality and quantity of feed, moreover the livestock performance (Mudzeng *et al*., 2020). The agro-industrial wastes, such as citrus pulp, can replace conventional feed ingredients, minimizing production costs by 20-40 %, lowering environmental pollution through waste volarization, and enhancing food security by improving feed availability (Morale *et al.*, 2019). Some agro-industrial wastes have moderate to high nutrients and exhibit low-toxicity, making them viable alternatives for ruminant feed. Moreover, the price of feed in winter increases due to the high demand for feed in commercial feed companies; thus, silage-making should be prioritized mainly in smallholder farming. In southern Africa, about 90% of the livestock producers are smallholder farmers who depend on livestock products to improve their socio-economic status, income generation and home consumption (Gwiriri *et al*., 2019). High temperatures and lack of rainfall in tropical regions result in forages of low quality that are less digestible due to the high concentration of lignin (Berhanu *et al*., 2019). The available forages in winter have reduced nutritional content, creating a substantial nutritional deficit that fails to meet the standard required for the small ruminants' production (Bakr, 2020). It is therefore necessary for farmers to utilize silage to provide good quality feed to their animals during periods of feed scarcity. As temperatures keep on increasing and rainfall is decreasing, sorghum is gaining attention and its use as the sole energy source increases in the tropical regions. While sorghum demonstrates its suitability for ensiling, with comparative nutritive value to maize (13% CP and 55%TDN) (Pereira *et al*., 2020; Rodrigues *et al*., 2020). It requires complementary supplementation for balanced ruminant diets due to limiting amino acids such as lysine and threonine (McCary *et al*., 2020; Soltan *et al*., 2021). Therefore, this study investigated the effects of graded inclusion levels of citrus species (lemon, tangerine, grapefruit and orange) pulp in

sorghum silage on the nutritive value and fermentation characteristics.

2.2 Significance of ruminants in South Africa

Ruminants in South Africa play a crucial role in the livelihood of people and alleviate poverty in rural areas. They also improve food security by providing meat, milk and skin at an affordable price, more than with small ruminants. Ruminants farming offers employment opportunities, which uplifts the economy and the lifestyle of the people, moreover in developing regions (Gwiriri *et al*., 2019; Kandiwa *et al*., 2020). Cattle, sheep and goats are adapted to different diet types (Schroeder *et al*., 2019) due to their anatomical and physiological digestive system. These animal species are used for rituals, they are a source of income, and they are for consumption and prestige by both women and youngsters (Wodajo *et al*., 2020). Ruminants provide protein high-quality sources (meat and milk) and produce premium wool/fibre valued for textile industries (Chadda *et al*., 2025). Furthermore, small ruminants can be kept and reared in a smaller space, require low input costs while providing quicker profits. Bonsmara cattle and Dorper sheep for instance, are well adapted to the southern African climatic conditions as they were produced in South Africa and do well under such conditions. They are large-framed breeds with high slaughtering weights and good mothering ability. Hence, they greatly contribute to the livelihoods of rural households and communities and also improve food security in South Africa.

Tropical regions are experiencing low rainfall as the climate changes and thus impact livestock production. Small ruminants can survive water shortages as they have efficient water-saving mechanisms, their intake and utilization of dry matter is maintained (between 2-2.1% of body mass) and efficient during drought periods (Akinmoladun *et al.*, 2019; Shinde & Mahanta, 2020). Additionally, ruminants such as goats are noted to be excellent at the removal of weeds by grazing on pasture lands (Karki, 2020) and this strategy is beneficial both to the farmer and the goats as it is a cheap strategy that provides essential nutrients to the goat (Ghanizadeh & Harrington, 2019;

Ntalo *et al*., 2022).

Ruminant production has tremendously grown in semi-arid Southern Africa, although it is facing major setbacks of feed scarcity and climate change (Mataveia *et al*., 2021). Goats' population in Southern Africa was roughly 35 million in the year 2020 (Mataveia *et al*., 2021). In 2023 South Africa had a total goat population of 5.1 million (Table 2.1). It has been predicted that by 2025, the ruminants will be producing more than 50% of the red meat in the South Africa region (Kandiwa *et al*., 2020).

Table 2.1 Ruminant livestock production and slaughter estimated numbers (in millions) in RSA in 2023

	Cattle	**Sheep**	**Goats**
Production number	12.2	21.4	5.1
Slaughter number	3.2	4.8	0.7

Source: FAOSTAT (2024)

2.3 Description of selected forage and citrus species pulps/fruits

2.3.1 Sorghum

Sorghum (*Sorghum bicolor* L. Moench) is a rich energy forage grown mainly for human and animal consumption with 94 360 and 25 954 458 tons of sorghum produced in 2023 in South Africa and Africa, respectively, with Africa exceeding all the other continents (FAOSTAT, 2024). The whole sorghum plant is used for ruminant feeding while the grains are for human consumption. It is well adapted to the different climatic conditions and the nutritive value ranges according to the climate the plant is grown in. Sorghum can produce high-quality yield in dry areas and areas that experience high rainfall from time to time. Its yield and quality are maintained under such weather conditions. Sorghum is an arid-region crop, requiring approximately 50% less water than maize under comparable conditions (Sabertanha *et al*., 2021). From a single sorghum plant, there can be more than one harvesting (Sabertanha *et al*., 2021). Its production and preservation are more

encouraged in the arid to semi-arid regions due to its capability to grow in such conditions. The stem of sorghum has a higher concentration of fibre and is more lignified while its leaves have a high concentration of crude protein. It is characterized by starch as the source of the carbohydrate with 800 g/kg of insoluble fibre (Léder, 2004). The high fiber content enhances its suitability for ruminant diets, as the insoluble fiber ungoes efficient microbial rumen fermentation. While whole plant sorghum is further associated with the presence of phenolic compounds such as tannins and flavonoids that assist with inflammations, growth parameters and health status, they can also lower the nutritive value of feeds (Xiong *et al*., 2019). Sorghum has low digestibility which affects its utilization and feed intake and hence, Alnaimy *et al*. (2017) stated that it is necessary to supplement sorghum feed with highly digestible feedstuff such as citrus pulp with roughly 74% of total digestible nutrients (TDN). Due to the poor production of sorghum in dry seasons, it is better to initiate its preservation either as hay or silage for the consumption of ruminants.

2.3.2 Citrus

Citrus fruits such as tangerine, lemon, orange and grapefruit belong to the *Rutaceae* family and their production remains high when compared with other fruits (Hussain *et al*., 2021). The fruits are distinguished by their bright colours, segmented membranes, aroma and inedible peels. They are grown and consumed worldwide for their distinctive taste, aroma and anti-inflammatory and antioxidant characteristics (Hussain *et al*., 2021). They have been linked to various health benefits due to their content of pectin, flavonoids, dietary fibre, vitamins, minerals and other beneficial compounds (Bampidis & Robinson, 2006). Lashkari *et al*. (2014) described citrus pulp as residues remaining after juice extraction and it is composed of peels, seeds and pulps. Andrade (2020) and Bakr (2020) noted that citrus fruit waste after juice extraction has been a major problem for various companies and they later affect climate change and the livelihoods of communities when discarded inappropriately. Their disposal has been an issue for years yet they have good nutritional value that can be used to enhance ruminant feeds. It has been noted that due to their high moisture, they

tend to spoil quickly causing the deterioration of the pulps when intended to be reused either for ruminant feed or other uses (Andrade, 2020). Due to the attractive and distinctive aroma, taste and colour, pulps are anticipated to encourage increased consumption by ruminants (Roongruangsri *et al*., 2013).

2.3.2.1 Tangerine (*Citrus reticulata)*

Tangerine, naartjie and mandarin are common names for *Citrus reticulata*. Naartjie is an Afrikaans name for the sweet, mostly seedless and easy-to-peel citrus fruit that is commonly grown in both tropical and temperate climates (Roongruangsri *et al*., 2013). The ripe tangerine has a small size and sweet taste, constitutes less than 20% of the global citrus production and contains essential oils, acids, water-soluble carbohydrates and carotenoids (Roongruangsri *et al*., 2013). Hussain *et al*. (2021) reported that tangerine has the highest carbohydrate concentration when compared to other citrus fruits. In South Africa, tangerine ripens in the mid-autumn to winter season and its pulp has low fibre content as compared to other citrus species' pulp due to their soft and light-weighted peels. Generally, the DM of the citrus fruit pulp is low resulting in losses of DM. The DM content of tangerine (Table 2.2) is lower than the standard and acceptable DM (>300g/kg) for ensiling (McDonald *et al*., 1991; Grizotto *et al*., 2020). Citrus pulp usage in ruminant feed is considered cost-effective, because it is an underutilized resource. Moreover, its inclusion in animal diets may contribute to mitigating climate change by reducing waste and lowering greenhouse gas emissions compared to conventional feed ingredients (Lashkari *et al*., 2014).

2.3.2.2 Lemon (*Citrus limon*)

The lemon is ranked the third-produced citrus fruit globally (Di Matteo *et al*., 2021) and has a sour taste, yellow peels and grows on thorny trees. Lemon pulp has a tart flavour (Xi *et al*., 2017) and a pleasant smell, which might increase feed intake and acceptance by ruminants. The high antioxidant content found in lemon pulp has an impact on the ruminants' health. The higher moisture content of roughly 80% found in lemon pulp and other citrus pulp can be used to increase the water intake by animals that are in areas experiencing water drought (Za'za, 2008; Alnaimy *et al*., 2017; Todaro

et al., 2017; Bakr, 2020). Lemon peels are low in crude protein and high in carbohydrates (Jiang *et al*., 2022). Twinomuhwezi *et al*. (2023) found that the pectin concentration was high in lemon peel, which serves to greatly reduce feed dust and waste by gelling together the feed particles. The lemon pulp contains vitamins and minerals (Di Matteo *et al*., 2021) essential for the health and metabolism of livestock animals.

2.3.2.3 Orange (*Citrus sinensis*)

Orange fruit is the most known and highest-produced citrus fruit globally (Bampidis & Robinson, 2006), amounting to 1 610 000 tons of production in 2023 (FAOSTAT, 2024). The orange pulp is therefore available in high quantities although it is a seasonal fruit. The orange fruit has a sweet taste, orange peels and thick peels. Alzawqari *et al*. (2016) stated that orange pulp is rich in flavonoids and phenolics that improve the intake and utilisation of nutrients by ruminants. Orange pulp has a high concentration of soluble fibre that enhances digestibility (Watanabe *et al*., 2010). The orange pulp contains vitamin C and antioxidants that improve health, performance and nutrient digestion (Bakr, 2020). Volanis *et al*. (2006) reported lambs fed orange pulp showed increased intake due to improved palatability of the pulp.

2.3.2.4 Grapefruit (*Citrus paradisi*)

Murthy *et al*. (2020) noted the origins of grapefruit as a cross between orange and pomelo citrus fruits. The grapefruit is characterized by red juice vesicles with thick peels, has a similar look to orange fruit (Murthy *et al*., 2020) and is grown in both tropical and coastal areas in South Africa. Grapefruit has a bitter taste which is attributed to the limonoids phytochemical (Manners, 2007) and is mostly medium to large-sized. The weight of the grapefruit pulp constitutes roughly 65% of the original weight of a complete fruit (Alnaimy *et al*., 2017). This weight and the soluble carbohydrates result in greater organic matter. Grapefruit is unique in the presence of folic acids, ascorbic acids, carotenoids and flavonoids (Murthy *et al*., 2020). The grapefruit pulp has high readily fermentable carbohydrates that improve the feeding efficiency and utilisation by ruminant animals. A study by Salami *et al*. (2020) reported that grapefruit pulp has greater palatability due

to its appearance and odour. Grapefruit has been found to have low pH that will promote production of lactic acid which is an excellent requirement for silage making when used as an additive (Lee *et al*., 2019).

Table 2.2 Nutritive composition (g/kg DM unless specified) of selected forage and citrus species pulp

	DM	OM	CP	NDF	ADF	ADL	EE	References
Sorghum	339.0 g/kg	937.3	131.5	446.1	201.7	-	13.5	Pereira *et al*. (2020)
Tangerine pulp	21.45 %	96.2 %	4.8 %	14.8 %	13.2 %	-	1.0 %	Beyzi *et al*. (2018)
Lemon pulp	237.9 g/kg	952.8	75.6	216.1	194.5	79.3	28.4	Ülger *et al*. (2020)
Orange pulp	130.0 g/kg	964.5	78.0	155.1	164.0	76.1	8.1	Lashkari *et al*. (2014) Ulger *et al*. (2020)
Grapefruit pulp	260.1 g/kg	-	91.4	166.5	130.8	-	23.7	Andrade *et al*. (2020)

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, EE: ether extract

2.4 Silage preparation

Silage is the end product of a process whereby the raw materials are subjected to anaerobic conditions while preserving or enhancing the chemical composition. Campbell (2014) stated that for successful anaerobic conditions for preservation, the forage material must be tightly squeezed and sealed to eliminate air particles. Elferink *et al.* (2000) indicated that the ensiling process may be divided into four stages as depicted and summarized in Figure 2.1. During the ensiling process, the water-soluble carbohydrate of the fresh material is converted into lactic acid by the lactic acid bacteria (LAB) and further into acetic acid. As the acids are produced, the pH goes down and in turn halts the activities of harmful microbes such as Clostridia microorganisms which cause spoilage (McDonald *et al*., 1991). Many articles have been published about the silage-making process (Elferink *et al*., 2000; Campbell, 2014). The primary aim of silage processing is to optimize the retention of primary nutrients in the forage crops for later use in livestock feeding (Salami *et al*., 2019). Silage is mostly fed and suitable for dairy animals because it retains the fresh state of the forages which is required for the production of milk (Campbell, 2014).

Stage 1: Aerobic stage

- Initial few hours
- Air particles present
- pH: 6.5-6
- Yeasts and enterobacteria (Facultative aerobic microorganism)

↓

Stage 2: Fermentation stage

- Weeks
- Air particles absent
- Development of LAB
- Production of LA and AA
- pH: 5-3.8

↓

Stage 3: Stable stage

- Weeks
- Microorganisms' population lowers
- pH: <4.5

↓

Stage 4: Feed out or aerobic spoilage stage

- Exposure to air particles
- Yeasts degrade LA and AA
- Harmful microorganisms' population increases
- pH increases
- Spoilage occurs

Figure 2.1 The silage process (Elferink *et al*., 2000; Campbell, 2014)

2.5 Laboratory feed evaluation methods

2.5.1 Nutritive (chemical, physical and minerals) analyses

Feed evaluation methods are important in animal feeds to ensure cost-effective production and maintain animal health. Different methods have been employed to determine different parameters that are significant in ruminant nutrition. Chemical composition analyses are the primary method used to determine the true nutritional status of feeds/forages (Mould, 2003; Mahmoud *et al*., 2017). This assists in knowing the neutral detergent fibre (NDF), acid detergent lignin (ADL), ether extract (EE), ash and crude protein (CP) content of feeds. Mahmoud *et al*. (2017) noted that they are used as indicators of chemical composition in feed. Feed evaluation parameters allow indirect assumptions about amino acid content, digestibility, ensiling, and livestock feed utilisation efficiency. It has been noted that different factors such as growth stage and environmental effects can impact the chemical composition of forages and feeds (Mburu *et al*., 2018). Moreover, the nutritional composition of the feed will determine the class of livestock animals it should be offered to (Mould, 2003). Malomo & Ihegwuagu, (2017) further stated that chemical composition analyses are less costly and simple subjecting them to a necessity before feeding.

The particle sizes, water retained and packed volumes of feed are imperative and often overlooked or under-analysed. These parameters are essential as they affect the retention time or time spent in the digestive system of livestock animals (Kaske & Engelhardt, 1990). It has been known that large particle sizes of feeds require regurgitation to be fully digested and absorbed. The tiny, ground and pelleted feed has been noted to decrease feed digestibility (Le Liboux & Peyraud, 1999). Therefore, it is essential to determine the physical parameters of feed as they impact the rumen function. Also, the digestive system of the livestock species differs and that necessitates different feed particle measurements. Water retention is the ability of the feed samples to contain water for a prolonged period of time, thus positively affecting quality and animal health. While packed volume is the

volume the feed fills which relates to the particle size and density. Packed volume impact on the feed intake, efficiency and ultimately performance, when other factors are kept constant.

Mineral analysis is mainly for the health aspect and are divided into two groups of macro and micro minerals (Soetan *et al*., 2010). The total mineral in the body of livestock animals constitutes 5% of weight (Márquez-Madrid *et al*., 2017). The correct concentrations of minerals in feed as stipulated in NRC (2007) have an impact on the health while also affecting the reproduction and productivity of animals. The determination of minerals [calcium (Ca), sodium (Na), iron (Fe) and zinc (Zn), to name a few] is necessary and assists with supplementation when feeding livestock animals. Minerals impact maintenance, breeding and performance of livestock (Soetan *et al*., 2010). Depending on soil conditions, plant species, and maturity, minerals such as phosphorus (P) and micro minerals such as copper (Cu), Zn, and selenium (Se) are deficient in forages (Gwelo *et al*., 2015; Sprinkle *et al*., 2018), hence the analyses of minerals are important. Other varying factors that have been reported to influence the concentration of minerals in forages are harvesting stage, soil mineral content, forage type, topography and environment (Mirzaei, 2012; Marijanušić *et al*., 2017). Furthermore, the mineral requirements of animals differ according to the health status of animals, age, gender, animal species and production level and such should be considered when formulating feeds (NRC, 2007).

2.5.2 *In vitro* dry matter degradability (IVDMD)

In vitro dry matter degradability (IVDMD) analyses determine feed quality as it allows feed formulators to determine the extent and speed at which the feedstuffs are degradable. This method utilizes rumen microbial digestion as it can degrade highly fibrous matter (Sanders, 2024). The IVDMD has been recently favoured over *in sacco* and *in vivo* methodologies because it is labour-efficient, precise, quick, affordable, replicable and appropriate for a large sample size (Harper & McNeill, 2015). The fibre content is the major constituent that affects the degree of IVDMD (Harper & McNeill, 2015). Insoluble carbohydrates such as starch and glucose and structural

carbohydrates such as lignin and cellulose are digested rapidly and slowly, respectively (Andualem *et al*., 2016). Variations in IVDMD results could allude to the differences in rumen fluid, chemical composition and sample preparation method amongst others (Harper & McNeill, 2015; Mahmoud *et al*., 2017). Several researchers investigated the IVDMD of forages and silages (Sebata *et al*., 2011; Mokoboki *et al*., 2019; Chelopo *et al*., 2023). Mould (2003) emphasized that particle size in feed samples for IVDMD must closely represent what livestock actually consume to avoid misleading digestibility estimates.

2.5.3 Fermentation analyses

Fermentation characteristics analyses are imperative in the feed samples that have undergone the ensiling process. This helps to determine the effect ensiling had on the samples and further assists in determining the suitability of the feed to be fed to livestock animals. Fermenting the samples improves palatability, nutritional status and shelf life (Li *et al*., 2018), as well as storing feed for the dry seasons (Chelopo *et al*., 2023). Fermentation characteristics are assessed through multiple metrics, includes potential of hydrogen (pH), lactic acid (LA), acetic acid (AA), water soluble carbohydrates (WSC), yeast and mould (YM) and carbon dioxide (CO_2) production (Nkosi *et al*., 2016). Each parameter provides insights into fermentation efficiency, stability, and feed safety. Different factors have been used to alter the fermentation process to acquire the desired results. For example, the application of lactic acid bacteria (LAB) can be used to speed up and enhance the fermentation process and to produce higher proportions of organic acids (Bai *et al*., 2021). Absorbents such as maize stover or wheat bran can be used to lower the moisture content to create an appropriate ensiling environment (Elahi *et al*., 2019). Temperature, density, inoculants, and ensiling duration have been reported to influence silage fermentation characteristics in varying ways, with the effects also depending on the chemical composition of the fresh material (Nkosi *et al*., 2016; Anésio *et al*., 2017; Li *et al*., 2018). Analyzing fermentation characteristics is significant for assessing silage quality as it assists evaluate the potential effects of the ensiling process.

2.6 Impact of sorghum silage in ruminants

Sorghum silage is a crucial feed as it preserves and provides essential nutrients to ruminants in dry seasons. Sorghum silage depending on its chemical composition and the physiological status of the livestock, maintains and improves the performance of ruminants. Campanili *et al*. (2017) reported that sorghum silage improves grain ruminal degradability and fibre digestibility without negatively affecting feeding behaviour. Furthermore, sorghum silage due to its low digestibility and higher lignin content reduces feed utilisation and ultimately the performance of the animals. Sabertanha *et al*. (2021) reported that sorghum silage did not have an impact on the blood metabolites of sheep while Osmari *et al*. (2011) reported that sorghum silage improved the milk quality of goat. Nascimento *et al*. (2020) also reported increased feed efficiency when lambs were fed sorghum silage. The study done by Wu *et al*. (2021), reported better growth performance and fatter carcasses in lambs fed sorghum silage. Colombini *et al*. (2012) outlined no effects on the milk production of dairy cows fed sorghum silage. Generally, sorghum silage has an impact on the performance of small ruminants although supplementation of limited nutrients is necessary to meet the standard requirements.

2.7 Review Summary

Ruminants are important to the livestock sector in South Africa and other developing countries, but they face difficulties due to a lack of adequate feed, which reduces animal productivity. Sorghum (*Sorghum bicolor* L. Moench) is a rich energy forage grown in semi-arid and arid areas mainly for the consumption of humans and livestock. Its capability to grow in different climatic conditions, characterized by increased WSC, high production rate, ease of cultivation and low buffering capacity makes sorghum a good silage material. Dietary inclusion of citrus pulps in silage might accelerate the fermentation process and result in the reduction of pH (Mapiye *et al*., 2020), thus, improving the quality of the silage. Citrus pulp might increase silage palatability and

intake due to the pleasant odour and high digestibility. The quality of the silage is based on improved silage fermentation characteristics, improved acid profile and reduced protein degradation. Nonetheless, sorghum production yield is higher, suitable for mechanization and is easily ensiled. Although it is low in protein content, its energy value is high. Therefore, supplementing sorghum with citrus pulp to produce silage could complement the protein content and other limiting nutrients in sorghum.

2.8 References

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CHAPTER 3: NUTRITIVE VALUE OF FRESH CITRUS SPECIES PULP

3.1 Abstract

Citrus species pulp has varying physical and chemical compositions and can potentially improve the availability and quality of ruminant feed. The objective of this study was to evaluate the nutritive value and physical characteristics of fresh citrus species pulp. The study took place at the North-West University research farm (25°49′22″S and 25°36′54″E). The citrus species fruits were purchased from the local commercial retail shop and were juiced using the Taurus Liquafruits Pro Juice Extractor. The remaining peel, seeds, juice residue and membranes were combined to make tangerine (TP), lemon (LP), orange (OP) and grapefruit (GP) pulps. The dried and ground citrus species pulps were analyzed for chemical composition and physical characteristics. The data were analyzed in a completely randomized design (CRD) by ANOVA using the GLM procedure of SAS (2010). The LP, GP and TP had high ($P<0.05$) DM, while OP had high ($P<0.05$) CP and ADL than LP, GP and TP. Ash, EE as well as P, K, Ca, Na, Mg, Zn, Mn, Fe, Cu and B concentrations were higher ($P<0.05$) in LP than other pulps. The NDF, ADF, Cel, WR and PV were higher ($P<0.05$) in OP relative to their counterparts. All the chemical estimates (DMD, DE, ME and TDN) were higher ($P<0.05$) in TP compared to other pulps. Nonetheless, the citrus species pulp had low CP, DM and micro-minerals, however, they had acceptable fibre fraction as potential ruminant feed ingredients. Their low DM might lead to DM losses and spoilage during preservation, thus necessitating an absorbent for improved shelf life and nutrient preservation. However, there is a need to assess the ensiling process of the citrus species pulps to improve the chemical composition and physical characteristics of other forages to compensate for limiting nutrients during ensiling.

Keywords: Orange, tangerine, lemon, grapefruit, livestock, feed, nutrients

3.2 Introduction

The growing human population worldwide is expected to reach approximately 10 billion people by 2050 (FAOSTAT, 2018), requiring constant and increasing animal products to meet the demand. Livestock feed is required in huge amounts every day, and the commercial feed industry struggles to meet the demand and sourcing the ingredients (Kim *et al*., 2019) due to climate change and urbanisation, which result in high feed costs. Hence, research focuses on exploring innovative additives to optimise production and enhance the use of non-conventional feedstuff. Among these, low-grade fruits and fruit by-products have emerged as promising candidates, with prior studies indicating their potential to reduce feed costs, lower environmental pollution and enhance profitability in livestock-producers (Bakr, 2020). The citrus fruits are abundant across the world, and their by-products are of huge concern as they contribute to environmental pollution, with global annual citrus production reaching approximately 160 million tons in 2021 (FAOSTAT, 2022). In 2022, South Africa produced 640 000 tons of tangerines, 770 000 tons of lemons, 1 740 000 tons of oranges and 420 000 tons of grapefruits (FAOSTAT, 2023). The citrus fruits are therefore of great interest to be incorporated into livestock feed due to their availability and nutritive value. There is however, variations in the chemical composition of different species of citrus fruits.

Citrus pulp is the residuals of juice extraction, and it is composed of peels, seeds and pulps (Lashkari *et al*., 2014). The citrus pulp consists of an average of 50.0% of the original fruit weight, has a high moisture content, and contains moderate nutrients that can enhance the nutritive value of feed (Czech *et al*., 2020). They further contain anti-inflammation and anti-cancerous properties due to their antioxidants and mineral composition (Czech *et al*., 2020). The pulp of citrus has traditionally been utilized on a fresh, but this method is unfavourable due to rapid spoilage. Ad a

result, it is now commonly processed into dried pulp, silage or citrus molasse (Bakr, 2020). Livestock that can benefit from the citrus waste include sheep, cattle (beef and dairy), owing to the waste' high energy content (Arthington *et al*., 2002). Research has also explored the inclusion of citrus waste in non-ruminant diets, with studies (e.g., Mourão *et al*., 2008) reporting beneficial effects.

The current study evaluated the chemical composition of the citrus species pulp as a potential feed ingredient for ruminant feed. Nutritional evaluation of the pulp will characterize their potential a feed resource. Currently, there is limited information on the chemical (proximate and mineral) composition and physical characteristics of individual citrus species' pulp. Therefore, there is a need to determine the chemical composition, chemical estimates and physical characteristics of fresh citrus species pulps individually. The study hypothesised that individual citrus species pulps have different chemical composition, chemical estimates and physical characteristics.

3.3 Methodology

3.3.1 Laboratory site

The research project was conducted at the North-West University research farm in Mmabatho (25°49′22″S and 25°36′54″E), North-West, South Africa. The area is partially semi-arid with an altitude of 1,290 metres above sea level and annual average rainful of 500 mm. Summer temperatures range between 25.0 - 38.0 ℃, while winter temperatures range between 11.0 - 18.0 ℃.

3.3.2 Citrus species pulp preparation and treatments

Four citrus fruit species (tangerines, lemons, oranges and grapefruits) were bought from the commercial retail shops. The fruits were juiced using Taurus Liquafruits Pro Juice Extractor, separating the juice and pulps, the pulps contained the peel, seeds and membranes. The pulps were further ground using a 2.0 mm sieve to determine their chemical (proximate, fiber fractions and mineral) composition. The citrus species pulps were categorized tangerine pulp (TP), lemon pulp (LP), orange pulp (OP) and grapefruit pulp (GP), with five replicates per treatment.

3.3.3 Chemical composition analysis

The treatments (represented as TP, LP, OP and GP) were subjected to laboratory analyses to determine dry matter (DM) as outlined by AOAC (2005) with moisture (M) as the lost mass from the DM analyses. Ash was determined by exposing one gram of the samples to 600 ℃ in a muffle furnace overnight as expressed by AOAC (2005). The samples were extracted with petroleum ether in the ANKOM Xt10 extraction system (ANKOM Technology, Macedon, and NY) to determine the ether extract (EE). One gram of each sample was mixed with the Kjeldahl tablets and sulphuric acid for digestion. Boric acid and sodium hydroxide were used to titrate according to AOAC (2005) for the analyses of crude protein (CP). ANKOM2000 Fiber Analyzer (ANKOM ANKOM Technology, New York) was used to determine neutral detergent fibre (NDF) acid detergent fibre (ADF) according to Van Soest *et al*. (1991), where samples (0.45–0.5 g) were refluxed with neutral and acid detergent solutions for 1 hour and 1 hour 15 minutes, respectively. A heat-stable bacterial α-amylase was included in the determination of NDF. Acid detergent lignin (ADL) was determined by subjecting samples to sulphuric acid (72.0 %) for 3 hours as outlined by van Soest *et al*. (1991).

3.3.4 Mineral analysis

Samples of the citrus species pulps (TP, LP, OP and GP) were analyzed for micro- and macro minerals. The Agri-Laboratory Association of Southern Africa (AgriLASA, 1998) methodology was followed in the analysis. The samples were ashed and analyzed for P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn, B and Al utilising an inductively coupled plasma (ICP) mass spectrometer (Perkin-Elmer, 1982, NexION 300Q).

3.3.5 Physical characteristics

The water retention and packed volume of the citrus species pulps were analyzed as described by Seoane *et al*. (1982) with the modifications of Matlabe *et al*. (2015). The samples (0.5 grams) were weighed into test tubes and dried at 105 °C overnight. The dried samples were transferred into the centrifuge tubes, mixed with 30 ml of distilled water and left for an hour. Each solution was then centrifuged at 1200 revolutions per minute (RPM) for 2 minutes, followed by paper filtration of the supernatant. The pellet was weighed together with the residues on the filter paper. The packed volume was analyzed by weighing 2.0 grams of the grounded samples and oven-dried at 105 °C overnight. The samples were then centrifuged at 1200 RPM speed for 20 minutes. The volume occupied by the sample in the centrifuge tubes was measured.

3.3.6 Chemical estimates formulas

The following chemical estimates were calculated using the formulas:

Hemicellulose and cellulose were calculated using the following formulas as described by Javier-Astete *et al*. (2021)

$$\text{Hemicellulose}\left(\frac{\text{g}}{\text{kg}}\text{DM}\right) = \text{NDF} - \text{ADF} \qquad (3.1)$$

$$\text{Cellulose}\left(\frac{\text{g}}{\text{kg}}\text{DM}\right) = \text{ADF} - \text{ADL} \quad (3.2)$$

Dry matter digestibility (DMD) was calculated using the ADF concentration as described by Boga *et al*. (2014).

$$\text{DMD}\left(\frac{\text{g}}{\text{kg}}\right) = 88.9 - (0.779 \times \text{ADF}) \quad (3.3)$$

Digestible energy (DE) was calculated according to Khalil *et al*. (1986) as in equation (3.4)

$$\text{DE}\left(\frac{\text{Mcal}}{\text{kg}}\right) = 0.027 + 0.0428 \times (\text{DMD}) \quad (3.4)$$

Metabolizable energy (ME) was determined using the following formula described by Khalil *et al*. (1986)

$$\text{ME}\left(\frac{\text{Mcal}}{\text{kg}}\right) = 0.821 \times \text{DE} \quad (3.5)$$

Total digestible nutrient (TDN) was predicted using the following formula as described by Bath (1989).

$$\text{TDN}\left(\frac{\text{g}}{\text{kg}}\text{DM}\right) = 82.38 - (0.7515 \times \text{ADF}) \quad (3.6)$$

3.3.7 Statistical analysis

Data on chemical composition characterized through proximate, fiber fractions ad mineral profiles, physical characteristics and chemical estimates, of fresh citrus species pulps were analysed in a completely randomized design (CRD) by ANOVA using the GLM procedure of SAS (2010). Treatment means were compared using the Duncan's multiple range test, where significance differences were declared at $P<0.05$. Data were fitted to the model:

$$\text{Yij} = \mu + \text{Ti} + \text{Eij} \quad (3.7)$$

Where: Y_{ij} was the individual observations of the i_{th} treatment and the j_{th} replicate, μ was the overall mean, T_i was the effect of the i_{th} treatment and ε_{ij} was the random variation or residual error.

PROC CORR (SAS, 2008) was utilized to determine Pearson's correlation coefficients amongst the chemical composition, chemical estimates and physical characteristics of the citrus species pulps.

3.4 Results

Results of the chemical composition of citrus species pulps are demonstrated in Table 3.1. Higher ($P<0.05$) DM was detected in LP and GP than OP. However, DM content for GP was comparable to that observed in LP and TP. The ADL content was higher ($P<0.05$) in OP than in TP, LP and GP species. The highest ($P<0.05$) ash content was observed in LP, while the lowest ($P<0.05$) was observed in OP. Higher ($P<0.05$) EE was detected in LP, followed by OP, GP and TP, respectively. The OP had significantly higher ($P<0.05$) CP compared to GP, LP and TP. Higher ($P<0.05$) NDF and ADF content were observed in LP with the lowest ($P<0.05$) content in TP. There were no significant differences ($P>0.05$) found on Hemi of the treatments. Higher ($P<0.05$) Cel was reported in LP and lower ($P<0.05$) content in OP and TP.

Table 3.1: Proximate composition of citrus species pulps (g/kg DM unless specified)

Parameters	LP	OP	GP	TP	SEM	P-value
DM (g/kg)	222.6[a]	185.0[c]	210.0[ab]	200.0[b]	6.6	0.007
Ash	48.9[a]	28.4[d]	47.6[b]	32.2[c]	0.4	<.0001
EE	31.8[a]	25.9[b]	21.7[c]	18.0[d]	0.8	<.0001
CP	49.8[bc]	55.9[a]	49.0[c]	50.7[b]	0.3	<.0001
NDF	207.5[a]	153.3[b]	158.2[b]	104.1[c]	2.0	<.0001
ADF	166.8[a]	108.1[c]	128.4[b]	79.0[d]	4.8	<.0001
ADL	8.9[c]	34.9[a]	15.9[b]	17.4[b]	1.8	<.0001
Hemi	40.7	45.1	29.7	25.1	5.1	0.05
Cel	157.9[a]	73.4[c]	112.5[b]	61.6[c]	5.0	<.0001

[a,b.c,d] Means within a row with different superscripts are different (*P<0.05*), LP: lemon pulp, OP: orange pulp, GP: grapefruit pulp, TP: tangerine pulp, DM: dry matter, EE: ether extract, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, Hemi: hemicellulose, Cel: cellulose SEM: standard error of means, P-value: probability value.

The results on macro minerals of the citrus species pulps are presented in Table 3.2 below. Significant differences (*P<0.05*) were evident on all the parameters (P, K, Ca, Mg and Na) for LP, OP, GP and TP species. The P content was higher (*P<0.05*) in LP with lower (*P<0.05*) content in TP. The LP and GP had higher (*P<0.05*) K content while OP and TP had lower (*P<0.05*) content. Higher (*P<0.05*) Ca, Na and Mg content were detected in LP followed by GP, TP and OP, respectively.

Table 3.2: Macro minerals of the citrus species pulps (% DM unless specified).

Parameter	LP	OP	GP	TP	SEM	P-value
P	0.17^{a}	0.12^{b}	0.12^{b}	0.10^{c}	0.002	<.0001
K	2.2^{a}	1.6^{b}	2.3^{a}	1.5^{b}	0.02	<.0001
Ca	0.7^{a}	0.3^{d}	0.7^{b}	0.4^{c}	0.004	<.0001
Na (mg/kg DM)	144.8^{a}	35.4^{d}	97.8^{b}	49.0^{c}	4.5	<.0001
Mg	0.14^{a}	0.07^{d}	0.13^{b}	0.08^{c}	0.001	<.0001

[a, b, c, d] Means within a row with different superscripts are different (*P<0.05*), LP: lemon pulp, OP: orange pulp, GP: grapefruit pulp, TP: tangerine pulp, P; phosphorus, K: potassium, Ca: calcium, Na: sodium, Mg: magnesium, SEM: standard error of means, P-value: probability value.

The results on micro minerals of the citrus species pulp are presented in Table 3.3 below. All macro minerals differed (*P<0.05*) significantly across treatments. While LP had high (*P<0.05*) Fe concentration, Fe in OP, GP and TP was similar (*P>0.05*). The LP also had higher (*P<0.05*) Cu and Zn concentration compared to OP. The Mn concentration was similar (*P>0.05*) in OP and GP, but higher in LP than in other treatments/species. Results revealed high (*P<0.05*) B concentration in LP followed by GP, OP and TP, respectively. No significant (*P>0.05*) differences were observed on Al amongst the citrus species pulps.

Table 3.3: Micro minerals of the citrus species pulp (mg/kg DM)

Parameter	LP	OP	GP	TP	SEM	P-value
Fe	28.4[a]	20.0[b]	20.6[b]	21.1[b]	1.9	0.02
Cu	4.7[a]	2.1[d]	3.5[b]	2.4[c]	0.1	<.0001
Zn	8.7[a]	5.0[c]	5.4[b]	5.7[b]	0.1	<.0001
Mn	5.4[a]	2.8[c]	2.6[c]	3.8[b]	0.1	<.0001
B	18.5[a]	14.6[c]	16.9[b]	14.0[d]	0.1	<.0001
Al	7.0	4.8	8.5	5.5	1.3	0.2

[a, b, c, d] Means within a row with different superscripts are different (*P<0.05*), LP: lemon pulp, OP: orange pulp, GP: grapefruit pulp, TP: tangerine pulp, Fe: iron, Cu: copper, Zn: zinc, Mn: manganese, B: boron, Al: aluminium, SEM: standard error of means, P-value: probability value.

Results of physical characteristics of citrus species pulp are shown in Table 3.4. Significant (*P<0.05*) differences were observed in WR and PV of all citrus species pulps. The highest (*P<0.05*) WR value was in OP compared to TP, GP and LP. The highest (*P<0.05*) PV was revealed in OP, although it was comparable (*P>0.05*) to the rest of the citrus species pulp (GP, TP and LP).

Table 3.4: Physical characteristics of citrus species pulps

Parameters	LP	OP	GP	TP	SEM	P-value
WR (g/g)	4.5[c]	5.4[a]	4.5[c]	4.9[b]	0.02	<.0001
PV (mL/g)	3.6[b]	3.8[a]	3.7[ab]	3.6[b]	0.05	0.04

[a,b,c] Means within a row with different superscripts are different (*P<0.05*), LP: lemon pulp, OP: orange pulp, GP: grapefruit pulp, TP: tangerine pulp, WR: water retention, PV: packed volume, SEM: standard error of means, P-value: probability value.

Results on the chemical estimates of citrus species pulps are demonstrated in Table 3.5 below. The chemical estimates differed (*P<0.05*) significantly across LP, OP, GP and TP species. The TP had the highest (*P<0.05*) DMD, DE, ME and TDN content than the other species, while LP had the lowest (*P<0.05*).

Table 3.5: Chemical estimates of citrus species pulps

Parameters	**LP**	**OP**	**GP**	**TP**	**SEM**	**P-value**
DMD (g/kg)	759.1[d]	804.7[b]	788.9[c]	827.5[a]	3.7	<.0001
DE (Mcal/kg)	3.5[d]	3.7[b]	3.6[c]	3.8[a]	0.02	<.0001
ME (Mcal/kg)	2.9[d]	3.1[b]	3.0[c]	3.1[a]	0.01	<.0001
TDN (g/kg DM)	698.4[d]	742.5[b]	727.3[c]	764.4[a]	3.6	<.0001

[a,b.c,d] Means within a row with different superscripts are different (*P<0.05*), LP: lemon pulp, OP: orange pulp, GP: grapefruit pulp, TP: tangerine pulp, DMD: dry matter digestibility, DE: digestible energy, ME: metabolizable energy: TDN: total digestible nutrients, SEM: standard error of means, P-value: probability value.

The correlation coefficients of chemical composition, chemical estimates and physical characteristics of the citrus species pulps are shown in Table 3.6. Correlation at significance level ($P<0.05$), which had an R-value between 0.65 and 0.84 was classified as "moderate" while those with an R-value between 0.85 and 1.00 were classified as "high". Variables that are positively correlated and included in the high category are: ash and Ca (0.98), NDF and ADF (0.93), DMD and ME (1.00) as well as CP and Ca (0.87). Ash and Mg were highly positively correlated (0.98).

Variables that showed strong negative correlation were CP and ADL (-0.88), NDF and EE (-0.89), DMD and NDF (-0.94), CP and WR (-0.88), DE and NDF (-0.93) and NDF and ME (-0.93).

Variables that are positively correlated and classified as moderate were DM and Cel (0.65), DM and Ca (0.67), EE and cellulose (0.71), ADF and K (0.84), ADF and Mg (0.71), cellulose and Ca (0.78), cellulose and Mg (0.79) and P and Na (0.84).

Variables that were negatively correlated and classified as moderate were EE and DMD (-0.80), EE and DE (-0.79), ADL and Ca (-0.82), CP and Mg (-0.78), DMD and Mg (-0.71), EE and ME (-0.80), ADL and Mg (-0.70) and Mg and DE (-0.71).

Table 3.6: Correlation coefficients amongst chemical composition, chemical estimates, physical characteristics, and minerals of the citrus species pulps.

	DM	Ash	EE	CP	NDF	ADF	Hemi	ADL	Cel	DMD	DE	ME	PV	WR	P	K	Ca	Mg	Na
DM	-	0.68	0.29 NS	-0.62	0.42 NS	0.56	-0.26 NS	-0.67	0.65	-0.56	-0.56	-0.57	-0.41 NS	-0.65	0.18 NS	0.63	0.67	0.60	0.54
Ash		-	0.40 NS	-0.78	0.68	0.77	-0.06 NS	-0.76	0.85	-0.77	-0.77	-0.77	-0.26 NS	-0.90	0.60	0.98	0.98	0.98	0.88
EE			-	0.08 NS	-0.89	-0.80	0.48	-0.13 NS	0.71	-0.80	-0.79	-0.80	0.04 NS	-0.21 NS	-0.02 NS	0.50	0.24 NS	0.30 NS	0.10 NS
CP				-	-0.15 NS	-0.33 NS	0.42 NS	-0.88	-0.51	0.33 NS	0.33 NS	0.34 NS	0.47	-0.88	-0.36 NS	-0.69	0.87	-0.78	-0.73
NDF					-	0.93	0.43 NS	-0.32 NS	0.88	-0.94	-0.93	-0.93	0.04 NS	-0.46	0.31	0.76	0.54	0.60	0.43 NS
ADF						-	0.08 NS	-0.45	0.97	-1.00	-1.00	-1.00	-0.09 NS	-0.57	0.36 NS	0.84	0.66	0.71	0.54
Hemi							-	0.25 NS	0.01 NS	-0.08 NS	-0.08 NS	-0.08 NS	0.36 NS	0.17 NS	-0.04 NS	0.02 NS	-0.17 NS	-0.10 NS	-0.17 NS
ADL								-	-0.65	0.45	0.45	0.46	0.38 NS	0.88	-0.14 NS	-0.68	-0.82	-0.70	-0.55
Cel									-	-0.97	-0.97	-0.97	-0.18 NS	-0.72	0.34 NS	0.89	0.78	0.79	0.61
DMD										-	1.00	1.00	0.09 NS	0.57	-0.36 NS	-0.84	-0.66	-0.71	-0.54
DE											-	1.00	0.09 NS	0.57	-0.36	-0.84	-0.66	-0.71	-0.55

				NS			***	
ME	-	0.10 NS	0.58	- 0.35 NS	- 0.84	- 0.66	- 0.71	- 0.54
PV		-	0.48	0.14 NS	- 0.13 NS	- 0.32 NS	- 0.18 NS	- 0.13 NS
WR			-	- 0.36 NS	- 0.83	- 0.93	- 0.86	- 0.75
P				-	0.64	0.59	0.73	0.84
K					-	0.93	0.97	0.87
Ca						-	0.97	0.89
Mg							-	0.94
Na								-

DM: dry matter, EE: ether extract, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, Hemi: hemicellulose, ADL: acid detergent lignin, Cel: cellulose, DMD: dry matter digestibility, DE: digestible energy, ME: metabolizable energy, PV: packed volume, WR: water retention: P; phosphorus, K: potassium, Ca: calcium, Mg: magnesium Na: sodium, Significance level: $P<0.05$ = NS.

3.5 Discussion

Chemical composition

The DM is the dried sample matter until a constant mass is achieved and it assists in determining the true nutritional quality of the feed sample. Feed utilization and the overall livestock performance can be anticipated from the DM content. Furthermore, in case of the limiting nutrients, it provides an opportunity to correct and supplement before offering the feed to livestock animals. Additionally, DM determination is critical in silage production, as it provides essential insight into the ensiling potential and assist predict the quality of the silage. According to Nkosi *et al*. (2021), the DM content of fresh materials is a critical parameter in silage production, as it directly influences fermentation duration, container selection, storage conditions and what additives to supplement with.

High moisture content reduces the shelf life and the nutritive value ruminant feed (Özdemir & Ülger, 2022). spoilage and bacteria can infest the agro-industrial by-products that are considered as potential feed ingredients. Therefore, it is crucial to process wastes and or by-products to preserve their nutritive value and shelf life. In the present study, the DM of the citrus species pulps ranged between 185.0-222.6 g/kg, falling below the recommended minimum value (250.0 g/kg) for the ensiling (Nkosi *et al*., 2016). Previous studies reported similar outcomes in citrus species pulps (Beyzi *et al*., 2018; Ülger *et al*., 2020). The low DM of the citrus species pulps is of great concern as it limits proper fermentation of the samples and leads to high DM losses due to anticipated aerobic fermentation and increased clostridial activity (Ülger *et al*., 2020; Nkosi *et al*., 2021). In the present study, the lemon pulp had higher DM, while the orange pulp had lower DM compared to the rest of the species pulps. This could be due to different proportions (number and size) of peels, seeds and juice residue from one species to another (Beyzi *et al*., 2018).

Generally, the citrus pulps have been noted to have a high moisture content of an average of 800.0 g/kg (Bakr, 2020) due to their juice residues and the pulps, which have been noted to cause difficulties in storing or ensiling resulting in quick spoilage and cause environmental pollution. Hence the need to dry them before ensiling or adding an absorbent (Nkosi *et al*., 2021). Citrus pulps may enhance feed intake due to their good smell and palatability (Bakr, 2020). Preserving the citrus pulps at their moisture content may be more convenient in dry areas, as they reduce the water requirements of livestock animals and have slight potential to supplement the water requirements.

The citrus species pulp in the present study had varying ash content of between 28.4–48.9 g/kg DM. Gomes *et al*. (2017) reported an ash content of 17.00 g/kg DM of citrus pulp and Kordi and Naserian (2021) reported an ash content of 53.00 g/kg DM of citrus pulp. The high ash content was reported on lemon pulp and low content on orange pulp in the present study. The results found in the present study, imply that the citrus species pulps may contain moderate to high amounts of minerals when compared to the two above-cited studies (Gomes *et al*., 2017; Kordi & Naserian, 2021). The different values in ash content among studies or the species could be attributed to the environmental effects where the citrus was grown and the genetic makeup of the individual species. According to Palangi *et al*. (2013), the processing procedure and the growth conditions have an impact on different values of ash content.

Ether extract is the extraction of fat-soluble substances, and it is vital as it serves as an energy source in livestock feed. It further assists in digestion and improves the overall nutrient composition (Pond *et al*., 2004). In the present study, lemon pulp contained a high EE content of 31.8 g /kg DM, and this is supported by the results of Özkan *et al*. (2017) who reported higher EE (114.0) in lemon pulp when compared to orange, tangerine and grapefruit pulps of 32.50 35.10

and 41.70 g/kg DM, respectively. Villanueva *et al*. (2017) reported EE content of 24.00 g/kg DM in fresh orange residue, similar to the current study. Beyzi *et al*. (2018) reported lower EE content than in the current study for orange, tangerine and lemon pulps at 8.100, 9.800 and 28.40 g/kg DM, respectively. The citrus pulps generally contain increased EE content due to their higher concentration of unsaturated fatty acids (UFA) (Allam *et al*., 2020). It was noted by de Ondarza *et al*. (2024) that the EE content in feeds should be less than 60.0 g/kg DM for ruminants. Thus, equating the EE of the citrus species pulp in the present study adequate for ruminants' nutrition.

The CP is regarded as the most important nutrient in livestock feeds, as it plays a role in the maintenance, growth and deposition in the desirable products. It has been noted that a CP equal to or more than 70.0 g/kg DM is required for the maintenance of livestock animals (Özkan *et al*., 2017; Souza *et al*., 2022). The citrus species pulps CP content in the current study ranged between (49.0-55.9 g/kg DM), which was below the recommended range for livestock maintenance. Different studies reported CP content ranging from 40.0 to 90.0 g/kg DM of different citrus species pulps (Palangi *et al*., 2013; Özkan *et al*., 2017; Ülger *et al*., 2020). This emphasizes the necessity for protein supplementation when citrus pulp is incorporated into livestock feeds.

Neutral detergent fibre (NDF) is the fibrous component of feed, which is mainly composed of carbohydrates. The present study reported NDF of the citrus species pulp of 104.1–207.5 g/kg DM with the lowest content in tangerine pulp and highest content in lemon pulp. Previous studies reported similar trends of NDF content (Beyzi *et al*., 2018; Ülger *et al*., 2020), while Souza *et al*. (2022) reported high NDF content of citrus pulp of 368.0 g/kg DM. The low NDF content in tangerine in the present study could be attributed to the soft peels and low concentration of seeds when compared to other citrus species pulps. The ripe degree of fruits and the storage duration are some of the factors that could cause variations in the NDF. It has further been noted that NDF

content of 400.0 g/kg DM and less is recommended in ruminant nutrition enhancement of rumen microbes, thus digestibility and productivity (Hoffman *et al*., 2001). Therefore, with the NDF content of citrus species pulps in the present study below 400.0 g/kg DM, it is evident that the pulps contain readily digestible fibre and soluble carbohydrates that is ideal for ruminant feed.

Acid detergent fibre (ADF) determines the amount of hemicellulose and lignin substances available in the feed sample and assists in determining the extent to which the feed sample is digestible. A lower amount of ADF suggests that the feed sample is highly digestible by the rumen microbes in a shorter period while higher amounts suggest the opposite (Owens *et al*., 2010). In the present study, the ADF content of the citrus species pulps ranged between 79.0 and 166.9 g/kg DM. Previous studies reported slightly higher ADF content in citrus species pulps of 151.0 - 183.0, 145.9 - 205.7 and 263.0 g/kg DM (Palangi *et al*., 2013; Özkan *et al*., 2017; Kordi & Naserian, 2021), respectively. As mentioned earlier, the differences among studies could be attributed to the different harvesting periods, processing methods and forage cultivars. Furthermore, this can be affected by different proportions of the pulps (peels, seeds, juice residue) and the environmental conditions.

The ADL consists mainly of lignin and is indigestible. A high amount of ADL in feed decreases intake in livestock, which further affects growth and productivity. It has been noted that the rumen microbes are unable to access the nutrients locked by the lignin. The ADL content of citrus species pulps in the present study is low, ranging between 8.8–34.8 g/kg DM with the lowest content in lemon pulp and the highest content in orange pulp. This may have little to no impact on the nutrient utilisation/digestion and accessibility by the rumen microbes. Santos *et al*. (2014) also reported a similar ADL content of 8.0 g/kg DM of citrus pulp, while Allam *et al*. (2020) reported

22.0 g/kg DM of dried orange pulp. The citrus species pulps can be used in livestock feed without negatively affecting feed intake and utilization.

Hemicellulose is composed of complex carbohydrates, which are polysaccharides that are found mostly in the cell wall of forages. Hemicellulose functions to keep the forages upright and gives the overall structure through its incorporation in the cell wall. Due to their complex carbohydrates, they are not easily digested by the rumen microbes. In the present study, the hemicellulose content of the citrus species pulps ranged between 25.1 and 45.1 g/kg DM with the highest content in orange pulp and the lowest content in tangerine. In contrast to the present study, Ulger *et al*. (2020) reported lower contents of 22.0, 11.0 and 17.0 g/kg DM in lemon, orange and tangerine pulps, respectively. However, the findings of the present study are in agreement with those observed by Allam *et al*. (2020), who reported 25.5 g/kg DM hemicellulose in dried orange pulp.

Cellulose is one of the complex carbohydrates that form part of the plant's branches and stem. Cellulose is broken down and hydrolysed by cellulases that are produced in the rumen compartment. In the present study, the cellulose content of orange pulp was 73.4 g/kg DM. Contrary to the results in the present study, Allam *et al*. (2020) reported 216.1 g/kg DM cellulose content in dried orange pulp. The cellulose content in citrus pulps provides energy and it is digested by rumen microbes to produce volatile fatty acids. Environmental conditions play a crucial role in the cell wall constituents' amounts; hence different amounts of cellulose are reported in different studies.

Minerals

The citrus species pulp is regarded as an important source of minerals for ruminants (Idamokoro *et al*., 2022). Varying concentration of minerals are required depending on the physiological status, age, gender, level of productivity of an animal. The P, K, Ca, Mg, Na and S are the major minerals that are regarded as the most important minerals required by livestock for their growth, health and metabolism. They are required by the ruminants in higher amounts. Phosphorus is essential for skeletal development, energy transfer and cell structure, while Ca functions to build and maintain bone structure, it also support muscle contraction, nerve impulse, blood clotting and enzyme function (Veum, 2010). The present study reported P and Ca contents of the pulps ranging from 0.10 - 0.17% and 0.3 - 0.7%, respectively. Lemon pulp produced the highest P and Ca content compared with other citrus species pulps. Idamokoro *et al*. (2022) reported P and Ca of 160.0, 133.3 and 483.3, 593.3 mg/100 g in whole *citrus clementine* and *citrus limon*, respectively. Low calcium concentration in diets leads to bone conditions, fractures, reduced mobility and rickets, and low P concentration in feed causes reduced body condition score, growth and productivity in the animal being fed. The Ca:P ratio is significant at 1:1 to 7:1, and toxic at ratios above 7:1 (NRC, 2000). The citrus species pulp in the present study were within the mentioned range.

The K and Na support the regulation of electrolytes and water (Heena *et al*., 2022), and in turn, enhance the livestock production when the right amount is included in feed. Adequate Na in livestock diets promotes muscles and nerves for animals to function accordingly and synchronously. The K and Na results in this study were 1.5 - 2.3% and 35.4 - 144.8 mg/kg with the high concentration in lemon pulp. Tayengwa and Mapiye (2018) reported an average of 9.3 and 1.2 g/kg DM of K and Na in citrus pulp, respectively. The NRC (2000) recommend K content

between 6.0 to 30 g/kg DM for growing cattle and gestation cows. Therefore, the K content of the citrus species pulp in this study is suitable for cattle and can be incorporated their diets. The NRC (2000) further reported the required Na content of growing, finishing cattle and gestation cows to be 0.06 - 0.08%, and early lactating cows to be 0.1%. The contents of the Na of the citrus species pulp in the present study, render them insufficient and they require top-up/supplementation in the cattle diet. The low Na content might lead to reduced growth due to the low Na, which limits feed consumption (NRC, 2007).

Magnesium also plays a role in bone rigidity and induces better bone health. This study reported Mg content of 0.07–0.14% in citrus species pulps content with the highest content in lemon pulp and the lowest content in orange pulp. Idamokoro *et al*. (2022) reported Mg content of 136.0 and 160.0 mg/100g in whole *citrus clementina* and *citrus limon* to be respectively. The Mg in lemon and grapefruit pulps in the current study, render them sufficient for the diet of gestating cows as they require a minimum of 0.12% (NRC, 2000). The Mg of the orange and tangerine pulp are lower than the requirement of the gestating, growing and finishing cattle (0.1 and 0.12%, respectively). The low Mg concentration in orange and tangerine pulps can have an effect on the growth and production of cattle.

The Fe mineral is significant to supports the immune system, development and regulation of body temperature (Heena *et al*., 2022). The Fe is also significant to form part in the formation of red blood cells. Low blood cell counts may cause a weak immune system and lead to health concern issues such as anaemia (Saxena & Srivastava, 2021). The Cu supports the red and white blood cells. Both Fe and Cu play a role in bone formation for the stimulation of collagen. The current study's findings were comparable to those of Heena *et al*. (2022), which indicated that citrus pulp micro-minerals were more abundant in Fe than in any other micro-minerals. The Fe and Cu of the

citrus species pulp in this study ranged from 20.0 to 28.4 and 2.1 to 4.7 mg/kg DM, respectively, with the highest content in lemon pulp. Idamokoro and Hosu (2023) reported Fe and Cu of 5.2 and 3.8, and 0.2 and 0.3 mg/100g) for *citrus limon* pulp + peel and *citrus clementina* pulp + peel, respectively. According to the NRC (2000), cattle across all physiological status, require a minimum of 50.0 and 10.0 mg/kg DM for Fe and Cu, respectively. Results in this study show that the citrus species pulp have low Fe and Cu concentration, and when fed to ruminants, might not meet the required concentration.

Manganese (Mn) plays a role in the synthesis of proteins and lipids without being utilized and it also promotes the formation of vitamin K. Zinc prevents disease infections in animals, enhances the immune system and repairs bodily tissues. Also, it plays a role in the metabolism and growth rate. The Zn and Mn of the citrus species pulp in the present study were 5.0 - 8.7 and 2.6 - 5.4 mg/kg DM, respectively with the highest concentration in lemon pulp. Tayengwa and Mapiye (2018) found the average concentration of Zn and Mn in the citrus pulp to be 14.0 and 8.0 mg/kg DM, respectively. Both the Zn and Mn concentrations of the citrus species pulps in this study do not meet the minimum requirements of the growing and finishing cattle as they need a minimum of 30.0 and 20.0 mg/kg DM, respectively (NRC, 2000).

Both B and Al elements are regarded as toxic elements that can cause detrimental effects when fed in high concentration in animal diets. The maximum Al amount in the diet of small ruminants is 1000 mg/kg (NRC, 2007), any amount greater than that can cause reproductive issues. For sheep, the maximum B amount is 150.0 mg/kg (NRC, 2007). The present study reported the B and Al of between 14.0 -18.5 and 4.8 - 8.5 mg/kg DM in citrus species pulp of, with the highest concentration in lemon and tangerine pulp, respectively. Heena *et al*. (2022) reported a B concentration 0.01 mg/g DM in dropped citrus fruits of. The B and Al concentrations in the citrus

species pulp in the present study are sufficient and would have no effects on the small ruminants. Generally, lower and higher minerals amount than required by ruminants can cause fertility issues, slow growth, health conditions, reduced performance and mortality if not corrected (NRC, 2007). Factors that cause variations in the concentrations of minerals in citrus species pulp could allude to the stage of ripeness, the processing procedure, the region where the study occurred (weather variations), citrus genotype and so forth.

Physical characteristics

The physical characteristics in ruminant feed are of crucial importance as they affect the nutrient intake and consequently the growth of animals. Water retention (WR) and packed volume (PV) were measured to determine the quality of citrus pulps, as they correlate with rumen fill. The higher water retained in the sample would result in increased rumen fill and reduced feed intake. Forages with less than 6.5 g/g WR influence high DM intake by ruminants (Mokoboki *et al*., 2005). In the present study, the citrus species pulps had WR ranging between 4.5 and 5.4 g/g, falling below the value (6.5 g/g) that encourages increased feed intake. The citrus species pulps had high moisture content, which could have influenced the WR. In contrast, the forage acceptable range of packed volume is 2.98 mL/g or less (Mokoboki *et al*., 2005). This study reported packed volume ranging between 3.6 and 3.8 mL/g for citrus species pulps. These results could be due to the different genetic makeup of citrus pulps and processing methods. The low fibre (low cell wall contents) and dry matter content could have influenced the high packed volume.

Chemical estimates

High dry matter digestibility (DMD) in feed ingredients translate to high feed intake due to the low fibrous matter (Yucel, 2019). It is mostly affected by the concentration of fibre and cell wall contents in the feed. The present study reported 759.1 to 827.5 g/kg DMD of citrus species

pulps, with the lowest contents in lemon pulp and the highest in tangerine pulp. The low fibrous content resulted in the highest DMD, while the high fibrous content resulted in the lowest DMD. Mayouf and Arbouche (2014) emphasised that a DMD of 500.0 g/kg was adequate for the maintenance of livestock. The observed high DMD in citrus species pulps in the current study was expected as they displayed lower fibrous content, which can enhance the DM feed intake and improve animal productivity (Indah & Permana, 2020).

Digestible energy is the amount of energy available in the feedstuffs that is absorbed by animals. The digestible energy is important to provide the necessary energy for the growth, metabolism and production of ruminants. In the present study, the DE content ranged between 3.5 - 3.8 Mcal/kg for citrus species pulps with the highest content observed in tangerine pulp. The DE is affected by factors such as processing method, lignin content and overall management of feedstuffs/plants (Frei, 2013). Higher DE is desired, as it renders the feedstuffs of higher energy impacting positively on the growth and performance of ruminants. The present study reported an ME content of 2.9 - 3.1 Mcal/kg of citrus species pulps with the lowest content in lemon pulp. These values were similar to those reported by Tayengwa *et al.* (2021) and Sharif *et al*. (2018) who reported ME of 2.7 and 3.1 Mcal/kg in dried citrus pulp, respectively. The ME has been noted to have a negative correlation with the fibre content (Lashkari & Taghizadeh, 2013). Increased ME increases digestion and feed utilization. The required DE and ME for growing and finishing lambs are 2.5 and 3.2 Mcal/kg according to NRC (1985). Therefore, both the DE and ME contents in the present study meet the minimum requirements of growing and finishing ruminants.

The TDN gives the estimate of the total nutrients that an animal can absorb and utilize from the feed. The present study reported TDN range of between 698.4 and 764.4 g/kg DM in citrus species pulps, with the highest content observed in tangerine pulp. Alnaimy *et al*. (2017) reported

825.0 g/kg DM TDN in citrus pulp, which is similar to that in our study. Gursoy *et al*. (2021) indicated that high amounts of TDN equate to increased quality of feed, which might improve the voluntary intake and growth of ruminants.

The correlation results amongst the chemical composition, chemical estimates and physical characteristics are scanty in literature. The measured CP content and DMD were negatively correlated to both water retention and NDF. This is because feedstuffs with high CP content and high DMD tend to have high intake (Mero & Uden, 1998). These results are similar to those of Jayanegara *et al*. (2016), who stated that fibre fraction especially ADF and NDF negatively correlated with DMD. The negative correlation between ADL and Ca (-0.82) contrasted with the result from Leuchner *et al*. (2017), who reported that ADL positively correlates with calcium absorption in ruminants. The correlation between NDF and EE is coherent with the result from Weld and Armentano (2017), who stated that EE could reduce NDF digestion in the rumen. When EE is absorbed in the small intestine, the digestible NDF that escapes from the rumen is fermented in the large intestine.

Potassium is active in the metabolism and synthesis of glycogen and proteins and it is also active in the regulation of the body's water content. Potassium in the current study shows a strong positive correlation with Na. This relationship between potassium and sodium is favourable in meat, considering that Na is found in scarce amounts. The correlation between Ca and Mg in this study is associated with ATP activity and substrate availability so Mg^{2+} assumes the role of substrate in a complex that requires the presence of ATP (Baaij *et al*., 2015). Magnesium is considered the most abundant element after potassium.

3.6 Conclusion

All citrus species pulp were within acceptable ranges in fibre fraction, chemical estimates and physical characteristics for livestock feed ingredients; however, both their CP and DM were fairly low. Generally, the orange pulps performed better than other citrus species pulps based on their CP. The low CP resulted in a negative correlation between nutrients (DM, ash) and chemical estimates (DMD). However, tangerines displayed better DMD and TDN. Overall, the citrus species pulps had lower concentrations of minerals (both micro- and macro-) for ruminant feeding. Their moisture content is very high for storage, therefore, there is a need to process them to reduce the water content prior to using them as supplements and or silage additives.

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CHAPTER 4: NUTRIENTIVE VALUE AND FERMENTATION CHARACTERISTICS OF SORGHUM (*SORGHUM BICOLOR* L. MOENCH) SILAGE SUPPLEMENTED WITH GRADED LEVELS OF CITRUS SPECIES PULPS.

4.1 Abstract

The preservation of sorghum with agro-industrial waste such as citrus pulp can amplify the nutritional status and the fermentation profile of the ruminants. The present study aimed to determine the effect of citrus (*Citrus sp.*) species pulp inclusion levels on the nutritional and fermentation profiles of sorghum (*Sorghum bicolor L. Moench*) silage. Citrus species pulp was mixed with sorghum at ratios of 5:95, 10:90, 15:85, 20:80 and 25:75% then were ensiled for 42 days. The silage treatments (SG: sorghum: grapefruit, SL: sorghum: lemon, ST: sorghum: tangerine and SO: sorghum: orange) were analysed for chemical composition, i*n vitro* dry matter degradability (IVDMD), nitrogen solubility and fermentation characteristics. The data were analysed in a 5 x 4 factorial arrangement in a completely randomized design (CRD). The Cel of the silages was decreasing ($P<0.05$) with increasing inclusions whilst, DMD, DMI, DE, ME and TDN were increasing ($P<0.05$) with increasing inclusion levels. The SO silage had higher ($P<0.05$) P, Na, Fe, Cu, Zn, Mn and IVDMD at all incubation hours. The Ca concentration was high ($P<0.05$) in SL silage, while Mg, Mn, pH were high ($P<0.05$) in SG silage, but the DM was lower ($P<0.05$) compared to other treatments. The ST silage had higher ($P<0.05$) CP, total N but lower ($P<0.05$) ADL than other silages. The fermentation characteristics optimized at 18.6%, with pH, WSC and YM at 23.2, 22.18 and 21.86%, respectively. The silages exhibited enhanced nutritive value and improved fermentation characteristics, particularly at higher inclusion levels. The results suggest that inclusion of citrus species pulps in sorghum silage can improve the silage

quality. However, supplementation with nitrogen/crude protein source remains important to meet nutritional requirements.

Keywords: Ensiling, orange, sorghum, inclusion, microbes, fermentation, ruminants

4.2 Introduction

Silage is a good method to use during dry periods where forages are of low quality and commercial feeds are prohibitively expensive (Bernardes *et al*., 2018). It also assists when there is excess feed in some periods of the year to store for periods when there is less or none. This aids in saving the excess feed available at the time for future use. Mineral composition, IVDMD and nitrogen solubility are some of the nutritional factors that are important in feed evaluation to improve the overall health status and the performance of ruminants. Fermentation characteristics are important parameters to determine the quality of silage and preservation conditions. Anaerobic conditions are critical in the correct method of preserving the silages (Bernardes *et al*., 2018), although the nutritive composition of the silage ingredients prior to ensiling also plays a huge role in the outcomes of the product. Anaerobic conditions during the ensiling period ensure the stability of good quality silage of low pH and low microorganisms population. The yeasts and moulds multiply quickly when exposed to the atmospheric environment leading to deterioration of the silages either through silo bag holes or when feeding out (Kung, 2010). Although not all types of yeasts and moulds are bad or toxic or lead to deterioration quickly, some assist in preserving the silages further (Muck *et al*., 2018).

Citrus pulp has a highly acidic profile owing to the ascorbic and malic acids (Ülger *et al*., 2020) that have a great influence in reducing the pH of the silages. The high moisture of the citrus

pulp is unsuitable to ensile alone and requires absorbents that has lower moisture to balance it (Lee *et al*., 2019) and to reduce the effluent losses that might arise from individual citrus pulp ensiling. According to Kordi and Naserian (2021), the high moisture in citrus pulp is considered a huge risk and a problem as the moisture promotes the presence of undesirable microorganisms such as *Clostridia* that lower the quality of the silage and promote quick deterioration once opened. High moisture also increase the pH, and the DM losses and consequently reduce the nutritive value of the silage (Grizotto *et al*., 2020). Sorghum silage is a high-energy source; therefore, it is unsuitable to be fed alone. It also has high water-soluble carbohydrates, and it is highly fermentable. To combat such problems in the current study, we opted to use old mature whole-plant sorghum to reduce the high moisture content found in citrus and to use low inclusion levels of citrus species pulp. There is limited information on the mineral, nitrogen solubility and IVDMD of citrus species pulp-sorghum silage. Therefore, the objective of the current study was to determine the effect of graded inclusion levels of citrus species pulp on the nutritional and fermentation profile of sorghum (*Sorghum bicolor* L. Moench) silage. We hypothesised that varying inclusion levels of citrus species pulp will affect the nutritional and fermentation profile of sorghum (*Sorghum bicolor* L. Moench) silage.

4.3 Methodology

The study site and citrus species pulp preparation and treatments are as described in Chapter 3.

4.3.1 Sorghum planting procedure and harvesting

Sorghum (*Sorghum bicolor* L. Moench) was planted at the North-West University research farm. The soil was tested to determine its physical status and appropriate fertilizers were applied. Sorghum seeds were outsourced from the BRASUDA Seed Company, Pretoria, Gauteng Province, South Africa. Some 84 rhizobium strains were used to inoculate the seeds prior to planting.

Sorghum was planted as monoculture during cropping season in December 2022 in a monoculture on a total field area of 32 m x 12 m (384m^2). Weeding was done every week to prevent nutrient competition between the sorghum and unwanted weeds. Sorghum was under an irrigation system and harvested as a whole plant manually in May 2023 during the post-maturity stage.

4.3.2 Silage preparation

Sorghum and citrus species pulp were chopped to acquire a 10 mm chop length. Sorghum and citrus species pulps were mixed at these inclusion levels: 95:5 (95 percent of Sorghum with 5 percent of citrus species pulp), 90:10, 85:15, 80:20, and 75:25% in vacuum bags. The quantity of each silage was 500 grams. The experimental treatments were sorghum: lemon pulp (SL), sorghum: orange pulp (SO), sorghum: tangerine pulp (ST) and sorghum: grapefruit (SG), and each was replicated 6 times. The experimental treatments were thoroughly mixed, compacted and the air eliminated from the bags using Orved VM12 vacuum master equipment to seal and ensure airtightness. Experimental treatments were analysed for chemical composition prior to ensiling for 42 days. The silages were subsequently opened 42 days later and were sampled to determine silage chemical composition, *in vitro* dry matter degradability, nitrogen solubility and fermentation profile.

4.3.3 Analyses

Chemical composition analyses of silage treatments (SL, SO, ST and SG) are described in Chapter 3. Chemical estimate calculations of SL, SO, ST and SG silages are described in Chapter 3.

DMI was calculated using the NDF concentration and 2.5% of animal body weight as described by Boga *et al*. (2014) and is as follows:

$$\text{DMI}\left(\frac{\text{kg}}{\text{day}}\right) = 120 \div (\text{NDF\%}) \quad (4.1)$$

4.3.3.1 *In vitro* dry matter degradability (IVDMD)

The ANKOM Daisy11 Incubator has a thermostatic chamber set at 39 ℃ and revolving four jars. It was utilized to analyze the *in vitro* dry matter degradability of silage treatments as described by ANKOM (2005). Silage samples (0.45–0.5 g) were weighed, put in ANKOM F57 filter bags, heat-sealed and placed in the revolving jars. The buffer solutions with 6.8 pH were prepared and mixed in a 1:5 ratio, with a total of 1.6 litres of the mixed buffers placed in each revolving jar. Rumen fluid was collected from an approximate 550 kg cannulated Bonsmara cow that was on a buffalo grass and lucerne hay diet. Two prewarmed thermostatic flasks were utilized to collect the rumen fluid and were whisked prior to being strained through double layers of warm muslin cloth. Each of the four revolving jars received 0.4 litres of rumen inoculum, 1.6 litres of buffer solution and a maximum of 50 ANKOM F57 filter bags. The revolving jars were purged with carbon dioxide (CO_2) to maintain the rumen-like environment conditions. The ANKOM F57 filter bags were withdrawn after 2, 4, 8, 12, 24, 36, 48 and 72 hours of incubation and cleaned for 20 minutes under running tap water using an ANKOM 2000 Fiber Analyzer. They were then oven-dried at 105 °C overnight prior to being weighed for DMD. The following formula was used to calculate *in vitro* DM degradability:

$$\%\text{IVDMD} = \frac{\left[100 - \left(\text{W3} - (\text{W1} \times \text{C1})\right)\right]}{(\text{W2} \times \text{DM})} \times 100 \quad (4.2)$$

Where W1 is the tare weight of the bag, W2 is the weight of a sample, W3 is the final bag weight following *in vitro* treatment, C1 is the blank bag correlation factor (final oven-dried weight / original blank weight), and DM is the dry matter.

4.3.3.2 Nitrogen Solubility

Buffer nitrogen solubility was analysed based on the method described by Licitra *et al*. (1996) with modification on utilising ANKOM F57 filter bags for the filtration procedure (Cudjoe & Mlambo, 2014). The treatment samples (0.45–0.5 grams) were weighed into the ANKOM F57 filter bags and heat-sealed. Borate phosphate solution (1.5 litres), 10% sodium azide and the ANKOM F57 filter bags were poured into each of the four revolving jars of the ANKOM Diasy[11] incubator set at 39 ℃. The ANKOM F57 filter bags and the solution were withdrawn 3 hours later, ANKOM F57 filter bags were squeezed of the solution and oven dried at 105 ℃ overnight. The samples were weighed after cooling. Nitrogen (N) residue was determined using the standard macro-Kjeldahl method (AOAC, 2005) and determined as insoluble N content. Soluble N content was calculated as the distinction between the total and insoluble N contents. The buffer solubility index was calculated as a ratio between the soluble N and the total N contents.

4.3.3.3 Fermentation profile

To determine the fermentation profile of the silage treatments, 40-gram of each treatment was used to analyse fermentation characteristics. Each 40 grams of the silage samples was blended with 0.36 litres of purified water for 4 minutes in a stomacher bag. The pH was determined instantly with a pH meter (Corning Model 4 pH-temperature, Ingold Messetechnik AG, Udorf, Switzerland). The samples were left at a temperature of 10 °C for 24 hours (Suzuki & Lund, 1980), blended for 4 min and filtered through a Whatman No. 4 filter paper (G.I.C. Scientific, Midrand, South Africa). The filtrate was utilized to determine water-soluble carbohydrates (WSC) and lactic acid (LA) concentrations. The phenol-sulphuric acid procedure determined the WSC according to Dubois *et al*. (1956), and the LA was determined by the colourimetric procedure of Barker and Summerson (1941) with modifications of Pryce (1969). Yeast and mould determinations were carried out on a

potato dextrose agar spread plate acidified with LA (85%). The plates were incubated at 28 °C, the yeasts were counted at 48 hours, and the counting mould was counted at 96 hours.

4.3.4 Statistical analysis

Data on the effects of varying inclusion levels of citrus species pulp on the nutrition and fermentation profiles of sorghum silage was determined in a 5 x 4 factorial arrangement in a completely randomized design. The following model was used:

$$Yijk = \mu + Vi + Lj + (V \times L)ij + \varepsilon ijk \quad (4.3)$$

Where Y_{ijk} is the response variable; μ - The overall mean; V_i – species effect; L_j= inclusion levels effect and $(V \times L)_{ij}$ was the species and inclusion level interaction effect and ε_{ijk} - random error.

Differences in treatment means were compared with the Duncan method, and significance was declared at $P<0.05$.

The citrus species pulp-sorghum silage parameters responses were modelled using the formula:

$$Y = a + b1X + + b2X2 \quad (4.4)$$

Where Y = response variables; a = intercept; b_1 and b_2 = coefficients of quadratic equations;

X levels of inclusions and $-b_1/2b_2$ = X value for optimal response. The quadratic model was

used because it gave the best fit.

4.4 Results

4.4.1 Chemical composition of sorghum pre-ensiled with incremental inclusion levels of citrus species pulp.

In Table 4.1, the citrus species pulp in sorghum had a significant influence on all parameters measured. Levels, species and the interaction between levels and species influenced ($P<0.05$) all parameters, except for ADL.

The ST at 15, 20 and 25% levels had higher ($P<0.05$) DM content when compared to all other treatments, which did not differ ($P>0.05$) significantly from each other. The 5% of SL had higher ($P<0.05$) DM content than other silage treatments. Inclusion of SG and SO in sorghum at level high ($P<0.05$) DM content compared to other levels of the same species, which did not differ ($P>0.05$) significantly from each other. The SL at 10, 20 and 25% inclusion levels had higher ($P<0.05$) ash content when compared to all other species in the same levels. The 5 and 15% of SG had the highest ($P<0.05$) ash content than all other species in the same levels. The 15% level of SG and 20% of SL had higher ($P<0.05$) ash content than other treatments.

The 10% inclusion of SG and ST had higher ($P<0.05$) EE content than all other species in the same level. The SG at 5, 10 and 15% levels depicted the highest ($P<0.05$) NDF fraction when compared to all other species in the same levels. Inclusion level of SL at 20% had higher ($P<0.05$) NDF content compared to other treatments. The 5, 10 and 15% levels of SG and 20% of SL had higher ($P<0.05$) NDF content relative to other treatments. Compared to other treatments, inclusion of SG in sorghum at 15% depicted the highest ($P<0.05$) ADF fraction. The ADL fraction was however similar ($P>0.05$) across the SL, ST and SO treatments at 15% inclusion level. There were no significant ($P>0.05$) differences on the ADF and EE content in SG, SL, ST and SO at 5, 20 and 25% inclusion levels in sorghum.

Table 4.1: Chemical composition of sorghum pre-ensiled with incremental inclusion levels of citrus species pulp (g/kg DM unless specified)

Parameter	Species	5%	10%	15%	20%	25%	SEM	L	S	L*S
DM (g/kg)	SG	341.7^{Cb}	383.4^{Aa}	331.3^{Bb}	341.7^{Bb}	320.9^{Bb}	13.2	<.0001	<.0001	<.0001
	SL	539.6^{Aa}	320.8^{Bb}	300.0^{Bb}	320.9^{Bb}	300.0^{Bb}				
	ST	414.6^{Bb}	320.9^{Bc}	393.8^{Ab}	487.5^{Aa}	487.5^{Aa}				
	SO	289.6^{Db}	372.9^{Aa}	300.0^{Bb}	331.3^{Bb}	300.0^{Bb}				
ASH	SG	49.4^{Ab}	46.3^{Bb}	55.1^{Aa}	48.5^{Bb}	46.1^{Bb}	1.4	<.0001	<.0001	<.0001
	SL	38.3^{Cd}	52.9^{Ab}	47.1^{Bc}	60.0^{Aa}	53.4^{Ab}				
	ST	43.3^{Ba}	44.5^{Ba}	48.6^{Ba}	44.1^{Ca}	47.7^{Ba}				
	SO	44.0^{Ba}	40.4^{Ca}	45.3^{Ba}	39.5^{Da}	44.0^{Ba}				
EE	SG	15.1^{Aa}	18.0^{Aa}	15.8^{Aa}	11.5^{Aa}	11.7^{Aa}	1.7	0.002	<.0001	0.004
	SL	13.7^{Aa}	6.9^{Ba}	17.5^{Aa}	8.9^{Aa}	14.0^{Aa}				
	ST	12.6^{Aa}	17.3^{Aa}	16.6^{Aa}	14.8^{Aa}	9.5^{Aa}				
	SO	10.0^{Aa}	11.5^{Ba}	9.8^{Ba}	8.9^{Aa}	5.3^{Aa}				
NDF	SG	448.1^{Aa}	466.5^{Aa}	468.8^{Aa}	413.8^{Bb}	393.2^{Ab}	11.9	0.04	<.0001	<.0001
	SL	373.9^{Bc}	423.2^{Bb}	344.0^{Cc}	473.5^{Aa}	410.6^{Ab}				
	ST	378.4^{Ba}	373.4^{Ca}	381.2^{Ba}	370.9^{Ca}	374.1^{Aa}				
	SO	403.0^{Ba}	412.8^{Ba}	393.4^{Ba}	377.4^{Ca}	402.9^{Aa}				
ADF	SG	286.5^{Aa}	251.2^{Aa}	290.8^{Aa}	274.3^{Aa}	262.3^{Aa}	11.5	0.04	<.0001	0.02
	SL	247.6^{Aa}	273.1^{Aa}	199.4^{Bb}	273.7^{Aa}	245.1^{Aa}				
	ST	241.3^{Aa}	237.7^{Aa}	238.8^{Ba}	238.2^{Aa}	234.5^{Aa}				
	SO	259.5^{Aa}	264.1^{Aa}	229.1^{Ba}	244.8^{Aa}	262.1^{Aa}				
ADL	SG	39.5	39.0	38.2	50.4	33.6	6.8	0.5	0.02	1.0
	SL	34.1	36.3	23.9	41.2	33.7				
	ST	31.5	31.8	33.9	35.5	32.8				
	SO	44.1	49.4	46.8	45.3	41.9				

$^{A-D}$ = Means within a column with different superscripts differ ($P < 0.05$) across treatments, $^{a-d}$ = means within a row with different superscripts differ across treatments, DM: dry matter, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, EE: ether extract, L: level, S: species, L×S: interaction between level and species, SEM: standard error of means, 5%, 10%, 15%, 20% and 25%: inclusion levels, SG: sorghum: grapefruit, SL: sorghum: lemon, ST: sorghum: tangerine, SO: sorghum: orange.

4.4.2 Chemical composition of sorghum silage containing graded levels of citrus species pulp

The citrus species pulp in sorghum silage had a significant influence on all parameters. Significant (*P<0.05*) differences were found in the effect of levels on ash, CP, NDF, ADF, and ADL parameters. The interaction between levels and species influenced (*P<0.05*) DM, CP and ADL (Table 4.2).

Inclusion levels of SG at 5, 10 and 15% and of SL at 10% showed the highest (*P<0.05*) ADL fraction compared to all other species in the same levels. Higher (*P<0.05*) ADL fraction than all other levels of the same species was seen in SG included at 5 and 15% in sorghum silage. Cmpared to other treatments, inclusion of SO at 15% level in sorghum had the highest (*P<0.05*) ADL fraction. There were no significant (*P>0.05*) differences in all inclusion levels on ash, EE, NDF and ADF across all the citrus species pulp in sorghum.

Table 4.2: Chemical composition of sorghum silages containing increasing inclusion of citrus species pulp (g/kg DM)

Parameter	Species	5%	10%	15%	20%	25%	SEM	L	S	L*S
DM (g/kg)	SG	326.7^{Ca}	343.3^{Ba}	344.2^{Aa}	370.9^{Ba}	353.7^{Ba}	20.2	0.2	<.0001	<.0001
	SL	423.7^{Bb}	347.2^{Bc}	425.7^{Ab}	504.7^{Aa}	533.2^{Aa}				
	ST	365.2^{Cb}	463.3^{Aa}	402.7^{Ab}	378.5^{Bb}	394.1^{Bb}				
	SO	518.7^{Aa}	374.6^{Bb}	369.4^{Ab}	357.7^{Bb}	362.0^{Bb}				
ASH	SG	47.4	52.4	54.8	57.5	59.8	1.0	<.0001	<.0001	0.1
	SL	43.4	48.0	50.0	51.2	53.5				
	ST	42.1	43.1	44.2	46.5	47.8				
	SO	45.6	47.0	48.4	49.0	51.6				
EE	SG	18.2	18.8	11.3	16.0	12.4	2.7	0.3	0.01	0.2
	SL	21.5	19.3	21.3	25.4	18.8				
	ST	21.9	18.2	18.4	20.0	24.0				
	SO	14.4	15.9	19.5	24.8	21.4				
CP	SG	40.7^{Bc}	42.4^{Bb}	42.4^{Cb}	43.1^{Db}	45.1^{Ca}	0.5	<.0001	<.0001	<.0001
	SL	43.1^{Bc}	44.2^{Bc}	45.4^{Bc}	49.7^{Bb}	51.8^{Aa}				
	ST	46.5^{Ab}	46.8^{Ab}	50.8^{Aa}	52.1^{Aa}	52.4^{Aa}				
	SO	42.0^{Bd}	43.5^{Bc}	46.1^{Bb}	46.8^{Cb}	48.4^{Ba}				
NDF	SG	533.4	473.3	417.8	403.7	372.2	15.1	<.0001	<.0001	0.8
	SL	417.2	360.0	336.0	321.7	301.3				
	ST	461.6	419.4	379.4	350.7	331.9				
	SO	467.2	430.3	391.5	384.8	369.8				
ADF	SG	366.0	315.1	268.1	250.6	233.8	12.6	<.0001	<.0001	0.2
	SL	273.1	267.9	251.0	208.3	180.3				
	ST	289.5	256.1	229.1	205.6	189.2				
	SO	278.0	254.5	242.5	225.9	208.3				
ADL	SG	55.9^{Aa}	45.6^{Ab}	53.5^{Aa}	44.2^{Ab}	38.9^{Ab}	2.7	<.0001	<.0001	0.01
	SL	45.1^{Ba}	51.4^{Aa}	41.5^{Ba}	38.6^{Aa}	33.7^{Aa}				
	ST	35.5^{Ca}	29.1^{Ba}	29.6^{Ca}	30.0^{Aa}	31.6^{Aa}				
	SO	34.8^{Cb}	32.5^{Bb}	43.6^{Ba}	33.1^{Ab}	29.8^{Ab}				

$^{A-D}$ = Means within a column with different superscripts differ ($P < 0.05$) across citrus species pulp for each level, $^{a-d}$ = means within a row with different superscripts differ across inclusion level for each species, DM: dry matter, EE: ether extract, CP: Crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, L: level, S: species, L×S: interaction between level and species, SEM: standard error of means, 5%, 10%, 15%, 20% and 25%: inclusion levels, SG: sorghum: grapefruit silage, SL: sorghum: lemon silage, ST: sorghum: tangerine silage, SO: sorghum: orange silage.

4.4.3 Chemical estimates of citrus species pulps in sorghum silage

The results of chemical estimates of citrus species pulp with different inclusion levels in sorghum silage are displayed in Table 4.3a below. No significant differences were observed in hemicellulose (HEMI) (*P<0.9222*), cellulose (Cel) (*P<0.3859*), dry matter digestibility (DMD) (*P<0.2445*), dry matter intake (DMI) (*P<0.8866*), digestible energy (DE) (*P<0.2569*), metabolizable energy (ME) (*P<0.2433*) and total digestible nutrients (TDN) (*P<0.2449*) among silages treatments with different citrus species pulp inclusion levels. Table 4.3b displays the effects of different inclusion levels (5, 10, 15, 20 and 25%) on chemical estimates. Inclusion levels affected (*P<0.05*) all the parameters except for HEMI. The CEL decreased (*P<0.05*) with the increasing inclusion levels of citrus species pulps in sorghum silage, while DMD, DMI, DE and ME increased with the inclusion levels. Table 4.3c displays the effects of citrus species pulps on chemical estimates of sorghum silage. The citrus species pulps affected (*P<0.05*) the chemical estimates of sorghum silage. The SO, SN and SL silages had similar (*P>0.05*) CEL content, which was lower (*P<0.05*) than those observed in SG. Citrus species pulps had no effect (*P>0.05*) on the DMD, DE, ME and TDN in SO, SN and SL. However, DMI was improved (*P<0.05*) with the citrus species pulps in SG followed by SL, SN, and SO, respectively.

Table 4.3a: Chemical estimates of increasing inclusion levels of citrus species pulp in sorghum silage (g/kg DM)

Parameter	Species	5%	10%	15%	20%	25%	SEM	L	S	L*S
HEMI	SG	167.4	158.3	149.7	153.1	138.4	16.6	0.1	<.0001	0.9
	SL	144.1	92.17	85.10	113.4	120.9				
	ST	172.1	163.3	150.3	145.2	142.6				
	SO	189.3	175.8	149.0	158.9	161.5				
CEL	SG	310.1	269.5	214.6	206.3	195.0	12.9	<.0001	<.0001	0.4
	SL	228.0	216.5	209.4	169.7	146.6				
	ST	254.0	227.1	199.5	175.6	157.7				
	SO	243.2	222.0	199.0	192.8	178.5				
DMD (g/kg)	SG	603.9	643.6	680.2	693.8	706.8	9.8	<.0001	<.0001	0.2
	SL	676.3	680.3	693.5	726.7	748.5				
	ST	663.5	689.5	710.6	728.9	741.6				
	SO	672.5	690.7	700.1	713.0	726.7				
DMI (kg/day)	SG	22.6	25.5	28.7	29.7	32.4	1.1	<.0001	<.0001	0.9
	SL	28.8	33.4	35.8	37.4	40.0				
	ST	26.3	28.6	31.7	34.4	36.2				
	SO	25.7	27.9	30.7	31.2	32.6				
DE (Mcal/kg)	SG	28.5	30.2	31.8	32.4	33.0	0.4	<.0001	<.0001	0.3
	SL	31.6	31.8	32.4	33.8	34.7				
	ST	31.1	32.2	33.1	33.9	34.5				
	SO	31.5	32.3	32.7	33.2	33.8				
ME (Mcal/kg)	SG	23.5	24.8	26.1	26.6	27.1	0.3	<.0001	<.0001	0.2
	SL	26.0	26.1	26.6	27.8	28.5				
	ST	25.5	26.4	27.2	27.8	28.3				
	SO	25.8	26.5	26.8	27.3	27.7				
TDN	SG	548.8	587.0	622.4	635.5	648.1	9.5	<.0001	<.0001	0.2
	SL	618.6	622.5	635.2	667.2	688.3				
	ST	606.2	631.3	651.7	669.3	681.6				
	SO	614.9	632.5	641.5	654.0	667.3				

HEMI: hemicellulose, CEL: cellulose, DMD: dry matter digestibility, DMI: dry matter intake, DE: digestible energy, ME: metabolizable energy, TDN: total digestible nutrients, L: level, S: species, L×S: interaction between level and species, SEM: standard error of means, 5%, 10%, 15%, 20% and 25%: inclusion levels, SG: sorghum: grapefruit silage, SL: sorghum: lemon silage, ST: sorghum: tangerine silage, SO: sorghum: orange silage

Table 4.3b: Effects of citrus species pulps inclusion levels on the chemical estimates of sorghum silage (g/kg DM)

Parameter	Citrus species pulps inclusion levels					SEM	P-value
	5%	10%	15%	20%	25%		
HEMI	168.2	147.4	133.5	142.7	140.7	16.6	0.1
CEL	258.5^{a}	233.7^{b}	205.6^{c}	186.1^{d}	170.5^{d}	12.9	<0.0001
DMD (g/kg)	654.0^{e}	676.0^{d}	696.1^{c}	715.6^{b}	729.9^{a}	9.8	<0.0001
DMI (kg/day)	25.9^{d}	28.9^{c}	31.7^{b}	33.2^{b}	35.2^{a}	1.1	<0.0001
DE(Mcal/kg)	31.0^{d}	31.6^{c}	32.5^{b}	33.3^{a}	33.9^{a}	0.4	<0.0001
ME (Mcal/kg)	25.2^{d}	26.0^{c}	26.7^{b}	27.4^{a}	27.9^{a}	0.3	<0.0001
TDN	597.1^{e}	618.3^{d}	637.7^{c}	656.5^{b}	670.3^{a}	9.5	<0.0001

$^{a-e}$: Values in a row with differing superscripts differ significantly ($P < 0.05$), SEM: standard error of means, HEMI: hemicellulose, CEL: cellulose, DMD: dry matter digestibility, DMI: dry matter intake, DE: digestible energy, ME: metabolizable energy, TDN: total digestible nutrients, 5%, 10%, 15%, 20% and 25%: inclusion levels, SEM: standard error of means, P-value: probability value.

Table 4.3c: Effects of citrus species pulps on the chemical estimates of sorghum silage (g/kg DM)

Parameter	Citrus species pulps in sorghum silage				SEM	P-value
	SO	SN	SG	SL		
HEMI	167.0[a]	155.6[a]	153.4[a]	111.2[b]	16.6	<0.0001
CEL	207.0[b]	206.0[b]	239.1[a]	194.0[b]	12.9	<0.0001
DMD	700.6[a]	704.3[a]	665.7[b]	705.1[a]	9.8	<0.0001
DMI (kg/day)	29.6[d]	31.1[c]	77.8[a]	35.1[b]	1.1	<0.0001
DE(Mcal/kg)	32.7[a]	32.9[a]	31.2[b]	32.9[a]	0.4	<0.0001
ME (Mcal/kg)	26.8[a]	26.9[a]	26.6[b]	27.0[a]	0.3	<0.0001
TDN	642.0[a]	645.6[a]	608.4[b]	646.4[a]	9.5	<0.0001

[a-d]: Values in a row with differing superscripts differ significantly ($P < 0.05$), SEM: standard error of means, HEMI: hemicellulose, CEL: cellulose, DMD: dry matter digestibility, DMI: dry matter intake, DE: digestible energy, ME: metabolizable energy, TDN: total digestible nutrients, SG: sorghum: grapefruit silage, SL: sorghum: lemon silage, ST: sorghum: tangerine silage, SO: sorghum: orange silage, P-value: probability value.

4.4.4 Macro- and micro-minerals of citrus species pulps in sorghum silage

4.4.4.1 Macro-minerals of citrus species pulps in sorghum silage

Table 4.4a below displays the results for macro-minerals of varying levels of citrus species pulps in sorghum silage. Citrus species pulps, inclusion levels, and level*species interaction affected (*P<0.05*) all the macro-minerals in sorghum silage.

Inclusion levels of citrus species pulps at 5 and 15% in SO, at 10, 15 and 20% in ST, and at 10, 15, 20 and 25% in SL as well 20% citrus species pulp inclusion level in SL had the highest (*P<0.05*) P concentration compared to their counterparts. The K concentration was increased (*P<0.05*) at 5% in ST, 10% in SL ,15, 20 and 25% in SG, and 10% in SO compared to other treatments.

The 10 and 25% inclusion levels of citrus species pulps in SG, SL and SO had the highest (*P<0.05*) Ca concentration compared to ST within their respective levels. The 10, 15, 20 and 25% inclusion levels in SL had the highest (*P<0.05*) Ca concentration compared to other treatments. The SO at 5, 10 and 25% inclusion level had the highest (*P<0.05*) concentration of Ca compared to their counterparts. Among the species, a 10% inclusion level in SG and SO as well as 20% in ST had higher (*P<0.05*) Ca concentration than to other levels of the same species.

Compared to other species at the same level, SG had higher (*P<0.05*) Mg concentration at 5, 10 and 15% inclusion levels. In SO, inclusion at 5 and 25%, and at 25% in SG and ST resulted in a higher (*P<0.05*) Mg concentration when compared with other species at the same level. Compared to other levels of the same species, inclusion level at 10% in ST and SL had the highest (*P<0.05*) concentration of Mg. The 10 and 15% inclusion levels in SG and 5% in SO produced the highest (*P<0.05*) Mg concentration than other levels of the same species.

The 5, 15, 20 and 25% inclusion levelsof citrus species pulps in SO had the highest ($P<0.05$) Na concentration than other species in their respective levels. Inclusion at 25% in SG and SO, 10% in SL and 15 and 20% in ST had the highest ($P<0.05$) Na concentration compared to their counterparts.

Table 4.4a: Macro-minerals of sorghum silage with increasing inclusion levels of citrus species pulps (% DM unless specified)

Parameter	Species	5%	10%	15%	20%	25%	SEM	L	S	L*S
P	SG	0.06^{Cb}	0.07^{Ba}	0.07^{Ba}	0.07^{Da}	0.07^{Ba}	0.001	<.0001	<.0001	<.0001
	SL	0.07^{Bc}	0.07^{Bc}	0.07^{Bc}	0.10^{Aa}	0.08^{Ab}				
	ST	0.07^{Bc}	0.09^{Aa}	0.08^{Ab}	0.09^{Ba}	0.07^{Bc}				
	SO	0.08^{Ab}	0.07^{Bc}	0.08^{Aa}	0.08^{Ca}	0.08^{Aa}				
K	SG	1.38^{Bd}	1.47^{Bb}	1.56^{Aa}	1.37^{Ad}	1.41^{Ac}	0.01	<.0001	<.0001	<.0001
	SL	1.24^{Db}	1.62^{Aa}	1.08^{Dc}	0.96^{De}	1.00^{Dd}				
	ST	1.45^{Aa}	1.03^{De}	1.19^{Cc}	1.24^{Bb}	1.09^{Cd}				
	SO	1.31^{Cb}	1.44^{Ca}	1.21^{Bc}	1.17^{Cd}	1.14^{Be}				
Ca	SG	0.14^{Bb}	0.17^{Aa}	0.15^{Bb}	0.15^{Bb}	0.15^{Ab}	0.002	<.0001	<.0001	<.0001
	SL	0.11^{Cc}	0.17^{Aa}	0.18^{Aa}	0.16^{Ab}	0.16^{Ab}				
	ST	0.11^{Cb}	0.09^{Bc}	0.11^{Db}	0.12^{Ca}	0.11^{Bb}				
	SO	0.16^{Ab}	0.17^{Aa}	0.14^{Cd}	0.15^{Bc}	0.16^{Ab}				
Mg	SG	0.18^{Ab}	0.19^{Aa}	0.19^{Aa}	0.15^{Ac}	0.15^{Ac}	0.001	<.0001	<.0001	<.0001
	SL	0.15^{Bb}	0.18^{Ba}	0.15^{Bb}	0.15^{Ab}	0.14^{Bc}				
	ST	0.15^{Bb}	0.16^{Ca}	0.15^{Bb}	0.15^{Ab}	0.15^{Ab}				
	SO	0.18^{Aa}	0.16^{Cb}	0.15^{Bc}	0.15^{Ac}	0.15^{Ac}				
Na (mg/kg DM)	SG	43.5^{Bb}	41.0^{Cb}	41.5^{Bb}	41.0^{Cb}	55.0^{Ba}	2.8	0.02	<.0001	<.0001
	SL	50.0^{Bb}	76.0^{Aa}	28.5^{Cc}	34.0^{Cc}	33.5^{Cc}				
	ST	45.5^{Ba}	27.0^{Cb}	48.5^{Ba}	55.0^{Ba}	34.0^{Cb}				
	SO	92.0^{Ab}	66.5^{Bc}	97.5^{Ab}	91.5^{Ab}	115.5^{Aa}				

$^{A-D}$ = Means within a column with different superscripts differ (*P<0.05*) across citrus species pulp for each level, $^{a-e}$ = means within a row with different superscripts differ across inclusion level for each species, P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, Na: sodium, S: sulphur, L: level, S: species, L×S: interaction between level and species, SEM: standard error of means, 5%, 10%, 15%, 20% and 25%: inclusion levels, SG: sorghum: grapefruit silage, SL: sorghum: lemon silage, ST: sorghum: tangerine silage, SO: sorghum: orange silage.

4.4.4.2 Micro-minerals of sorghum silage with increasing inclusion levels of citrus species pulp

The results for micro-minerals of sorghum silage with incremental inclusion levels of citrus species pulps are shown in Table 4.4b below. Citrus species pulp, inclusion levels, and level*species interaction affected all the micro-minerals in sorghum silage.

Compared to other species within the same levels, inclusion of citrus species pulp in SO at 5, 10, 15 and 20% led to the highest ($P<0.05$) Fe concentration. The 10 and 25% inclusion levels in SL increased ($P<0.05$) the Fe concentration. Compared to other levels of the same species, inclusion of pulps in SO at15% and in SG at 20% in SL and ST at 15 and 25%, had the highest ($P<0.05$) Fe concentration.

The 10% pulp inclusion level in SL, and 15–25% in SO, generated the highest ($P<0.05$) Cu concentration than other species pulps in the same levels. The 15% pulp inclusion level in SG, 25% in SL and SO also produced the highest ($P<0.05$) concentration of Cu than other levels of the same species.

The inclusion of citrus species pulps in SG, SL, ST and SO at 15%, 10%, 25%, and 5 and 20%, respectively, had the highest ($P<0.05$) Zn concentration than other species in the same levels. The inclusion of citrus species pulps in SG, SL, ST and SO at 15% 10%, 25% and 5%, respectively, produced the highest ($P<0.05$) Zn concentration than other levels of the same species. At 5% inclusion level in SO, 15% in SG and ST, 20 and 25% in SL and ST, the highest ($P<0.05$) Mn concentration was observed than in other species at the same respective levels. At 15% in SG, 5% in SO, 5–20% in ST and 5–10% in SL, the highest ($P<0.05$) Mn concentrations was improved compared to other levels of the same species.

The inclusion of pulps in SO at 5 and 25%, SG at 10%, SO and SL at 15% as well as in SO and ST at 20% had higher ($P<0.05$) B concentrations compared to other species in the same respective levels. At 10 and 25% in SG, 15 and 20% in SL, 20–25% in ST and 25% in SO inclusion levels had the highest ($P<0.05$) concentration of B compared to the other levels of the same respective species. At 5, 15 and 20% in SO, 10 and 25% in SL produced high ($P<0.05$) concentrations of Al than other species in the same respective levels. The 10% in SL and SO had the highest ($P<0.05$) Al concentration compared to other species at the same level. The 20% in SG, 15% in SL, 25% in ST together with 15% in SO had the highest ($P<0.05$) Al compared to other levels of the same species.

Table 4.4b: Micro-minerals of sorghum silage with increasing inclusion levels of citrus species pulps (mg/kg DM)

Parameter	Species	5%	10%	15%	20%	25%	SEM	L	S	L*S
Fe	SG	95.9^{Bb}	88.2^{Bb}	95.5^{Cb}	124.5^{Ba}	97.5^{Cb}	3.2	<.0001	<.0001	<.0001
	SL	84.3^{Bd}	114.2^{Ab}	128.2^{Ba}	99.6^{Dc}	135.0^{Aa}				
	ST	88.7^{Bb}	81.7^{Bb}	104.4^{Ca}	109.3^{Ca}	112.8^{Ba}				
	SO	145.3^{Ac}	118.6^{Ad}	218.4^{Aa}	171.8^{Ab}	112.4^{Bd}				
Cu	SG	4.8^{Ac}	5.7^{Bb}	6.5^{Ba}	4.7^{Cc}	4.8^{Cc}	0.2	<.0001	<.0001	<.0001
	SL	4.7^{Ad}	8.1^{Ab}	6.1^{Bc}	5.8^{Bc}	16.9^{Ba}				
	ST	4.4^{Aa}	4.3^{Ca}	4.1^{Ca}	3.9^{Ca}	4.8^{Ca}				
	SO	5.4^{Ae}	6.3^{Bd}	15.5^{Ac}	18.9^{Ab}	27.2^{Aa}				
Zn	SG	22.9^{Dc}	27.2^{Cb}	31.2^{Aa}	20.2^{Cd}	20.1^{Dd}	0.3	<.0001	<.0001	<.0001
	SL	27.5^{Bb}	31.8^{Aa}	25.7^{Bc}	24.7^{Bd}	23.1^{Ce}				
	ST	24.2^{Cc}	26.2^{Db}	24.6^{Cc}	24.2^{Bc}	28.2^{Aa}				
	SO	35.5^{Aa}	30.8^{Bb}	24.0^{Cd}	26.0^{Ac}	26.5^{Bc}				
Mn	SG	29.3^{Cb}	30.2^{Ab}	31.4^{Aa}	22.1^{Cc}	21.1^{Cd}	0.3	<.0001	<.0001	<.0001
	SL	31.4^{Ba}	32.1^{Aa}	24.9^{Bc}	29.9^{Ab}	28.9^{Ab}				
	ST	32.2^{Ba}	31.2^{Aa}	31.4^{Aa}	30.5^{Aa}	28.2^{Ab}				
	SO	45.8^{Aa}	29.5^{Ab}	25.5^{Bc}	24.1^{Bc}	24.7^{Bc}				
B	SG	2.9^{Cd}	3.7^{Aa}	3.3^{Cc}	3.6^{Cb}	3.8^{Ca}	0.04	<.0001	<.0001	<.0001
	SL	3.0^{Bd}	3.4^{Bc}	3.8^{Aa}	3.9^{Ba}	3.6^{Db}				
	ST	2.8^{Cc}	2.9^{Cc}	3.6^{Bb}	4.3^{Aa}	4.3^{Ba}				
	SO	3.1^{Ae}	3.5^{Bd}	3.8^{Ac}	4.3^{Ab}	5.2^{Aa}				
Al	SG	90.0^{Bb}	72.0^{Bc}	76.0^{Cc}	101.0^{Ba}	91.0^{Cb}	2.0	<.0001	<.0001	<.0001
	SL	57.0^{Cd}	93.0^{Ac}	143.0^{Ba}	97.0^{Bc}	131.0^{Ab}				
	ST	59.0^{Cc}	50.0^{Cd}	70.0^{Db}	73.0^{Cb}	83.0^{Da}				
	SO	124.0^{Ac}	92.0^{Ae}	260.0^{Aa}	180.0^{Ab}	104.0^{Bd}				

$^{A-D}$ = Means within a column with different superscripts differ ($P<0.05$) across citrus species pulp for each level, $^{a-e}$= means within a row with different superscripts differ across inclusion level for each for species, Fe: iron, Cu: copper, Zn: zinc, B: boron, Mn: manganese, Al: aluminium, L: level, S: species, L×S: interaction between level and species, SEM: standard error of means, 5%, 10%, 15%, 20% and 25%: inclusion levels, SG: sorghum: grapefruit silage, SL: sorghum: lemon silage, ST: sorghum: tangerine silage, SO: sorghum: orange silage.

4.4.5 *In vitro* dry matter degradability (IVDMD*)* of citrus species pulps in sorghum silage

The results for *in vitro* dry matter degradability of sorghum silage with incremental inclusion levels of citrus species pulps are shown in Table 4.5 below. Citrus species pulp, inclusion levels, and level*species interaction affected all withdrawal periods of sorghum silage.

Inclusion levels of citrus species pulps in SO and ST at 5, 15, 20, and 25% had the highest IVDMD at a 12-hour withdrawal period than all other species in the same levels. At 25 and 20% in SG and at 25% inclusion levels in ST, the IVDMD values during the 12-hour withdrawal period were higher ($P<0.05$) compared to other levels of the same species. Inclusion of the pulps in SO, SG and ST at 25% had the highest ($P<0.05$) IVDMD during the 24-hour withdrawal period than all other species in the same levels. Compared to other treatments, 25% in SG and ST showed higher ($P<0.05$) IVDMD value at a 24-hour withdrawal period. All ST and SO inclusion levels showed the highest ($P<0.05$) IVDMD at a 36-hour withdrawal period than all other species in the same levels. Inclusion levels of the citrus species pulps at 25% in SG and 15–25% in SL depicted high ($P<0.05$) IVDMD value at a 36-hour withdrawal period. At 25% in ST had a higher ($P<0.05$) IVDMD value at a 36-hour withdrawal period compared to other treatments.

All the inclusion levels in ST and SO, and 25% in SG had higher ($P<0.05$) IVDMD at a 48-hour withdrawal period than other treatments. At 25% in SG and 15–25% in SL depicted high ($P<0.05$) IVDMD value at a 48-hour withdrawal period. Inclusion levels of the citrus species at 20 and 25% in ST had higher ($P<0.05$) IVDMD values at a 48-hour withdrawal period compared to the 5% level in the same species. At 15% in SO had a higher ($P<0.05$) IVDMD value at the 48-hour withdrawal period compared to 5 and 10% inclusion levels of the same citrus species pulps. Inclusion levels of the citrus species pulps at 10% in ST and SO, and 15% in ST, SO and SL had high ($P<0.05$) IVDMD at a 72-hour withdrawal period. At 5% in SO depicted a higher ($P<0.05$)

IVDMD value at a 72-hour withdrawal period compared to SL of the same level. Inclusion levels of citrus species pulps at 15% in ST had higher ($P<0.05$) IVDMD value at a 72-hour withdrawal period compared to other treatments. The inclusions of citrus species pulp at 25% in SG had a high ($P<0.05$) IVDMD value at a 72-hour withdrawal period. At 25% in SL and 20% in SO had higher ($P<0.05$) IVDMD value at a 72-hour withdrawal period compared to 5 and 10% levels of the same respective species.

Table 4.5: *In vitro* dry matter degradability of incremental inclusion levels of citrus species pulp in sorghum silage (g/kg DM)

Parameter	Species	5%	10%	15%	20%	25%	SEM	L	S	L*S
2 Hrs	SG	236.2^{Abc}	205.5^{Bc}	192.3^{Bc}	271.9^{Ab}	315.3^{Aa}	1.4	<.0001	<.0001	0.001
	SL	176.4^{Bb}	202.6^{Bb}	247.3^{Aba}	195.6^{Bb}	236.0^{Bab}				
	ST	239.7^{Ab}	237.2^{Abb}	224.0^{Bb}	226.1^{Bb}	293.3^{Aa}				
	SO	223.4^{Ab}	271.2^{Aa}	280.9^{Aa}	267.8^{Aa}	288.0^{Aa}				
4 Hrs	SG	262.5^{Ab}	250.0^{Bb}	177.9^{Cc}	280.6^{Aab}	321.2^{Aa}	1.5	<.0001	<.0001	<.0001
	SL	189.7^{Bb}	221.7^{Bb}	272.0^{Ba}	210.1^{Bb}	260.9^{Bab}				
	ST	258.3^{Ab}	247.5^{Bb}	283.4^{Bb}	264.3^{Ab}	343.1^{Aa}				
	SO	247.8^{Ac}	301.0^{Ab}	333.0^{Aab}	301.1^{Ab}	360.8^{Aa}				
8 Hrs	SG	268.1^{Bb}	254.6^{Bbc}	219.9^{Cc}	325.6^{Aa}	354.6^{Aa}	1.5	<.0001	<.0001	0.001
	SL	229.2^{Bb}	237.1^{Bb}	284.4^{Ba}	219.5^{Bb}	251.4^{Bab}				
	ST	337.0^{Aab}	307.6^{Ab}	336.4^{Aab}	329.2^{Aab}	370.0^{Aa}				
	SO	328.4^{Ab}	342.5^{Aab}	362.1^{Aab}	358.5^{Aab}	386.6^{Aa}				
12 Hrs	SG	336.1^{Bb}	319.4^{Cbc}	283.4^{Cc}	386.9^{Ba}	418.3^{Ba}	1.3	<.0001	<.0001	<.0001
	SL	286.5^{Cb}	324.2^{Cb}	380.8^{Ba}	352.5^{Bab}	370.7^{Ca}				
	ST	425.4^{Ab}	422.4^{Bb}	427.6^{Ab}	436.4^{Ab}	501.2^{Aa}				
	SO	436.1^{Ab}	475.6^{Aa}	461.6^{Aab}	467.3^{Aab}	451.9^{Bab}				
24 Hrs	SG	432.1^{Bc}	400.3^{Dd}	396.6^{Dd}	489.1^{Bb}	534.1^{Aa}	0.8	<.0001	<.0001	<.0001
	SL	423.7^{Bc}	434.0^{Cc}	479.9^{Cb}	490.8^{Bab}	509.3^{Ba}				
	ST	491.4^{Ab}	490.7^{Bb}	508.5^{Bb}	497.1^{Bb}	547.4^{Aa}				
	SO	508.8^{Ab}	536.4^{Aa}	542.9^{Aa}	529.1^{Aab}	548.8^{Aa}				
36 Hrs	SG	432.6^{Bc}	440.0^{Bc}	404.1^{Cd}	488.1^{Bb}	520.9^{Ba}	1.0	<.0001	<.0001	<.0001
	SL	430.5^{Bb}	431.9^{Bb}	498.7^{Ba}	499.9^{Ba}	504.6^{Ba}				
	ST	568.9^{Ab}	560.6^{Ab}	571.3^{Ab}	580.8^{Aab}	604.1^{Aa}				
	SO	579.7^{Ab}	587.3^{Aab}	579.3^{Ab}	608.6^{Aa}	590.4^{Aab}				
48 Hrs	SG	464.1^{Bc}	435.3^{Ccd}	409.7^{Cd}	505.0^{Bb}	579.5^{Aa}	1.1	<.0001	<.0001	<.0001
	SL	480.6^{Bb}	474.6^{Bb}	532.5^{Ba}	530.3^{Ba}	535.2^{Ba}				
	ST	573.5^{Ab}	599.5^{Aab}	603.2^{Aab}	606.6^{Aa}	608.4^{Aa}				
	SO	569.4^{Ab}	587.0^{Ab}	621.9^{Aa}	615.2^{Aab}	604.8^{Aab}				
72 Hrs	SG	579.5^{Abb}	568.4^{Bb}	467.9^{Bc}	590.9^{Bb}	647.1^{Aa}	1.9	<.0001	<.0001	0.001
	SL	562.4^{Bb}	531.4^{Bb}	603.4^{Aab}	606.7^{Bab}	638.4^{Aa}				
	ST	608.0^{Aba}	630.3^{Aa}	650.8^{Aa}	654.2^{Aba}	654.8^{Aa}				
	SO	618.5^{Ab}	625.4^{Ab}	653.9^{Aab}	681.7^{Aa}	654.8^{Aab}				

$^{A-D}$ = Means within a column with different superscripts differ (*P<0.05*) across citrus species pulp for each level, $^{a-d}$ = means within a row with different superscripts differ across inclusion level for each species, 2, 4, 8, 12, 24, 36, 48 and 72: *in vitro* dry matter degradability incubation hours, L: level, S: species, L×S: interaction between level and species, SEM: standard error of means, 5%, 10%, 15%, 20% and 25%: inclusion levels, SG: sorghum: grapefruit silage, SL: sorghum: lemon silage, ST: sorghum: tangerine silage, SO: sorghum: orange silage.

4.4.6 Nitrogen solubility of citrus species pulps in sorghum silage

The results of buffer nitrogen solubility are presented in Table 4.6 below. The inclusion of citrus species pulp in sorghum silage affected total N (*P<.0001*), with no significant differences observed on soluble N (*P<0.094*), insoluble nitrogen (*P<0.094*) and solubility index (*P<0.094*). The ST had a higher (*P<0.05*) total N across all the inclusion levels than other citrus species pulp-sorghum silages. The SG, SL and SO displayed similar (*P>0.05*) total N at the inclusion of 5 and 10%, however, SG had the lowest (*P<0.05*) total N at 15, 20 and 25% inclusion levels. Nonetheless, total N increased with increasing inclusion levels of each citrus species pulp.

Table 4.6: Buffer nitrogen solubility of incremental inclusion levels of citrus species pulps in sorghum silages

Parameter	Species	5%	10%	15%	20%	25%	SEM	L	S	L*S
	SG	6.5^{Bc}	6.8^{Bb}	6.8^{Cb}	6.9^{Db}	7.2^{Ca}	0.1	<.0001	<.0001	<.0001
Total N	SL	6.9^{Bc}	7.1^{Bc}	7.3^{Bc}	8.0^{Bb}	8.3^{Aa}				
(g/kg DM)	ST	7.4^{Ab}	7.5^{Ab}	8.1^{Aa}	8.3^{Aa}	8.4^{Aa}				
	SO	6.7^{Bd}	7.0^{Bc}	7.4^{Bb}	7.5^{Cb}	7.8^{Ba}				
	SG	386.5	271.1	299.7	389.3	470.6	55.9	0.03	0.04	0.1
Soluble N	SL	419.8	279.6	326.8	199.6	296.2				
(g/kg N)	ST	262.8	91.45	257.2	276.5	398.2				
	SO	241.9	333.0	292.7	226.5	337.8				
	SG	613.5	728.9	700.3	610.7	529.4	55.9	0.03	0.04	0.1
Insoluble N	SL	580.2	720.4	673.2	800.4	703.8				
(g/kg N)	ST	737.2	908.5	742.8	723.5	601.9				
	SO	758.1	667.0	707.3	773.5	662.2				
	SG	38.7	27.1	30.0	38.9	47.1	5.6	0.03	0.04	0.1
Solubility I	SL	42.0	28.0	32.7	20.0	29.6				
(%)	ST	26.3	9.1	25.7	27.7	39.8				
	SO	24.2	33.3	29.3	22.7	33.8				

$^{A-D}$ = Means within a column with different superscripts differ (*P<0.05*) for across citrus species pulp for each level, $^{a-c}$ = means within a row with different superscripts differ across inclusion level for each species, Total N: total nitrogen, Soluble N: soluble nitrogen, Insoluble N: insoluble nitrogen, Solubility I: solubility index, L: level, S: species, L×S: interaction between level and species, SEM: standard error of means, 5%, 10%, 15%, 20% and 25%: inclusion levels, SG: sorghum: grapefruit silage, SL: sorghum: lemon silage, ST: sorghum: tangerine silage, SO: sorghum: orange silage.

4.4.7 Fermentation characteristics of citrus species pulps in sorghum silage

The results for fermentation characteristics of sorghum silage with differing inclusion levels of citrus pulp are presented in Table 4.7 below. Citrus species' pulp inclusion levels and the level*species interaction affected the fermentation characteristics of the sorghum silage.

Inclusion levels of citrus species pulp at 5% in SL, ST and SO had higher ($P<0.05$) pH than other treatments. Compared to other species, SL and ST also had higher ($P<0.05$) pH at 10–25%. At 5% in SG and SO together with 5 and 10% in SL and ST had high ($P<0.05$) pH. Inclusion levels of citrus species pulps at 5 and 25% in SL, 10 and 20% in ST and 15% in SG had the highest ($P<0.05$) WSC percentage than other species at the same level. At 15% in SG, 25% in SL, 10% in ST and SO produced high ($P<0.05$) WSC percentage. Compared to SO species at the same level, SL, ST and SG had the highest ($P<0.05$) Y&M count at 5% inclusion levels. At 10% in SO and SG had the highest ($P<0.05$) Y&M count than other species at the same level. Inclusion levels of citrus species pulps at 15 and 20% in SO produced higher ($P<0.05$) Y&M count when compared to other treatments. At 25% in ST and SG had higher ($P<0.05$) Y&M count than other species at the same levels. Inclusion levels of the citrus species pulps at 5–15% in SG, 5% in SL and ST, and 10–15% in SO had produced the highest ($P<0.05$) Y&M count than all other levels of the same respective species.

4.4.8 Optimal fermentation characteristics of citrus species pulps in sorghum silages

Table 4.8 shows the optimal responses on fermentation characteristics of citrus species pulps in sorghum silages. The silage pH from different citrus species pulp was optimised at an average inclusion level of 24.3% (SG), 22.5% (SL) and 22.8% (SO) in sorghum silage. The silage WSC from different citrus species pulp were optimised at an average inclusion level of 13.4 g/kg DM (SG), 16.1 g/kg DM (SL), 12.9 g/kg DM (ST) and 46.3 g/kg DM (SO) in sorghum silage. The silage Y&M counts from different citrus species pulps were optimised at an average inclusion level of 7.8 log10 CFU · g−1 (SG), 44.5 log10 CFU · g−1 (ST) and 13.2 log10 CFU · g−1 (SO) in sorghum silage, respectively.

Table 4.7: Fermentation characteristics of sorghum silage with increasing levels of citrus species pulps

Parameter	Species	5%	10%	15%	20%	25%	SEM	L	S	LxS
pH	SG	4.5^{Ba}	4.2^{Bb}	3.9^{Bc}	4.0^{Bc}	3.9^{Bc}	0.1	0.0001	0.0001	0.01
	SL	4.7^{Aa}	4.5^{Aa}	4.3^{Ab}	4.3^{Ab}	4.1^{Ab}				
	ST	4.8^{Aa}	4.75^{Aa}	4.4^{Ab}	4.4^{Ab}	4.2^{Ac}				
	SO	4.7^{Aa}	4.2^{Bb}	4.1^{Bb}	4.0^{Bb}	3.8^{Bc}				
WSC (g/kg DM)	SG	13^{Bb}	11^{Dc}	30^{Aa}	2.5^{De}	10^{Cd}	0.03	0.0001	0.0001	0.0001
	SL	15^{Ad}	23^{Bb}	17^{Bc}	6.4^{Ce}	24^{Aa}				
	ST	11^{Cd}	33^{Aa}	13^{Cc}	16^{Ae}	1.7^{Db}				
	SO	11^{Cd}	15^{Ca}	12^{Dc}	10^{Be}	14^{Bb}				
Y&M log10 CFU · g−1	SG	7.4^{Aa}	7.4^{Aa}	7.3^{Ba}	6.2^{Bb}	5.5^{Ac}	0.2	0.0001	0.0001	0.0001
	SL	7.34^{Aa}	6.8^{Bb}	6.4^{Cb}	5.5^{Cc}	4.4^{Bd}				
	ST	7.2^{Aa}	6.3^{Cb}	6.3^{Cb}	6.3^{Bb}	5.6^{Ac}				
	SO	6.4^{Bc}	7.5^{Aa}	7.8^{Aa}	7.0^{Ab}	4.6^{Bd}				

$^{A-D}$ = Means within a column with different superscripts differ ($P<0.05$) for each level, $^{a-e}$ = means within a row with different superscripts differ for species, pH: the potential of hydrogen, WSC: water-soluble carbohydrates, Y&M: yeast and mould count, L: level, S: species, L×S: interaction between level and species, SEM: standard error of means, 5%, 10%, 15%, 20% and 25%: inclusion levels, SG: sorghum: grapefruit silage, SL: sorghum: lemon silage, ST: sorghum: tangerine silage, SO: sorghum: orange silage.

Table 4.8: Quadratic equations for optimal responses on fermentation characteristics of citrus species pulps in sorghum silages

Variable	Silages	Formulae	Optimal	Y-level	R^2	Average	P-Value
pH	SG	$Y=4.9+-0.1x+0.002x^2$	24.3	3.7	0.9	18.6	0.1
	SL	$Y=4.9+-0.1x+0.001x^2$	22.5	4.4	1.0		0.04
	ST	$Y=5.0+-0.04x+0.00x^2$	0.0	0.0	0.9		0.1
	SO	$Y=5.1+-0.1x+0.002x^2$	22.8	4.0	0.9		0.1
WSC g/kg DM	SG	$Y=0.4+0.2x+-0.01x^2$	13.4	1.8	0.2		0.8
	SL	$Y=2.4+-0.1x+0.004x^2$	16.1	1.4	0.1		0.9
	ST	$Y=0.02+0.4x+-0.01x^2$	12.9	2.4	0.6		0.4
	SO	$Y=1.2+-0.002x+8.6E\text{-}5x^2$	46.3	1.2	0.01		1.0
Y&M log10 CFU · g−1	SG	$Y=7.0+0.1x+-0.01x^2$	7.8	7.4	1.0		0.03
	SL	$Y=7.5+-0.01x+-0.004x^2$	-1.1	7.5	1.0		0.01
	ST	$Y=7.5+-0.1x+0.001x^2$	44.5	5.5	0.8		0.2
	SO	$Y=3.9+0.6x+-0.02x^2$	13.2	7.9	1.0		0.01

pH: the potential of hydrogen, WSC: water-soluble carbohydrates, Y&M: yeast and mould count, R^2: Coefficient of determination, P-value: probability value

4.5 Discussion

4.5.1 Chemical composition

According to Mirzaei-Aghsaghali and Maheri-Sis (2008), agro-industrial waste has become essential in the ruminant industry and their incorporation in diets enhances the carcass characteristics, milk and meat quality which are essential in human nutrition for their wellbeing. Therefore, this study analyzed the nutritional profile of sorghum silage containing graded/varying inclusion levels of citrus species pulp was.

The DM analyses are significant for ensiling as they can predict the rate of fermentation (Chen *et al*., 2019) and contribute to the silage quality and overall nutritive value. The DM of less than 40% but greater than 25% prior to ensiling is regarded as an excellent DM that positively influences the ensiling process (Nkosi *et al*., 2016). Such DM satisfactorily compresses the silage to extract any air particles in the sample leading to improved nutritive value and well-preserved silages. The pre-silage in the present study reported DM of between 289.6–539.6 g/kg which are good for ensiling and are comparable to those reported by Nkosi *et al*. (2016). Most of the pre-silages had acceptable DM with few having high DM, which could be due to long sorghum stem proportion. Results in this study show that ensiling resulted in DM of between 326.7 and 533.2 g/kg with the DM ranges of (326.7–370.9 g/kg), (347.2–533.2 g/kg), (365.2–463.3 g/kg) and (257.7–518.7 g/kg) in SG, SL, ST and SO, respectively. The results indicated that DM decreased in sorghum silage with the increasing inclusion levels of citrus species pulp. The results reported are similar to those reported by Ulger *et al*. (2020) where DM declined with increasing inclusion levels of citrus pulp. The DM in ST and SO decreased with the increasing inclusion levels of citrus species pulp. This could be alluded to the fact that the inclusion of citrus pulps having higher moisture ought to decrease DM and cell wall proportions. While the DM in SG remained statistically the same, the DM in SL increased with the increasing inclusion levels of the pulp,

which could result to the initial high DM of lemon pulp (Table 2.2 and 3.1). Generally, the decrease in DM with ensiling the citrus species pulps is related to the preservation period and citrus species pulp lower DM content, which was true to a fraction of this study's findings. One of the possible causes of DM increase is the degradation of the cell wall (complex sugars/carbohydrates) by the available bacterial enzymes.

Ash is one of the main measures of feed quality, and in the present study, the ash content in sorghum silage with increasing levels of citrus species pulp was between 42.1–59.8 g/kg DM, which increased as the inclusion levels increased. Research conducted by Baba *et al*. (2020) reported partially related results of the ash content (36.6–43.4 g/kg DM) of *Sorghum almum* (with lablab) silage with citrus pulp. It has been observed from the present study that the preservation of sorghum with citrus species pulp increased the ash content of the silages, this indicates that inclusion of the citrus species pulp at different levels may contain a desirable amount of minerals. Ash is desirable for livestock feeding as the livestock species require minerals in their diet for good health maintenance and improvement.

Ether extract (EE) is essential at a low concentration of approximately equal to or less than 60.0 g/kg DM of EE for ruminant diets (de Ondarza *et al*., 2024). An increased EE might lead to high fat deposition in ruminants. The EE in this study ranged between 11.3 and 25.4 g/kg DM. These results are similar to those of Ülger *et al*. (2020) who reported EE contents (19.5–26.3 g/kg DM) of maize silage with citrus species pulp. Contrary to the current study, Souza *et al*. (2022) reported EE contents of 24.6–34.5 g/kg DM of maize silage with increasing levels of citrus pulp. An increase in EE content with the inclusion of citrus species pulp in sorghum silage in the present study indicates that it is a high-energy density feedstuff. Furthermore, it is anticipated to boost the health and the metabolism of ruminant animals as the EE can enhance nutritive value and palatability while increasing feed particle integration (Pond *et al*., 2004).

Crude protein (CP) is one of the significant nutrients in livestock production. The CP is made up of amino acids that are essential for muscle development and bone development amongst other uses (Hou *et al*., 2022). Ruminants require a minimum of 70.0 g/kg DM CP (Souza *et al*., 2022) for their maintenance and growth. The present study reported the CP content in silages between 40.7–52.4 g/kg DM, rendering it a poor protein source for livestock production. Among the silages in the current study, the highest (52.4 g/kg DM) CP was detected in ST. Nonetheless, there was an increasing trend with increasing inclusion levels of citrus species pulp across all the silages. The reported CP of the silages was largely influenced by the CP of the fresh citrus species pulp as they were low (Chapter 3). Gomes *et al*. (2017) and Ulger *et al*. (2020) reported the CP of citrus pulp-infused silage to be below 100.0 g/kg DM. The difference in CP could be attributed to different sizes and numbers of peels, seeds, grains, and sorghum stalks in the silages. Guo *et al*. (2019) reported that early cut-stage silages produced high CP and better silage quality, however, sorghum used in this study was harvested post-maturity and that could have impacted the CP of the silages. The CP in the present study may not retain the rumen microbiota leading to impaired growth when fed. For maximum rumen microbial growth, Pathak (2008) noted that 11% of the protein is needed as it enhances the biological processes and production processes. Lower values of CP may restrain the production of microbial protein as the complete CP is not degraded by the microbes due to resistance.

The NDF is the fibre fraction of feed consisting of complex carbohydrates such as cellulose and lignin and affects voluntary DM intake (Harper & McNeill, 2015). Low NDF content is recommended as it stimulates more feed intake and utilisation by ruminants. Additionally, low fibrous matter improves the digestibility and growth of ruminants. An NDF of greater than 400.0 g/kg DM is not recommended as it “impairs rumen motility” and causes a reduced rate of degradation of the fibre matter (Hoffman *et al*., 2001). The ST and SO at 15–25% inclusion levels, SL at 10–25% and SG at 25% inclusion level are the only silages that had acceptable NDF for

ruminants, thus can be offered to animals without adverse effects. The present study reported the NDF of silages between 301.3–533.4 g/kg DM. Gomes *et al*. (2017) reported higher NDF content (585.7 and 552.5 g/kg DM) of the wilted and unwilted elephant grass with the inclusion of citrus pulp, respectively. The NDF of below 400.0 g/kg DM is reported to have a minimum to no impact on the DM intake by livestock. The ADF is one of the constituents of NDF and the present study reported the ADF content of silages of between 180.3–366.0 g/kg DM. All the silages in the present study had similar concentrations of ADF across all the inclusion levels. The study conducted by Kordi and Naserian (2012) reported 230.9 and 240.0 g/kg DM of ADF on citrus pulp silage and citrus pulp silage added to barley grain. The ADF concentration in the current study could be attributed to the high soluble carbohydrates/sugars content abundant in the citrus species pulp (Zheng *et al*., 2016).

Lignin is the anti-nutritional factor that limits nutrient digestion and absorption (Gomes *et al*., 2017). It locks in the highly digestible matter, rendering highly digestible mmatter inaccessible to the rumen microbes. The present study produced a low ADL content of silages ranging between 29.1–55.9 g/kg DM. The ST and SO had the least ADL content with the highest ADL content in SG across the inclusion levels. The low ADL in the silages could mostly be affected by the lower ADL of the citrus species pulp. The low ADL in silages of this study may improve digestion and absorption of nutrients when fed to ruminants.

4.5.2 Chemical estimates

Chemical estimates such as DMI and DMD are necessary in cases where true animal performance analyses are not feasible, and they equate to forage quality (Osbourn, 1978). They are further used to rank and select forages according to their quality (Osbourn, 1978) for ruminant nutrition. These estimates are calculated from the chemical composition analyses. In this study, there was no variation in the effect of species* level interaction on all the chemical estimates. The chemical estimates ranging between 92.2–189.3, 146.6–310.1, 603.9–748.5, 22.6–40.0 and 548.8–

688.3 g/kg DM, for HEMI, Cel, DMD, DMI, and TDN, and 28–35 and 23–29 Mcal/kg for DE and ME, respectively. Contrary to these results, Anjos *et al*. (2022) reported the HEMI content of Massai grass with different levels of pelleted citrus pulp silages of 223.3–303.0 g/kg DM. The hemicellulose is the major carbohydrate and serves as the energy source in the ruminant's feed (Weimer, 2022). The Cel constitutes the cell of the forages and is mostly found in the secondary wall. The study conducted by Anjos *et al*. (2022) reported a CEL content of 298.6–378.3 g/kg DM of the Massai grass with different levels of pelleted citrus pulp silages. Although not significant, the citrus pulp dilutes/ reduces the fibre content of sorghum silage, as it has low HEMI and cellulose content, which also increases the DMI and DMD. The low fibre amounts are recommended as they increase feed intake leading to improved growth and metabolism.

The DE and ME are essential and are used to estimate the energies of the feedstuffs in comparison with the required energies of the livestock animals across different ages, physiological status, gender and health. De Oliveira *et al*. (2023) reported the ME of sorghum silage with the addition of discarded melon fruit of between 2.4–2.6 Mcal/kg, which is lower than the value recorded in this study. The NRC (1985) mentioned that animals (replacement ram lambs) require 2.8 and 2.3 Mcal/kg of DE and ME, respectively. The ME and DE of the present study might be good for livestock animals in extreme climate conditions such as extreme cold (snow) as this energy helps to produce heat to keep the animals warm.

Though non-significant, numerically the highest TDN values were observed at 25% inclusion level. Compared to our study, Anjos *et al*. (2022) reported low TDN of the Massai grass with different levels of pelleted citrus pulp silages of 458.6–512.7 g/kg DM, whereas Souza *et al*. (2022) reported the TDN of maize silage with increasing levels of citrus pulp of between 640.2–727.4 g/kg DM. The citrus pulp contains higher nutrient digestibility (Mahrous *et al*., 2019) hence the increase of TDN with increasing inclusions of citrus species pulp in sorghum silage. This might

be due to the lower fibrous content of citrus pulp. Lower TDN is associated with high NDF as the fibre reduces the nutrient utilization by the rumen microorganism.

The citrus species pulp in sorghum silage had different impacts on the chemical composition and the chemical estimates. This could be due to the genetic differences of the citrus species. The tangerine pulp exhibited higher CP in sorghum silages compared to other species. Low CP and high ADL were observed in SG silage. Low HEMI was in SL silage. High CEL was in SG silage. Low DMD, TDN, DE and ME were observed in SG silage. High DMI was on SL silage. Lemon pulp-maize silage had higher DM and tangerine-maize silage had higher CP and low NDF in orange-maize silage in the research conducted by Ulger *et al*. (2020). Thus, this indicates that the genetic makeup of the citrus species pulp had different effects on the chemical composition and chemical estimates of the sorghum silages. Furthermore, the genotypic effect could be affected by the environment leading to different chemical compositions and chemical estimate results. There is limited information on the chemical composition and chemical estimates of citrus species pulp silages.

Inclusion levels are important as they indicate different amounts of the specified ingredient being added to another. They assist in determining the maximum amount or level that can be added to enhance productivity without detrimental effects. Different effects are expected when analysing the chemical composition as the different levels might be used, but if not many differences occur between or amongst the levels, not many effects can be detected. Also, the homogeneity of the said ingredient should be maintained for accurate results. The inclusion levels of the citrus species pulp had a big effect on the chemical composition and the chemical estimates. In the current study, the impact was observed on the increase in DM and CP as the inclusion levels increased. Also, the chemical estimates (DMD, DMI, DE, ME, RFV and TDN) increased as the inclusion levels were elevated. Except for the CEL, the values of HEMI were statistically the same throughout the

inclusion levels. Back to the chemical composition, no significant differences were observed in the ASH, EE, NDF and ADF in our current study.

Souza *et al*. (2022) reported an increase in DM and CP with increased inclusion levels of citrus pulp. Kordi and Naserian 2012 reported an increasing CP and NDF of the citrus pulp with an increasing wheat bran which contradicts the current study. Chaudhry and Naseer (2006) reported increasing citrus pulp in poultry litter-corn forage silage resulting in decreasing DM and CP. Baba *et al*. (2020) reported a decrease in DM, increasing CP and NDF with increased inclusion levels of citrus pulp in sorghum almum-Lablab silage. The expected results are that the DM and NDF would decrease as the cell wall contents decrease due to the high concentration of soluble carbohydrates found in citrus pulps while the opposite occurs for the CP. Also, ensiling results in DM losses and the microorganisms indulge on the available nutrients.

Essentially, the amount of acids produced and microbial population in the silage affect the feed quality of the silages. High and correct feed values (DMD, DMI, ME, DE, RFV and TDN) are essential for improved health status, growth rate, production and reproduction.

4.5.3 Macro and micro minerals of citrus species pulps in sorghum silage

Minerals play a very important role in maintaining productive and reproductive health, through interrelated systems of immune function, oxidative metabolism, and energy metabolism in ruminants, that are directly or indirectly involved in growth, production, and reproduction (Palomares, 2022).

Generally, citrus fruits are a valuable source of phosphorus hence there was a significant increase in P content with increasing levels of various citrus pulps. Both phosphorus and calcium participate in the formation of strong bones and teeth involved in chewing the feed thereby improving digestion (Kubkomawa, 2019). The current study reported the P and Ca of the citrus species pulp-sorghum silages of between 0.06 to 0.10 % and 0.09–0.18 %, respectively. Whereby

the highest P content was recorded in the SL silages amongst the inclusion levels when compared with other silages, while the 10% and 25% of SG, SL and SO inclusion levels produced the highest concentrations of Ca when compared to ST within their respective levels. Morales-Querol *et al*. (2022) reported a P content of 75% *S. bicolor* + 25% citrus fruit pulp silage of 0.043 % DM, which is much lower than the one observed in the current study. According to the NRC (1985), 0.20% of P is recommended for the maintenance of ewes and a maximum of 0.60 % of P for small ruminants (NRC, 2007). The P content of the citrus species pulp-sorghum silage in the present study was below the required amount for the small stock. Bone fractures, mobility difficulties and performance reduction might arise from ruminant animals fed a silage diet with low P or Ca values. Morales-Querol *et al*. (2022) reported a higher Ca content of 0.36% DM with a 75% *S. bicolor* + 25% citrus fruit pulp silage. The NRC (1985) recommends 0.4% Ca for ewes during the last month of gestation.

The Mg is also one of the largest cations in the ruminant's diet and has various roles in bones, muscles and tissues as explained by Kubkomawa (2019), while, the element K is involved in preventing coronary diseases, transmits nerve impulses and contracts muscles (Gurung *et al*., 2020). The present study resulted in Mg and K content of between 0.14–0.19 % and 0.96–1.56 %, respectively, of citrus species pulp-sorghum silages. The highest Mg content was obtained in SG and the lowest Mg was found in SL silages, while the SG silage had the highest K content at 15 to 25%. The 75% *S. bicolor* + 25% citrus fruit pulp silage from the research conducted by Morales-Querol *et al*. (2022) had 0.203% DM of Mg which is slightly higher than that reported in the current study. According to the NRC (2007) growing lambs, late gestating and early lactating ewes demand 0.12, 0.15 and 0.18% of Mg respectively. The same authors also reported that feedlot cows and bulls need 0.60–3.00% K for maintenance. The Mg and K content in the current study was adequate for the mentioned group of animals and positively influenced proper metabolism, growth and development, of cattle.

The present study reported the Na content of citrus species pulp-sorghum silages of 27.0–115.5 mg/kg DM with SO silage having the highest Na content at most inclusion levels when compared with other silages. The NRC (2000) reported that non-lactating beef cattle require a maximum of 800 mg/kg DM of Na and lactating beef cattle require 1000 mg/kg DM of Na. Severe effects can occur on the ruminants fed a low Na concentration diet such as poor development, stagnant growth, poor reproduction, decreased body score and ultimately death. Sodium sources such as sodium bicarbonate, sodium chloride and animal by-products can be used to increase the sodium concentration in citrus species pulp-sorghum silages. There is little information on the mineral composition of citrus pulp incorporated in the sorghum silage or forage silage.

Iron (Fe) is an essential trace element in the ruminant's body as it is mainly involved in the blood for the transportation of oxygen and CO_2 (NRC, 2007). The present study had the Fe content ranging between 81.7–218.4 mg/kg DM of the silages with the highest content being in the SO silages across the inclusion levels when compared with the SG, ST and SL silages. An amount of 50.0–1000 mg/kg DM of Fe is required and causes little to no effects on the beef cattle (NRC, 2000). The values found in the present study for all the silages at all inclusion levels are suitable for the production and growth of beef cattle and it is assumed that they improve the performance and the health status of beef cattle.

Both copper (Cu) and zinc (Zn) are associated with the synthesis of enzymes and a lack of either of them in the diet may cause unequal growth of bone structure, heart conditions and dull skin, fibre and wool (Palomares, 2022). Regarding the acceptable Cu concentration in the diet of cattle, it is stipulated as 10.0–100.0 mg/kg DM (NRC, 2000). For a healthy beef cow and bull, it is recommended to consume a minimum of 30.0 mg/kg of Zn and should not exceed 500.0 mg/kg DM of Zn (NRC, 2000). The silages with lower Zn content could cause excess saliva, poor reproduction, poor growth, and diarrhoea. To avoid such complications in ruminant animals, zinc oxides, sulphates and mineral licks can be supplemented in diets to enhance the Zn content (NRC,

2007). Moreover, the physiological status, age, gender and health status might implicate the Cu and Zn requirements of the animals. The Cu content of SG and ST silages would be rendered inadequate as they were below 10.0 mg/kg DM, which may warrant supplementation, and for SL and SO silages, they were sufficient for beef cattle at 25% and 15–25% inclusion levels respectively. On a general scale, the present study resulted in an increase in Cu of the sorghum silages with the inclusion of citrus species pulp while there was a partial decrease in Zn content for SG, SL and SO.

Manganese (Mn) is one of the trace elements involved in enzyme-enhancing roles such as in bone growth and blood clotting (Palomares, 2022). The NRC (2000) set the acceptable range of the Mn concentration in the diet of growing and finishing cows and bulls between 20 and 1000 mg/kg DM. Based on the above-recommended range, the SG, SL, ST and SO silages at all inclusion levels (21.1–45.8 mg/kg DM) had the acceptable Mn concentration for the proper growth, development and maintenance of growing and finishing cattle.

Boron (B) has been noted to be an essential element as it positively affect livestock health and performance, but the recommended values have not been clearly stated along with their possible effects on ruminant animals, though, Bhasker *et al*. (2017) stated that the maximum amount of boron required in animal diet is 17.7 mg/kg DM. The present study reported the B content of SG, SL, ST and SO silages ranging between 2.8–5.2 mg/kg DM which are lower values than those reported by Basker *et al.* (2017). The B concentrations in the current study would not affect the performance and growth of the sheep animals, as they are below the maximum tolerable concentration.

At high concentrations greater than 1000 mg/kg DM (NRC, 2007), Aluminium is toxic. The silages in the present study had an Al content of between 50.0–260.0 mg/kg DM. Higher concentrations of Al in ruminants' diets can cause reduced feed consumption, absorption in the internal organs such as liver and kidney and grass tetany depending on the concentration or

amount, the health status, duration of the consumption of high/rich Al diet and possibly the effects of severe weather conditions (Asín *et al*., 2019; Squadrone *et al*., 2021; Yipel & Yipel, 2024).

Generally, except for K, Mg and Fe, all the silages had a mineral concentration level which was below the threshold.

4.5.4 *In vitro* dry matter degradability

In vitro dry matter degradability (IVDMD) analyses are essential as they determine the degree to which the feedstuffs are digestible by the rumen microbes (Bao *et al*., 2022). Different hours (2, 4, 8, 12, 24, 36, 48 and 72) of incubation were applied in the current study to determine the duration it takes for the rumen microbes to degrade the silages. Cattle ruminate for a period of approximately 15 ±8 hours in a day, however, this depends on the chemical constituents of the feed and the health status of the ruminants (Millen *et al*., 2016). All the silages in the present study (SG, SL, ST and SO) had an increasing trend of digestion rate from 2 up to 72 hours of incubation. At 2, 4 and 8-hour withdrawals, the digestion was low, this could be because the rumen microbes had limited time to degrade the samples. The degradability rate was between 176.4–386.6 g/kg DM with higher IVDMD at increasing inclusion levels. Furthermore, the reduced fibre fractions at the higher inclusion levels of citrus species pulp in sorghum silages enhanced IVDMD. From 12 to 72 hours of incubation, the IVDMD exponentially increased, also with the increasing inclusion levels of the citrus species pulp in sorghum silage between 396.6–681.7 g/kg DM. Overall, the high inclusion levels had low fibre content and high IVDMD and this is supported by the results of Valente *et al*. (2016) and Bao *et al*. (2022). This suggests that IVDMD is dependent on the fibre content of forages, rather than the CP content. Buthane (2020); Mhlongo (2021) and Msiza (2021) noted that protein content is related to the IVDMD, opposite to the current study the CP levels were assumed not to be the contribution factor behind high substrate degradation as it was below the recommended values (Ravhuhali *et al*., 2020). The authors stated that the microbes require 60 g/kg DM CP and above for the degradation of the substrates. The high degradability of the silage

in the present study indicates that the feed might influence the high consumption and improve the growth and performance of the ruminant animals.

4.5.5 Nitrogen solubility

The nitrogen solubility method is used to assess the concentration of soluble nitrogen in the artificial ruminal pH and temperature solution (borate-phosphate buffer N) and gives an estimate of the true protein fraction of the feed (Cudjoe & Mlambo, 2014). This method has been cultivated to replace the *in-situ* bag and *in vitro* methodologies, as they impact animal welfare and ethics, need intense skills and are economically inviable (Mnisi, 2015). Total N is essential as it predicts the total amount of nitrogen that can potentially be absorbed by the ruminants when provided in the feed. It is further related to protein concentration, as it promotes improved performance and growth, and it is usually low in forages during the dry season. The citrus species pulp-sorghum silages had low CP content and low total N content (6.513–8.380 g/kg DM). High total N content was observed on the ST silage and low content on the SG silage and was increasing with the increasing levels for all the citrus species pulp in the sorghum silage. These results suggest that the silages have low degradable protein content (>50% solubility index) which can cause adverse effects in ruminants if not well-supplemented. It is anticipated that due to the low soluble N and solubility index values, the citrus species pulp-sorghum silages would be less degradable as the microbes would not find sufficient easily fermentable carbohydrates to attach to. The low soluble N amount could affect the rumen microbial activities consequently affecting the digestibility and the overall metabolism of the ruminants. The content of the total N of the silages in the present study renders them of poor protein source. Therefore, there is a need to supplement these silages with higher total N and soluble N sources to improve the nutritive value of the feed and also the productivity of ruminants.

4.5.6 Fermentation

Fermentation is the process whereby the sample undergoes anaerobic degradation while preserving the nutritional composition of the sample (Kung, 2010). The microbial population found in the sample converts the complex carbohydrates into simple carbohydrate sugars then to alcohol and carbon dioxide (da Silva *et al*., 2017). Fermentation can be amplified by adding supplementing ingredients, chemical additives, and inoculants to improve the fermentation process and the end results (Oskoueian *et al*., 2021). Fermentation characteristics determined in this study were WSC, pH and Y&M, and they are essential in determining the quality status of the silage. Silage quality outcomes are affected by several factors such as the silage preservation method, ensiling period, silage raw materials, silage density and the temperature where the silages are stored (Knický, 2005).

The pH is a measure of ensiling characteristics, and it is used to determine how acidic or basic the samples are, acidic samples are considered good-quality silage. The pH greatly declines in the first few days (7 days) and thereafter remains constant and stable under the anaerobic conditions. The pH of the silages is a good indicator of how well or badly the silage was preserved. Anésio *et al*. (2017) and Chen *et al*. (2019) noted that a silage pH of between 3.6 and 4.5 is a well-preserved silage and this range is highly accepted by ruminant animals. The SG (3.9-4.5) silage showed the typical and accepted range of pH throughout all inclusion levels while other silages had a pH of 3.8–4.8 in the present study. Silages of $SL_{5,10}$; $ST_{5,10}$ and $S0_5$ were the only silages that were higher than the acceptable range of pH that is deemed a well-preserved and excellent silage in the present study, of which the reason is unknown. In the current study, the pH of the silages varied with increasing inclusion levels of citrus pulp and within each citrus pulp variety, with the general trend of decreasing pH with increasing inclusion levels of the citrus species pulp in sorghum silage (SG, SL, ST and SO). Generally, most silages of the current study had pH within the range that indicates well-preserved silages. The relationship of increasing citrus species pulp

inclusions in sorghum silages with decreasing pH of the present study is in agreement with results reported by Baba *et al*. (2020) and Müller *et al*. (2018) of *Sorghum almum* -Lablab silage and soybean silage both with increasing inclusions of citrus pulp respectively. This trend was expected as the citrus pulps contain a highly acidic profile of malic, citric and ascorbic acids and the current results are supported by Ülger *et al*. (2020) and Souza *et al*. (2022). According to Tolentino *et al*. (2016), the possibility of improper enclosing and compression leading to the presence of air particles and ultimately low-quality silages of increasing and unstable pH might have contributed to high pH.

Water-soluble carbohydrates are the simple and highly digestible carbohydrates found in forages such as glucose, fructose and sucrose and are easily accessible and available for digestion. Water-soluble carbohydrates are essential for efficient and intensive fermentation as they are responsible for the production of organic acids and enhance the fermentation process. According to Horst *et al*. (2020), the WSC content of plants is affected by maturity; and the climate in tropical regions is one of the conditions that limits the readily fermentable carbohydrates (simple sugars) concentration to reach minimums. The WSC in silages are utilised to determine the degree to which the fermentation process will occur, in the sense that higher values indicate a moderate to vague fermentation. In the current study, the WSC of silages was of an average of 15 g/kg DM, which is lower than the range (60–70 g/kg DM) as recommended by Lunden-Pettersson and Lindgren (1990). Compared to the current study Ramakatana (2019) reported a higher 30 g/kg DM WSC of buffalo grass and orange pulp at 30 days of ensiling, whereas, Beyzi *et al*. (2018) reported lower WSC content (<6 g/kg DM) of orange, lemon, maize and tangerine alone silages. Additionally, the WSC in the present study can be accounted for by microorganisms' population degrading soluble carbohydrates (Malebana *et al*., 2019; Rodrigues *et al*., 2020) during the fermentation process.

Yeast and mould are the microorganisms present in the silages that assist in fermenting the samples, however, undesirable microorganisms can develop affecting the nutritive quality of

silages. The literature stated that the presence of air particles leads to unfavourable conditions for microorganisms' growth that compete with lactic acid bacteria for soluble carbohydrates and increase pH value in silages (Anésio *et al*., 2017; Liu *et al*., 2024; Gu *et al*., 2025). Additionally, the increased concentration of yeast and mould scavenge the available sugars, protein and other nutrients found in the silages to maintain their growth, thus reducing the quality of the silages. The silage of such conditions is rendered unsuitable for the consumption of livestock animals, as it contains harmful microorganisms such as *Clostridium* that can greatly alter the growth, health and productivity of animals. Silages with high yeast and mould counts lead to fast deterioration that leads to the reduction of the nutritive value when exposed to oxygen. Oxygen, substrate and temperature are some of the factors that affect aerobic stability. Well-preserved silages of no oxygen limit the effluent losses, whereby the concentration of yeast count of an acceptable amount is less than 6 $\log_{10}$ CFU.g^{-1} (Kung, 2010). The low inclusion levels of citrus pulp species in sorghum silage in the present study had a higher Y&M count than the increased inclusion levels. In the current study, low WSC and higher Y&M, practically show that the Y&M population increased due to adequate WSC in the fresh silage materials. The high Y&M concentration observed could be attributed to the high sugars of both sorghum and citrus species pulp as they serve as energy sources in the form of carbon for the microorganisms leading to their rapid growth (Yang *et al*., 2006). Kung (2010) stated that high temperatures where the silages are stored can detrimentally increase the microorganisms' population. Most of the silages of the current study had higher Y&M count which indicates silages are susceptible to quick spoilage when exposed to atmospheric conditions. However, Y&M improved with increasing inclusion levels of the citrus species pulp in sorghum silages across all the experimental treatments. The Y&M content was within the accepted values at a higher inclusion of 25% across all the treatments.

4.6 Conclusion

The silages had better nutritive value in different nutrients (sections). The citrus species pulp in sorghum had a significant influence on the nutritive values of both the pre-ensiled and silage. Citrus species pulp, inclusion levels, and level*species interaction affected all the micro minerals, *in vitro* DMD, and silage fermentation of sorghum silage.

In detail, regarding chemical composition and chemical estimates, the SG silage had acceptable DM and the ST silage had better CP and ADL. The inclusion level of 25% produced better chemical composition of the silages. Generally, except for K, Mg and Fe, all the silages had the mineral concentration level which was below the threshold. The macro- and micro-minerals were better at 15 and 10% of citrus species pulp inclusion. The SO and ST silages had superior IVDMD (greater than 50% DM) in comparison with the other silages. The 25% level produced better results on IVDMD of citrus species pulp in sorghum silages. The ST silage had a preferable total N and 10% produced a better nitrogen solubility index. All the silage pH, WSC and Y&M counts were optimized at an average of 23.2, 22.18 and 21.86% citrus pulps inclusion levels in sorghum silage. Although these raw materials produced good quality silage, there is a need for supplementation, due to lower CP and minerals (except for K, Mg and Fe) to improve the overall growth, metabolism and productivity of ruminants. There is a need to assess the aerobic stability across all the silages, furthermore, there is also a need to run metabolic trials to assess the feeding value of the current study silages.

The ensiling process, duration of ensiling, additives, microorganisms and pH could be some of the factors that could have caused the decrease in the mineral content in the silages.

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CHAPTER 5: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Tropical regions obtain low-quality forages or no forages in dry seasons (winter periods) thus affecting ruminant production and especially the communal farmers (Morales-Querol *et al*., 2019). There is an abundance of agro-industrial products that are underutilised while posing a threat to the environment (air, soil and water bodies) and living conditions of communities (Rana *et al*., 2024). Chapter 3 centred around the theme of assessing the quality and physical characteristics of raw citrus species pulps. Variations amongst the citrus species pulp on the chemical, chemical estimates and physical characteristics were observed. The obtained chemical composition (DM, CP and NDF) was similar to Ülger *et al*. (2020). The macro mineral ratios (P: Ca and Mg: K) are sufficient for ruminant nutrition as they are in the adequate range according to the NRC (2000) with micro minerals (Fe, Cu, Zn and Mn) not adequate for ruminant nutrition as they are below the stipulated values (NRC, 2000). The WR of the citrus species pulp were adequate, while PV was slightly higher than the required concentration as stated by Mokoboki *et al*. (2005). The reported DMD is adequate for ruminant nutrition and was supported by the findings of Mayouf and Arbouche (2014), the DE and ME are adequate, while ME was similar to that of Tayengwa *et al*. (2021) and Sharif *et al*. (2018). The lemon pulp had desirable chemical composition and mineral concentration as compared to the other species, while orange pulp performed superiorly in the CP and WR contents and tangerine pulp had the highest chemical estimate values. The DM, CP and micro minerals of the citrus species pulps need to be supplemented with high DM, CP and micro minerals sources for them to serve as the potential ruminant feed and for preservation of the silage. In order to maximize the production potential of livestock exposed to the pulps, the above-mentioned factors should be supplemented to meet the nutritional feeding requirements.

Ensiling is a process that has long been practiced and it is essential for farmers who experience feed fluctuations due to seasonal changes. Chapter 4 evaluated the effect of inclusion

levels of citrus species pulp in sorghum (*Sorghum bicolor* L. Moench) silage on the nutritional and fermentation profiles. The citrus species pulps in sorghum silages at several inclusion levels were analysed and diverse results were obtained for chemical composition, *in vitro* dry matter digestibility (IVDMD), nitrogen solubility and ensiling characteristics. Ulger *et al*. (2020) obtained results similar to those of our study for the DM trend, EE and CP. Partially, some of the NDF content of the silages at increasing inclusion levels were within the acceptable range. The DM and NDF decreased with the increasing inclusion of citrus species pulp in sorghum silages. Whilst ASH, EE and CP increased as the inclusion levels increased. Silage SG had acceptable DM, ST silage had high CP and SL silage had acceptable NDF. The P of all silage in the current study was lower than the recommended values by the NRC (1985) but similar to that reported by Morales-Querol *et al*. (2022). Magnesium and K had an acceptable range as stipulated by the NRC (2007) and Mg content was similar to that of Morales-Querol *et al*. (2022). Both Fe and Mn had acceptable ranges as stated by the NRC (2000) and Al as stated by the NRC (2007). There was a significant increase in P, Fe and Al content with increasing levels of various citrus species pulps. A decrease in K and Mg with increasing levels was observed. The highest P content was recorded in the SL silages, and the highest Mg and K content was obtained in SG. The Fe and Al were highest in the SO silages. The IVDMD increased with increasing incubation times and inclusion levels and these results were similar to what was reported by Valente *et al*. (2016) and Bao *et al*. (2022). The SO silage had superior IVDMD when compared to other species. The citrus species pulp-sorghum silages had low total N content, thus suggesting that they have low degradable protein content (>50 % solubility index) which can cause adverse effects in ruminants. High total N content was observed on the ST silage and was increasing with the increasing levels for all the citrus species pulp in the sorghum silage. The SG silage had acceptable pH across all inclusions with the other silages having acceptable range at higher inclusion levels. Ülger *et al*. (2020) and Souza *et al*. (2022) had similar pH results with the current study. All silages had a lower-than-acceptable value for the WSC. The low inclusion levels of citrus pulp species in sorghum silage in the present study

had a higher YM count than the increased inclusion levels. The silages had high YM count above acceptable ranges up to 20% inclusion level. Generally the pH and YM were decreased with increasing inclusion levels. The silage pH, WSC and YM count optimized at an average of 23.2, 22.18 and 21.86% citrus pulps inclusion level on sorghum silage.

From the above-stated results, the citrus species had differing effects on the parameters, with high inclusions having a higher impact on the parameters. It can be concluded that the protein source, P, Ca, Na, Mn, B, Zn and Cu, total N, soluble N and WSC should be improved to produce appropriate ranges for ruminant nutritional feeding. Or rather the inclusion levels should be increased with the application of inoculants to attain better results. It is recommended, from the present study, that the above-mentioned enhancements be applied in animal feeding trials or pilot projects. Future research studies, may address the limitation of the CP content by applying the protein sources or use different absorbents to improve the nutritional profile and to determine the true effect of the citrus species pulp, thereafter, run a metabolic trial to assess the feeding value.

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LIST OF APPENDICES

Appendix 1: Ethics Certificate

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Research Ethics Regulatory Committee
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ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC)** on 04/04/2024, the NWU Animal Production Sciences Research Ethics Committee hereby **approves** your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Nutritive value, in vitro dry matter degradability and ensiling characteristics of citrus pulp in sorghum silage

Study Leader/Supervisor (Principal Investigator)/Researcher: Prof H.K Mokoboki

Student: Ditabo AB

Ethics number:

N	W	U	-	0	0	8	0	4	-	2	4	-	A	5
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: **2024/05/05** **Risk Category:** 2
Expiry date: 2025/04/04
Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the NWU-AnimProdREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-AnimProdREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-AnimProdREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
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- *In the interest of ethical responsibility, the NWU-SCRE and NWU-AnimProdREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected;*
    - *it becomes apparent that any relevant information was withheld from the NWU-AnimProdREC or that information has been false or misrepresented;*
    - *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *NWU-AnimProdREC can be contacted for further information or any report templates via upenyu.marume@nwu.ac.za or 018 389 2725.*

The NWU-AnimProdREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the NWU-AnimProdREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely



Prof Upenyu Marume
Chairperson NWU Animal Production Sciences Research Ethics Committee

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Appendix 2: Turnitin report

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Language Editing Certificate

Date: 14th April 2025

This serves to confirm that I have read and edited the dissertation of **Ms** Atharlia Bontle **DITABO** (Student number: **33059020**) entitled:

Nutritive value, in vitro dry matter degradability and ensiling characteristics of citrus pulp in sorghum silage

The candidate corrected the language errors identified.

The document is of an acceptable scientific and linguistic standard.

Best regards.

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Language Editor
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