In vitro evaluation of nutritional content and anthelmintic values of *Kigelia africana* fruit to domesticated ruminants

By

Ndwandwe K.C (201142603)

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Faculty of Science and Agriculture

University of Zululand

Supervisor: Dr F.N Fon

Declaration by student and supervisor

I, Khanyisani Cyril Ndwandwe declare that, this dissertation I now submit for assessment is entirely my own work and has not been taken from the work of others except where I have appropriately acknowledged and referenced the original source. I have read and understood the University's policies and rules applicable to postgraduate research, and I certify that I have, to the best of my knowledge and belief, complied with their requirements. I further certify that this research is original, and that the material submitted for examination has not been submitted, either in whole or in part, for a degree at this or any other university.

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Acknowledgments and dedication

I would like to dedicate this dissertation to my late father Mr Musawenkosi Ndwandwe, my ever enthusiastic mother Thembile Zanele Cele and my two supportive brothers Khulekani Mabaso and Nhlakanipho Ndwandwe.

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Abstract

Food security is at risk due to the ever growing world population. More effort should be put on improving agricultural production since farming provides the primary source of food for humans. Livestock farming; be it commercial or small scale, plays a major role in supplementing the protein component of human food especially with the expensive nature of plant proteins. In northern KwaZulu-Natal, both large (cow) and small (goat and sheep) ruminant farming plays a vital role in the rural communities as they do not only provide proteins, but are used for traditional ceremonies, bride price, prestige and clothing. These ruminants require adequate and constant nutrient availability to meet their best production standards but forage availability and quality declines, especially in winter, which results in poor animal performance. Besides forage limitation, water scarcity is also a major problem in this area with the recent drought that hit in 2015. Therefore, alternative indigenous forages with feed potential and high moisture should be investigated, as water from feed can assist in improving animal production in this area. *Kigelia africana* plant has been reported to produce fruits (sausage fruit) that are suspected to have high moisture, be rich in secondary compounds and persist throughout the dry season but are not used by animals as feed. Rather, it has been used by humans as flavour, to ferment traditional beers, treat worms and even as an aphrodisiac. Hence, the aim of the study was to explore *Kigelia africana* fruit (sausage fruit) as a potential feed supplement and its anthelmintic value in domesticated ruminants.

Five feed portions were made from the sausage fruits; 1. Exocarp (Ex), 2. Endocarp (En), 3. Endocarp plus seeds (En+SS), 4. Seeds (SS) and 5. Whole fruit (Wf). The nutritional value of feed portions was determined by measuring their chemical composition and the force required to break-open the fruit (shear force). For chemical composition, dry matter (DM), moisture content (MC), crude protein (CP), condensed tannins (CT), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose and hemi-cellulose were measured. Fibre components were analysed using the ANKOM filter bag method while proteins were analysed using the Kjeldhal method. Acid butanol Assay and the Warner-Blatzer shear device were used to analyse condensed tannins. Shear force was measured using the Warner Blatzer shearing device where maximum force required the feed (F Max), distance covered

by the blade at breakage (dL at break), and force during break (F at break) were measured. For *in vitro* digestibility the Daisy incubator from ANKOM Technology was used with inocula from rumen fluid and 1 g sample, incubated at 38ºC in an anaerobic chamber for 72h. Apparent (APD) and True degradability (TD) and microbial yields (MY) were measured. For anthelmintic activity, the Helminth motility test was conducted with extracts of phenolic compounds from Ex, En, En+SS and SS at five levels (control 0g, 5g, 10g, 15g and 20g) using Baerman's technique and L3 larva survival was counted at 10x magnification.

The results revealed that En+SS (21%) had the lowest ($p<0.05$) DM, followed by En, Wf, Ex and SS (49.65%). This implies that En+SS (79%) showed the highest ($p<0.05$) MC, followed by En, Wf, Ex and SS (50.3%). In terms of fibre composition, SS and Ex had the highest cellulose content (32% and 42% respectively). Ex had the highest ($P<0.05$) NDF, ADF and ADL (70.67%, 59.18% and 17.69% respectively) compared to other feed portions. The highest protein content was observed in SS (12.55%), followed by Wf (3.9%) and En+SS (3.8%) while the lowest was seen in Ex (2.9%). There were higher contents of lignin and cellulose on the Ex than on the En, which was associated with plant mechanisms to protect themselves against herbivory. Ex required the highest FMax (1060.1N) to break the feed while En had the lowest shear force of 540N, which were all lower than the maximum force that can be generated by most ruminant's jaws. For digestibility studies, En and En+S had the highest ($p<0.05$) TD of 554.46 g/kg and 539.32 g/kg respectively while Wf, Ex and En were not statistically different but Wf was relatively higher than both. For its anthelmintic potential, it was found that *Kigelia africana* fruit treatments had an overall of 96% larva mortality percentage when compared to the control. There were no significant differences between L3 larva survival and mortality at different treatment levels of all *Kigelia africana* extracts but for the control. The result from the chemical composition of the different feed portions and whole fruit, shows that *Kigelia africana* has feed potential as its constituents are comparable to most types of hay especially with a crude protein of above 8%. Its high moisture content could be of potential benefit especially in winter where water sources are scarce. The force required to break the Ex was a major limitation to gain access to En and SS that were relatively easier to break. Hence the removal of the hard exocarp can reduce *Kigelia africana* fruit's shearing force and make it accessible for chewing. Both APD and TD showed

digestible values higher than most hays, hence, it has feed potential for ruminants. Strong anthelmintic properties were also demonstrated in all extracts with high mortality of larva. Further in vivo experimentation is required to establish the anthelminthic activity prior to rural farmer's application.

Key words: *Kigelia africana,* nutritional value, sausage tree fruit, shear force, anthelmintic potential and *in vitro* digestibility

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Glossary of abbreviations

ADF	Acid detergent fibre
ADFD	Acid detergent fibre digestibility
ADL	Acid detergent lignin
ADLD	Acid detergent lignin digestibility
APD	Apparent Digestibility
Cellu D	Cellulose digestibility
Cellu	Cellulose
CP	Crude protein
CT	Condensed tannins
dL	Change in length
DM	Dry matter
En	Endocarp
En+SS	Endocarp plus seeds
EPG	Egg per gram
Ex	Exocarp
F	Force
FMax	Maximum force
GINs	Gastro intestinal nematodes
Hemi- Cellu	Hemicellulose
Hemi D	Hemicellulose digestibility.
MC	Moisture content
MY	Microbial yield

N	Newtons
NDF	Neutral detergent fibre
RI	Rumen inoculum
SS	Seeds
TD	True digestibility
Wf	Whole fruit

Chapter 1

1.1 Introduction

Ruminants require constant nutrient intake throughout their life to maintain high production standards. In winter, forage availability and nutrient quality decline and ruminants suffer nutrient shortages particularly energy, water and proteins. This problem is severe among small scale farmers in rural communities where they depend on natural pastures for their animals. Purchasing of supplementary feed is not really an option for local farmers as it is often very expensive. Hence, there is need for alternative indigenous forages that are cheaper, available and have high nutritional value. Crop residues have often been the main sources of indigenous supplements especially in rural communities. In winter or dry season, feed shortages are not the only problem being faced by rural or commercial farmers as water shortages have also been a major problem of late especially in South Africa (Molefi, 2017). Therefore, forages with potential to provide both nutrients and high feed water can be of great importance in livestock production. *Kigelia africana* fruit also known as the sausage fruit seem to contain enough moisture that can be exploited by domesticated ruminants throughout the dry season but have not been seen eaten by these animals except for a few from the wild. However, no specific reason has been associated with them not being eaten but for the observation that the seed coat (exocap) is very hard (high shear force required to break the fruit) and hardly fall off if not harvested or pushed down by heavy wind. Azu et al. (2010b) reported that *Kigelia africana* fruit pulp and extract has been exploited in a variety of ways; traditionally, dietary, nutraceutically and pharmaceutically. Apparently monkeys, baboons, bush pigs and porcupines are the only animals reported to utilize this fruit as feed (Lim, 2012). Its hard shell appears to be a rationale towards its limited consumption by ruminants. Exploring various ways by which ruminants can exploit sausage tree fruit as a feed can greatly subsidise the cost of running livestock production systems. *Kigelia africana* fruit has been reported to be relatively poisonous when fed immature. Azu et al. (2010b) reported that some animals (e.g. Baboons, porcupines and wild pigs) administered with 6400mg/kg *Kigelia africana* fruit extract suffered with jerks and writhes. However, humans still use it to flavour and ferment traditional beers. It seeds are roasted and mixed with beer and used as an aphrodisiac (to enlarge male sexual

organs). Its fine endosperm appears to be rich in carbohydrates, which are a huge source of energy for ruminants. This evidence can possibly promote the utilization of this fruit to feed ruminants (Chivandi *et al*., 2011).

The extensive use of this fruit as an antibiotic by locals in Southern Africa or worm medicine for humans is a major reason to think that this fruit possesses anthelmintic potential (Ndhlala, 2017; Singh, 2017). This comes at a time when the world has been fighting anthelmintic resistance where almost all the drugs on the market are facing some sort of resistance. This has forced some of the chemicals used in livestock drug production to be banned in Europe in 2006 since they posed a major risk of chemical residues in food and antibiotic resistance can potentially be passed on to consumer's pathogens. Therefore, there is an urge to explore plants, plants extracts and natural plant compounds, which can enhance livestock treatment and productivity. The utilization of natural plant products as a new substitute for pharmaceutical entities or bioactive compounds may not only control human disease but can also enhance ruminant productivity and health for food safety and quality while still conserving the environment (Makkar *et al*., 2009).

1.2 Problem Statement

Grazing ruminants require high and an unceasing nutrient intake to achieve maximum production, maintenance and reproduction throughout the year (Freer and Dove, 2002). However, harsh environmental conditions during winter reduce forages available and nutrient quality for ruminants, which also reduces nutrient intake for ruminants. Therefore, there is need for alternative forages that are cheaper and available for supplementation especially to the rural poor farmers. In terms of quality, protein and carbohydrates are the major nutrients required to maintain high ruminant production (Saha et al., 2010). Water sources are also said to be limited during winter and feed with potential high moisture can play a major role in improving production. Therefore, indigenous forages with potential to provide both nutrients and high moisture can be advantageous. There is also a major milestone in animal production where anthelmintic drugs are losing their effectiveness due to merging nematode resistant strains (George, 2017; Raza, 2018; Sangster, 2018; Wang, 2018). Beside nematode resistance problems, a health concern has been reported against possible presence of anthelmintic drug residues on animal products which are consumed by

humans. Canny (1973) stated that nearly all fruits that grow up to large size like *Kigelia africana* fruit are found to be a major source of carbohydrates while Gabriel and Olubunmi (2009) also stated that *Kigelia africana* fruit contains secondary metabolites (Ashishie, 2018; Spiegler, 2018) which are believed to have anthelmintic potential as demonstrated by many different scholars (George, 2017; Raza, 2018; Sangster, 2018). However, this fruit has not been utilized to feed ruminants. Therefore, this study will evaluate the potential of the fruit as feed especially during winter as well as investigating its anthelmintic properties.

1.3 Main objective

To assess the nutritional content, moisture content, anthelmintic properties, digestibility and the shear force of different *Kigelia africana* fruit portions.

1.3.1 Specific objectives

1.3.1.1 To evaluate the chemical composition and shear force required by ruminants to consume different portions of *Kigelia africana* fruit.

1.3.1.2 To investigate the *in vitro* digestibility and anthelmintic potential of *Kigelia africana* fruit.

1.4 Hypothesis

1.4.1 It is hypothesized that *Kigelia africana* portions will vary in chemical composition and shear force.

The storage tissues and crows of the leaves provide the main sources of nutrients and dry material during *Kigelia africana* fruit formation. This fruit is the major source of carbohydrates, which is required by ruminants for reproduction, maintenance and production. (Canny, 1973). Jung and Vogel (1986) reported that lignification is always proceeded by deposition of carbohydrates; this implies that lignin concentration is usually higher in the primary than the secondary wall. Therefore, it is right to suggest that the different portions of the fruit Viz. Exocarp (Ex), Endocarp (En), Endocarp plus seeds (En+SS), seeds (SS) and whole fruit (Wf) will have different chemical composition and strength because of fibre distribution hence will require different shear force to break the fruit.

1.4.2 It was hypothesized that *Kigelia africana* fruit portions will vary in their digestibility as well as their anthelmitic potential

Kigelia africana fruit contain high lignin content on its cell wall and high lignin content may negatively affect fibre digestibility (Lebo et al., 2001). Semwal et al. (2014) reported that *Kigelia africana* fruit possess numerous secondary compounds, which provide ethno-medicinal and antibacterial properties in humans hence can potentially have the same effect if applied in animals.

1.5 Intended body of knowledge

It is to provide a possible cheaper source of energy, water and possible protein supplement to ruminants especially in the long, dry winters when ruminants experience nutrient deficiencies. It will also provide a safe plant-based anthelmintic which poses no threat to the consumer's health status and environment. *Kigelia africana* fruit persist through winter and can withstand water scarce environments where there is inadequate forage availability for ruminants, thereby providing a nutrient source during dry periods, which form the greatest constrain in livestock production in the country. Consumption of this fruit by ruminants may improve ruminant production by eradicating internal parasites (helminths and nematodes), which pose a huge threat in livestock production in South Africa.

Chapter 2

2. Literature review

2.1 *Kigelia africana* plant

Kigelia africana (Lam Benth) is a tropical African plant extensively grown and distributed throughout central, south, and West Africa. It belongs to the family *Bignoniaceae* and it is generally called the sausage tree because of the shape of its huge fruits (Azu *et al*., 2010b; Chivandi *et al*., 2011). Umfongothi (Sausage tree in isiZulu) is mainly found along streams, river banks, open woodlands and on high rainfall savannah where it occurs on loamy clay red soils (Gabriel and Olubunmi, 2009).This smooth grey barked plant can grow up to 20m long with a yellowish pale-brown stem that is susceptible to cracking. The bark can be 6mm thick on a 15cm branch and on older trees barks peel off. The tree is deciduous in areas with long dry seasons but evergreen where rainfall occurs throughout the year. It produces 30 to 50cm long pinnate leaves, which are either in whorls of three or opposite. Leaflets are oval shaped, about 20cm long and 6cm broad. The bark and root extracts of this tree contain phytochemicals (dihydrisocoumarin, kigelin, lapachol and naphthoquinone), which explains the extensive usage for medicinal purposes (Chivandi et al., 2011; Ndhlala, 2017; Singh, 2017). *Kigelia africana* tree yields orange to reddish or purplish green flowers, which are bell shaped and often found on panicles. Flowers do not hang down but are horizontally oriented with strong supporting stems for birds to successfully land on which are vital for pollination. *Kigelia africana* solely relies on bats for pollination because the flower scent is prominent mainly at night thus bats come for pollen and nectar. Its flowers are very large (up to 11cm long) with a curved tip. The male and female parts are found in one flower (Gabriel and Olubunmi, 2009).

This tree produces large cylindrical fruits (5 to 10kg in weight and 30 to 80cm in length), which hang down on a long rope like peduncles. This woody indehiscent fruit, which is grey-brown in colour and heavily marked with lenticels contains hundreds of ovoid seeds with leathery testa which are embedded in the fibrous pulp of the fruit. In Malawi, the seeds are roasted for human consumption (Semwal *et al*., 2014). Kokwaro (1976) cited by Azu *et al*. (2010b) reported that roasted *Kigelia africana* seeds are mixed with beer to serve as an aphrodisiac (enlarges sexual organs). The antibacterial fatty acids

isolated from *Kigelia africana* fruit and the cytotoxic gamma-sitosterol from the root bark gives the trees potential in managing bacterial infections (Chivandi *et al*., 2011).

2.1.1 Development of *Kigelia africana* fruit

Once pollen has been brushed onto the open stigma, within a few seconds the lobes straighten because pollens are very susceptible to drying, thus lobes close after straightening creating a flat, receptive surface. The following day, the corolla of a fertilized flower is shed leaving an obvious stigma rising from the copular calyx. At this time the stigma lobes remain closed. Inside the calyx cup the superior ovary then grows as a small stretching peg-like structure and appears outside the cup. It then matures to form a thin cylindrical structure of 2 to 3cm in diameter and between 10 and 15cm long, thereafter it thickens to form a characteristic sausage shape and continues to grow for weeks (Canny, 1973; Lim, 2012).

2.2 Moisture content of the fruit

Forage scarcity in the dry seasons and during droughts is the main constraint of ruminant production systems in shrub lands and savannah areas of South Africa. Production losses are mainly due to reduced energy, protein and water availability. Reduction in ruminant's water availability results in reduction of dry matter intake, which also affects productivity of the animal (Minson, 2012; Defar, 2017). Ruminant bodies obtain water through drinking water, metabolic water and water from feed. There are plant tissues with high moisture content that can possibly subsidize water intake during the dry periods (King, 1983; Legese, 2017). High moisture character of *Kigelia africana* fruit appears to have been influenced by the habitat where these plant species occur. The sausage tree is found mainly in wetlands, across river banks and in open woodlands. This plant remains evergreen throughout the year in high rainfall areas and is deciduous in areas with a prolonged dry season (Gabriel and Olubunmi, 2009). *Kigelia africana* fruit is available throughout the dry season in the tropics and sub tropics. Apparently with its high moisture content, it can aid in improving feed and water availability to ruminants in dry seasons where feed and water are scarce (Dada *et al*., 2010).

2.2.1 Dry matter

The dry matter (DM) of a feed characterizes everything contained in the feed (protein, fats, carbohydrates, vitamins and minerals) except water. Dry matter is basically the percentage form of total weight of feed minus weight of water in the feed. Air drying and oven drying are generally used to determine dry matter of a particular feed. In fermented materials like silage, the dry matter determination is tricked by the presence of volatile fatty acids. Volatile fatty acids introduce a variable amount of error since some of the acids are lost during drying by evaporation. However, analysis of forage without ensiling provides accurate results (Saha *et al*., 2010). The levels of nutrients in a feed sample based on dry matter are referred to as dry matter basis. Different forages contain different water contents, thus removing water content of the forage eliminates the dilution effects of forage water content. Dry matter basis allows for a reasonable and direct comparison of nutritional contents across different forages. Dry matter intake is one of the most useful terms in animal nutrition as it denotes the amount of dry matter consumed by an animal. Dry matter intake increases as digestibility increases, though the presence of anti-nutritional factors like condensed tannins and alkaloids may reduce intake. High percentages of neutral detergent fibre also reduce feed intake (Uttam *et al*., 2010).

Dry matter comparison among feedstuff is important because it reveals the actual amounts of various nutrients available to the animal consuming the feed. However, “As fed” signifies the raw forage as it is fed to the animal including the moisture content. While as fed is a precise presentation of the feed being offered, it does not provide a precise indication of the nutrient composition of the non-water feed components, particularly when the moisture content of the forage is high (Misra and Singh, 2002).

2.2.2 Shearing force

All ruminants possess similar dental arrangement consisting of a thick dental layer of connective tissue also called a dental pad, which is situated on the upper jaw and four paired incisors on the lower jaw. Cattle have been reported to use its tough tongue against incisors to grasp feed particles. It is because of this procedure that cattle can grind thick and solid guava fruits. Although some ruminants grind feed using incisors and dental pad, there is not much variation in bite force between small and large ruminants. If bite force had to correspond with body mass then small bodied animals

would have had to find softer and more fragile forages for consumption. Strategically, sheep reduce the force required to fracture forages by making a fold on the lamina while pulling the lamina at an angle across the incisors. Cattle and other ruminants insert incisors and move their mouths sideways to thoroughly fracture forages (Griffiths and Gordon, 2003). The shearing force or cutting force seems to be affected by the feed moisture content as demonstrated by studies of Zhou *et al*. (2012) who further reported that a greater shearing force is witnessed with decreased moisture content in rice stems. Therefore, the lower the moisture content of a rice stem the higher the shearing force.

2.2.3 Physical composites of feeds that determine shearing force

The physical characteristic of forages also influences ruminant's ability to utilize it as feed. Fresh forages which require lower grinding energy are frequently exploited for feed, especially when it is free of toxins and other deleterious factors. Evaluation of the physical properties of feed is essential because chemical composition alone cannot precisely determine forage intake mechanism by ruminants (Griffiths and Gordon, 2003).

There are five plant based terms that are often used in determining foraging behaviour as discussed by Griffiths and Gordon (2003);

a) Fracture force in tension, it is a measure of the herbage strength which is assessed from the maximum force required to fracture a plant organ.
b) Maximum force in shear, which is also a measure of herbage strength required to crack the plant organ, and is determined from the highest peak on the force displacement curve.
c) Tensile strength is the crack force in tension per unit cross-sectional of the plant specimen.
d) Toughness is the energy required to shear the tested sample per unit cross-sectional area of the plant specimen.
e) Forage resistance is the accumulated force required for cutting/ chewing all plant organs incorporated within a bite.

Griffiths and Gordon (2003) further discussed two animal based factors that define the mechanics of foraging behaviour which are;

a) Bite force, which denotes three-dimensional forces exerted to sever a bite (This force does not denotes the force applied during chewing).
b) Biting effort is the power exerted by an animal in severing a bite and it includes components of resistance, bite force and head acceleration.

2.2.4 Chemical composites that determine shearing force

Lignin is the second most abundant compound following cellulose (Moore and Jung, 2001). It is somehow, referred to as a structural compound anti-nutritional factor due to its negative effect on forage nutritional availability. Lignin gives strength and rigidity to plant cell walls (Knudsen, 1997). Lignin also prevents water losses thus retaining plant moisture by reducing cell wall permeability. All these functions are necessary for successful plant growth but at the same time they limit foraging digestibility. Lignin prevents physical and biochemical damage of the walls through cementing and anchoring the cellulose micro fibrils and other matrix polysaccharides hence stiffening the walls (Moore and Jung, 2001). Cellulose and hemicellulose also affect the shearing force e.g. they play a major role in maize stem strength. Shearing force increases with increasing cellulose and hemicellulose contents (Zhou *et al*., 2012). This implies that it increases with plant maturity.

2.3 Chemical analysis of *Kigelia africana* fruit

2.3.1 Nutritional value

Human beings around the globe depend on agriculture as a main source of food, where animals provide the cheapest source of proteins as plant protein sources are often expensive. About 73% increase in meat and milk production will be required to feed the continuously growing world's population in 2050 (Godfray *et al*., 2010). South Africa is one of the developing countries with the highest growing human population, which is a serious problem in terms of food security for this growing population. To solve this problem, more attention should be focused on increasing feed resources to boost livestock production systems (IFAD, 2011). Therefore, it is imperative that more plant materials should be explored and utilised as nutrient sources for ruminants that are a major role player in protein supplies. In humid and sub-humid tropics, trees subsidize nutrient recycling and improve soil fertility, hence, more nutrients are available for forage growth. *Kigelia africana* is a deciduous tree that sheds its leaves

mainly in autumn contributing to soil mulching which also increases soil fertility. Its leaves are assumed to contain significant amounts of protein, phosphorus and lipids. This plant produces large fruits, which are yet to be exploited by ruminants as a source of feed. There is limited information on sausage tree fruit consumption by most domesticated livestock animals except that in West Africa, monkeys, baboons, wild pigs and porcupine have been reported to utilize this fruit for feed (Lim, 2012). Seeds from ripe fruits are also assumed to be energy-rich as they are roasted in warm ash and consumed by humans. In several parts of sub-Saharan Africa, sausage tree leaves are consumed by pregnant women to improve quality and volume of breast milk (Chivandi et al., 2011; Lim, 2012; Arkhipov et al., 2014). These seeds are potentially rich in some essential fatty acids like linolenic and linoleic acids (Semwal *et al*., 2014). Since ruminants cannot synthesize these fatty acids, it might be worth including the fruit in their diet. These fatty acids are responsible for the synthesis of specialised omega-3-fatty acids and omega-6-fatty acids (Jackson and Beckett, 2012), which are important for managing and preventing heart diseases (Dong-Soon, 2016).

2.3.2 Digestibility

Digestibility refers to the degree at which a particular ingested feed is absorbed as it passes through the gastro-intestinal tract. Digestibility varies depending on type of animal or type of feed ingested. Dry matter digestibility is the percentage of dry matter in a feed that is digested by animals at a certain level of feed intake. On the other hand, digestible energy refers to the amount of energy in the feed that can be available to be utilized by an animal. Digestible energy is the difference between gross energy and faecal energy. Digestible energy is considered suitable for poultry feed quality because poultry animals do not urinate thus they have sole energy loss through faeces (Uttam *et al*., 2010). In ruminants fibrous feeds are digested in the rumen through the process of fermentation. This process involves rumen microbes (Bacteria and protozoa) which aid in cellulose digestion. During fermentation carbon dioxide and methane are released, therefore one can measure the microbial activity (fibre digestion) by measuring the gas production. Gas production values will give an estimation of the microbial activity in digesting a particular feed (Moore and Jung, 2001).

2.3.2.1 Factors affecting forage digestibility

Roughages are easily digested by ruminants through the fermentation process occurring in the rumen but not by monogastric animals which lack the microbes. This process involves rumen microbes (bacteria, protozoa and fungi) breaking down fibre components of feed. Non-ruminants can utilize concentrates easily as they have relatively low fibre content (Griffith, 2017). Very young and very old animals are inefficient in digestion. Very young ruminants might have an underdeveloped gastro-intestinal tract which leads to poor utilisation of roughages while old animals might have poor feeds and declined health status which reduces the ability of these animals to efficiently digest forages. Heavy working animals have a poor feed conversion ratio while light working animals perform better because they spend enough time for proper digestion. Animals fed high quantities of feed tend to have poor digestion as feed passes through the gastro-intestinal tract very fast (Herron, 2015; Griffith, 2017). Apart from differences in animal physiology that affects digestibility, plant based factors are also very important.

Plant based factors involve age of a plant where old plants tend to have high lignin content (Lebo et al., 2001). Lignin content negatively affects digestibility of forages. Early cut fodder has relatively high protein and mineral concentration with low fibre content which makes it easily digestible. Feed preparation has been done to improve digestibility of feed stuffs, which often decreases the particles size to increase surface area for digestion. This includes grinding, soaking, smocking, boiling, steaming and pelleting (Uttam et al., 2010; Owens and Basalan, 2016). The digestion of forages involves complex enzyme activities that digest feed into soluble forms that are absorbed by the animal body. Protein requires enzyme peptidase and pepsin to break large protein molecules into soluble amino acids. Lipids require bile juice and enzyme lipase to digest large lipid molecules to fatty acids and glycerol molecules (Wilkinson, 2015). Fibre digestion requires rumen microorganisms to digest cellulose and starch. These organisms will differ depending on the type of feed eaten by an animal. Feed rich in cellulose will result in cellulose digesting micro-organisms while starch digesters will be abundant when an animal's diet is compost of starch. Cellulose digesters possess an enzyme called cellulase while starch digesters have enzyme amylase. The activity of these enzymes results in production of three volatile fatty acids: acetic acid, Butyric acid and propionic acid, which are absorbed and metabolised by the animal to

yield energy. Furthermore, hydrogen, methane and carbon dioxide are also produced as rumen gases during fermentation. Measuring the volume of rumen gases can provide an estimate of the enzyme activity which is equivalent to digestibility. The more the gases are produced the higher the enzyme activity in fibre digestion (Minson, 2012; Owens and Basalan, 2016).

2.3.3 Condensed tannins

Condensed tannins are groups of large water-soluble phenolic compounds consisting of polymers or oligomers or monomeric units of flavon-3-ols. They are produced by numerous plants in different plant tissues and their production also varies with plant resource availability and environment. They are categorised into condensed and hydrolysable tannins depending on their structural compounds (Min *et al*., 2003). Only condensed tannins are of interest in animal nutrition since hydrolysable tannins are easily hydrolysed with acids, alkali, enzymes and even in hot water. Only a small amount of condensed tannins has been reported to be absorbed by ruminants while the rest has been reported to bind on macro-molecules like proteins, starch and fibre thereby creating soluble and insoluble complexes. The molecular mass and molecular configuration determines its affinity to proteins. High condensed tannin concentrations (>7% DM) in ruminant forages has been reported to inhibit growth and reduce feed intake as well as interfering with the morphology and proteolytic activities of microbes. However, consumption of smaller amounts of condensed tannins (<6% DM) has been reported to improve ruminant production (growth rate, milk and wool production). This is due to the formation of condensed tannins-to-protein complexes, which resist degradation of proteins in the rumen thereby increasing protein absorption in the lower digestive tract (Hoste *et al*., 2006). Also, the binding to gastrointestinal tracts nematodes is said to destroy these worms hence decreasing parasitism and improving animal production.

2.3.4 Carbohydrates

Carbohydrates are the end products of photosynthesis for every plant that possesses chlorophyll in its leaves. Throughout the season carbohydrates are transported from the leaves (where they are manufactured) to other plant tissues like flowers and fruits where they are utilised (Wardlaw, 1968). *Kigelia africana* fruit is believed to be a potential source of energy for domesticated ruminants. Shortages of feed resources

caused by seasonal and environmental changes like reduced rainfall and temperature during winter is a major problem to many small holding farmers. Therefore, alternative sources of carbohydrates will be very important for supplementation. When carbohydrate expenditure exceeds its production, leaves are shed and the plant recovers when favourable environmental conditions returns. Starch is the storage form of soluble carbohydrates in all woody plants. Whenever high sugar levels build up starch accumulates in plant tissues, when plants suffer low sugar levels starch is then transformed into sugars. Stored carbohydrates initiate a number of mechanisms in woody plants. These include development of cold hardiness, protection against herbivores, respiration, growth and mortality inhibition. Drastic decrease in carbohydrate reserves are observed during the development of new shoots which emphasises the importance of soluble carbohydrates in plant development (Latt *et al*., 2001). Therefore, a proper understanding of carbohydrate distribution and variation during plant maturity or fruit maturity will give more insight into when or which part of a plant to harvest to feed animals.

2.3.5 Energy

Sun is the ultimate source of all energies for the earth's biological processes. Generally, radiant energy from the sun is converted to food energy by green leafy plants that have chlorophyll during the process of photosynthesis. Sunbeams striking the leaf blades may be absorbed by leaf pigments, transmitted through the leaf or reflected. Physical and chemical features of leaves together with radiation wavelength determines absorption, transmission and reflection mechanisms on leaf blades (Ting, 1982). Ruminants then obtain that energy by feeding on plant's carbohydrate, oils, and vitamin reserves which are found in various tissues of the plant. That energy is further passed on from animals to scavengers through decomposition (Messel, 2014). Carbohydrates are the principal energy source for both plants and animals. Starch is the storage form of energy in plants while in animals it is stored as glycogen (Ting, 1982). Ruminants acquire over half of the energy they need for maintenance, production and growth from dietary carbohydrates. The principal source of energy is glucose which is also a precursor for many other oligosaccharides such as lactose (synthesized in mammary glands). Carbohydrates in leaves are mostly cellulose, which can only be digested by rumen microbes to yield volatile fatty acids (VFA) in

ruminants (Nafikov and Beitz, 2007). There are three VFA produced in the rumen a) acetic acid which is oxidized in the liver to produce ATP, b) propionic acid which is responsible for gluconeogenesis in the liver (Glucose synthesis) since there are no glucose molecules reaching small intestines for absorption and c) butyric acid which is released by the rumen as ketone beta-hydroxybutyric acid and can be oxidised for energy in numerous body tissues (Bowen, 2009).

2.3.6 Protein

In plants, protein functions as a structural component of membranes and is responsible for the storage of nitrogen and carbon (glycoproteins). They also function as enzymes where they regulate cellular reactions. *Kigelia africana* fruit's total nitrogen content may be high enough to meet the protein requirements of animal feed but needs proper investigation to confirm. The presence of condensed tannins in these fruits can bind with proteins, when consumed in the rumen, to form tannin-protein complexes which can escape rumen digestion into the abomasum where they can be displaced into free proteins or amino acids. These proteins are absorbed in the small intestines and are used by the animal body for production, reproduction and body maintenance (Clements, 1940). In animals, proteins function as hormones (insulin) while some function in movement as contractile proteins (muscles) (Ting, 1982). Protein supply for human consumption consists of about 70% plant proteins and 30% animal protein (Federoff, 2015). Animal proteins are more similar to human protein hence they are readily and rapidly utilised by human bodies. Plant proteins are structurally unique and different from human proteins therefore plant proteins usually lack at least one amino acid to be fully utilized by the human body. Plant proteins also lack cysteine: methionine balance, which is a key amino acid to optimize growth and excellent health status of the animals. This implies that feeding plant protein to ruminants is a way of structurally simplifying protein to be easily and readily consumed and utilized by animals (Massey, 2003). Among ingested proteins, there are degradable proteins which are degraded in the rumen and bypass proteins which escape the rumen and are absorbed in the abomasum or small intestines. Bypass proteins can be used by ruminants for the synthesis of new body tissues although 70% of ruminant proteins come from microbial proteins (Mahgoub *et al*., 2012).

Fruit seeds are known to be rich in storage proteins where they store nitrogen and carbon, therefore *Kigelia africana* fruit might be an excellent source of storage proteins for ruminants. Where they occur in roots or tubers, they function as nitrogen and carbon sources during seed germination or tuber regrowth and as a source of amino acids during protein synthesis (Ting, 1982). Although structural proteins have other functions, they specifically provide structure to the plant cell wall mainly as a component of membranes. Almost all structural proteins just like ordinary proteins function as enzymes. Some structural proteins function to transport substances in and out of the cell. Some are involved in the electron transport chain (Ting, 1982). However, these plant proteins are very important for farm animals as they provide essential amino acids which cannot be synthesized by the animal. Therefore, research on the type of amino acids present in the sausage fruit can be advantageous to farm animal feed formulation.

2.3.7 Fibre

Kigelia africana fruit pulp is fibrous and is assumed to be rich in fibre which is the main source of energy for ruminants. Ruminant's diet is mainly composed of fibre since one of its four stomach compartments contains microbes that can digest fibre. Therefore the sausage fruit can be very good forage for ruminants if the fibre composition is analysed and established for advice on feeding. However, lignin seems to reduce fibre degradation in the rumen (Moore and Jung, 2001). Ruminant and non-ruminant nutritionists define fibre as a plant-based polymeric compound that is not digested by the action of mammalian enzymes (Mahgoub *et al*., 2012). Fibre is found as structural components of cell walls of any plant materials. It is composed of soluble fibre (pectans, β-glucans, Galactans and fructans) and insoluble components like cellulose, hemicellulose and lignin. These cell wall polysaccharides are reduce ruminant enzyme digestion but are successfully fermented by rumen microbes. Among these rumen microbes there are fibre digesters which tolerate high pH environments and starch digesters which tolerate low pH environments. Therefore, feeding high cereal grain diets may hinder fibre digestibility since it lowers the rumen pH. For efficient fibre digestibility, grains or concentrates can be added slowly to kickstart the digestive processes without inactivating fibrolytic microbes (Gressley *et al*., 2011). Ruminants also secrete saliva during ingestion that function as a buffer and prevent extreme

rumen pH changes (Galyean and Goetsch, 1993). Insoluble fibre includes neutral detergent fibre, which represents the plant cell wall. Another unit that represent less digestible fibre is the acid detergent fibre that yields hemicellulose when subjected to acid detergent solution. Generally, fibre component of the feed is also referred to as plant cell wall. Microbes ferment these plant cell walls for nutrient purposes. During fermentation of plant cell walls volatile fatty acids are produced and metabolised to yield energy (Mahgoub *et al*., 2012).

2.4 Anthelmintic potential of plants

In Zululand most ruminants are reared extensively and rely on natural pastures for grazing where the climate favours the development of gastro-intestinal parasites (GIPs). Common parasites found in this area include; *Haemonchus contortus*, *Oesophagostomum columbianum and Cooperia sp*. These GIPs affects nutritional management due to incompetent feed utilisation in ruminants hence may result in huge economic losses especially in rural community farms (Cenci *et al*., 2007). In the study conducted by Van Wky *et al*. (1999), they observed a higher degree of helminths resistance across pastures in KwaZulu-Natal. Anthelmintics like benzimidazole, levamisole, ivermectin and rafoxanide were losing effectiveness towards *Haemonchus contortus* strains in small ruminants. Hence, any new anthelmintic drug or plant that can improve this resistance (which poses a serious risk to ruminant production) might be very important.

2.4.1 Anthelmintic resistance

Anthelmintic resistance against gastro-intestinal nematodes (GINs) has been reported as a worldwide problem in livestock production systems (Coles et al., 2006; Charlier et al., 2014; Geurden et al., 2014). A higher number of nematodes are now showing multiple resistance throughout the world including South Africa and America (Jackson and Coop, 2000). Anthelmintic resistance occurs when there is a higher frequency of individuals in a population which are capable of tolerating doses of certain anthelmintic drugs than in a normal population of the same species. This resistance is heritable (Coles *et al*., 2006), which is dangerous in production systems. Anthelmintic resistance can be caused by either pre-adaptive phenomenon or through relative mutation. Relative mutation occurrences are not common while pre-adaptive phenomenon

seems to be the case for many helminths where the occurrence of resistant populations appears to be a predictable consequence of intensive chemotherapy. In the absence of alternative methods to control helminths, high levels of resistance involving highly pathogenic nematodes like the *Haemonchus contortus* species may make it impossible to sustain sheep farming (Jackson and Coop, 2000). It is possible that the gene or genes that carry resistance already exist within a phenotypic range of a particular species. These genes are present in small proportions even before the worms are exposed to a drug for the first time. Under this condition the introduction and continuous use of an anthelmintic drug develops some survival benefit to individuals carrying a resistance gene or genes. The proportion at which the selection process occurs is influenced by numerous factors. These factors are;

- Parasite strain – some parasites are resistant to numerous anthelmintic drugs hence control measures for such parasites are very limited.
- Host management – poor host management results to reduced animal body response towards parasites.
- Host type – different animals respond differently towards parasites due to dissimilarities in their immunity.

These factors are also influenced by environmental conditions where environments are unfavourable for host adaptation; this will definitely favours parasite development. (Jackson and Coop, 2000).

2.4.2 Anthelmintics or vermifuges replacement

Waller (1997) reported a survey on approximately 60 farms in South Africa which showed that 90% of the farms had parasite strains resistant to compounds from at least one anthelmintic group. These results were further confirmed by Van Wyk *et al*. (1999). Some farmers in South Africa have abandoned sheep farming due to chemotherapeutic failure to control worms. More research is being carried out on developing new drugs (anthelmintic) to counter the many challenges posed by resistant strains of microorganisms. Antibiotic vermifuges are occasionally associated with opposing effects on hosts which includes depletion of beneficial gut, hypersensitivity, mucosal microorganism, immune suppression and allergic reactions. However, some indigenous plants have shown anti-parasite activity as an alternative anthelmintic drug. This alternative anthelmintic appears to be advantageous to both

the animal and farmers controlling GINs since it is harmless and environmental friendly (Jeyachandran and Mahesh, 2007). *Kigelia africana* may be a good replacement for "costly" vermifuges in controlling GIPs because of its relatively high phenolic compounds. The presence of natural chemicals in ruminant forages that can control GIP may subsidize the use of synthetic anthelminthics hence reducing the production costs (Kimenju *et al*., 2013). Plants with anthelmintic properties have also been included in the British pharmacopoeia e.g, oil from chenopodium that originates from chenopodium ambrosioides has been used in numerous occasions to control nematode parasite infections in mono-gastric animals in the United Kingdom (Githiori *et al*., 2006). The administration of extracts from Acacia and Artemisia spp. in animals infected with blood parasites (such as Trypanosoma and Plasmodium spp.) has resulted in a reduction in the number of blood parasites. Tobacco leaves have also been used to control ectoparasites (Okpekon et al., 2004; Githiori et al., 2006). Therefore, there is a need to investigate the anthelminthic properties of *Kigelia Africana.*

2.4.3 Helminth species

There are many parasites affecting ruminants, some to a greater extent and others minimal. The main interest of this study is on those parasites with a major impact on ruminant production. *Haemonchus contortus* commonly known as barber pole worm is the major parasite affecting ruminants in the Zululand area. *Haemonchus contortus* is a cylindrically shaped worm, narrowing at both ends, and has a complete digestive system. This worm is a worldwide threat, but is more predominantly found in sub-temperate and temperate regions under warm and wet conditions (Leite-Browning, 2006). There are several ways to determine whether the levels of *H. contortus* are high or low in an animal. Classification based on eggs per gram (EPG) of faeces is one of the simplest methods where 1-1500 EPG is regarded as low while 500-1500 is moderate and greater than 1500 is considered as high or heavy hence requiring treatment. At levels of moderate to low faecal egg counts, plant ethnomedicinal properties has shown a higher efficiency on treatment compared to higher parasite infestation levels (Githiori *et al*., 2006). Barper pole infestation signs include low packed cell volume (PCV), dehydration, diarrhoea, dark coloured faeces, wool break and anaemia while weight losses and weaknesses are observed with chronic cases

of barper pole worm (Leite-Browning, 2006; Scheuerle, 2009). Infested ruminants suffer lower growth rates, reduced productive performance and increased rate of illnesses, which result in subsequent deaths. There are very few anthelmintics ratified by food and drug agencies against barber pole worm. Therefore plant based anthelmintics can play a vital role in the replacement of anthelmintic drugs like levamisole and ivermectine (Leite-Browning, 2006).

2.4.4 Plants' secondary compounds

There is a group of diverse plant phenolics derived from condensation of acetate unites and those modified from aromatic amino acids. Young nutrient-stressed plants (low carbon) revealed very low secondary compound concentration compared to older nutrient rich plants (Bennett and Wallsgrove, 1994). The plant family *Bignoniaceae*, has been noted for their high concentrations of secondary metabolites (iridoids, naphthoquinones, flavonoids, terpenes, tannins, steroids, saponins and caffeic acid) in fruits, stems, leaves and roots (Azu *et al*., 2010a). These secondary metabolites may present these plants with excellent anthelmintic potential. In some cases the type of compounds a particular plant can present are estimated by studying its taxonomic belonging, hence *Bignoniaceae* family can be studied to find proximate compounds present in *Kigelia africana* plant (Priya *et al*., 2013).

Plenty of research has been done on evaluating the effect of forage quality on gastro-intestinal parasites (Coop and Holmes, 1996; Coop and Kyriazakis, 2001; Field et al., 2002; Holmes and Coop, 2018) However, there is no clear picture whether improved physiological response against parasites is due to improved nutrition or presence of secondary metabolites. Numerous studies display strong anthelmintic potential from secondary metabolites compared to improved nutrition. It has been suggested that improved nutritional content, in particular protein, will improve immune response of the host towards parasites (Githiori *et al*., 2006). Plants that possess secondary metabolites with a great range of biochemical characteristics are believed to have great potential against gastro-intestinal parasites (Ademola, 2016). Tannin is one of the major secondary metabolites that has been investigated when studying plant anthelmintic potential since it contains a great range of biochemical characteristics (Hoste et al., 2006; Ademola, 2016). These tannins could either be condensed tannins or hydrolysable tannins.

2.4.4.1 Condensed tannins

Among the secondary compounds contained by *Kigelia africana* fruit are condensed tannins (Lim, 2012; Priya et al., 2013; Wurger, 2013). Taylor and Murant (1966) reported that *in vitro* analysis of aqueous extracts from the roots and stems of raspberries, which are rich in condensed tannins, reduced the population of the plant nematode *Longidorus elongates*. The effectiveness of anthelmintic activity of this plant was related to time and dose. Paolini *et al*. (2003) reported third stage larval mortality of nematodes due to the presence of condensed tannins in their environment. This was also seen when Quebranco extract (commercially rich in condensed tannins) were administered to parasitized sheep and goats. It resulted in a reduction of abomasa nematode fecundity which led to a decrease in number of eggs and adult worms in small intestines of parasitized host by 50%. Min et al. (2003) reported that when the diet was changed from perennial rye grass (which does not contain CT) to *L. corniculatus* (32 g CT/kg DM) in sheep, the populations of the rumen proteolytic bacteria decreased due to high consumption of condensed tannins (≥ 4 5% Dry matter).

Table 2.1: Shows sheep quantity of proteolytic bacteria in the rumen before and feeding with *L. corniculatus* feed which contains 32gCT/kgDM (Min et al., 2003).

Proteolytic bacteria	**Quantity of microbes before feeding *L. corniculatus* (per ml)**	**Quantity of microbes after feeding *L. corniculatus* (per ml)**
***Speciesclostridium proteoclasticum* b316**	$1.6x10^8$	$5.1x10^7$
***Eubacterium* sp.C12b**	$2.7x10^8$	$1.5x10^8$
***Streptococcus bovis* B315**	$2.7x10^6$	$1.6x10^6$
***Butyrivibrio fibrosolvens* C211**	$102x10^6$	$1.0x10^6$

This decrease in enzyme population reduces dry matter digestibility and promotes low feed intake. In some cases it may affect rumen metabolism by affecting the digestive tract of sheep which may result in reduced absorption of certain nutrients. This implies that, high condensed tannin consumption will result in poor animal performance (Githiori *et al*., 2006). Therefore, the application of condensed tannins for treatment must be done with great care to prevent negative effects.

2.4.4.2 Iridoids

Iridoids are compounds that has been extracted from *Kigelia africana* and consists of 9-carbon chain structures which are similar to those found in other plant species under the family *Bignoniaceae*. *In vitro* assays using these compounds exhibited excellent anti-inflammatory effects especially verminosied. The main iridoids are found in plant roots, stem and fruit bark and are called verminoside, minecoside and specioside. In comparison with the well-known antiamoebic drug metronidazole, Iridoids showed a 2-fold antiamoebic activity (Jackson and Beckett, 2012). Therefore, these compounds have been shown to have pharmaceutical potentials.

2.4.5 Effect of helminths on ruminant nutrition

Parasite losses constitute one of the highest production losses in the livestock industry. These losses could either be direct by affecting the host or indirect by impairing nutritional assimilation in the gastro-intestinal tract. GINs result in inefficient utilization of absorbed nutrients. With a high degree of gastro-intestinal infection, mineral (especially phosphorus) absorption and retention processes are severely disturbed. Poor protein metabolism is observed with endogenous protein losses into the gastro-intestinal tract leading to a high leakage of plasma proteins. This protein loss in GIT is mainly caused by either mucosal injury or lymph obstruction resulting in direct seepage of protein-rich lymph (Umar and DiBaise, 2010). When a gastro-intestinal tract's amino acid requirement increases due to parasitism (during repair processes) the consequence of the latter will be compensated by other tissue's protein metabolism. Poor protein synthesis observed in sheep muscles and wool results from reduced availability of absorbed amino acids from metabolism for peripheral tissues (Coop and Kyriazakis, 2001). Parasitized (*Ostertagia* and *Trichostrongylus* infections)

sheep has been observed to display poor feed intake and increased urinary nitrogen excretion, hence, a negative nitrogen balance. The results from another study appeared to be even worst where sheep were observed with 4000 *O*. *circumcincta* larvae (Ceriac, 2017). Apparent digestibility of dry matter, organic matter, crude fibre and protein (nitrogen) were all very low (Parkins and Holmes, 1989; Mavrot et al., 2015). Intestinal nematodes have been found to reduce phosphorus metabolism, which often occurs by impairing its absorption in the GIT hence decreasing retention which reduces the overall mineralization of the skeleton (Coop and Holmes, 1996). Therefore, parasitized animals will suffer if not treated, hence, the importance of exploring indigenous feeds with dual potential of acting as a feed and an anthelmintic.

2.4.6 Role of nutrition on host resilience

During nutrient allocation, an animal's body prioritises maintenance of body protein because it assures short term animal survival. Reproduction and growth are given second highest priority. Providing additional nutrients to improve animal resilience over parasites is also very important. Nitrogen within GIT of ruminants is either degraded in the rumen or consumed by rumen microbes or passes undegraded forming bypass protein (Leng, 1990). Both microbial and bypass protein are absorbed in the small intestine as metabolisable proteins. When the amount of metabolisable protein was improved by feeding ample undegradable protein amounts, sheep experimentally infected with intestinal nematode larva showed improved resilience against nematodes (Knox et al., 2006). This improvement in resilience has led to supplementation of ruminant diets with urea (source of degradable nitrogen). Protein supplementation seems to work efficiently in young, naïve animals where patho-physiological disorders like gastroenteritis are more likely to occur. Dietary proteins are more efficient than energy in improving animal resilience except in animals which suffer from severe malnutrition where increased energy supply will obviously improve resilience (Coop and Kyriazakis, 2001).

2.4.7 Effect of nutrition on host resistance

Hosts develop resistance after continuous exposure to a parasite and this resistance is influenced by the age of an animal, productive state of an animal and the type of animal breed (Geurden et al., 2014; Grencis, 2015; Babják et al., 2017). Reduced

intake is often seen in parasitized animals which consequently weakens their immune system resulting in production losses (Coop and Holmes, 1996). Provision of protein supplements particularly undegradable proteins does boost the immune system against gastro-intestinal nematodes. This supplementation enhances expression of immunity such that the development of larva is suppressed while nematode population survival and productivity is reduced. Lactating ewes usually display reduced immune response since nutrient requirements will be prioritized for milk production rather than expression of immunity. However, rapid immune response is regained during dry season or winter when the number of larvae in a pasture decreases to almost 80% (Field *et al*., 2002).

Nutritional status and GINs are two major factors affecting ruminant production in the Zululand area. This study aims to explore *Kigelia africana* fruit's potential to provide a cheap and effective anthelmintic while providing a possibly good protein and energy supplement as well as moisture (feed water). Therefore, this study aims to solve nutritional deficiency problems faced by farmers in dry seasons and a drop in production standards due to GIN infestation.

Low forage availability has observed during dry periods. Ruminant production are also reported to decline during this period as production requirements for animals are hardly met. Poor feed conversion ratio has also been largely caused by Gastro intestinal nematodes (GINs) infections. These parasites consume nutrients that are supposed to be utilized for production, growth and reproduction, hence, compromising ruminant production industry. The principal problem towards controlling GINs is that, most problematic helminths have developed resistance against vermifuges. Therefore, *Kigelia Africana* fruit aims to provide high moisture and energy rich supplement that will also control GINs.

Chapter 3

Chemical analysis and shearing force of *Kigelia africana* fruit

3.0 Abstract

The aim of this study was to investigate the nutritional value and shearing force of the *Kigelia africana* fruit. Five samples (portions) were made from this fruit, Exocarp (Ex), Endocarp (En), Endocarp plus seeds (En+SS, 1:1), Seeds (SS) and Whole fruit (Wf). The different chemical components determined for each feed were dry matter (DM), moisture content (MC), crude protein (CP), condensed tannins (CT), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose and hemi-cellulose. Fibre components were analysed using the ANKOM filter bag method while proteins were analysed using the Kjeldhal method. Acid butanol Assay and Warner-Blatzer shear device were used to analyse condensed tannins and shearing force, respectively. The results revealed that SS and Ex had the highest cellulose content (32% and 42% respectively). Ex had the highest ($P<0.05$) NDF, ADF and ADL (70.67%, 59.18% and 17.69% respectively) compared to other extracts. The highest protein content was observed in SS (12.37%) while the lowest was seen in Ex. There were higher contents of lignin and cellulose on the Ex than on the En. Ex had the highest FMax (1060.1N) while En had the lowest shearing force of 540N. Removal of the hard exocarp reduced *K. africana* fruit shearing force requirements and eased chewing. Therefore, *Kigelia africana* fruit can be suggested as a potential feed supplement for ruminants due to its chemical constituents and high moisture especially in winter when water sources are scarce. High jaw shear force illustrate that fruit shear force is not the major limitation towards fruit utilisation by domesticated ruminants specifically cattle. However, there is a need to further investigate the digestibility of this plant both *in vitro* and *in vivo* to confirm its application as well as its anthelminthic potential.

Key words: Sausage tree, nutritional value, fibre, water content, shearing force

3.1 Introduction

The world's food production should increase by more than 75% to be able to feed the 7.7 billion world population in the next 30 years (Godfray et al., 2010; Goujon, 2018). Well-targeted and determined measures should be employed in improving food security especially in developing an under-developed world that is experiencing an accelerated population growth (IFAD, 2011; Agarwal, 2018). This can only be achieved by improving both crops and animal products. Ruminants reared for meat and milk production are the major sources of protein for human beings globally because plant protein sources are often expensive and scarce (Bath, 2018). These animals especially goats, sheep, and cattle are often reared by the rural poor farmers which they received as either a gift from the groom as a bride price or in traditional ceremonies. However, small ruminants are much cheaper, easily marketed and require less management as they can browse or graze in the open fields and veld around the communities (Freer and Dove, 2002; Mahgoub et al., 2012).They possess a distinctive digestive system that allows them to derive nourishment from forages and utilize land which is unsuitable for crop production and bio fuelling. This digestive system also reduces the direct competition for grain that can be used for human consumption (Guyader et al., 2016). However, in tropical areas, cattle still suffer low live weight gain during the dry season (Baumgard and Rhoads, 2012). This scenario creates a major obstacle in producing young animals of high carcass weight to meet market specifications. Energy and protein are the key nutrients required for ruminant production (Peyraud et al., 2014) but pastures on which they graze are often limited in nutrient supply because the energy is locked in complex cellulose structures or forage is protein deficient. Water is also a major problem in desert regions as well as sporadic droughts such as the one that was recently reported in the southern part of Africa (Mapato, 2018). During that period, many commercial, as well as community, farmers were forced to sell their animals because animals were losing condition rapidly and eventually dying (Gressley et al., 2011; Minson, 2012). Therefore, exploring alternative forages that can increase both energy and proteins, as well as increase water content from feed, can be a major step in sustaining these animals.

Hence, it is crucial to explore supplements like *Kigelia africana* fruit, which does not pose any competition to human food sources as a potential supplement to improve ruminant production during dry periods. *Kigelia africana* plant known as sausage tree

bears large fruits which may be 15 to 30cm long and about 10kg in weight (Gabriel and Olubunmi, 2009). It has never been exploited for its feed potential in domesticated animals except by wild animals like porcupines, baboons and wild pigs that have been observed to have a bite of these fruits. However, humans, especially in west and central Africa, have been utilizing *Kigelia africana* plant in numerous ways. Its seeds have been roasted and mixed with beer to enhance milk production in pregnant women and also to enlarge male sexual organs (Gabriel and Olubunmi, 2009).

This fruit, upon observation, contains a fibrous endocarp and has relatively high water content. This is a fascinating factor in ruminant production since ruminants are excellent utilizers of fibre through fermentation occurring in the rumen (Minson, 2012). Furthermore, water is a key factor in ruminant production which implies that none of these production systems will thrive or be economical without a water source more so a cheaper one. The water requirements of normal cattle weighing 400kg is 40 to 80L/day while normal dry sheep will consume 2 to 4L/day (Curtis, 2017). However, water requirements may vary with the production stage of an animal (i.e. lactating cattle consume 50 to 100L/day) and the type of diet involved (concentrate, or hay) (Sitnikov et al., 2016; Sordillo, 2016). Also, environmental temperature has a great influence on the water consumption of an animal. During hot weather conditions water intake will increase as the animal will be trying to cool down its body temperature (Ward and McKague, 2007).

For maximum production, clean sufficient water should be available to the animal at all times and this seems to be the challenge during the dry season or severe drought periods (Misra and Singh, 2002). Gressley et al. (2011) reported that ruminants get water in three ways, drinking water, metabolic water and water from feed. Hence, *Kigelia africana* fruits may play a very important role in reducing water stress in ruminants during the dry season where small streams and rivers dry off by its relatively high moisture content. For normal ruminant production systems, water stress levels should be kept as low as possible and meet the nutritional requirements of the animal. The primary requirements for animal production are carbohydrates and proteins (Bowen, 2009).

The majority of ruminant production losses have been reportedly due to reduced energy, protein and water unavailability in the Zululand area in KwaZulu-Natal

Province, South Africa. Ruminants obtain their nutrients from feeding on nutrient rich forages. Availability of these forages during winter (low rain fall) is the major constraint towards ruminant production systems in the Zululand region. *Kigelia africana* fruit has been observed to have a high moisture content which may also complement water requirements of the animal while its fibrous pulp may yield enough energy. To the best of our knowledge this fruit has never been reported to be utilized by ruminants before as a feed supplement for domesticated animals. One of the reasons that might be hindering the use of this fruit especially by domesticated animals may be its hard exocarp. The presence of this hard exocarp protects the relatively high fibrous and moisture endocarp which is more succulent and available to ruminants. This is probably the reason why most animals do not have access hence its limited application.

It will be right to investigate the strength that is required to break open this fruit which is also called the shear force (Szczyglak, 2012). The shear force will probably give more information about which animals will be able to break open these fruits without assistance and those that cannot. The shear force will also be able to predict how the feed should be processed prior feeding. It is right to suggest that proper knowledge of the nutritional composition of this fruit as well as its shear force will be able to inform farmers about their use as a potential feed supplement. Therefore, the objective of this study was to investigate the nutrient quality of *Kigelia africana* for ruminants and the shear force required to break open these fruits for utilization. It was hypothesized that the nutritional composition will meet the basic requirements for ruminant forages as well as the shearing force required to break open the fruit by both small and large ruminants.

3.2 Materials and methods

3.2.1 Kigelia Africana fruit collection and preparation

Fully matured *Kigelia africana* fruit were harvested at University of Zululand premises which Is located at KwaDlangezwa area, northern KwaZulu-Natal, South Africa. The average rainfall varies from 670 to 950 mm while temperature ranges from 23°C to 29°C. The sausage fruit harvested were separated into five different parts (portions) called; Exocarp (Ex), Endocarp (En), Seeds (SS), Endocarp plus seeds (En+SS) and the whole fruit (Wf) prior to chemical analysis.

3.2.2 Chemical Analysis

3.2.2.1 Dry matter and moisture content analysis

Fresh fruit extract was thoroughly cleaned by washing and transferred into a clean plate weight (W_0) and weighed (W_1). Fruit samples (Ex, En, SS, En+SS and Wf) with three pseudo replicates each, were oven dried at 60°C for 4 days and cooled in a desiccator before being weighed (W_2). Dry matter percentage was determined using the following formula;

$$\text{Dry Matter (DM)}\% \left(\frac{\text{W2}}{\text{W1}}\right) \times \frac{100}{1}$$

$$\text{Moisture content(MC)}\% = \left(\frac{\text{W1} - \text{W2}}{\text{W1}}\right) \times \frac{100}{1}$$

3.2.2.2 Protein analysis

Protein was analysed using the kjeldhal method (Aggarwal, 2018) with three (3) pseudo replicates each. *Kigelia africana* fruit portions (1g) were boiled in sulphuric acid giving complete oxidation of organic material where protein was completely degraded thereby releasing ammonium ions (NH_4^+). The solution was alkalised to convert NH_4^+ to NH_3^+ and steamed with distilled water to release NH_3 which was trapped by boric acid. NH_3 molecules were measured by titration according to the method described by Massey (2003). The protein concentration was calculated by multiplying nitrogen concentration by a protein factor of 6,25.

3.2.2.3 Determination of condensed tannins

Condensed tannins were determined by using the Acid Butanol Assay for proanthocyanidins (Gressner, 2005), where 6 ml of acid butanol reagent was added in a 13×100 screw cap culture tube, containing 1.0g sample. The mixture was then vortexed after the addition of 0.2ml of iron reagent. Tubes were then inserted into a boiling water bath for 50 minutes with tube caps loosened. Thereafter, the ice bath was used to cool the tubes and the absorbance was read at 550nm. Finally condensed tannins (CT) for 3 pseudo replicates were calculated as follows:

$$\mathrm{CT} = \left(\frac{\text{Absorbance} \times \text{Dilution Factor} \times 78.26}{\%DM}\right)$$ (Iqbal, 2011)

3.2.2.4 Determination of Neutral Detergent Fibre (NDF)

Kigelia africana fruit sample fibre components were determined using AOAC methods using ANKOM Technology (Damiran, 2002) with three (3) pseudo replicates for each extract. The fruit portions were ground to pass through a 2 mm sieve. Filter bags were weighed individually (W_0) before transferring about 0.45g to 0.50g into each filter bag (W_1) while ensuring samples were placed below 4mm of the filter bag. A blank filter bag C_1 was included in the run as a control. A heat sealer was used to seal the filter bags encapsulating samples within the 4mm mark. There were five samples (EX, En, SS, En+SS and Wf) with four replicates of each and three control bags summing up to 23 bags that were used. To uniformly spread samples, filter bags were flicked and shook thereby eliminating sample clumps. Only three bags were placed on each of eight Bag Suspender Trays (which carries the maximum of 24 bags). Trays were stacked on the centre post of the Bag Suspender while each level was rotated at 120°C in relation to the tray below it. Before switching the Power button ON, assurance was made if the Exhaust Hose was connected to the instrument and securely positioned in the drain. The Bag Suspender with bags was placed in the Vessel while the Bag Spender weight was put on top of the ninth tray to keep the bag suspender submerged. Exactly 100ml/bag of Neutral detergent solution (NDS) and 4.0ml of Alpha-amylase was added to the solution in the vessel. Right after the addition of Alpha-amylase agitation, the heat were turned on for 75 min before switching OFF the heat. The valve was opened slowly to exhaust the solution. With the drain valve closed, about 1900-200ml of 70-90°C rinse water containing 4.0 ml of Alpha-amylase was used to rinse the samples for 5 minutes. The same five-minute rinsing procedure was repeated three times.

After rinsing, the bags were removed and gently compressed to remove excess water and submerged in a 250 ml beaker with acetone for 3-5 minutes. Bags were removed and placed on a wire-screen to dry completely by evaporating acetone before oven drying them at 102 ± 2°C for 4 hours. Filter bags were removed from the oven and placed in a collapsible desiccant pouch to cool before weighing (W_3) them. The formula below was used to calculate NDF percentage;

$$\%\text{NDF} = \left(\frac{W_3 - (W_1 \times C_1)}{W_2}\right) \times \frac{100}{1}$$

3.2.2.5 Determination of Acid Detergent Fibre (ADF)

Acid Detergent fibre (ADF) analysis was performed according to the ANKOM filter bag technique (for A200 and A2001) (Damiran, 2002). *Kigelia africana* fruit portions were ground to pass through a 2mm sieve. Filter bags were firstly weighed individually giving (W_0) before transferring 0.45g to 0.50g directly into a filter bag while ensuring samples were placed below 4mm of the filter bag. Three blank filter bags (C_1) were included in the run as a control. There were five samples (EX, En, SS, En+SS and Wf) with four replicates of each and three control bags summing up to 23 bags for each run. The heat sealer was then used to seal the filter bags encapsulating samples within the 4mm mark. To uniformly spread samples, filter bags were flicked and shook thereby eliminating sample clumps. Up to 3 bags were placed on each of eight Bag Suspender Trays (which carried the maximum of 24 bags).

The following instructions on the ANKOM^{2000} were followed: Acid detergent solution (2000ml) was transferred into the ankom machine to float the rack. The vessel lid was closed; water temperature was kept above 70°C before START button was pressed. ADF extraction and rinsing was same as described in section 3.2.2.4 but for the utilisation of NDS solution. Thereafter sample bags were oven dried at 60°C for 4 hours. Filter bags were removed from the oven and placed on collapsible desiccant pouch where they were flattened to cool down at ambient temperature before weighing (W_3);

$$\%\text{ADF} = \left(\frac{W_3 - (W_1 \times C_1)}{W_2}\right) \times \frac{100}{1}$$

3.2.2.6 Determination of Acid Detergent Lignin (ADL)

Filter bags from ADF analysis were further analysed for lignin content. The bags were submerged in a 3 L beaker containing 250 ml of 75% H_2SO_4 for 3 hours while a 2L beaker was used for agitation at 30 min intervals. After 3 hours, the bags were rinsed with tap water until the acid was completely removed. A final rinse with 250 ml of

acetone was done before bags were oven dried at 60°C for 3 hours before weighing them (W_3).

$$\%\text{ADL} = \left(\frac{W_3 - (W_1 \times C_1)}{W_2}\right) \times \frac{100}{1}$$

3.2.2.7 Determination of Ash content

Bags from ADF analysis were further analysed for ash content. Bags were folded from top to bottom and from left to right before sealing them. Bags were then ashed on pre-weighed crucibles at 525°C. After 3 hours, crucibles were allowed to cool and weighted. The residue was considered as the ash content of the feed.

3.2.3 Shear force of *Kigelia africana* fruit.

A knife was used to peel off the exocarp from the endocarp just before analysis to separate the different portions required for shear force measurement. Shearing force analysis of the Ex, En and Wf were done using the Warner Blatzer shearing device from Avatar Solutions, in Pretoria**.** A sample was placed firmly by an adjustable slotted blade base. A notch shaped blade (100mm Width, 60mm depth and 41mm height) was used for *Kigelia africana* fruit specimens. The specimen was placed on the base plate and the blade moves downwards at a constant speed and cut through the specimen. Results for all tested parameters were displayed on Zwick ProLine Z030 software. The result supplied Maximum force (FMax) which is the maximum force required to cut through the specimen. dL at Fmax symbolises the distance travelled by the blade at maximum force while dL at break represents the distance covered by the blade to break the specimen and F at break is the force during breaking of the specimen. Bite simulation was also done where the blade was set to perform two bites. Springiness, gumminess and chewiness were determined though Zwick ProLine Z030 software.

3.2.4 Statistical analysis

Comparisons between means of chemical components (DM, Moisture, NDF, ADF, ADL, Cellulose, Hemicellulose, ASH, CP, CT, and ADL) and Shear force values (dL, dL at break, Fmax, F at break chewiness, gumminess and springiness) were performed by one way analysis of variance (ANOVA) using the statistical packages

for social sciences SPSS (IBM 2013). Means of replicates were separated for least significance at 5% level ($LSD_{0.05}$) in order to find statistical differences.

3.3 Results

Exocarp had the highest (P<0.05) NDF, ADF, cellulose and hemicellulose than other fruit extracts (Table 3.1). However, there was no significant difference (P<0.05) observed on the ADL component of fibre in all five samples. Moisture content appeared to be highest in En+SS while SS had the least. Dry matter was highest in SS (P<0.05) whereas En+S had the lowest dry matter content. There was a significant difference (P<0.05) between CP content across *Kigelia africana* fruit portions with seeds having the highest value (12%) while Ex had the lowest (2%).

Table 3.1: Chemical composition of *Kigelia Africana* fruit components.

Extract	Wf	Ex	En	En+SS	SS	S.E.D	*P*-Values
MC (%)	73.52^{c}	66.60^{b}	76.46^{d}	79.00^{e}	50.81^{a}	2.83	0.00
DM (%)	26.48^{a}	34.03^{b}	22.75^{a}	21.00^{a}	49.65^{c}	2.82	0.00
CP (%)	3.90^{a}	2.50^{a}	3.05^{ab}	3.80^{bc}	12.50^{d}	0.99	0.00
CT (%)	4.25^{b}	4.60^{b}	3.08^{ab}	3.29^{ab}	1.73^{a}	0.31	0.01
NDF (%)	47.04^{b}	70.67^{c}	39.00^{a}	41.64^{ab}	64.19^{c}	3.41	0.00
ADF (%)	35.90^{a}	59.18^{c}	29.32^{a}	31.78^{a}	46.56^{b}	3.01	0.00
ADL (%)	11.46^{ab}	17.69^{b}	2.61^{a}	5.33^{ab}	13.81^{ab}	1.80	0.02
H- cellu. (%)	11.15^{a}	11.45^{a}	9.69^{a}	9,65^{a}	17.63^{b}	0.82	0.00
Cellu. (%)	24.44^{a}	41.45^{b}	26.70^{a}	26.48^{a}	32.75^{ab}	1.82	0.01
Ash (%)	0.42^{b}	1.25^{d}	0.18^{a}	0.72^{c}	0.55^{b}	0.10	0.00

CT= Condensed tannins, NDF= Neutral detergent fibre, ADF= Acid detergent fibre, Wf= Whole fruit, Ex= Exocarp, En= Endocarp, En+SS= Endocarp plus seeds, SS= Seeds, S.E.D = standard error of difference. ^{a,b,c,d} Numbers in the same row with different superscript letters are different (P<0.05)

CT appeared to be different (P<0.05) across fruit portions with Ex having the highest concentration and SS had the lowest concentration. There was a significant difference (P<0.05) between NDF values of *Kigelia africana* fruit portions with Ex having the highest value followed by S, Wf, En+SS and En. There was a significant difference (P<0.05) in ADF content of *Kigelia africana* fruit with Ex having the highest content while En had the lowest. Ex appeared to have the highest cellulose content while Wf had the least.

There was a significant difference between the Fmax values (P<0.05) across *Kigelia africana* fruit extracts. Ex appeared to be the strongest portion as it took 9.8mm for a blade to reach FMax at 1060. 1N followed by Wf with 963,0N and En with the least FMax (547N) at 20mm distance. At breaking point, Ex had the highest force (209,6N) recorded followed by Wf (190.0N) and En (108.4N) with least.

Table 3.2: Shows shearing force of *Kigelia africana* extracts with blade travelling at different distances.

Parameter	Wf	Ex	En	P-Value
dL at FMax (mm)	20.0	16.1	9.5	0.00
FMax (N)	963.0	1060.1	547.0	0.00
dL at Break	48.6	45.0	35.0	0.11
F at Break	190.0	209.6	108.4	0.08

Wf= whole fruit, Ex= Exocarp, En= Endocarp, dL= distance covered by the blade during cutting and F Max= maximum force, dL at break distance covered by the blade at breakage, F at break= force during break and ^{a,b,c,d} = Numbers in the same row with different superscript letters are different (P<0.05).F Max= maximum force, dL at break distance covered by the blade at breakage, F at break= force during break

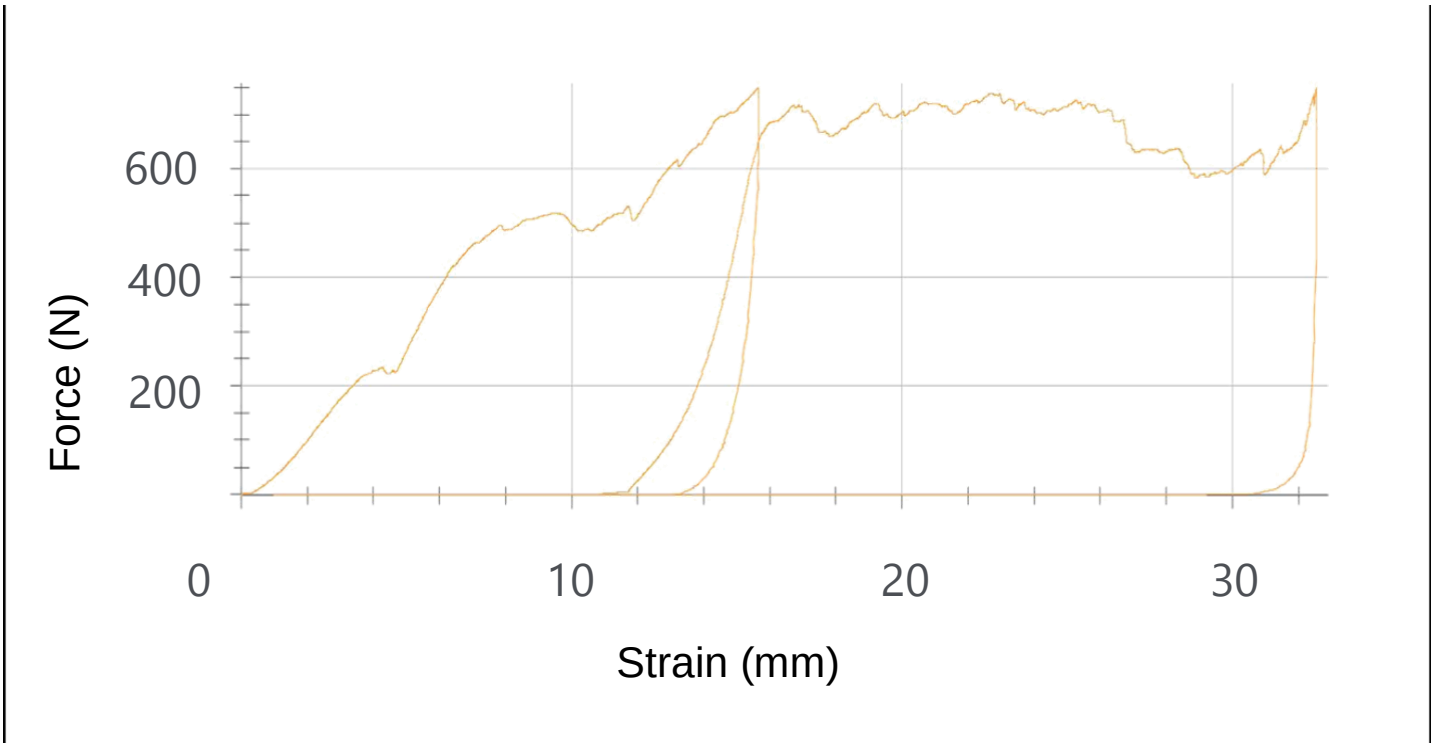

Figure 3.1 Simulation of two bites on the endocarp extract.

It took about 15.66mm to reach the 750N on the first bite on the endocarp **(Figure 3.1)** but it took 32.55mm to complete the second bite demanding 750N. En appeared to have strong chewiness capacity accompanied by very low springiness. Force per millimetre increases in the second bite due to a long distance travelled by the blade to complete the second bite as a result the cohesive strength on the second bite is also doubled.

Table 3.3: Properties of *Kigelia africana* fruit endocarp during bite simulation.

Index	Si (N/mm)	Fi3 (N)	Si3 (mm)	Ei1 (mJ)	Springiness	Gumminess (N)	Chewiness (N)
1	50.55	750	15.66	6255.28	139	1553.91	2154.50
2	65.70	750	32.55	12395.14			

Index= Bites, Si= force applied per millimetre, Fi3= Hardiness, Si3= Distance covered in a single bite, Ei1= Cohesive strength, N= Newtons

3.4 Discussion

The experimental results showed that *Kigelia africana* fruit has a very high moisture content which was more that 50% in all the different portions that were investigated. This was probably due to its high water holding capacity. Its ability to hold water was probably due to the fleshy, juicy skin surrounding the fibre mesh in which sporadic small seeds were found (Pritchard, 2004). High moisture (85.5%) content was also reported by Lim (2012) which supports En+S having the highest moisture content while seeds had the lowest moisture content. Therefore, if animals can have access to the water in these fruit through feeding, it can assist in increasing water intake as water from feed (Misra and Singh, 2002; Ward and McKague, 2007). This can play a major role in decreasing water stress especially during the dry season or winter or even in drought periods where water sources are scarce or have dried up. The principal function of the fruit exocarp is to protect the fleshy part of the fruit and its seeds. It does secrete secondary compounds which play a huge role in the prevention of herbivory thus protecting the inner seeds which enclose the developing embryos of fertilized eggs. If protection is the major function of the exocarp, it can be understood as the primary reason for its high fibre content (NDF, ADF and cellulose) which will definitely increase the strength, making it hard to break (Hossain, 2016). The highest CT content was also reported in the Exocarp, which can also be understood as a mechanism to deter browsers from utilising the fruit (Mack, 2000). These secondary

compounds provide protection against plant inversion by bacteria, fungi and other disease causing organisms. *Kigelia africana* fruit exocarp also had high condensed tannins compared to endocarp and seeds. The study of Grace (2002) and Henke (2017) reported that high tannin concentration in plants was found to deter herbivores from browsing sausage fruit. Others reported the extensive use of *Kigelia africana* fruit traditionally as medication in treating microbial infections and primary and secondary infections due to the presence of condensed tannins (Jackson and Beckett, 2012; Wurger, 2013). The endosperm, which provides nutrition to the growing embryo, is found within the seeds (Xiangnan, 2016); this explains why the highest value of protein content was observed in the seeds of *Kigelia africana* fruit. According to Wurger (2013), the *Bignoniaceae* family has an average of 2.8% condensed tannin concentration. Exocarp appeared to have higher NDF, ADF, cellulose and hemicellulose while these parameters were low in the endocarp. Lignin, cellulose and other components of fibre were commonly found in the outermost layer where they provide rigidity and protection to the fruit (Moore and Jung, 2001). Ash measurements represent the amount of minerals present in any experimental feed sample. Wurger (2013) reported that *Kigelia africana* had an ash content of 0.176% which is not much different from the results shown in this study. The highest ash contents were observed in the exocarp and seeds. This was associated with the requirements of these minerals to assist building up the strength of the exocarp or to furnish the embryo during growth (Zafilaza, 2017).

There are numerous studies that have described both positive and negative impacts of tannins (Earl and Semlitsch, 2015; Henke, 2017) depending on concentration. This implies that the high tannin value found in these fruits might be detrimental or beneficial based on type of phenolics and consumption levels. A high amount of condensed tannins (above 6%) tend to bind with proteins and cause strong insoluble protein-tannin complexes. These insoluble complexes normally occur at slightly acidic to neutral pH (3.5 to 7.5), however, if the pH is reduced to less than 3.5 proteins dissociate making it available in the abomasum especially to ruminants. These complexes may sometimes result in a reduction of protein digestion in the rumen which leads to low microbial protein synthesis (Min et al., 2003) due to a lack of feed protein for microbial growth or binding of tannins to microbial proteins hence affecting fibrolytic microbial activity (Lorenz et al., 2014). Lorenz et al. (2014) and Jung and Vogel (1986)

reported that high secondary compounds and lignin contents are abundant in plant exocarps where they play a huge roll in inhibition of herbivory, hence, this explains why *Kigelia africana* fruit exocarp and whole fruit appeared to have high condensed tannins rather than crude protein. Seeds, on the other hand, appeared to be richer in protein since they contain less CT value compared to all the fruit extracts. This is because seeds contain endosperm which provides nutrients for the tissue growth of new plants (Calderon, 2011).

Kigelia africana Exocarp showed the highest shearing force followed by whole fruit and endocarp with the least. This is probably due to the high fibrous threads found in the exocarp compacted together by lignin. This is in line with the findings by Chen (2007) who reported a strong relationship between shearing force and fibre components. Shearing force of maize stover increased with cellulose and lignin content with lignin having a much stronger influence as reported by Griffiths and Gordon (2003) and Chen (2007). The fibre constituents have a direct link to chewiness (Mouth feel the sensation of laboured chewing due to sustained elastic resistance from the food) and gumminess (stickiness or cohesiveness). The chewiness and gumminess for exocarp and whole fruit were unsuccessful as the Warner Blatzer shear device fail to perform bite simulation on whole fruit due to exocarp heavy-duty on the blade. However, the bite simulation of the endocarp exhibited relatively fair maximum force requirements on both bites. Chemical analysis showed that Ex had a lower moisture content than En, this led to Ex having a higher shearing force compared to En. These findings are supported by Esehaghbrygi (2009) where shear force decreased with increase in wheat stem moisture content.

According to Akimoto (2003) cattle have a bite force of 3280N while horses had 6350N. These values illustrate that cattle can be fed *Kigelia africana* fruits or hard exocarp can be removed to ease chewing burden by only supplementing using endocarp. The endocarp appeared to have tolerant springiness (degree of recovering from deformation). High gumminess, which refers to the stickiness property of the extract is due to high moisture content on the endocarp, which also results in high cohesiveness allowing enough feed deformation before breakage during chewing (Alakhrash, 2016). *Kigelia africana* fruit seems to meet the basic nutritional composition of most forage diets as well as the shearing force required to break open this fruit for consumption is far lower than the maximum shear force that can be generated by a cow or sheep.

3.5 Conclusion

In conclusion, *Kigelia africana* fruit has a potential to be used as a feed supplement for domesticated ruminants due to its high moisture content, fibre and relatively lower protein. Its considerate shearing force values will allow ruminants to access high protein content of the seeds as well as moisture although it was found that breaking the exocarp could even reduce the shear force. This fruit also appeared to have considerably moderate condensed tannin concentration which may also play a role in the inhibition of gastro-intestinal parasites. Therefore, there is need to further estimate the digestibility of this plant as well as its anthelmintic potential.

Chapter 4

In vitro digestibility and anthelmintic value of *Kigelia africana* fruit harvested in Zululand

4.0 Abstract

Meeting ruminant's nutritional requirements has been the primary goal for livestock farmers. Any compromise to this may result in massive production losses. Absorption of nutrients in the animal body is largely affected by digestibility of that particular feedstuff. Therefore, there is a need to investigate the digestibility of any potential feed and not just the chemical composition. The aim of this study was to investigate the *in vitro* digestibility and anthelmintic value of *Kigelia africana* fruit for domesticated ruminants. Fresh *Kigelia Africana* fruits were harvested at the University of Zululand premises, separated into five portions (parts) called Exocarp (Ex), Endocarp plus Seeds (En+SS), Endocarp (En), Seeds (SS) and Whole fruit (Wf). *In vitro* digestibility was done using a Daisy incubator from ANKOM Technology with inoculum from rumen fluid, incubated at 38ºC in an anaerobic chamber for 72h. Apparent and True degradability and microbial yields were measured. For anthelmintic activity, A Helminth motility test was conducted with extracts of phenolic compounds from Ex, En, Wf and SS using Baerman's technique and L3 larva was observed at 10x magnification. Each extract was dosed at four treatment levels Viz. 5, 10, 15 and 20 g. There were differences ($P< 0.05$) in TD values among *Kigelia africana* portions with En and En+S having the highest values (539.32g/kg and 554.46g/kg respectively) while the least was observed in Ex and SS (321.00g/kg and 252.62g/kg, respectively). MY was significantly higher for En and En+SS 163.57g/kg and 161.67g/kg respectively. Though there was no difference ($P>0.05$) in larva mortality between *Kigelia africana* treatments, treatments displayed 96% mortality rate on average when compared to the control. The strong anthelmintic properties displayed by *Kigelia africana* treatments were associated with high concentration of CT as earlier anticipated but may be linked to other unknown secondary compounds in the extracts that need to be explored.

Keywords: Sausage tree, Digestibility, Vermifuges, anthelmintic value

4.1 Introduction

Ruminant production largely depends on the nutritional status (quality and availability) of the feed. For maximum production, an animal's nutritional requirements should be met (Griffith, 2017). When these requirements are not met, production and health status of the animal may be compromised which also leads to a drop in meat, milk and egg production quality and quantity (Gressley et al., 2011). To obtain these nutrients, ruminants need to fully digest the ingested feed, absorb nutrients and excrete the waste material to make space for more feed to be consumed. The actual intake of a particular feed by an animal minus the excreted faeces gives the digestible material in that particular feed. Different forages have different chemical constituents, hence their digestibility will also differ (Herron, 2015).

Digestion in animals begins with mechanical digestion by teeth in the mouth, grinding of food in the gizzard for poultry birds, fermentation in the fore stomach of ruminants (rumen, reticulum, omasum) to enzyme break down of feed in the simple stomach (abomasum) and small intestine to soluble forms which are absorbed by the villi in the small intestines (Uttam et al., 2010). There are many factors affecting digestibility of feedstuffs. Some are animal-based while others are plant-based and some depend mainly on how the particular feed was prepared. Animal-based factors are dependent on the type of animal as mono gastric animals cannot digest feedstuffs with high fibre content while ruminants can easily utilise fibrous feed by the aid of rumen micro-organisms (Saha et al., 2010). Plant-based factors include the quality and quantity of crude protein and fibre which also vary with the plant age.

Anti-nutritional factors are major factors to consider as they greatly affect digestibility, be it in monogastric, ruminants or hindgut fermenters. Older plants tend to have high lignin content. Lignin is an anti-nutritional factor, which plays a vital role in promoting the rigidity of a plant cell, but reduces the digestibility of nutrients in feed stuffs. Cellulose is another cell wall component, which cannot be digested by non-ruminants but can in ruminants. This is because non-ruminants possess only one stomach which is only able to digest feedstuffs with low fibre content.

Forages like hays are fermented in the rumen (Knudsen, 1997). Volatile fatty acids (Acetic acid, butyric acid and propionic acid) and rumen gases (Methane and Carbon dioxide) are the end products of the fermentation process. Digestibility can be estimated by measuring the volatile fatty acids and gases produced. High microbial activity will yield a high volume of gases (Owens and Basalan, 2016). Apparent digestibility (APD) is estimated by subtracting nutrients contained in faeces from nutrients contained in the feed; hence, it does not include nutrients lost as methane gas or metabolic waste product excreted in faeces. However, True digestibility (TD) is measured to correct these unaccounted losses. TD accounts for endogenous losses and microbial amount of nutrients actually lost in faeces and it is therefore more complex as compared to APD (Griffith, 2017).

In vivo digestibility analysis is also used where the feed taken in and excreted in manure is measured. The difference between the two will be what was absorbed by an animal body. The main problem here is that animals used are so expensive to manage and take care of. However, *in vitro* digestibility studies uses digestion jars to simulate rumen digestion (Owens and Basalan, 2016). This method is said to be far cheaper and less time consuming because over 100 samples can be evaluated at the same time. Digestibility measures what is actually absorbed by an animal body for production, growth and maintenance.

Gastro-intestinal nematodes (GIN) are parasites (helminths) that infest the gastro-intestinal tract of different farm animals (monogastric, ruminant or hindgut fermenters). GINs in the alimentary canal of ruminants consume nutrients apportioned to ruminants (Sordillo, 2016) which often results in a poor feed conversion ratio that may subsequently lead to massive production losses (Mavrot et al., 2015). Ruminants are primarily reared on natural pastures in community fields making them more vulnerable to nematode infestation. This has led to the elevated use of vermifuges (anthelmintics) to control helminths. Recent studies show that controlling these helminths has never been easy especially with helminths gaining strong resistance against widely used vermifuges (Scheuerle, 2009). A study conducted by Van Wky et al. (1999) on *Haemonchus contortus* shows that out of 80 farms 79 were resistant against benzimidazole, 73 against ivermictin 23 against levamisole. Resistance and high cost of these anthelmintics seems to be a major problem for many small scale livestock

farmers in northern KwaZulu-Natal. Plant-based anthelminthics appear to be the most likely solution to this problem since they are environmentally friendly.

A study by Hoste et al. (2006) revealed that *Acacia nilotica* and *Hedysarum coronarium* plant extracts reduced faecal egg count and improved weight gain of naturally infected sheep. *Acacia karoo* had no effect on live weight gain but reduced *Haemonchus contortus* infection by 34%. This is because *Acacia karoo* had more condensed tannins than *Acacia nilotica*. *Rhamnus alaternus L*. commonly known as Italian Buckthorn had a total phenolic content of 60.1mg/g and showed helminth inhibition activity of 94% in 60 min (Jamous, 2017a). *Kigelia Africana* plant has been reported to have condensed tannin concentration (Jackson and Beckett, 2012), and no research has been done on its effect on gastro-intestinal nematodes. However, this fruit has been positively used for its phytochemicals, as a study by Grace (2002) showed that *Kigelia africana* bark and roots extracts displayed strong antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and antifungal effects against *Aspergillus niger*, *Aspergillus flavus*, Candida *albicans* and *Pullularia pullulari*. This was associated with the presence of secondary compounds in *Kigelia africana* plant, which belong to the *Bignoniaceae* family (Chivandi et al., 2011). This plant is commonly known as a sausage tree. In Africa, it is mostly found in open woodlands and wet areas and on the river banks (Grace, 2002; Dada et al., 2010; Lim, 2012). Traditional healers in West Africa are reported to be using the plant for medicinal purposes as they treat a wide range of skin ailments like fungal infections and eczema. Pregnant women are also reported to roast and consume seeds to enhance milk production while men ferment and mix it with beer to enlarge male sexual organs (Jeyachandran and Mahesh, 2007; Jackson and Beckett, 2012).

A random work by Saini (2008), showed the extensive use of *Kigelia africana* to control bacterial and fungal infections. Due to the presence of these secondary compounds, it is a very good reason to investigate its application on helminth infestation in ruminants. Therefore, the aim of this study was to evaluate *in vitro* digestibility and anthelminthic properties of *Kigelia africana* fruit as a potential feed supplement and a cheap and effective anthelminthic in ruminants. It was hypothesized that the digestibility of *Kigelia africana* will be high enough to act as a potential feed supplement and the fruits will also possess anthelminthic properties.

4.2 Materials and methods

4.2.1 In vitro digestibility

4.2.1.1 Sample collection and preparation

Kigelia africana fruits were harvested at the University of Zululand main campus premises which is situated in KwaDlangezwa, about 19km south of Empangeni and about 142km north of Durban off the N2 National Road on the KwaZulu-Natal North Coast. Four fruits were harvested from four different sausage tress. For each harvested fruit, five (5) portions (samples) were separated, Viz. Exocarp (Ex), Endocarp (En), endocarp plus seeds (En+SS), seeds (SS) and the whole fruit (Wf). These five samples were then dried as described in chapter 3 section 3.2.1 and ground to pass through a 1mm sieve.

4.2.1.2 Filter bag preparation and sample preparation for *in vitro* fermentation using the ANKOM Technology

F50 filter bags were rinsed in acetone for 5 minutes to remove surfactant that may reduce microbial digestion ability before drying to use for the experiment. Bags were weighed to get W_1 and the balance was zeroed before a 0.5g sample (W_2) was directly weighed into the filter bag. Bags were heat sealed and placed in a Daisy incubator digestion jar. Each jar contained 25 bags plus a sealed blank bag as a correction factor (C_1).

4.2.1.3 Rumen inoculum preparation

Rumen fluid from Nguni cows reared in natural pastures was collected at Eshowe abattoir shortly after slaughter. Rumen fluid with some particulate matter was transferred into a five litre container, which had been pre-heated and flushed with CO_2 in an airtight thermos flask (38°C). This was then transferred to the laboratory of the University of Zululand for further processing. While in the Laboratory, the rumen content was transferred into a blender that had been purged with CO_2 and pre-heated with warm water before blending at high speed for about 30 seconds. The blending activity aimed to dislodge rumen microbes attached onto a fibrous mat. Blended rumen material (RI) was then filtered through four layers of cheese cloth into a warm beaker that had been flushed with CO_2 prior use.

4.2.1.4 *In vitro* digestibility of *Kigelia africana*

Digestibility was done using the ANKOM Technology method as described by Vogel (1999) where the experiment had three (3) pseudo replicates for each extract. The ANKOM machine contains four different 2 litre jars for incubation but only two were used. Each jar was filled with 1600ml of salivary buffer (from a mixture of solution A and B (1:5) (266ml of Reagent A (78.4g $NaHCO_4$, 29.6g $NaHPO_4$, 4.56g KCl, 3.76g NaCl, 1.04g $MgCl.6H_2O$) was mixed with 1330ml Reagent B (5.03g $CaCl_2.2H_2O$)) that had been pre-warmed to 39°C . Total of 22 filter bags (4 Wf, 4 Ex, 4 En, 4 S, 4 En+S and 2 blank (C)) was transferred into each jar and mixed thoroughly before adding 400 ml of RI into each digestion jar which was continuously purged with CO_2. The machine was then switched on for 48 hrs of incubation at 39°C. After 48hrs, jars were removed, the fluid was drained and samples were oven dried at 60°C to constant mass. For apparent digestibility, the following formula was used to calculate;

$$\mathrm{APD} = \left(\frac{100 - (W_3 - (W_1 \times C_1))}{W_2}\right) \times 100$$

Where:
W_1 is the bag tare weight
W_2 is the sample weight
W_3 is the bag weight after in vitro treatment.
C_1 is the correction factor (final oven dried blank/ original blank.

The remaining soluble material attached to the residue fragments was removed using a neutral detergent solution in other to calculate true digestibility values. NDF and ADF digestibility were analysed by subjecting the bags into an acid detergent solution and 70% H_2SO_4, respectively. The difference between true digestibility (TD) and apparent digestibility (APD) was microbial yield (MY).

4.2.2 Worm mortality test

4.2.2.1 Feed portion extraction and concentration

The sausage fruits were harvested from four different trees and dried as described in section 4.2.1.1. The ground *Kigelia africana* fruit samples (Ex, En, SS and Wf each with three pseudo replicates) were subjected to Buchi R soxhlet extraction, where 10g sample was weighed directly into thimbles. For extraction of phenolics, 70% ethanol was used as a solvent (10g sample per 100ml 70% ethanol) where thimbles containing samples were boiled in Soxhelet extractor; solvent containing sample phenolics settled on the borosilicate glass reagent bottles placed on the bottom of the columns. Clear solvent that evaporates during boiling was discarded. After 48hours solvent containing sample phenolics was removed and temporally stored in 500ml reagent bottles. A similar procedure was repeated four (4) times for each extract resulting in four (4) separate 10g of each extract per 100ml solvent. For each extract this was mixed together in 500ml reagent bottles.

For concentration, a 100ml mark was made on new clean borosilicate glass reagent bottles, which were used for concentration of the solvent (previously stored in 500ml reagent bottles) from 40g extract per 400ml solvent to 40g extract per 100ml solvent. This was achieved by continuously boiling the solvent containing phenolics while removing the clear evaporated solvent until the remaining solvent containing phenolics reaches the 100ml mark on borosilicate glass reagent bottles. The final product which 40g extract per 100ml solvent was stored in the refrigerator at 5°C.

4.2.2.2 Faecal egg count

Faecal egg count was done before culturing. Samples with low EPG score were not cultured. Faecal egg count for three faecal samples from a Merino sheep prior culturing were 2500 EPG, 2300EPG and 2600 EPG. Such infestation gave a go-ahead to culturing of faecal samples.

This ensures that collected faecal samples contain enough eggs to hatch. Collection bags were used to collect faecal samples from non-dewormed sheep feeding on pastures at University of KwaZulu-Natal uKulinga Research Farm. Clean collection bags were worn on the animals and removed early in the morning the following day. A cooler box with ice was used to store faecal samples as warm environment can

allow eggs to hatch. Faecal samples were crushed by hand and later, a blender was used to dissolve them into a sugar to salt solution at 4:6 ratio. A sieve was used to completely sieve out faecal debris. Using a McMaster slide, clear solution was observed under a microscope at 10x magnification. A microscope counter was used to count eggs from each chamber (Cringoli et al., 2004). Faecal egg count for three faecal samples of a Merino sheep prior to culturing were 2500 EPG, 2300EPG and 2600 EPG. Such infestation gave a go-ahead with culturing for faecal larvae mortality.

4.2.2.3 Faecal culture

Faecal samples collected for egg count were used for faecal culture. For culturing, the method explained by Bellaw and Nielsen (2015) was used, where 5g of faecal samples were weighed and mixed with vermiculite on 1:1 ratio in a petri dish. Where the mixture appeared too dry, a little bit of water was added to each petri dish. Petri dishes containing vermiculite and faecal samples were incubated at 27°C for 12 days. During the incubation period, petri dishes were watered daily to keep the culture moist. This is because warm and moist environments are suitable for hatching and the growth of nematodes.

4.2.2.4 Treatment preparation and dosing

Each extract (Ex, En, SS, and Wf) solution was diluted to give 5g, 10g, 15g and 20g *Kigelia Africana* extracts in 100ml. Faecal culture was treated on the 13th day by dosing each petri dish 5ml of each *Kigelia africana* extract at different doses (5g, 10g, 15, and 20g). Each dose level had 5 pseudo replicates. For control, three (3) petri dishes were dosed with 5ml 70% ethanol without extract. A bit of vermiculite was added whenever the culture appeared to be too wet to prevent larva from drowning. After treatment petri dishes were incubated for 36 hours prior harvesting.

4.2.2.5 Harvesting of the L3 Larva

Baerman's technique as explained by Bellaw and Nielsen (2015) was used to isolate L3 larva that had survived treatment, where rubber tubes were attached to labelled funnels. Each tube was sealed tight at the end using elastic bands. The funnels were then placed onto a stand. Treated faecal culture was removed from petri dishes and wrapped with a single clean layer of cheesecloth. The double layer prevented larva

from escaping. Sealed cheesecloth containing faecal culture was place on each funnel. Warm distilled water at 27°C was used to completely submerge the cheesecloth containing faecal culture. Larva that survived treatment were expected to swim past the cheesecloth and settle at the bottom of the tube.

4.2.2.6 Worm mortality test

After 24 hours, elastic bands were loosened and 6ml was collected at the bottom of the solution to sample for live L3 Larva. The 6ml solution containing L3 larva was transferred into a test tube. A pasture pipette was used to draw the contents from the bottom of the test tube into a Macmaster slide. A microscope and counter were used to observe and count larva in both chambers. Any larva present in the slide were regarded as alive as they were able to swim past the cheesecloth, those dead were assumed to be killed by an unfavourable environment after isolation.

4.2.3 Statistical analysis

Comparisons of means of digestibility values and helminth mortality test values were performed by one-way analysis of variance (ANOVA) using the statistical packages for social sciences SPSS (IBM 2013). Means were separated using least significant difference at 5% ($LSD_{0.05}$) probability level. Students Newman's test was used to establish difference between different treatments (Ex, En,SS and Wf) at the same level of treatment (5g, 10g, 15 or 20g) where $P< 0.05$.

4.3 Results

There were differences ($P< 0.05$) in APD values of *Kigelia africana* whole fruit and its portions (Table 4.1). Whole fruit and Ex had the lowest APD values while En+SS and SS were much higher (Table 4.1). TD, Ex and SS showed the lowest values while En and En+SS had the highest values. There was a significant ($P< 0.05$) difference in MY values of *Kigelia Africana* fruit extracts with En and En+SS having the highest values. There was no difference ($P>0.05$) in ADFD of *Kigelia africana* fruit extracts though seeds had the lowest ADFD compared to all other fruit extracts. There were also differences observed in HemiD values across extracts, with En and En+SS having the highest values while SS had the lowest. CelluD appeared to be very low in Ex while it was high in En+SS.

Table 4.1*: In vitro* digestibility of *Kigelia africana* fruit and its fragments

Extract	APD (g/kg)	TD (g/kg)	MY (g/kg)	ADFD. (g/kg)	Hemi D (g/kg)	Cellu D (g/kg)
Wf	274.48[a]	384.62[a]	110.14[a]	116.20[a]	268.42[ab]	47.69[a]
Ex	226.40[a]	321.00[a]	94.60[a]	97.26[a]	223.74[a]	14.88[a]
En	375.76[b]	539.32[b]	163.57[b]	105.28[a]	434.05[c]	19.59[a]
En+SS	392.80[b]	554.46[b]	161.67[b]	183.26[b]	371.20[bc]	115.20[b]
SS	182.45[a]	252.62[a]	70.17[a]	90.01[a]	162.62[a]	24.22[a]
SED	41.12	59.23	18.16	25.14	61.06	25.30
***P*-Values**	0.01	0.00	0.00	0.13	0.08	0.11

APD= Apparent digestibility, TD= True digestibility, MY= Microbial yield, ADFD= Acid detergent fibre digestibility, ADLD= Acid detergent lignin digestibility, Hemi D= Hemicellulose Digestibility, Cellu D= Cellulose Digestibility, SED = standard error difference, Wf= Whole fruit, Ex= Exocarp, En= Endocarp, En+SS= Endocarp plus Seeds, SS= seeds and [A,b,c,d] = Numbers in a column with different superscript letters are significantly different (P<0.05).

There was a significant difference (P<0.05) observed in L3 larva survival across *Kigelia africana* fruit extract treatments at different doses (Table 4.2). All the fruit extracts (Wf, Ex, En and SS) had lower numbers of larva that survived compared to the control. There was no significant difference (P>0.05) on larva survival between Ex, En, SS and Wf at different treatment levels (5g, 10g, 15g and 20g) but was much lower than the control with the highest (P<0.05) amount of L3 larva survival.

Table 4.2: Effect of *Kigelia africana* fruit extracts and doses on L3 Larva survival

Number of larva survived at different treatment levels					
Treatment	**5g**	**10g**	**15g**	**20g**	**P- Value**
Ex	6.00±1.7^{a}	5.00±1.9^{a}	6.00±2.3^{a}	8.00±5.3^{a}	0.64
En	4.00±2.1^{a}	4.00±1.8^{a}	5.00±1.3^{a}	13.00±8.5^{a}	0.63
SS	4.00±1.5^{a}	6.00±2.7^{a}	3.00±1.3^{a}	8.00±4.4^{a}	0.04
Wf	6.00±1.0^{a}	8.00±3.1^{a}	16.00±4.4^{a}	6.00±1.8^{a}	0.84
Co	199±44.7^{b}	199±44.7^{b}	199±44.7^{b}	199±44.7^{b}	-
P- Value	0.00	0.00	0.00	0.00	

Wf= Whole fruit, Ex= Exocarp, En= Endocarp, SS= seeds, Co= Control and ^{A,b,c,d} = Numbers in a column with different superscript letters are significantly different (P<0.05).

There was a significant difference (P<0.05) between larva percentage mortality in all treatments and the control (Table 4.3).

Table 4.3: Percentage larva mortality at different doses of *Kigelia africana* fruit treatments.

	Percentage mortality(%) at different treatments			
Treatment	**5g**	**10g**	**15g**	**20g**
Ex	98.50±0.86^{b}	96.66±0.93^{b}	97.16±1.17^{b}	96.15±2.70^{b}
En	98.00±1.04^{b}	98.17±0.88^{b}	97.67±0.67^{b}	93.45±4.29^{b}
SS	97.83±0.73^{b}	97.17±1.33^{b}	98.33±0.67^{b}	96.00±2.18^{b}
Wf	97.00±0.50^{b}	96.00±1.53^{b}	92.13±2.24^{b}	96.83±0.88^{b}
Co	0.00±22.45^{a}	0.00±22.45^{a}	0.00±22.45^{a}	0.00±22.45^{a}
P- Value	0.00	0.00	0.00	0.00

Wf= Whole fruit, Ex= Exocarp, En= Endocarp and SS= seeds, Co= Control and ^{A,b,c,d} = Numbers in a column with different superscript letters are significantly different (P<0.05).

Mortality percentages were highest in Ex, En, SS and Wf when compared to the control with the least. At different treatment levels (5g, 10g, 15g and 20g), mortality percentage was higher in all extracts (Ex, En, SS and Wf) than the control with the least mortality.

4.4 Discussion

The results from both APD and TD showed that Wf and its portions (Ex, En, SS, En+SS) can be degraded or fermented by rumen microbes. It was also noticed that TD for En and En+SS were more than 50% whereas TD for Ex and Wf was about 40%. The main difference between them was that Ex and Wf had more fibrous material which was more difficult to digest unlike En and En+SS with less fibre but more solubles. Seeds were also found to have the least TD. This was associated with the hard high fibre because seeds were ground with the hard seed coat. The amount of fibre in Ex was much higher than in Wf and that was reflected in their digestibility where Wf was about 9% higher in TD than Ex. The lignin levels were also highest in the seed which is also one of the reasons that could have limited its degradability (Lebo et al., 2001). Different studies have reported that less than 50% of most of the forages or hays consumed by ruminants are being digested (Reinecke, 2016; de Paiva et al., 2017; Griffith, 2017; Lipolis, 2017; Chowdhury, 2018). For example, studies by Che (2017) showed that corn gluten meal was only 55% digested while the study of Brown (2017) and Brown (2016) showed that *Acacia karoo* forages were only 49% digested. Work by Shumbusha (2017) also revealed that sweet potato vines were over 50% digestible. These values denote that *Kigelia africana* fruit digestibility is within the digestibility range of different forages in ruminants and needs more experimentation to establish its utilisation (Sitnikov et al., 2016; Mapato, 2018).

Microbial yield is the fraction of feed which is converted to biomass or ATP (Lipson, 2015). En degradability yielded higher MY while Ex had the least. This is because Ex has high lignin and fibre content which has a negative effect on digestibility of feedstuff (Gressley et al., 2011) or is less soluble to boost that initial energy required for microbial population growth. There was a significant difference between ADFD of *Kigelia africana* fruit portions where En+SS had the highest degradability compared to other samples. It was due to more soluble matter present in fruit fibrous pulp, that boosted microbial growth which then increased fibre breakdown (Dada et al., 2010;

Wilkinson, 2015). There was no significant difference among CelluD values across *Kigelia africana* extracts. This is because cellulose and hemicellulose form part of the indigestible fraction of the feed (Griffith, 2017). Ex and En are made of fibrous pulp which is composed of various fibre components (Jackson and Beckett, 2012). Low celluD on Ex and SS may be linked with high lignin content within these two portions. A study by Parente (2017) showed that babassu fruit endocarp was highly digestible compared to its exocarp.

High CT concentrations in feed have the potential to reduce microbial activity. They do so by forming insoluble tannin-protein complexes which reduce microbial protein availability hence reduce microbial activity (de Paiva et al., 2017). Min et al. (2003) reported that when diet was changed from perennial rye grass (which does not contain CT) to *L. corniculatus* (32 g CT/kg DM) in sheep, the populations of rumen proteolytic bacteria decreased. This was associated with insoluble tannin-protein complexes that reduce microbial activity, hence reducing microbial protein availability. This was probably one of the reasons why TD and MY in Ex were much lower since it had relatively higher levels of condensed tannins. This implies that CT can actually affect degradation rate which can possibly lead to poor utilisation of feed, low availability of nutrients for growth, production, reproduction and body maintenance (Minson, 2012). However, with the presence of tannins, the digestibility of Wf and its portions were still relatively high compared to other forages reported in the literature (Chivandi et al., 2011). Although tannin does have a negative effect on digestibility, smaller quantities have been reported to be beneficiary to ruminants (Paolini et al., 2003; Wurger, 2013). Smaller quantities in the feed have been reported to have anthelmintic activities hence its effect on L3 larva that was monitored in this study. The *Kigelia africana* has been used as an antibacterial (gram positive and gram negative), antifungal, antimicrobial and widely in the phytochemistry industry (Arkhipov et al., 2014).

A recent study by Fomum and Nsahlai (2017) showed that phenolic compound extracts from different plants reduced worms in merino sheep at the University of Kwazulu-Natal Ukulinga farm that were infested with 87.27% *Haemonchus Contortotus*, 7.34% *Trichostrongylus* and 5.39% *Oesophagostomum spp*. In this study, Kigelia Africana fruit treatment contributed to a more than 96% mortality rate on gastro-intestinal nematodes (GINs) of a merino sheep. More larva mortality was observed in 5g treatment level. Results showed no major differences between

treatment level and the larva mortality. This implies that there is no significant difference between number of larva survived at 5g and those that survived at 20g treatment level. This implies that 5g was the right dose after which, there was no further dosing could then promote toxicity in the animal. The wide distribution of secondary compounds in *Bignoniaceae* family plant species (Jeyachandran and Mahesh, 2007; Azu et al., 2010b; Jackson and Beckett, 2012; Chenia, 2013) is a very important factor to note as an inherited characteristic of the fruit. Condensed tannins play a crucial role in phytochemical use of *Kigelia africana* (Hoste et al., 2006; Kilmister et al., 2014; Lorenz et al., 2014). Larva mortality rate percentage is not linked to condensed tannin concentration only because SS (Low CT concentration) had the same effect on mortality percentage as Ex and Wf, which had much higher CT concentration. Therefore, the presence of other secondary compounds like alkaloids, iridoids, flavonoids and napthoquinones might have played a similar role as CT in increasing L3 larva mortality percentage (Grace, 2002; Makkar et al., 2009; Jackson and Beckett, 2012).

4.5 Conclusion.

Kigelia Africana fruit portions showed modest true digestibility except for Ex and SS. This means En, En+S and Wf can serve as a potential source of nutrients for ruminants. These portions also appeared to have strong anthelmintic value regardless of the dosage level. The average L3 larva mortality rate was above 95% in fruit portions irrespective of the dosage level but 5 g application seems to be the preferred dosage. Ruminants may have a hard time cracking the hard coat of *Kigelia Africana* fruit which can result in high energy demands but removing the exocarp can leave the endocarp which is more digestible and can be easily utilized by ruminants as a cheap potential feed source.

Chapter 5

5.1 General discussion

Kigelia africana fruit portions that appear to have high moisture content prior to this research were confirmed to have more that 60% moisture, if harvested fresh. A study by Pritchard (2004) reported that *Kigelia africana* seeds are desiccant-sensitive which explains the high moisture content in these fruits. Exocarp had the highest neutral detergent fibre (NDF) and dry matter content which is due to the presence of strong fibrous material accompanied by anti-nutritional factors that play a huge role in protection of the inner nutritious seed and prevention of herbivory (Calderon, 2011; Brown, 2017; Graupner, 2017). Lignin provides structural rigidity to plants (Eloy, 2017; Griffith, 2017) and that was probably one of the main reasons why Ex digestibility was more inhibited. *Kigelia africana* fruit has a woody appearance and that explains the high acid detergent lignin in the exocarp. The hard coat of the seeds appears to be the main reason why high NDF and ADF content were also observed in seeds. This hard shell actually provides a protective layer to the seed endosperm (Bouman, 2017). Endosperm provides nutrients for embryonic tissue growth which requires protein (Chivandi et al., 2011; Bouman, 2017). This is why high protein content was observed in *Kigelia Africana* seeds. In contrast, exocarp had very low protein content but high condensed tannin concentration. This is due to the presence of anti-nutritional factors like condensed tannins and lignin in the exocarp that work in conjunction to provide fruit protection (Kumar and Singh, 1984; Hossain, 2016; Graupner, 2017). This protection is in the form of polymeric components of the cell wall that provide strength to plants. (Liu, 2016). Lignin content in vascular plants is high mainly on the stem and barks (Lebo et al., 2001) but the fruit bark is hard and appears woody with high fibre. This explains high shear force on the exocarp than endocarp. Esehaghbrygi (2009) reported that maize stalks with high moisture content required relatively low shear force compared to maize stalks with low moisture content. *Kigelia Africana* fruit endocarp also had high moisture content which explains its low shear force compared to the exocarp that appeared to have relatively low moisture content. Endocarp shear force was about half to that of the exocarp. High shear force on the exocarp can result in ruminants squandering high energy that could have been used for production (Minson, 2012). Therefore, removing fruit exocarp can improve consumption of the endocarp without difficulty.

Furthermore a study by Akimoto (2003) reported that cattle have a bite force of 3280N while horses have 6350N. This means cattle and horses can both utilize this fruit as feed. Upon utilization, nutrient degradability plays a huge role in absorption and utilization of nutrients in animal bodies (Herron, 2015; Griffith, 2017). Digestibility of feed stuff is largely influenced by the fibre constituents present in that particular feed stuff (Raffrenato et al., 2017). Hemicelluloses are easily digestible components of fibre compared to complex crystalline cellulose. However, lignin in plant cell walls is not hard but cements plant wall structure making it very difficult for rumen microbes to digest. Therefore lignin is inversely proportional to digestibility (Purwantari, 2009; Sitnikov et al., 2016). Likewise in this study exocarp, which was found to have high lignin content, had low apparent and true digestibility values. Endocarp and endocarp plus seeds appeared to have more than 50% digestibility coefficient. This is due to low lignin content in these two portions. These results are also supported by Purwantari (2009) where leaves of *soghurm stipodeum* grass had higher neutral detergent fibre digestibility than its stems. This is because stems are reported to have higher lignin content then leaves (Herron, 2015). Endocarp and endocarp plus seeds had higher microbial yield. This is because microbes were able to digest the relatively higher soluble material that provided the energy for rumen microbes before commencing the fibrous *Kigelia africana* fruit pulp digestion. Hemicellulose digestibility was high in endocarp plus seeds this is because rumen microbes can easily ferment hemicellulose and cellulose (Galyean and Goetsch, 1993; Herron, 2015).

Condensed tannins can be advantageous or disadvantageous depending on the concentration level in feed. High condensed tannin concentrations are reported to interfere with protein absorption. This occurs by formation of insoluble protein-tannin complexes which prevent digestion of proteins (Lorenz et al., 2014; Msimango and Fon, 2016; Griffith, 2017). *Kigelia africana* fruit exocarp had high condensed tannins for a chemical defence mechanism (Elhag, 2015; de Paiva et al., 2017). *In vivo* study by Coop and Kyriazakis (2001) reported that previously infected sheep were drenched with Quebrancho extract at 4%, 8% and 16% condensed tannin concentration. This resulted in reduced faecal egg count where 16% had much lower faecal egg count compared to both 8% and 4% CT concentration. *Kigelia Africana* fruit has been reported to be rich in secondary compounds thus it has been widely used in the ethno-

medical industry (Azu et al., 2010c; Lim, 2012; Min et al., 2015). *Kigelia africana* fruit extracts showed strong anthelmintic activity against faecal samples containing 87.27% *Haemonchus Contortotus*, 7.34% *Trichostrongylus* and 5.39% *Oesophagostomum spp.* Extracts had about 96% L3 larva mortality percentage. An *in Vitro* anthelmintic study by Jamous (2017b) revealed that plant extracts from the Rhamnaceae family showed about 70-80% anthelmintic activity against 20% *Teladorsagia circumcincta* and 80% *Trichostrongylus colubriformis* which are not very far from the results obtained from this study.

5.2 General conclusion

The results obtained from this study showed that *Kigelia africana* can serve as a potential feed supplement as demonstrated by the different parameters that were measured. The high moisture content displayed by *Kigelia africana* fruit showed that it can provide a significant amount of feed water especially if the endoderm is used. The protein content was as high as those found in much forages making it a potential supplement that can enhance microbial fermentation in ruminants. Endocarp and endocarp plus seeds also displayed a high digestibility coefficient which means more nutrients will be available for absorption by ruminants but most interestingly the bite force required to break open these seeds is much smaller than the force that can be generated by the jaws of a cow and a horse. It was also concluded that breaking the hard exocarp reduced the force required for chewing hence increasing the surface area for enzyme or microbial activity. Apart from providing moisture and nutrients, this fruit also showed strong anthelmintic properties which surpass some of the widely used vermifuges that are expensive yet have gradual inefficiency due to parasite resistance.

5.3 Recommendations

It will be right to recommend that this fruit can be used especially in winter or during drought to increase water supply as feed water.

The chemical constituents of the feed parts showed that the endocarp was the best portion that should be fed to ruminants due to its relatively high digestibility and lower shear force.

It is also recommended that this fruit can be used by rural poor farmers to treat their animals for gastro-intestinal nematodes, at a 5 g concentration but this should be properly investigated *in vivo* as this was only an *in vitro* study.

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