The medicinal properties of Moringa (*Moringa oleifera* Lam) leaves and the effect of its use as a supplement on goat growth performance and meat characteristics

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Declaration

I, Busani Moyo, hereby declare that this research is an outcome of my own investigation under the supervision of Prof P.J. Masika and Prof V. Muchenje; and has not been previously submitted to any University. Where reference to other researchers' work has been made and where assistance was rendered; has been duly acknowledged in the text.

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List of abbreviations

a*	redness of meat
ADF	acid detergent fibre
ADG	average daily gain
AOAC	Association of Official Analytical Chemists
b*	yellowness of meat
BCS	body condition score
BWT	body weight
CP	crude protein
DM	dry matter
FAMACHA	Faffa Malan chart
FLC	faecal larval count
GH	grass hay
GLM	generalised linear models
*L**	lightness of meat
LTL	*m. longissimus thoracis et lumborum*
MOL	*Moringa oleifera* leaf meal
MUFA	Monounsaturated fatty acids
n-3	omega-3 fatty acids
n-6	omega-6 fatty acids
NDF	neutral detergent fibre
NRC	National Research Council
PDIFF	probability difference
PCV	packed cell volume

PUFA	polyunsaturated fatty acids
SAS	Statistical Analysis System
SC	sunflower seed cake
SFA	saturated fatty acids
WBSF	Warner Bratzler shear force

Abstract

The medicinal properties of moringa (*moringa oleifera* lam) leaves and the effect of its use as a supplement on goat growth performance and meat characteristics

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The main objective of the study was to determine if feeding goats with *Moringa oleifera* leaves would lead to an increase in productivity and in value of the meat. The proximate, van Soet, atomic absorption spectrophotometric and soxhlet extraction methods were used to determine the nutritional value *M. oleifera* leaves of the South African. The *in-vitro* antimicrobial screening methods were used to determine antimicrobial activities *M. oleifera* extracts while *in vitro* and *invivo* models were used to determine the antioxidant activities of *M. oleifera* leaves. An evaluation of the potential of *M. oleifera* leaf meal as a feed supplement in terms of its effect on helminth load, goat growth performance, carcass characteristics, meat quality attributes, nutritional and consumer sensory characteristics of goat meat was done. A total of 24, eight month old goats were randomly allocated to dietary treatments of *M. oleifera* leaf meal (MOL), sunflower seed cake (SC) and GH (grass hay) which was the control. All the groups were fed on basal diet of grass hay *ad libitum* and 200g wheat bran per head per day. The MOL group was given an additional 200 g of dried *M. oleifera* leaves while the SC group was offered 170 g sunflower seed cake per head/day. The study showed that the dried leaves had crude protein levels of 30.3 %, polyunsaturated fatty acids (52.21 %), Saturated fatty acids (43.31), *n-3* (44.57 %), *n-6* (7.64 %), 19 amino acids, vitamin E (77 mg/100 g) and Beta-carotene (18.5 mg/100 g). The *M. oleifera* leaf extracts showed antibacterial activities against *Escherichia coli, Enterobacter cloace, Proteus vulgaris, Staphylococcus aureus* and *Micrococcus kristinae*. The

supplementation of goats with MOL and SC resulted in decreased feacal larval count and lower *Haemonchus contortus*, *Trichostrongylus colubriforms* and *Oesophagastum columbianum* worm burdens than those in the non-supplemented goats. Goats supplemented with SC and MOL had higher average daily weight gain and heavier carcasses than those in the GH group. Higher pH_1 scores were observed in chevon from GH diet than the supplemented ones. The MOL and SC supplemented goats had chevon with higher values for lightness (L*) 24 hr *post-mortem* than the one from the GH group. The redness (a*) values of chevon 24 hr *post mortem* was highest in MOL supplemented goats. Warner Bratzler shear force (WBSF) values of SC (30.1 N) and MOL (29.8 N) supplemented goats were lower than those from GH diet (32.6 N). Chevon from goats fed GH diet had significantly higher cooking losses (29.5 %) than that from MOL (25.4 %) and SC (25.6 %) fed groups. It was observed that chevon from MOL and SC supplemented groups had higher crude protein (23.57 and 22.95 %, respectively) than the one from the GH group (21.20 %). Cholesterol levels were higher in chevon from SC (42.84) supplemented goats than those from MOL (38.76) and GH (35.63 mg). Chevon from GH and MOL group had higher ($P < 0.05$) proportions of PUFA, *n*-3, PUFA/SFA ratio and lower *n*-6/*n*-3 ratio. Mean consumer scores for first bite, aroma, flavour and juiceness were higher in the MOL group than in the GH group ($P < 0.05$). The acetone extract exhibited higher concentrations of total flavonoids, flavonols, phenolics. The acetone extracts depicted higher percentage inhibition against DPPH, ABTS and nitric oxide radicals which were comparable with reference antioxidant (vitamin C and BHT). The *M. oleifera* leaf meal increased the antioxidant activity of GSH, SOD and catalase. *Moringa oleifera* leaves also exhibited medicinal properties by having anthelmintic, antibacterial activities and showed antioxidant properties. It was also observed that protein

supplementation improved the animal growth performance, the physico-chemical characteristics, nutritional and fatty acids composition of meat hence meeting the consumer needs.

Dedication

I genuinely dedicate the success of this research study to God, my wife Sipho Moyo, my sons (Busiso and Bukhosi), and the Moyo Families.

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Chapter 1: Introduction

1.0 Background

Goats play a vital role in the livelihoods of subsistence farmers by contributing significantly towards food security and poverty alleviation through the provision of animal protein, income generation and as well as being used for socio-cultural purposes (Rumosa Gwaze *et al*., 2009). Nevertheless, goat production and productivity in most subsistence farming areas which are based mainly on extensive system, characterized by poor management and low productivity, is faced with numerous challenges, such as inadequate nutrition, diseases and parasites (Campbell, 2003; Rumosa Gwaze *et al*., 2009). Inadequate supply of nutrients such as protein, vitamins and minerals causes nutritional deficiency diseases and hence impede animal growth performance (Brisibe *et al*., 2008). This in turn could predispose animals to the development of infectious diseases as their immune system need adequate supply of protein, vitamins and minerals (Jayarajan and Daly, 2011). Goats that are well fed are better able to resist diseases when exposed to infection than those which are already weakened due to malnutrition (Hart *et al*., 2007). To mount the immune response, energy and protein are required in the manufacture of antibodies and cells. Specific nutrients such as minerals (calcium, zinc, copper and iron), fatty acids and, vitamin A and E are also needed (Jayarajan and Daly, 2011). In addition, improving nutrition of goats, especially with protein and minerals, increases their resistance to helminth infection and improve digestibility of feeds (Brisibe *et al*., 2009).

Natural rangelands in South Africa can no longer provide sufficient forage due to encroachment by settlements, crop production, unpalatable plant and grass species and frequent droughts (Meadows and Hoffman, 2002). As a result, farmers resort to supplementing their livestock with

the expensive concentrates, which are usually expensive and unaffordable to most resource-limited farmers (Roland-Host and Otte, 2007).

Supplementation of livestock with diets high in protein is often important to improve livestock performance, and this needs to be done according to the requirements of the animal (Sarwatt *et al*., 2002). Various conventional protein supplements such as soybean meal, sunflower seed cake, cotton seed cake and fish meal have been widely and successfully used (Reyes-Sánchez *et al*., 2006). However, these protein sources are scarce and their prices have been escalating recently (Roland-Host and Otte, 2007). There is increasing competition between humans and livestock for these protein sources due to their rapid population growth. Furthermore, the grasses are of low quality, with the crude protein ranging between 2- 6 % (Stapelberg *et al*., 2008) during winter. The problem is further worsened by overgrazing and invasion of unpalatable, nutritionally inferior grass and plant species which reduce the grazing value of the veld such as *Cymbopogon plurinods, Sporobolus pyramidalis, Sporobolus natalensis* and *Pogonathria squarrosa* (Sarwatt *et al*., 2002). There is need, therefore, to identify and explore use of non-conventional feed sources that have the capacity to yield the same protein output as conventional feeds and perhaps at affordable prices.

Use of non-conventional protein supplements has a potential to reduce livestock production costs and improve livestock productivity, thereby ensuring cheaper products such as milk and meat. At the same time it will reduce the requirements of conventional protein sources. The possible alternative cheap protein sources are the forage trees, many of these have long life spans and low maintenance demands (Reyes-Sánchez *et al*., 2006). They are used in livestock production for

various purposes that include feeding. Forage trees are sources of some essential nutrients such as vitamins and minerals (Sarwatt *et al*., 2002).

Besides being natural supplements, forage trees have been explored recently as sustainable alternatives for many diseases afflicting the global livestock industry and they seem to be quite efficacious (Brisibe *et al*., 2009). One such plant that is reported to have nutritional, therapeutic and prophylactic properties is *Moringa oleifera* Lam, commonly referred to as the drumstick tree (Fahey, 2005; Reyes-Sánchez *et al*., 2006). It is the most widely cultivated species of a monogeneric family, moringaceae, in the tropics and subtropics (Fahey, 2005; Monera *et al.*, 2008). The plant is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005). *Moringa oleifera* is a rapidly growing tree which is easy to establish, making it easy for farmers to cultivate and has good coppicing ability, as well as good potential for forage production. It can be used in the aforestation and reforestation programmes, therefore, it becomes an ideal tree to be grown because of its drought resistance and ability to grow on poor soils (Monera *et al.*, 2008). It is reported to grow well in areas receiving an annual precipitation of 500 to 1 500 mm and annual temperatures from 18.7 to 28.5˚C (Marcu and Pharm, 2005).

All plant parts of *M. oleifera* are edible and have long been consumed by humans as the plant is highly nutritive (Foidl *et al*., 2001; Amaglo *et al*., 2010). The plant has also been used as traditional medicine for centuries (Marcu and Pharm, 2005). Furthermore, its leaves can be used as a protein feed supplement for livestock (Fugile, 2005). Research in other countries has shown that this plant has important nutrients such as proteins, which range from 16 to 40 % (Foidl *et al.*, 2001; Marcu and Pharm, 2005). Advantages of using *M. oleifera* for protein source are

numerous, they include the fact that it is a perennial plant, with coppicing ability, which can be harvested several times in one growing season and has also the potential to reduce feed cost. It also has some tannins which play a significant role in the nutrition of animals, causing beneficial effects on nutrient utilization, health and production (Foidl *et al.*, 2001; Marcu and Pharm, 2005; Hoste *et al*., 2006). A feed with condensed tannins may affect feed intake, digestibility and rumen microbes' growth and morphology (Louvandini *et al*., 2006; Cenci *et al*., 2007; Waghorn, 2008). However, consumption of condensed tannins by animals has been reported to suppress the population of gastro-intestinal parasites (Minho and Abdalla, 2007; Xhomfulana *et al*., 2009). Considering that protein supplements are scarce and expensive, this underscores the necessity to assess *M. oleifera* as an alternative supplementary livestock feed and its potential medicinal effect on livestock production. Use of *M. oleifera* in communal areas has a potential to limit the challenge of feed shortage especially during winter seasons.

Moringa oleifera has been reported to possess both nutritional and medicinal properties (Foidl *et al.*, 2001; Marcu and Pharm, 2005). Such multi-purpose plants like *M. oleifera* have a potential to reduce the dependence on expensive conventional protein supplements and commercial drugs (Okoli, *et al*., 2002). Furthermore, some consumers now prefer organic products; therefore the use of such plants gives the resource-limited farmer greater advantage in the production of organic product (Muchenje *et al*., 2010).

Animal products, such as meat quality or characteristics are generally influenced by the type and quality of feed consumed by the animal. Poor nutrition in animals, which characterizes many communal areas of South Africa leads to meat of low quality. Use of supplementation has often

been suggested as an approach in improving goat nutrition and meat quality. Despite the shortage of feed, farmers should aim at producing high quality products that are acceptable to modern consumers who are concerned about safe meat with no undesirable effects on their health (Andersen *et al.*, 2005).

In South Africa, most research has concentrated largely on the improved breeds such as Boer goats, neglecting those kept by most rural farmers (Xhosa lop eared and Nguni goats) (Malan, 2000; Campbell, 2003). These goats (Xhosa lop eared and Nguni) provide meat (chevon) which is popular meat throughout South Africa, with greatest production and consumption in subsistence farming areas (Abia and Fry, 2001)**.** Chevon is acceptable to consumers because of its aroma and palatability. In addition, chevon is leaner and contains less cholesterol and fat than both lamb and beef (Baron and Andersen, 2002). Chevon has been reported to have lower lightness and higher redness than lamb, mainly due to the lower intramuscular fat of goat carcasses (Simela and Merkel, 2008).

However, no studies have been done on *M. oleifera*'s nutritional composition, medicinal properties and its effects as a supplement on the following parameters in goats: growth performance, carcass and non-carcass traits, meat's physico-chemical characteristics, fatty acid profile and consumer sensory scores in South Africa.

1.1 Justification of the Study

Despite the high crude protein content of *M. oleifera* leaves, there is paucity of information on the effect of *M. oleifera* leaf diet on goat growth performance, chevon physico-characteristics,

and fatty acid composition. In addition, its antioxidant and anthelmintic information is lacking. Such information is needed in devising alternative feeding strategies to improve goat meat yield and quality in the subsistence farming areas. Moreover, feeding locally adapted breeds such as Nguni, Xhosa lop eared and their crosses with *M. oleifera* have the potential to produce more acceptable and healthy goat meat with minimal use of anthelmintics and antibiotics. Most of the research on *M. oleifera* has been concentrated on human beings. Therefore, research on the use *of M. oleifer*a and evaluation of its chemical composition, antibacterial, anthelmintic, antioxidants properties and its effect as a supplement feed on growth performance, carcass characteristics and meat eating quality traits on livestock is imperative. The widespread claims of *M. oleifera's* nutritional and medicinal properties on humans are inspiring to further investigate some of these properties on livestock.

1.2 Main Objective

The main objective of the study was to determine if feeding goats with *Moringa oleifera* leaves would lead to an increase in productivity and in value of the meat.

1.2.1 Specific Objectives

- To determine the nutritional value of *Moringa oleifera* leaves of the South African ecotype;
- To determine the antibacterial and antifungal activities of the *M. oleifera* leaf extracts;
- To determine the effect of supplementing goats with *M. oleifera* leaf on helminth load;
- To determine the effect of supplementing *M. oleifera* leaves on growth performance, carcass and non-carcass characteristics of goats;

- To determine the physico-chemical characteristics of chevon from goats supplemented with *M. oleifera* leaf meal;
- To determine the nutritional, fatty acid composition and consumer sensory scores of chevon from goats supplemented with *M. oleifera* leaf meal; and
- To investigate the antioxidant properties of *M. oleifera* leaves.

1.3 Hypotheses

The following null hypotheses will be tested

- *Moringa oleifera* leaves have no nutritive value;
- *Moringa oleifera* leaf extracts have no anti-bacterial and antifungal activities;
- *Moringa oleifera* supplementation to goats has no effect on helmintic load;
- Supplementing goats with *M. oleifera* leaf meal has no effect on growth performance, carcass and non-carcass characteristics;
- Supplementing goats with *M. oleifera* has no effect on physico-chemical characteristics of chevon;
- Supplementing goats with *M. oleifera* has no effect on nutritional, fatty acid composition and consumer sensory scores of chevon; and
- *Moringa oleifera* leaves have no antioxidant activities.

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CHAPTER 2: Literature Review

2.1 Introduction

Goats are important to resource-limited farmers, as they fulfill multiple roles such as the provision of meat, milk, manure, skins, cashmere, mohair (Haenlein and Ramirez, 2007), and barter trade (Morand-Fehr *et al.,* 2004). Goats, together with other livestock, are used as investments and status symbols in some societies as they generate income after their sales (Sweet, 2008). In addition, goats play a pivotal role in socio-cultural and ceremonial purposes (Kosgey and Okeyo, 2007; Simela and Merkel, 2008). de Vries and Pelant (2008) and Rumosa Gwaze *et al*. (2009) reported that goats can be exchanged or loaned to neighbours to enhance kinship ties. They are also useful in controlling bush encroachment in natural rangelands of Southern Africa (Saico and Abul, 2007).

Manure from goats is an invaluable source of organic fertilizer for maintaining or improving agricultural production especially for resource-poor farmers who cannot afford expensive inorganic fertilizers. Manure is an alternative organic fertilizer which can be used for the production of organic products (Hansson and Fredriksson, 2004). Therefore, goats are used to meet food security needs and improve welfare among resource-limited farmers. However, the productivity of goats is affected by shortage of feed and high incidence of helminths and diseases (Rumosa Gwaze *et al*., 2009). There is, therefore, a need to find alternative feed supplements which could alleviate feed shortages, and also have curative properties, which could reduce the disease incidence in goat production. This could improve the amount and quality of goat meat produced to meet the consumer demand.

One of the forage plants that could be used as an alternative protein supplement is Moringa (*Moringa oleifera*). *Moringa oleifera* Lam is the most cultivated species of the monogeneric family Moringaceae (order Brassicales), it includes 13 species of trees and shrubs distributed in sub-Himalayan ranges of india, Sri-lanka, North Eastern and South Western Africa, Madagascar and Arabia (Anwar *et al*., 2007). It is believed to be native to sub-Himalayan tracts of Northern India, but it has become naturalized in many locations worldwide in the tropics and sub-tropics (Reyes-Sánchez *et al*., 2006; Mendrieta-Araica *et al*., 2011). It is widely cultivated in Africa, Thailand, Burma, Singapore, West Indies, Sri-Lanka, India, Mexico, Malaysia and the Philippines (Fahey 2005; Reyes-Sánchezi *et al*., 2006). It is a tree that is nutritious, having almost all essential nutrients in adequate amounts (Anwar *et al*., 2007).

The tree ranges from 7 to 12 m in height, but is usually trimmed to make it easier to harvest the leaves and pods. It has tuberous roots, soft spongy wood, thin slender trunk (24 cm thick), wide spreading, drooping and fragile branches. The leaves are imparipinnate-rachis 3 to 6 cm long with 2 to 6 pairs of pinnules. Each pinnule has 3 to 5 elliptical leaflets that are 1 to 2 cm long and 0.3 to 0.6 cm wide. The terminal leaflet is oval in shape and the flowers are borne profusely in auxiliary, drooping panicles 10 to 25 cm long. They are fragrant, white or creamy-white with yellow stamens and 2.5 cm in diameter. The pods, borne singly or in pairs, are pendulous, brown, triangular, tapering at both ends; 25 to 45 cm long and 1.8 cm wide. Each pod contains about 16 seeds embedded in the pith. The pod split length-wise into three parts when dry. The seeds are round with a brownish semi-permeable seed hull with three white papery wings, embedded in dry, white, tissue-like pith. *Moringa oleifera* is propagated either by planting stem cuttings 1 to 2 m long or by seeding (Aregheore, 2002).

Moringa oleifera is drought tolerant and is reported to tolerate an annual precipitation of 500 to 1 500 mm and annual temperatures from 18.7 to 28.5˚C. It grows in a wide range of soil types, preferring neutral to slightly acidic soil (pH range of 4.5 to 8.0). *Moringa oleifera* is a fast growing tree which also has fast re-growth after pruning, and capacity to produce high quantities of fresh biomass per square meter even at high planting densities (Foidl *et al*., 2001). The dry matter (DM) yield is high, from 4.2 to 8.3 tons/ha when harvested every 40 days (Aregheore, 2002). It is a typical multipurpose tree of significant economic importance because there are several industrial and medicinal applications and various products to be used as food and feed which can be derived from its leaves and fruits (Anwar *et al*., 2007).

2.2 Nutritional composition of *Moringa oleifera* Lam Leaves

Recent researches indicated that the leaves of *M. oleifera* are concentrated sources of several macro and micro nutrients including proteins, vitamins (A, B C and E) and minerals (calcium, iron, potassium and magnesium) as shown in Table 2.1 (Fugile, 2005; Oduro *et al*., 2008). There is quite a lot of literature on the nutritional value of *M. oleifera* Lam leaves; however, the literature reports varying nutritional chemical composition. In addition, the information has been recycled for a long time. The possible sources of variations of the available nutritional data are due to factors, that include natural variation, sample preparation and analyses, physiological stage at harvesting and lastly human errors. Natural variation could be due to differences in genetic background of the plant (ecotype, cultivar), the environment (soil, climate, pathogens) and cultivation methods (inputs, harvesting frequency). Variation due to sample preparation and analysis may depend on the time between collection and analysis, the mode of preparation and

Table 2.1: Chemical composition of dried *Moringa oleifera* leaves

Nutrient	Mean value for 100g Dry matter	Reference
Protein (g)	22.5-29	Fahey, 2005; Rweyemamu, 2006; Broin, 2008; Oduro *et al*., 2008
Minerals (g)	9-11	Aslam *et al*., 2005; Broin, 2008
Lipid (g)	2.23-8	Rweyemamu, 2006; oduro *et al*., 2008
Carbohydrate s(g)	38.2-39	Aslam *et al*., 2005; Rweyemamu, 2006
Fiber (g)	10-19.2	Rweyemamu, 2006; Nuhu, 2010
Calcium (mg)	2003-2009	Rweyemamu, 2006; Nuhu, 2010
Copper (mg)	0.57-1.0	Rweyemamu, 2006; Oduro *et al*., 2008
Iron (mg)	28.2-28.3	Rweyemamu, 2006; Oduro *et al*., 2008
Potassium (mg)	1324-1384	Rweyemamu, 2006; Oduro *et al*., 2008
Magnessium (mg)	368-422	Rweyemamu, 2006; Nuhu, 2010
Phosphorus (mg)	204-267	Rweyemamu, 2006; Nuhu, 2010
Manganese (mg)	8.4-9.1	Richter *et al.*, 2003; Rweyemamu, 2006
Zinc (mg)	2.5-3.29	Rweyemamu, 2006; Oduro *et al*., 2008
Vitamin A –B carotene (mg)	16.3-18.9	Rweyemamu, 2006; Nuhu, 2010
Vitamin B1 oxalic acid (mg)	1.60-1.63	Rweyemamu, 2006; Oduro *et al*., 2008
Vitamin B1 thiamin (mg)	2.54-2.64	Rweyemamu, 2006; Oduro *et al*., 2008
Vitamin B2 riboflavin (mg)	19.8-20.5	Richter *et al.*, 2003; Rweyemamu, 2006
Vitamin B3 nicotinic acid (mg)	8.2-8.6	Rweyemamu, 2006; Nuhu, 2010
Vitamin C ascorbic acid (mg)	17.3	Rweyemamu, 2006
Vitamin E tocopherol acetate (mg)	113	Rweyemamu, 2006

conservation between collection and analysis (drying, refrigeration and freezing) (D'Souza and Kulkarni, 2004). These can seriously affect nutrient content of the sample especially vitamin C which is lost during the drying process (Newton, 2006). As far as errors of human origin are concerned, they may be due to error of manipulation during analysis, mis-calculation of results and inappropriate botanical identification of the sample (Broin, 2008).

Moringa oleifera has been reported to possess quality proteins, its crude protein content varies from 16 to 40 % (Foidl *et al.,* 2001*;* Marcu and Pharm, 2005; Rweyemamu, 2006; Reyes-Sànchez *et al*., 2006; Mendieta- Araica *et al*., 2011). According to Makkar and Becker (1997) and Marcu and Pharm (2005), the amounts of essential amino acids are comparable to those in Soya beans. The number of amino acids found in the leaf meal varies from 9-12 (Table 2.2) and also their nutritional value varies (Richter *et al*., 2003). Leaves are a good source of beta carotene and minerals (Richter *et al*., 2003; Reyes-Sànchez *et al*., 2006). The calcium content is remarkably high for a plant with low phosphorus levels (Rweyemamu, 2006). Also, the content of iron is good as such it is used for anaemic patients (Anwar *et al*., 2007). *Moringa oleifera* leaves have high nutritive value, as such it has a potential to be used as feed supplements.

2.3 Medicinal uses of *Moringa oleifera*

2.3.1 Antimicrobial activity

For a long period of time, plants have been a valuable source of natural products for maintaining human and animal health, with more intensive studies for natural therapies which can be used to combat serious diseases, especially in the last decade (Zhang, 2004). The use of plant compounds for pharmaceutical purposes has gradually increased world-wide. According to

Zhang (2004), medicinal plants would be the best source to obtain a variety of drugs. Approximately 60-80 % of the world's population still relies on traditional medicines for management of diseases (Zhang, 2004). Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Eloff, 1998).

Bacteria are micro-organisms that have circular double-stranded DNA and cell walls. They are classified by several criteria, including morphology. They may be cylindrical (bacilli) or spherical (cocci) (Mckay *et al*., 2009). Basically there are two groups of bacteria namely the gram positive and gram negative bacteria (Tsang *et al*., 2005). The gram positive bacteria retain crystal violet dye after iodine fixation and alcohol decolaration, whereas gram negative bacteria do not. Gram negative organisms have thinner cell walls and the cell wall composition is different from that of gram positive organisms (Tsang *et al*., 2005). This difference accounts for general differences in how both virulence factors and antigenic determinants are expressed. In addition, difference accounts for some general distinctions in susceptibility to antibiotic drugs.

Bacteria are controlled using antibacterial drugs which have either bactericidal or bacteriostatic properties (Maukonen *et al*., 2006). Antibacterial drugs have many mechanisms of action including inhibiting cell wall synthesis, activating enzymes that destroy the cell wall, increasing cell membrane permeability and interfering with protein synthesis and nucleic acid metabolism (Mckay *et al*., 2009). Other antibacterial drugs are bacteriostatic which slow or stop *in vitro* and *in vivo* bacterial growth but depend on body defenses to kill bacteria (Mckay *et al*., 2009). According to Michel *et al*, (2008) quantitative methods are used to identify the minimum *in vitro*

concentration at which an antibiotic can inhibit growth (minimum inhibitory concentration or MIC) or kill (Minimum bacterial concentration or MBC).

Moringa oleifera is reported to reduce the activity of pathogenic bacteria and molds (Mehta *et al*., 2003). Recent studies have demonstrated that leaf extracts have antimicrobial activity including the inhibition of the growth of *Staphylococcus aureus* strains isolated from food and animal intestines (Mehta *et al*., 2003). Dahot (1998) and Mehta *et al*. (2003), also reported that *M. oleifera* leaf extracts inhibited the growth of bacteria (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas areginosa*) and fungal species that included *Aspergillus fumigates,* and *Aspergillus flavus.* Aside from the antimicrobial activities of the leaves, the stem bark extracts showed antibacterial effect against *Staphylococcus aurens* (Mehta *et al*., 2003; Garrity *et al*., 2006). It could be a potential bioceutical agent to substitute antibiotics in livestock production. Yang *et al.* (2006) reported that the inclusion of *M .oleifera* leaves in broiler diet reduced the population of the bacteria *Escherichia coli* in the ileum. The *M. oleifera* leaves, stem bark and roots are reported to contain compounds such as pterygospermin, benzyl glucosinate and benzyl isothiocyante and phytochemicals namely flavonoids, saponins, tannins and phenolic compounds which have powerful antibacterial and fungicidal effects (Nikkon *et al*., 2003; Fahey, 2005; Anwar *et al*., 2007). According to Fahey (2005), the medicinal use of *M. oleifera* is summarized in Table 2.2.

Table 2.2: **Medicinal uses of *M. oleifera* (Fahey, 2005) and Anwar *et al*., 2007)**

Plant Parts	Traditional use/Effect
Leaves	Antibacterial infection, Urinary tract infection, Herpes simplex virus, Helminthes, Trypanosomes, Bronchitis, External sores/ulcers, Fever, Anti-Tumor, Prostate, Radio protective, Anti-anemic, Antihypertensive, Diabetes/hypoglycemia, Diuretic, Colitis, Dysentry, Ulcer/Gastritis, rheumatism, headache, Antioxidant, Carotenoids, Iron deficiency, Protein and mineral deficiency, Lactation enhancer, Antiseptic catarrh, Scurvy and Tonic.
Stem Bark	Dental caries/toothache, Common cold, External sores/ulcer, Anti-tumor, Snake-bite, Scorpion bite, Colitis, Epilepsy, Hysteria, Headache, Anti-nutritional factors, Aphrodisiac, eye ointment, Splenomegaly, Pain killer, Birth control and Scurvy
Roots	Dental decay/toothache, common colds, External sores/Ulcers, Fever, Asthma, Cardiotonic, Diuretic, Hepatorenal, Flatulence, Anti-spasmodic, Epilepsy, Headache, Aphrodisiac, Gout, Hepatamegaly, Low back/Kidney pain, Anti-inflammatory, Scurvy and splenomegaly.
Exudate	Dental caries/Toothache, Syphilis, Typhoid, Earache, Fever, Asthma, Diuretic, Dysentry, Rhematism and Headache.
Flowers	Throat infection, common colds, antihelmintic, anti-tumor, diuretic, tonic, hysteria, abortion.
Seeds	Antihelmintic, Warts, antitumor, Ulcer, Skin cancer, ant-hypertensive, diabetes, joint pain.
Gum	Dental caries, astringent, asthma, syphilis and rheumatism

2.3.2 Anthelmintic Activity

In the tropics and sub-tropics, helminthes remain one of the most prevalent and economically important parasites of goats (Torres-Acosta *et al*., 2008). Several authors have highlighted that, gastrointestinal helminths are a major constraint to economic productivity of goats as they constitute the chief parasites responsible for disease-related production losses arising from goat mortality, severe weight loss and poor production (Walkden-Brown and Kahn, 2002; Githiori *et al*., 2004; Torres-Acosta *et al*., 2008).

Control of gastrointestinal anthelmintic infections is through use of conventional anthelmintics. However, these drugs are increasingly expensive with some side effects and sometimes unavailable or unaffordable to resource-limited farmers in developing countries, who as a result end up using adulterated drugs (Monteiro *et al*., 1998). Moreover, the widespread intensive use of conventional anthelmintic drugs by most commercial farmers has created multiple drug resistance that has led to a failure in controlling helminths (Wolstenholme *et al*., 2004; Jabbar *et al*., 2006). These constraints have necessitated alternative strategies of helminth control (Jabbar *et al*., 2006).

Supplementing livestock with condensed tannin-rich feeds can be a cost-effective alternative control strategy for reducing gastro-intestinal parasite burdens in livestock in communal areas (Waller and Thamsborg, 2004; Alonso-Diaz *et al.*, 2008; Xhomfulana *et al*., 2009). Condensed tannins directly affect the physiological functioning of gastrointestinal parasites, and may reduce egg hatch rate and larval development in faecal samples (Nguyen *et al*., 2005). In addition, it is thought that tannins could act indirectly by improving response of the host to gastro-intestinal

parasites (Waller and Thamsborg, 2004). On the other hand *M. oleifera* leaves are rich in nutrients, and its supplementation improves the body condition of animals fed on the plant. Increased intestinal protein supply is known to improve host homeostasis and its immune response to gastro-intestinal parasites (Hoste *et al*., 2006). The improved utilization of nutrients by hosts could thus contribute to the improvement in resilience usually observed in infected animals and could modulate host resistance (Nguyen *et al*., 2005; Minho and Abdalla, 2007).

Moringa oleifera has been perceived to have anthelmintic effects by resource-limited farmers in Uganda (Wasswa and Olila, 2006; Rastogi *et al*., 2009), however, there is little scientific evidence on the claim. However, the *in-vitro* work on ascaris worm, showed that *M. oleifera* was not all that effective as antihelmintic (Wasswa and Olila, 2006). Its anthelmintic effect could be attributed to the amount of condensed tannins the leaves possess or it could be due to proteins. The leaves also contain some copper which is reported to have some anthelmintic effect (Burke *et al*., 2007).

2.3.3 Antioxidants

Moringa oleifera has been reported to possess some antioxidant properties (Sreelatha and Padma, 2009; Atawodi *et al*., 2010). Antioxidants are substances that may protect animal cells against the damage caused by free radicals that cause oxidation in the body (Tsang *et al*., 2005). Free radicals are highly reactive molecules containing one or more unpaired electrons and can be formed when oxygen interacts with certain molecules such as peroxides (Nair *et al*., 2003). Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as

DNA, or the cell membrane. Cells may function poorly or die if this occurs. In addition, free radicals can damage cells, and may play a role in heart disease, cancer and other diseases (Tsang *et al*., 2005).

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to animals against infections and degenerative diseases (Sreelatha and Padma, 2009; Verma *et al*., 2009). These antioxidants safely interact with free radicals and terminate the chain reactions before vital molecules are damaged (Nair *et al*., 2003). Although there are several enzyme systems within the body which scavenge free radicals, the principle micronutrient antioxidants are vitamin E, bêta-carotene, and vitamin C (Nair *et al*., 2003). Additionally, selenium, a trace mineral that is required for proper functioning of one of the body's antioxidant enzyme systems, is sometimes included in this category (Peschel *et al*., 2006). The body cannot manufacture these micronutrients and they need to be supplied in the diet (Peschel *et al*., 2006). In nature antioxidants are grouped as endogenous or exogenous. The endogenous group includes enzymes (and trace elements as part-of) like superoxidase dismutase (zinc, manganese and copper), glutathione peroxide (selenium) and catalase and proteins like albumin, transferrin, ceruloplasmin, metalliothioein and haptoglobin (Nair *et al*., 2003). The most important exogenous antioxidants are dietary phytochemicals such as polyphenols, quinines, flavonoids, catechins, coumarins, terpenoids and the smaller molecules like ascorbic acid, beta-carotene and vitamin-E (Nair *et al*., 2003).

Moringa oleifera leaves have been reported to contain various phytochemicals, viz carotenoids, vitamins, chlorophyll, xanthins, minerals, amino acids, sterols, alkaloids, flavonoids and

phenolics (Siddhuraju and Becker, 2003; Anwar *et al*., 2007; Verma *et al*., 2009). The presence of vitamins C, E, carotenoids, flavonoids and selenium make *M. oleifera* a potential antioxidant. Aqueous and methanolic extracts of *M. oleifera* leaves have been reported to possess antioxidant properties (Siddhuraju and Becker, 2003; Sreelatha and Padma, 2009; Khalafalla *et al*., 2010). Ashok Kumar and Pari (2003) reported that the administration of *M. oleifera* extract decreased hepatic marker enzymes and lipid peroxidation with simultaneous increase in the level of antioxidants. It was speculated that the extracts exerted its protective effects by decreasing liver peroxide and enhancing antioxidants. In addition, Verma *et al*. (2009) reported that the leaves of *M. oleifera* have phenols, which are known to scavenge free radicals. The investigation of antioxidant polyphenols indicated the presence of phenolic acids (gallic, chlorogenic, ellagic and ferulic acid) and flavonoids (Kaempferol, quercetin, caffeoylquinic acids and rutin). This also corroborates the findings of Siddhuru and Becker (2003) who reported that the major bioactive compounds of phenolics were flavonoid group such as querctin and kaempferol. Furthermore, *M. oleifera* extracts have significant metal chelating properties and have the ability to protect against DNA nicking. In rats the extracts protected the tissues against oxidative stress (Verma *et al*., 2009).

Selenium and zinc are minerals, not antioxidant nutrients (Yazdanparast and Ardestani, 2007), however, they are components of antioxidant enzymes and *M. oleifera* leaves are reported to possess selenium and zinc (Fugile, 2005). These minerals are thought to help fight cell damage by oxygen-derived compounds and thus may help protect against cancer. It is best to supply selenium through feeds in limited quantities, as large doses of the supplement form can be toxic (Beytut *et al*., 2002). Deficiency of selenium and vitamin E causes White Muscle disease (Beytut

et al., 2002). In addition, their deficiency results in lower conception rates, dystocia, retained placenta, reduced milk production, reduced semen quality, poor growth rate and increased incidence of periodontal diseases (Beytut *et al*., 2002). Higher concentrations of other minerals (calcium, sulphur and copper) and feed contaminants (nitrate, unsaturated fats) may decrease absorption of selenium in the small intestines. Vitamin E deficiency clearly reflects forage quality. Prolonged storage of feeds results in degradation of vitamin E activity. Plants can also be a good source if grown in selenium-rich soils (Fugile, 2005).

Vitamin A protects cells from free radicals and its important sources are liver, dairy products and fish (Peschel *et al*., 2006; Mills *et al*., 2008). Vitamin C, also called ascorbic acid, is a water-soluble vitamin found in all body fluids, so it may be one of the body's first line of defence (Peschel *et al*., 2006). This powerful antioxidant cannot be stored by the body, so it is important that it should be fed regularly (Chong *et al*., 2007). Vitamin E, a fat-soluble vitamin also known as alpha-tocopherol, can be stored with fat in the liver and other tissues (Chong *et al*., 2007). Furthermore, vitamin E is promoted for a range of purpose, from delaying aging to healing sunburn (Chong *et al*., 2007). Important sources include wheat germ, nuts (almonds), seeds, whole grains, green leafy vegetables, broccoli, mangoes, corn and soybean oil and fish-liver oil (Mills *et al*., 2008).

Moringa oleifera, has also been reported to be rich in beta-sitosterol which acts against some forms of cancer. It has been found to reduce the growth of prostate and colon cancer cells. Beta-sitosterol is known to boost immunity, helps to normalize the blood sugar and supports the pancreas and has anti-inflammatory properties (Fugile, 2005). Interestingly, *M. oleifera* has

cytokines like zeatin or kinetin which have a potent of anti-aging and protective effects in animals (Fugile, 2005). Besides its antioxidant potential, *M. oleifera* is documented for treating diseases in humans (Anwar *et al*., 2007). Some of the antioxidants are of dietary importance.

2.4 Uses of *Moringa oleifera* as a feed supplement

Protein is the most limiting nutrient especially during the dry season (Devendra and Sevilla, 2002), therefore, protein supplementation is strongly recommended when finishing goats on rangelands (Marume, 2010). Supplementation with high-protein diets improves body weight gains and consequently higher carcass weights and better chevon quality than low-protein diets (Baublits *et al*., 2006). Conventional protein supplements such as protein blocks are expensive and unavailable to resource-limited farmers; therefore they have resorted to the use of alternative protein supplements (Devendra and Sevilla, 2002). Some of the supplements are highly fibrous and low in protein, vitamins and essential minerals (Ngongoni *et al*., 2007). Legume trees such as *Dichrostychs cinera*, *Julbernadia globiflora* and *Acacia karroo* (Mlambo *et al*., 2004; Marume, 2010), have been used as protein supplements.

Moringa oleifera has a greater potential as a protein supplements because of its easy growth and adaptability (Mendieta-Aracia *et al*., 2011). The leaves of *M. oleifera* are readily consumed by cattle, sheep, goats, pigs, rabbits, fish and chickens (Ben Salem *et al*., 2004; Mendieta-Araica *et al*., 2011). Foidl *et al*. (2001); Ly *et al*. (2001); Reyes-Sànchez *et al*. (2006) conducted some trials using *M. oleifera* leaves as livestock feed for beef, dairy cows, pigs and poultry. Both beef and dairy cattle were fed 15-17 kg each per day of fresh *M. oleifera* leaves and the beef cattle gained up to 33 % while dairy cows' milk yield increased by 43-65 %. In the work done by

Reyes-Sànchenz *et al.* (2006) the milk yield increased by 58 and 65 % when the dairy cows were fed 2 and 3 kg dried leaves per day, respectively. Furthermore, in an interesting experiment performed with cross-bred dairy cows *M. oleifera* leaf meal was compared with cotton seed cake as a concentrate component together with maize bran and minerals (Sarwatt *et al*., 2004).

The cows were fed a basal Elephant grass diet together with one of three concentrate mixtures. *Moringa oleifera* leaf meal substituted 43, 73 and 100 % of the cotton seed cake in these mixtures. The cows that were fed higher proportions of *M. oleifera* leaf meal yielded significantly more milk (Mendieta-Aracia *et al*., 2011) indicating that *M. oleifera* leaf meal can be used as an alternative feed in dairy cow diets.

However, milking should be done at least three hours after feeding to avoid the grassy taste of *M. oleifera* in milk (Fugile, 2005). When it was fed to Jersey cows, the calf's birth weight averaging 22 kg increased by 3-5 kg which results in higher dystocia cases in small cattle (Fugile, 2005). Thus, it may be advisable to induce birth 10 days prematurely to avoid dystocia problems. In addition, frequency of twin births also increased by 15 %, with *M. oleifera* feed (Richter *et al*., 2003).

Moringa oleifera is also reported to be used as poultry, fish and swine feed (Richter *et al*., 2003; Fugile, 2005; Kakengi *et al*., 2006; Olugebemi *et al*., 2010). However to increase the nutrient value of moringa leaves to poultry and chickens, the enzyme phytase should be added to break down the phytates, which lead to increased absorption of nutrients such as phosphoric compounds found in *M. oleifera* (Fugile, 2005). Richter *et al*. (2003) suggested that *M. oleifera*

Table 2.3: Amino acids composition of *Moringa oleifera* leaves

Name of amino acids	Quantity	Reference
Arginine (mg/g DM)	13.25-18.9	Rweyemamu, 2006; Oduro *et al*., 2008
Histidine (mg/g DM)	5.1-6.13	Rweyemamu, 2006; Oduro *et al*., 2008
Lysine (mg/g DM)	13.25-20.5	Richter *et al.*, 2003; Rweyemamu, 2006; Oduro *et al*., 2008
Tryptophan (mg/g DM)	4.25-7.53	Richter *et al.*, 2003; Rweyemamu, 2006; Oduro *et al*., 2008
Phenylanaline (mg/g DM)	13.8-13.9	Rweyemamu, 2006; Oduro *et al*., 2008
Methionine (mg/g DM)	3.3-3.5	Richter *et al.*, 2003; Rweyemamu, 2006; Oduro *et al*., 2008
Threonine (mg/g DM)	10-12	Rweyemamu, 2006; Oduro *et al*., 2008
Leucine (mg/g DM)	19.5-20.5	Rweyemamu, 2006; Oduro *et al*., 2008
Isoleucine (mg/g DM)	8.25-11.9	Rweyemamu, 2006; Oduro *et al*., 2008
Valine (mg/g DM)	10.6-14.5	Rweyemamu, 2006; Oduro *et al*., 2008
Cysteine (mg/g DM)	3.87	Rweyemamu, 2006; Oduro *et al*., 2008
Aspartate (mg/g DM)	15.8-20.5	Rweyemamu, 2006; Sanchez-Machado *et al*., 2010
Glutamate (mg/g DM)	17.1-28.4	Rweyemamu, 2006; Oduro *et al*., 2008
Serine (mg/g DM)	9.4-9.7	Rweyemamu, 2006; Oduro *et al*., 2008
Glycine (mg/g DM)	10.3-10.8	Rweyemamu, 2006; Sanchez-Machado *et al*., 2010
Alanine (mg/g DM)	12.5-14.1	Rweyemamu, 2006; Oduro *et al*., 2008
Proline (mg/g DM)	12.4-14.3	Rweyemamu, 2006; Sanchez-Machado *et al*., 2010

leaf meal can be used to substitute up to 10 % of dietary protein in Nile tilapia without significant reduction in growth.

In the study done by Sarwatt *et al*. (2002), it was shown that *M. oleifera* meal could be used as an alternative protein supplement because there was a significant increase in dry matter intake and NDF digestibility of poor quality feed when replacing Sunflower seed-cake. Also, Aregheore (2002) reported that the inclusion of *M. oleifera* at 20 and 50 % levels of total daily forage allowance could be used as a cheap protein supplement in batiki grass based diets for goats. Ben Salem *et al*. (2004) reported that dried leaves are rich in protein up to 40 %. In its use as livestock feed, farmers should take note that too much protein in pig feed increases muscle development at the expense of fat production, while in ruminants it can be fatal as it alters the nitrogen cycle (Fugile, 2005).

Use of *M. oleifera* leaves as feed seems to reduce the activity of pathogenic bacteria and molds thereby improving the digestibility of other feeds, thus enhancing the expression of natural genetic potential by the animals (Anwar *et al*., 2007). The other advantage of using *M. oleifera* is that, it will be green especially when other plants are not green (Fahey, 2005; Trees for life, 2005). Feeding fresh *M. oleifera* leaves is convenient but there is a large variation in production and chemical composition over the year. Therefore, *M. oleifera* leaf meal is an interesting product as it can be produced during periods of high yields and later used for feeding during the dry season when high quality feed resources are scarce (Mendrieta-Araica *et al*., 2011). Moreover, *in-vitro* protein digestibility of its leaves is high, ranging between 85-90 % (Aregheore, 2002). Leaves have low or insignificant levels of anti-nutritive factors such as

phenols, saponins, trypsin and amylase inhibitors, lectins, cyanogenic glucosides and glucosinolates (Ben Salem *et al*., 2004).

Moringa oleifera leaves contain some condensed tannins, however, Makkar and Becker (1997) reported that they were negligible (12 g kg $^{-1}$). Condensed tannins play a significant role in the nutrition of animals, causing either adverse or beneficial effects on nutrient utilization, health and production (Waller and Thamsborg, 2004; Hoste *et al*., 2006). Consumption of high concentrations of condensed tannins (>7 % of DM) has been associated with detrimental effects on ruminants, such as reduced feed intake, reduced digestibility, growth inhibition and interference with the morphology and the proteolytic activity of microbes in the rumen (Nguyen *et al*., 2005; Hoste *et al*., 2006; Xhomfulana *et al*., 2009).

However, the ideal (low or moderate) concentrations of condensed tannins (< 6 % of DM) have positive effects. Condensed tannins have the protein-binding ability, which protects dietary protein from degradation in the rumen by rumen microbes, which increases protein availability in the lower digestive tract (Nguyen *et al*., 2005; Louvandini *et al.*, 2006; Cenci *et al*., 2007). Their ability to form a complex with soluble proteins may be involved in bloat prevention. Condensed tannins also stimulate the salivary flow in the animal (Nguyen *et al*., 2005). Inclusions of *M. oleifera* in diets have been reported to influence the quality of meat (Mahecha *et al*., 2009).

2.5 Meat quality parameters

Supplementation of goats with *M. oleifera* has a possibility of producing chevon of better quality classified as organic meat, meeting both producer and consumer expectations. However,

information on meat quality from goats supplemented with *M. oleifera* is scarce. Modern consumers are increasingly concerned about eating quality meat with no undesirable effects on their health (Andersen *et al*., 2005). Meat quality parameters such as juiceness, tenderness, taste, appearance, colour, price and food safety all influence the consumer's decision to purchase meat (Vasta *et al*., 2008). Meat should have a desirable colour that is uniform throughout the entire cut. The colour is related to the level of the protein pigment, myoglobin, present in the muscle. Good quality meat should also have marbling (intramuscular fat) throughout the cut. The marbling increases juiciness, tenderness and flavour of the meat (Cividini *et al*., 2008).

Water holding capacity is a factor that also determines the juiciness of meat. It is defined as the ability of meat to retain its water during application of external forces such as cutting, heating, grinding or pressing (Lawrie, 2004). The quality of meat may vary according to the feeding system, which plays an important role in meat quality, because changes in fatty acids composition of body fats are primarily linked to the respective fatty acid contents in the diet. As such farmers can manipulate animal feed to improve the dietary quality of meat. Goats feeding in pasture systems have an increase in *n*-3 polyunsaturated fatty acids in meat in comparison to goats fed on grain-based diets (das Gracas Padre *et al*., 2006). Furthermore, the diets rich in forage, favour the growth of fibrolytic microorganisms responsible for the rumen production of conjugated linoleic acids (Mahecha *et al*., 2009). In addition, management practices such as diet, animal welfare and slaughter management may influence the meat pH and quality (Martinez-Cerezo *et al*., 2005).

2.5.1 Meat pH and meat quality

The pH of meat measures its acidity. Both the rate and extent of postmortem pH fall influences meat quality characteristics. Variation in pH influences meat consumption characteristics such as colour, juiceness, tenderness, taste and water holding capacity (Martinez-Cerezo *et al*., 2005; Mushi *et al*., 2009). The high ultimate pH of meat, as a consequence of depleted muscular glycogen reserves prior to slaughter, greatly affects meat quality. Various stress factors have been mentioned as responsible for glycogen depletion: time and manner of transportation of animals from the farm to the abattoir; diet restrictions; mixing animals of different lots; lairage time; climatic factors; pathological conditions and genetic factors (Muchenje *et al*., 2009a; Ding *et al*., 2010). In practice, any situation which provokes a substantial depletion of muscle glycogen reserves will give rise to meat with a high ultimate pH, if the animal is slaughtered before its energetic reserves are restored (Blanco *et al*., 2010).

Meat with high ultimate pH is dark, is more susceptible to bacterial spoilage and has reduced flavour. Nevertheless, this meat is associated with a higher rate of tenderisation or with a better ultimate tenderness (Serra *et al*., 2008). Meats with low pH are lighter in colour and have a decreased water-holding capacity resulting in less juice after preparation of meat (Mushi *et al*., 2009). On the other hand, more juice in prepared meat gives a juicer, more succulent and tender eating experience (Dhanda *et al*., 2003; Vasta *et al*., 2008). Conversely, a higher pH gives a darker colour and less drip loss (Vasta *et al*., 2008). It can be noted that, some diets also may influence the meat pH and colour. For example, sheep that grazed on *phalaris spp* had higher meat pH and the meat was darker with a rubbery texture (Silva *et al*., 1999).

2.5.2 Colour and meat quality

Colour of carcasses after slaughter can be used to determine the quality of meat. Several factors affect meat colour such as species/breed, age, sex, cut of meat, surface drying of the meat and surface spoilage (Mushi *et al*., 2009; Muchenje *et al*., 2009a; Muchenje *et al*., 2010). Meat colour is largely determined by the content of myoglobin and its derivatives, and meat discolouration depends on the presence or absence of air (Baron and Andersen, 2002). For instance, exposed meat changes colour due to reactions occurring between myoglobin and oxygen. In fresh meat myoglobin can exist in three different forms: the reduced form of myoglobin (deoxymyoglobin) is purplish, and the oxygenated form (oxymyoglobin) is bright red whereas the oxidized form (metmyoglobin) is brown. Fresh meat colour is affected by the relative abundance of these three forms (Baron and Andersen, 2002)**,** with more myoglobin in meat resulting in darker meat.

Meat colour is also influenced by the enzymes, age of the animal, the species, sex, diet, and even the exercise it gets (Baron and Andersen, 2002). For example, myoglobin, a protein, responsible for the majority of the red colour in meat, does not circulate in the blood but is fixed in the tissue cells and it is purplish in colour. When it is mixed with oxygen, it becomes oxymyoglobin, and produces a bright red colour which is measured objectively by a* coordinates (Priolo *et al.*, 2001). The remaining colour comes from the haemoglobin which occurs mainly in the circulating blood, but a small amount can be found in the tissues after slaughter (Priolo *et al*., 2001). According to Warris (2010)**,** during transportation, handling and pre-slaughter stress there is little lactic acid production that results in dark firm dry (DFD) meat, and this condition is measured by L* coordinates. The DFD meat is of inferior quality as the less pronounced taste

and the dark colour are less acceptable to the consumer and it has a shorter shelf life, due to the abnormal high pH value. Colour is also greatly affected by muscle pH. At high pH, muscles have a closed structure and hence appear dark (Mushi *et al*., 2009). Baron and Andersen (2002) added that good quality meat usually has a pH of 5.4–5.7 and muscle of a living animal has a pH of 7.1. According to Kannan *et al.* (2006), dark colour shows animals that were exposed to situations that exhaust glycogen levels.

According to Mancini and Hunt (2005), changes in a* (redness) and b* (yellowness) values over a period of time describe meat colour deterioration from red to brown, and reflect the myoglobin concentration and its redox state in meat (Muchenje *et al*., 2009). Moreover, it has been recently shown that over a period of storage, while b* values were positively related to sensory appreciation of meat colour degradation, a* values were negatively correlated to the sensory degradation of colour (Insausti *et al*., 2008).

Meat colour has great influence on the purchase decision of consumers (Carrasco *et al*., 2009). It is necessary to assess the effect of the feeding system on the meat colour in order to avoid meat rejection. The colour of meat may be influenced by the feed consumed by the animal (Borton *et al*., 2005). Furthermore, Borton *et al*. (2005) reported that meat colour may be influenced by diet, with grass fed animals having darker lean meat than grain fed ones. Also animals fed on pasture have a yellow fat because of the high levels of beta-carotene contained in grass (Muchenje *et al*., 2009a). They acquire carotenoid pigments from their feed and these pigments accumulate in the carcass fat. High pigment levels in feed such as fresh forage may cause the fat to become yellow at an early stage (Carrasco *et al*., 2009).

Consumers often perceive meat with yellow fat as having come from an old or diseased animal (Mapiye *et al*., 2010). In addition, forage-based rations, as well as different forage and seasonal changes, allow for carcasses with a darker lean appearance or fat that is yellow in appearance (Baublitis *et al*., 2004). The darker lean (Low L* values) may be attributed to increased myoglobin, decreased muscle glycogen or both (Priolo *et al*., 2001). According to Vasta *et al*. (2008), animals fed on tannin-containing feeds produce meat of a lighter colour. Also, the concentration of myoglobin increases as cattle grow old and older animals tend to have dark muscles. But young animals also may produce carcass with dark meat, if they have been severely stressed or exhausted prior to slaughter (Carrasco *et al*., 2009).

The amount of dietary iron consumed by the animals also influences the redness (a^*) and colour saturation. High iron levels increases haemoglobin and myoglobin concentrations in meat and subsequently increase the meat freshness (Mapiye *et al*., 2010). In addition meat may appear dark because it has a high pH and scatters less light than normal. It could be due to glycogen and its effect on pH of meat (Baublitis *et al*., 2004). Younger bulls that were fed forage–limited diet had less glycogen, a higher pH, and darker lean meat (Vestergaard *et al*., 2000). It is speculated that the decreased dietary energy on the forage-limited diet favoured an increase in oxidative muscle metabolism. An increase in oxidative muscle metabolism could possibly allow for the decreased necessity to store comparable amounts of glycogen in muscle with a higher glycolytic capacity (Baublitis *et al*., 2004). The resultant pH differences therefore, caused variations in yellowness (b*).

2.5.3 Meat tenderness

Tenderness can be attributed to a person's perception of meat, such as softness to tongue, resistance to tooth pressure and adhesion. It is a major factor affecting the consumer's assessment of meat quality (Mushi *et al*., 2009). The tenderness of meat is influenced by breed of the animal, age, sex, pre-slaughter treatment such as vitamin D injections or medication, handling, transportation to the slaughter place and the slaughtering method. Tenderness of meat decreases with increasing age, which is due to the changing nature of collagen, connective tissue protein of meat. Collagen is a contributing factor to variation in meat tenderness and texture, it becomes more complex and stronger with advancing age (Silva *et al*., 1999).

Marbled meat is more tender than steaks where fat is in a layer around the outside (McMillin and Brock, 2005). There is also a view that both stress before slaughter in particular, and lack of aging of the meat has more influence on toughness than most other factors, such as marbling (McMillin and Brock, 2005). There is a complex interplay between the effects of pasture species, protein intake, calcium status, stress before and at killing, breed, the age of the animal, and how the meat is treated after slaughter in affecting tenderness. Another factor that affects meat tenderness is the diet (Serra *et al*., 2008). Grain fed goats are usually slightly more tender because they are slaughtered at a slightly younger age (Scholtz, 2005). Most indigenous goat breeds grow naturally without any growth supplements such that by the time they reach slaughter weight they are mature and their meat becomes tougher (Scholtz, 2005). The tougher the meat, the more force required to shear it and this is objectively measured by the Warner-bratzler shear force (WBSF) test. Therefore this necessitates investigating the effect of feeding *M. oleifera* on the tenderness of meat.

Meat tenderness and flavour appear to be the most important sensory characteristics that determine meat quality (Tshabalala *et al*., 2003). Consumers tend to evaluate cooked meat on the basis of tenderness, juiciness and flavour. The more tender the meat, the more rapidly juices are released in the mouth after chewing (Scholtz, 2005). Consumer choose the meat of higher quality and as such there is need to supplement goats with plants like *M. oleifera* to encourage fast growth rate which will yield meat of better quality.

2.5.4 Fatty acid profiles

Goats are good sources of lean meat with desirable fatty acids, due to the fact that they deposit relatively higher proportions of polyunsaturated fatty acids compared to other ruminants (Mushi *et al.*, 2008; Safari *et al*., 2009). Fatty acid composition of meat is affected by the fatness level of the animal, which may be enhanced by the type of feed the animal consumed, age and genotype (Alfaia *et al*., 2006; Borton *et al*., 2005; Muchenje *et al*., 2009a). In turn, the fatty acid profiles affect the meat eating quality traits such as flavour and juiciness (Calkins and Hodgen, 2007).

The changes in fatty acid composition of body fats are primarily due to the respective fatty acid content of the diet (Cividini *et al*., 2008; Muchenje *et al*., 2009b). Goats fed on pasture, present meat with lower intramuscular fat and lower percentages of monounsaturated fatty acids in loin subcutaneous fat (Warris, 2010). As such nutritionists are now focused on increasing omega-3 fatty acids; particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that could have a profound influence on the health of consumers (Demirel *et al*., 2006). The fatty acid composition and cholesterol levels in meat have received increasing attention owing to their implications in human health and product quality (Muchenje *et al*., 2009b; Warris, 2010). The

ratios of PUFA/SFA and *n*-6/*n*-3 PUFA, and hypocholesterolaemic/hypercholesterolaemic (h/h) are widely used to evaluate the nutritional value of fat (Orellana *et al.*, 2009). Health-conscious consumers are preferring meat with higher ratio of unsaturated fatty acids composition which benefits in preventing cardiovascular and coronary diseases (Calder and Deckelburn, 2003; McMillin and Brock, 2005; Muchenje *et al*., 2009b). As a result consumers are advised to reduce the intake of fat, especially saturated fatty acids; *n*-6 fatty acids (Orellana *et al.*, 2009). Medical officials recommend consumption of omega 3- fatty acids, which play a crucial role in brain function as well as normal growth and development (Department of Health, 1994).

Moreover the types of feed given to animals affect their health. For instance feed with high level of vitamin E protects myoglobin from oxidation (Vasta *et al*., 2008). In addition, a feed with conjugated linoleic acids (CLA) protects animals against cancer, heart diseases; reduces animal body fat, stimulates the immune system and bone development, and alleviates wasting away diseases (Vasta *et al*., 2008).

2.5.5 Flavour and aroma

Flavour and aroma are attributes that are most easily detected and assessed by consumers as to being either acceptable or not. In small stock, comparisons have been between sheep and goats with distinct species characteristics (Sheradin *et al*., 2003; Tshabalala *et al*., 2003; Webb *et al*., 2005). Martinez-Cerezo *et al*. (2005) noted also that the muscle and the method of preparation had an effect on flavour and aroma. There appears to be an optimum subcutaneous fat thickness for optimum flavour, since meat with 1–4 mm subcutaneous fat is more acceptable than meat with either more or less subcutaneous fat. Goat meat flavour can be as acceptable (Babikerm *et*

al., 1990; Mushi *et al*., 2009) or not as desirable (Madruga *et al*., 2008) as meat from lamb/mutton. Age is another factor which affects the flavour of meat. Marlinez-Cerezo, *et al*. (2005) reported that younger goats had a more desirable flavour. However, the flavour of very young animals was found not to be as acceptable as older ones (Madruga *et al*., 2008; Mushi *et al*., 2009). Branched chain fatty acids (BCFA) have been specifically implicated in sheep and goat species related flavour (Ha and Lindsay, 1990).

Other BCFA implicated in goat-like flavour are 4- methyloctanoic, 4-methylnanoic (Madruga *et al.,* 2008) and 4-ethylheptanoic (Ha and Lindsay, 1990; Madruga *et al*., 2008). Alkaloids, pyridines and sulphur containing compounds are other notable flavour compounds that have been identified in goat meat, but are unlikely to play a major role in the development of goat flavour (Ha and Lindsay, 1990).

2 5.6 Juiciness

Juiciness of meat is directly related to the intramuscular lipids and moisture content of the meat (Calkins and Hodgen, 2007), but the water remaining in the cooked product is the major contributor to the sensation of juiciness during eating (Sheradin *et al*., 2003). Goat meat and its products are reportedly less juicy (Tshabalala *et al*., 2003; Sheradin *et al*., 2003; Mushi *et al*., 2009), which is attributed to the lower fat content of goat meat. The effect of age on juiciness is not clear since Banskalieva *et al.* (2000) found that kid chevon from 10 to 25 kg carcasses were juicier than chevon from 15 to 30 kg carcasses, while Mushi *et al*. (2009) found the chevon of older goats to be more juicy and palatable.

2.5.7 Consumer perception on goat meat

Chevon is almost universally acceptable, but with cultural traditions and social and economic conditions influencing consumer preference (Casey and Webb, 2010). Consumers tend to prefer mutton and beef which could be attributed to residual effects of habit and preferences for texture (Casey and Webb, 2010). A cross culture-education-ethnic study in multicultural South Africa revealed the perception that the use of goat meat is linked to (African) cultural activities (Mahanjana and Cronj´e, 2000). However, it became clear that consumer bias could change after being exposed to and having tasted the meat (Xazela, 2010).

Although chevon is not favoured in western countries, the demand for chevon exceeds supply in many developing countries in subtropical and arid regions (Casey and Webb, 2010). These regions account for more than 90 % of the world goat population of approximately 650 million. In most of these countries, the production of goats is beleaguered with much inefficiency at primary production and in post-production systems (Devendra, 2001). The problems have led to a product of inconsistent quality and a general lack of synchrony between market preferences and product supply (Devendra, 2001; Simela, 2005). Mature animals above two years dominate goat markets in the developing countries (Devendra, 2001). In such animals, the collagen in connective tissue has a reduced ability to gelatinise under the influence of heat and moisture (Casey and Webb, 2010), a reason for chevon being perceived as stringy, tough and strongly flavoured, which is typical of old animals in most species (Simela and Merkel, 2008).

From the preceding review it can be seen that *M. oleifera* has great potential to be used in livestock production as a livestock feed, as a dewormer and for treatment of various livestock

diseases. However, little is known about *M. oleifera*'s medicinal properties, its use as fodder and the effect of feeding it on the meat eating quality and consumer acceptance in South Africa.

2.6 Summary of Literature review

Goats play a vital role in communal areas; however their productivity is hampered by inadequate feeds and helminth infection. *Moringa oleifera* which is now grown in many countries is reported to have nutritional and medicinal benefits with some useful mineral, vitamins, amino acids. Despite *M. oleifera*'s nutritional and medicinal properties, its potential as a supplementary feed and its effect on chevon quality and medicinal property has not been determined especially in Southern Africa. The main objective of this study was to determine the effect of supplementation of goats with *M. oleifera* leaf meal and its medicinal properties.

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Chapter 3: Nutritional characterization of *Moringa oleifera* Lam. leaves

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Abstract

The objective of the study was to determine the nutritional value of *M. oleifera* leaves of the South African ecotype. Proximate analyses and Van Soest methods were used to determine the nutritional value of *M. oleifera* leaves. The dried leaves had crude protein levels of 30.3 %. Dried leaves had the following mineral contents: calcium (3.65 %), phosphorus (0.3 %), magnesium (0.5%), potassium (1.5 %), sodium (0.164 %), sulphur (0.63 %), zinc (13.0 mg/kg), copper (8.25 %), manganese (86.8 mg/kg), iron (490 mg/kg), selenium (363 mg/kg). The α-Linolenic acid (44.57 %) had the highest value followed by heneicosanoic (14.41 %), g-linolenic (0.20 %), palmiteic (0.17 %) and capric acid (0.07 %). Vitamin E had the highest concentration of 77 mg/100g. Beta-carotene had a concentration of 18.5 mg/100g in the dried leaves. The fiber content levels were NDF (11.4 %), ADF (8.49 %), ADL (1.8 %), and ADC (4.01 %). The condensed tannins had a value of 3.2 % while total polyphenols were 2.02 %. The values of amino acids, fatty acids, minerals and vitamin profiles reflect a desirable nutritional balance.

3.1 Introduction

Moringa (*Moringa oleifera*) tree is widespread, drought tolerant tree with a potential high dry matter in the tropics (Reyes-Sánchez *et al*., 2006). It is considered as one of the World's most useful trees, which is under utilised with multi purposes such as feed, medication and industrial uses (Khafalla *et al*., 2010). *Moringa oleifera* has a potential to improve nutrition, boost food

security and foster rural development (Hsu, 2006), however most people in South Africa, are not aware of the potential benefits of this plant.

Recently a high degree of renewed interest has been placed on the nutritional properties of *M. oleifera* in most countries where it was not native (Reyes-Sánchez *et al*., 2006; Oduro *et al*., 2008). This could be due to the claims that it increases animal productivity as it has nutritional, therapeutic and prophylactic properties (Fahey, 2005). Studies from other countries indicate that the leaves have immense nutritional value such as vitamins, minerals and amino acids (Anwar *et al*., 2007). As such the leaves have been used to combat malnutrition, especially among infants and nursing mothers. In addition, nutrition plays a crucial role in both humans and livestock as short term alternative to chemoprophylaxis.

Nutrition plays a major role in animal's ability to overcome the detrimental effects of parasitism and diseases (Anwar *et al*., 2007). A well nourished animal better resists diseases when exposed to infection than the malnourished animal. When an animal is exposed to pathogens, its immune system mounts a response to fight off infection. This includes raising antibodies to fight the infection, as well as using white blood cells to attack pathogens (FAO, 2002). To gain immunity the animal needs energy, proteins for manufacture of antibodies and cells, minerals (zinc, copper and iron) and vitamins (A and E) in communicating messages in parts of the animal's body to fight infections (Conroy, 2005).

There are considerable variations among the nutritional values of *M. oleifera*, which depend on factors like genetic back ground, environment and cultivation methods (Brisibe *et al*., 2009). As

such it necessitates determination of the nutritive value of *M. oleifera* of South African ecotype, which could assists in the formulation of diets according to nutrient requirements. The nutritional composition of *M. oleifera* of the South African ecotype has to our knowledge not previously been evaluated; this is the first report that includes the profiling of chemical composition, fatty acids, amino acids and vitamins for *M. oleifera*. Amino acids, fatty acids, minerals and vitamins are essential in animal feed. These nutrients are used for osmotic adjustment, activation of enzymes, hormones and other organic molecules that enhance growth, functioning and maintenance of life process (Anjorin *et al*., 2010).

Nutritional composition of the plant plays a significant role in nutritional, medicinal and therapeutic values (Al-Kharusi *et al*., 2009). Also, the chemical composition of the fibre fractions affects the digestibility of the feed which directly or indirectly affect the feed's utilization by animals. It was reported that nutritional content in the leaves of *M. oleifera* varies with location (Anjorin *et al*., 2010). This has prompted the study of nutritional composition of *M. oleifera* of South African ecotype. Therefore the objective of the study was to determine the nutritional composition of *M. oleifera* leaves of the South African ecotype.

3.2 Materials and Methods

3.2.1 Plant collection and preparation

The plant leaves were collected from Sedikong sa Lerato in Tooseng village Ga-Mphahlele (24˚26´57.10˝S, 29˚33´47.02˝E), Limpopo Province of South Africa. The mean annual rainfall of the area is approximately 300 mm and the mean annual temperature is 15 °C. The plant was authenticated at the University of Fort Hare, Department of Botany and a voucher specimen (BM

01/2009) was prepared and deposited in the Giffen Herbarium of the University of Fort Hare. The leaves were harvested green, air-dried under shade and milled into powder through 1 mm sieve using Restch Cross Beater Mill SK 100, Monitoring and Control laboratories (Pty) Ltd, Parkhurst, South Africa. They were stored in well-dried black plastic containers inside the storeroom at room temperature of 25 °C.

3.2.2 Nutritional composition determination

Dried powdered *M. oleifera* leaves were assessed for dry matter (DM), crude protein (CP), crude fat, calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P) zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), selenium (Se) and sodium (Na). The DM (967.03) and CP (988.05) were analysed according to the standard methods of Association of Official Agricultural Chemists (AOAC, 2005) procedures. Minerals were determined in dried macrofungi samples by an atomic absorption spectrophotometer (Varian Techtron Model AAS 1 000, Varian Associates, Palo Alto, CA). Minerals (Fe, Cu, Zn, Mn, Mg, Ca, and K) were determined by atomic absorption spectrophotometric (AAS) method. Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), acid detergent cellulose (ADC) and hemi-cellulose were determined following the techniques established by Van Soest *et al*. (1991) using an Ankom200 fibre analyzer and Ankom F57 filter bags (Ankom Technology Corp., Fairport, NY) and were expressed including residual ash.

3.2.3 Condensed tannins and total phenolics determination

Condensed tannins (CT) assays were performed calorimetrically with butanol-HCl method (Bate-Smith, 1981) using purified CT from *Desmodium intortum* as a reference standard. This

method is based on oxidative cleavage of the interflavan bonds in the presence of mineral acids in alcoholic solutions at about 95 °C to yield pink coloured anthocyanidins, which are measured at 550 nm.

Total phenolics were assayed calorimetrically according to Price and Butler (1977). In this method 6 ml of aqueous solution of phenolics, 50 ml of distilled water were mixed and 0.1 ml ferric chloride were added, immediately followed by timed addition of 3 ml of 0.008 M of ferricyanide solution. The absorbance at 720 nm was read after 10 min standing at room temperature. Distilled water was used as a blank. The method exploits an oxidation-reduction reaction in which the phenolate ion is oxidized. The ferric ions are reduced to the ferrous state and detected by the formation of the Prussian Blue complex ($Fe_4[Fe(CN)_6)]_3$) with a potassium ferricyanide-containing reagent.

3.2.4 Fatty acid profile determination

Total lipids from plant material were quantitatively extracted, with a Soxhlet extraction (AOAC, 2005). The extracted fats were stored in a polytop (glass vial, with a push-in top) under a blanket of nitrogen and frozen at –20 °C, pending analyses. Approximately 10 mg of extracted lipids were transferred into a Teflon-lined screw-top test tube by means of a disposable glass Pasteur pipette. Fatty acid methyl esters (FAME) were prepared for gas chromatography by methylation of the extracted fat, using methanol–BF_3 (Christie *et al.*, 2001). Fatty acid methyl esters were quantified using a Varian GX 3400 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thickness). Analysis was performed using an initial isothermic period (40 °C for 2 minutes). Thereafter the temperature

was increased at a rate of 4 ^{o}C/minute to 230 °C. Finally, an isothermic period of 230 °C for 10 minutes followed. Fatty acid methyl esters in *n*-hexane (1µl) were injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250 °C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the chromatograms.

Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, South Africa). The following fatty acid combinations and ratios were calculated: total saturated fatty acids (SFA), total mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and *n*-6/*n*-3 ratio. All other reagents and solvents were of the analytical grade and obtained from Merck Chemicals (Pty) Ltd Halfway House, South Africa.

3.2.5 Amino Acid Determination

The samples were hydrolyzed with 6 M HCL at 100 °C for 24 hours under vacuum and amino acids were analyzed using an amino acid analyser (Bassler and Buchholz, 1993).

3.2.6 Beta-carotene and Vitamin E determination

Beta-carotene was measured according to AOAC (2005) methods 974.29, 992.04 and 992.06 and the method of Thompson and Duval (1989). Vitamin E was measured according to the methods of McMurray *et al.* (1980), Cort *et al.* (1983) and Speek *et al.* (1985) on dried leaves.

3.2.7 Statistical analyses

Each nutrient analysis was done in triplicate. Data obtained was processed using SAS proc means (2003) which computed the means and standard errors.

3.3 Results

The dried leaves of *M. oleifera* had a CP content of 30.3 % (Table 3.1) with 19 amino acids (Table 3.2). The highest value of the amino acids was alanine which had a value of 3.033 % and the least content was cysteine with 0.01 %. Calcium had the highest value of 3.65 % followed by potassium (1.5 %) and phosphorus had the least value of 0.30 % among the macro-elements (Table 3.3). The highest value among the micro-minerals was iron with 490 mg/kg followed by selenium with 363 mg/kg. Copper had the least value of 8.25 mg/kg (Table 3.3). Dried *M. oleifera* leaves were found to contain 17 fatty acids; and α-Linolenic acid had the highest (44.57 %) value followed by heneicosanoic (14.41 %), g-linolenic (0.20 %) palmiteic (0.17 %) and capric acid (0.07 %) (Table 3.4). Vitamin E had the highest level with 77 mg/100g while Beta-carotene had 18.5 mg/100g. The fiber content being NDF, ADF, ADL, and ADC of the leaves were 11.4, 8.49, 1.8 and 4.01 %, respectively. The condensed tannins had a value of 3.2 % while Total polyphenols were 2.02 % (Table 3.1).

Table 3.1: **Chemical composition of dried leaves of Moringa (*Moringa oleifera* Lam.)**

Nutritive value	Dry leaves	Standard error
Moisture (%)	9.533	0.194
Crude protein (%)	30.29	1.480
Fat (%)	6.50	1.042
Ash (%)	7.64	0.433
Neutral detergent fibre (%)	11.40	0.425
Acid detergent fibre (%)	8.49	0.348
Acid detergent lignin (%)	1.8	0.204
Acid detergent cellulose (%)	4.01	0.101
Condensed tannins (mg/g)	3.12	0.104
Total polyphenols (%)	2.02	0.390

Table 3.2: Amino acids composition of dried Moringa (*Moringa oleifera* Lam.) leaves

Name of Amino acid	Quantity (Mean+/-%)	Standard error
Arginine	1.780	0.010
Serine	1.087	0.035
Aspartic acid	1.430	0.045
Glutamic acid	2.530	0.062
Glycine	1.533	0.060
Threonine*	1.357	0.124
Alanine	3.033	0.006
Tyrosine*	2.650	0.015
Proline	1.203	0.006
HO-Proline	0.093	0.006
Methionine*	0.297	0.006
Valine*	1.413	0.021
Phenylalanine*	1.640	0.006
Isoleucine*	1.177	0.006
Leucine*	1.960	0.010
Histidine*	0.716	0.006
Lysine*	1.637	0.006
Cysteine	0.010	0.000
Tryptophan*	0.486	0.001

*General Essential amino acids

Table 3.3: Mineral contents of dried Moringa (*Moringa oleifera* Lam.) leaves

Mineral	Dry leaves	Standard error
Macro elements (%)		
Calcium %	3.65	0.036
Phosphorus %	0.30	0.004
Magnesium %	0.50	0.005
Potassium %	1.50	0.019
Sodium %	0.16	0.017
Sulphur %	0.63	0.146
Micro-elements (mg/kg)		
Zinc (mg/kg)	31.03	3.410
Copper (mg/kg)	8.25	0.143
Manganese (mg/kg)	86.80	3.940
Iron (mg/kg)	490.00	49.645
Selenium (mg/kg)	363.00	0.413
Boron (mg/kg)	49.93	2.302

Table 3.4: Fatty acids composition of dried Moringa (*Moringa oleifera* Lam.) leaves

Name of Fatty acids	Quantity (mean+/- %)	Standard error
Ether extract	6.50	0.041
Capric (C10:0)	0.07	0.064
Lauric (C12:0)	0.58	0.402
Myritic (C14:0)	3.66	1.633
Palmitic (C16:0)	11.79	0.625
Palmitoleic (C16:1c9)	0.17	0.056
Margaric (C17:0)	3.19	0.155
Stearic acid (C18:0)	2.13	0.406
Oleic (C18:1c9)	3.96	2.000
Vaccenic (C18:1c7)	0.36	0.038
Linoleic (C18;2c9,12(n-6)	7.44	0.014
α-Linolenic (C18:3c9,12,15(n-3)	44.57	2.803
g-Linolenic (C18:3c6,9,12 (n-6)	0.20	0.013
Arachidic (C20:0)	1.61	0.105
Heneicosanoic (C21:0)	14.41	0.194
Behenic (C22:0)	1.24	0.383
Tricosanoic (C23:0)	0.66	0.025
Lignoceric (24:0)	2.91	0.000
Total Saturated Fatty Acids (SFA)	43.31	0.815
Total Mono Unsaturated Fatty acids (MUFA)	4.48	1.984
Total Poly Unsaturated Fatty Acids (PUFA)	52.21	2.792
Total Omega-6 Fatty Acids (*n*-6)	7.64	0.012
Total Omega-3 Fatty Acids (*n*-3)	44.57	2.805
PUFA: SFA (PUFA:SFA)	1.21	0.096
n-6/*n*-3	0.17	0.016
PUFA: MUFA (PUFA:MUFA)	14.80	7.168

3.4 Discussion

Moringa oleifera leaves have been reported to contain nutritious compounds (Sanchez-Machado *et al*., 2010). Noteworthy is the crude protein content of 30.3 % observed in this study although lower than Sunflower cake's CP of 35.88 % which is mostly used as protein concentrate (Mapiye *et al*., 2010). This makes the *M. oleifera* leaves a good potential source of supplementary protein in animal diets. Other studies have reported variable protein content ranging between 16, 22.42, 23.27, 27.4 and 40 % (Gidamis *et al.*, 2003; Sarwatt *et al*., 2004; Nouala *et al*., 2006; Reyes-Sánchez *et al*., 2006; Oduro *et al*., 2008; Sánchez-Machado *et al*., 2010). This level of crude protein content is of particular nutritional significance as it may meet animal's protein and energy requirements and boost the immune system against diseases (Kyriazakis and Houdijk, 2006; Brisibe *et al*., 2009). General growing ruminants like goats require 16 % CP for their growth (Liginbuhl and Poore, 1998). The CP supplied by *M. oleifera* is above the protein requirement of goats making it ideal for use as a protein supplement for such ruminant animals.

Moringa oleifera is reported to have high quality protein which is easily digested and that is influenced by the quality of its amino acids (Foidl *et al*., 2001). In this study, the dried *M. oleifera* leaves contained 19 amino acids which slightly differ with findings of Foidl *et al*. (2001) and Sánchez-Machado *et al*. (2010) who reported 18 and 16 amino acids respectively. In this study only glutamine was not detected from the common 20 amino acids, however, glutamine can be derived from glutamic acid (Misner, 2008). Out of 19 amino acids observed 10 are classified as essential amino acids namely Threonine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, histadine, lysine and Tryptophan. Alanine had the highest

value of 3.03 % which differed with Sánchez-Machado *et al*. (2010) who reported the value of 1.25 %.

In their work Sánchez-Machado *et al*. (2010) reported leucine having the highest value of 1.75 % which is lower than our findings of 1.96 %. Findings from this study showed the presence of Ho-proline, cystine and tryptophan which was detected in Sánchez-Machado *et al*. (2010)'s work. Cystine and Ho-proline had the least values followed by methionine which is commonly deficient in green leaves. Methionine and cystine are powerful antioxidants that help in the detoxification of harmful compounds and protect the body from radiation (Brisibe *et al*., 2009). Ho-proline is a major component of the protein collagen, it plays a key role in collagen stability. The variations in the amino acid composition could be influenced by protein quality; the origin of the plant (cultivated or wild) and physiological stage of the plant leaves. Usually cultivated plants are fertilized, which could influence the quality of proteins (Sarwatt *et al*., 2004).

Amino acids are organic compounds that combine to form proteins; as such they influence the quantity and quality of protein. Amino acids are classified as essential and non-essential which vary according to animal species and their production system (Swanepoel *et al*., 2010). Rumen microbes synthesise the essential amino acids from other amino acids or from nitrogen containing substances. The efficiency of rumen microbial growth and activity in the rumen is enhanced by the presence of adequate amino acids, peptides and most macro and micro minerals (Alfaia *et al*., 2009). Each amino acid has a specific function in the animal's body. In general, amino acids are required for the production of enzymes, immunoglobulins, hormones, growth and repair of body tissues and form the structure of red blood cell (Brisibe *et al*., 2009). Also,

they contribute to the formation of glucose, acting as a buffer when other precursors are in short supply (Swanepoel *et al*., 2010). Amino acids affect the function of other nutrients in the animal's body such as presence of lysine, which ensures adequate calcium absorption and aids in antibody production.

The dried leaves could serve as a protein supplementary source in animal and human diets. Proteins are also essential for continuous replenishment of the endogenous protein that is lost due to infections with gastro-intestinal helminthes (Coop and Holmes, 1996). The current study identified 17 fatty acids in the dried leaves of *M. oleifera*, of which 11 were classified as saturated fatty acids, however, these had lower values. Henicosanoic had the highest value of 14.41 % followed by palmitic (11.79 %) and capric which had the least value of 0.07 %. Three polyunsaturated fatty acids were detected namely α-Linolenic, linoleic and g-Linolenic with α-Linolenic having the highest value of 44.57 %. Sánchez-Machado *et al*. (2010) reported α-Linolenic having a higher value of 56.87 %. Of interest was α-Linolenic which is an *n*-3 fatty acid that belongs to the group of the essential fatty acids.

Our findings differ with Sánchez-Machado *et al.* (2010) who found 14 fatty acids, which could be attributed to age of the leaves, soil type and climatic conditions. Sánchez-Machado *et al*. (2010), however, reported that caprylic acid (0.96 %) palmitic acid (3.66 %) and arachidonic acid (0.12 %) had the lowest in value, whereas in the current study we found that capric, palmiteic and g-Linolenic had the lowest values. Of these three fatty acids, only lauric was found in our analysis. As observed in this study, *M. oleifera* leaves contain more dietary polyunsaturated fatty acids than the saturated fatty acids. A higher content of PUFA and lower

amount of SFA is desirable (Hoffman and Wilklund, 2006), as such, its inclusion in the diet is recommended as it prevents the occurrence of diseases thereby promoting good health. Wood *et al.* (2008) recommended more consumption of α-linolenic acid which promotes the endogenous synthesis of long chain *n*-3 fatty acids.

Polyunsaturated fatty acids are important for human and animal health. They are of interest because they are precursors of long chain *n*-3 PUFA in the eicosanoids biosynthesis, which are viewed as important bioregulators of many cellular processes (Khotimchenko, 2005). They are linked to the development and functionality of the immune system. Consumers have preference of food low in saturated fatty acids (SFA) because they are associated with an increased risk of cardio-vascular diseases and some cancers (Griffin, 2008; Alfaia *et al*., 2009). Human nutritionists urge consumers to increase intake of polyunsaturated fatty acids (PUFA), particularly the *n*-3 PUFA at the expense of *n*-6 PUFA (Hoffman and Wiklund, 2006; Alfaia *et al*., 2009). The quantity and composition of fatty acids in the animals' body are related to the presence of some of their precursors in the diet (Wood *et al*., 2003).

The observed low concentration of acid detergent fibres and neutral detergent fibres in the study compared with most forage plants is of interest because, fibre fraction defines the extent and rate of feed digestibility (Rubanza *et al.,* 2005). The values of NDF and ADF of 11.4 and 8.49 % differed with the findings of Foidl *et al*. (2001) that showed NDF and ADF values of 21.9 and 11.4 %, respectively, suggesting that the leaves used in this study could be of high digestibility. These variations of NDF and ADF values may be due to differences in agro-climatic conditions, physiological stage of trees, and possibly due to different stages of maturity of leaves. The

observed concentrations of Acid detergent lignin (ADL) in the current study were however, consistent with values reported by Foidl *et al.* (2001).

Another interesting aspect of the results reported here is the low percentages of anti-nutritional factors in the leaves, which though present were negligible. The value of condensed tannins was 3.12 % while Foidl *et al.,* 2001 reported 1.4 % of tannins and did not detect the condensed tannins. Drying is reported to reduce or remove extractable condensed tannins by 15-30 % relative to fresh foliage (Vitti *et al*., 2005). The decrease of condensed tannins after drying may be due to decomplexation between tannins and proteins, and depolymerisation and oxidation of tannins (Makkar, 2003). The content of total phenols (2.02 %) in this study was lower than previously reported values of 2.7 and 4.3 % (Gupta *et al*., 1989; Foidl *et al*., 2001). At these concentrations simple phenols do not produce any adverse effects when consumed by animals (Foidl *et al*., 2001). However, these phenols have been reported to have multiple beneficial biological effects that include antioxidant activity, anti-inflammatory action, inhibition of platelet aggregation, antimicrobial activities and antitumor activities (Thurber and Fahey, 2009).

It is also of remarkable interest that the dried *M. oleifera* leaves have high deposit of mineral elements. Calcium was observed to be higher compared to other plant sources (Nkafamiya *et al*., 2010). It is required for formation and maintenance of bones and teeth thus preventing osteoporosis. It is also needed for normal blood clotting and nervous function. Interestingly, even iron, which is commonly deficient in many plant-based diets, was found in abundance in this plant's leaves. Iron is a necessary component of haemoglobin and myoglobin for oxygen transport and cellular processes of growth and division (Kozat, 2007). Iron is also an essential

trace element for normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats (Umar *et al*., 2007). Iron also has a role in energy metabolism as it facilitates transfer of electrons in the electron transport chain for the formation of Adenosine triphosphate (ATP) (Kozat, 2007).

The presence of zinc in fairly high amounts is of special interest in view of the importance of the inclusion of zinc in the diet of animals and humans. Results from this study had higher levels of zinc (31.03 mg/kg) than the findings of Barminas *et al*. (1998) who reported 25.5 mg/kg in dried *M. oleifera* leaves. Zinc is essential for the synthesis of DNA, RNA, insulin and function and/or structure of several enzymes (Brisibe *et al*., 2009). Zinc is also required for cell reproduction and growth especially sperm cells. In addition zinc is known for its anti-viral, anti-bacterial, anti-fungal and anti-cancer properties (Brisibe *et al*., 2009).

The *M. oleifera* dried leaves contained copper which is considered to have strong effects on the immune system (Anwar *et al*., 2007). Copper is involved in stimulating body defence system, as it is active in neutrophil production and affects phagocyte killing ability. It is required for antibody development and lymphocyte replication (Burke and Miller, 2006). Copper in combination with zinc, plays a significant role in superoxide dismutase activity and the removal of oxygen free radicals. It is, therefore, a key component in the protective mechanism of cellular membranes against superoxide free radicals damage (Guo *et al*., 2010). In addition, the copper containing enzyme, ceruloplasmin has been shown to exhibit antinflammatory activity, which may prove beneficial in mastitis cases (Guo *et al*., 2010). Copper has been found to reduce internal parasite namely *Haemonchus contortus* load in sheep and goat (Burke and Miller, 2006).

Moringa oleifera leaves have sulphur that is necessary for efficiency of rumen microbial growth and activity (Brisibe *et al*., 2009). *Moringa oleifera'*s mineral composition plays a significant role in nutritional, medicinal and therapeutic values (Al-kharusi *et al*., 2009).

The results showed that the dried powdered *M. oleifera* leaves have high levels of vitamin E and Beta-carotene. *Moringa oleifera* powder has been reported to be rich in beta-carotene, thiamine, riboflavin, niacin, pyrodixine, biotin, ascorbic acid, cholecalciferol, tocopherol and vitamin K (Broin, 2006). As such in our study we investigated the presence of Beta-carotene and vitamin E in the dried leaves. The reason being that under normal conditions, healthy ruminants synthesise adequate amounts of B vitamins as well as vitamin C and K (Rinehart, 2008). Beta-carotene is the most potent precursor to vitamin A. The animals are able to convert beta-carotene into vitamin A within their body (Panday and Tiwari, 2002). *Moringa oleifera* is reported to be rich in vitamin C which increases iron absorption in the animal's body (Anwar *et al*., 2007). Vitamin A is necessary for many functions in the ruminants including vision, bone growth, immunity and maintenance of epithelial tissue. In addition, vitamin A also maintains adequate levels of iron in plasma that supply the different body tissues including the bone marrow (Thurber and Fahey, 2009). Supplementation of diets with both iron and vitamin A may increase the iron status as measured by haemotological indices like haemoglobin and haemocrit (Babu, 2000).

Beta-carotene rich *M. oleifera* leaves can thus be an important source of vitamin A, and can be used for releasing the bound iron status and thus help in reducing anaemia as well as prevalence of vitamin A deficiency. Vitamin A and E are some of the specific nutrients that assist animals to develop disease resistance. Our findings are in agreement with Fuglie (2001) where the amount

of vitamin E was 113 mg/100g in dried leaves. Vitamin E is known to help maintain and increase the storage of vitamin A and iron in the body. *Moringa oleifera* powder is, however, rich in vitamin such that it is one of the richest plant sources of vitamin (Anwar *et al*., 2007).

Vitamin E with selenium contain antioxidants that work co-dependently in the body to help destroy free radicals (Thurber and Fahey, 2009). The interaction of selenium and immune function focuses around the selenoprotein, glutathione peroxidase. Glutathione peroxidase inactivates oxygen radicals such as hydrogen peroxide and prevents them causing cellular damage. Also supplementing dairy cattle with adequate levels of selenium (0.3 ppm of dry mater) reduce the prevalence, severity and duration of mastitis (Rock *et al*., 2001). Looking at all the properties of the plant leaves, this probably explains the traditional use of the plant as a herbal tonic in India, because of its high levels of readily available essential nutrients and mineral resources which may be required for the maintenance of electrical potential of nervous tissues and cell membranes. It can as well be used for the treatment of blood related disorders that is necessary for the improvement of the overall well-being of the body.

The nutritional variations observed among the studies could be attributed to the genetic background of the plant, in terms of ecotype and cultivar, environmental factors that include the soil and climate (Sánchez-Machado *et al*., 2010). Also, the cultivation method used which encompasses the frequency of harvesting and physiological stage of the plant or leaves. Mode of conservation between collection and analysis (drying, refridgeration, freezing) might influence the leaves' nutritional composition (Barminas *et al*., 1998; Broin, 2006).

3.5 Conclusion

In conclusion, the results derived from nutrient characterization of *M. oleifera* are clear indications that the plant leaves are rich in nutrients and have a potential to be used as a feed additive with multiple purposes. These include serving as a protein, fatty acid, mineral and vitamin resource for animal and human feed formulations. High nutritional content found in the dried leaves are important nutritional indicators of the usefulness of the plant as a likely feed resource. Given that, *M. oleifera* leaves contain some compounds such as tannins, polyphenols and proteins speculated to have antimicrobial properties, it is therefore imperative to determine the microbial effects of *M. oleifera* extracts.

3.6 References

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Chapter 4: Antimicrobial activities of *Moringa oleifera* Lam leaf extracts

(Submitted to *African Journal of Biotechnology*)

Abstract

The objective of the study was to determine the antibacterial and antifungal activities of the *M. oleifera* leaf extracts using *in vitro* antimicrobial screening methods. The acetone extract of *M. oleifera* leaves at a concentration of 5 mg/ml showed antibacterial activities against *Escherichia coli, Enterobacter cloace, Proteus vulgaris, Staphylococcus aureus* and *Micrococcus kristinae*. *Micrococcus kristinae* was the most susceptible as its growth was inhibited at 0.5mg/ml. On the other hand *M. oleifera* acetone extract did not exhibit any inhibition on *Streptococcus faecalis, Bacillus pumilus, Klebsiela pneumonia, Bacillus cereus* and *Pseudomonas aeruginosa*. The acetone extract was bactericidal on *E. coli* and *Micrococcus kristinae*. It was also bacteriostatic on *S. aureus, Enterobacter cloace* and *Proteus vulgaris*. However, the water extract showed no activity at the highest concentration (5 mg/ml) tested. Furthermore both the acetone and aqueous extracts did not exhibit any antifungal activity against the fungal species of *Candida albicans, Pennicillium notatum, Aspergillus flavus* and *Aspergillus niger* even at the highest concentration of 10 mg/ml. The ability of acetone extract to inhibit the growth of some strains of bacteria is an indication of its antibacterial potential which may be employed in the management of microbial infections.

4.1 Introduction

Over the years, plants have been used as valuable sources of natural products for maintaining animal and human health. Plants have been reported to contain large varieties of chemical substances that possess important preventative and curative therapies (Nascimento *et al*., 2000). About 80 % of individuals from developed countries use traditional medicines which have compounds derived from medicinal plants (Igbinosa *et al*., 2009). Despite the presence of various approaches to drug discovery, plants still remain the main reservoir of natural medicines (Mahomed and Ojewole, 2006).

Interest in plants with antimicrobial properties has been revived as a result of antimicrobial resistance to conventional drugs. This resistance could be attributed to indiscriminate use of commercial drugs or not taking an antibiotic prescription according to the instruction for example not taking all the prescription in the treatment of infectious diseases (Aliero and Afolayan, 2006). In addition, certain antibiotics present the undesirable side effects such as nausea, depression of bone marrow, thrombocytopenic purpura and agranulocytosis leading to the emergence of previously uncommon diseases (Marchese and Shito, 2001; Poole, 2001). This has given scientists the impetus to search for newer and alternative microbial compounds from medicinal plants (Aliero and Afolayan, 2006). Besides, the high cost of conventional drugs, particularly in resource-limited communities has led to the increased use of plants as an alternative for the treatment of infectious diseases. Plant extracts and phytochemicals with antimicrobial properties are of great significance in therapeutic treatments. Their antimicrobial properties are due to compounds synthesized in the secondary metabolism of the plant. The

screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of novel antibiotic protypes (Afolayan, 2003).

Bacteria and fungi are of both human and veterinary importance as outlined below. *Bacillus cereus* has been implicated in food-borne intoxication (Granum and Lund, 1997). *Escherichia coli*, *S. aureus* and *P. aeruginosa* cause diseases like mastitis, abortions and upper respiratory complications (Fraser, 1986). *Streptococcus faecalis* is a pathogenic bacteria commonly found in the intestines of birds (Granum and Lund, 1997). *Aspergillus niger* has been reported to cause lung diseases aspergillosis and otomycosis. Similarly *A. flavus* is a human and livestock pathogen associated with aspergillosis of the lungs and sometimes causing corneal, otomycotic and nasoorbital infections. *Aspergillus flavus* produce significant quantity of aflatoxin (Samson *et al*., 2001: Klich, 2007). *Penicillium notatum* induces hypersensitivity and pneumonitis in animals (Klich, 2007). *Candida albicans* is reported to cause vaginitis and yeast Mastitis (Gurib-Fakim, 2006). These necessitate searching for antibiotics that could be used against microbes.

Moringa oleifera Lam leaves have been reported to possess antimicrobial properties and this explains the reason for its wide use in the treatment of human diseases (Lockett *et al*., 2000; Anwar *et al*., 2007). To the best of our knowledge, there is little or no information on the antimicrobial activities of the South African ecotype of *M. oleifera*. The objective of the current study was, therefore, to determine the antibacterial and antifungal activities of *M. oleifera* extracts using *in vitro* antimicrobial screening methods.

4.2 Materials and Methods

4.2.1 Plant material and extract preparation

The *M. oleifera* leaves were collected at Sedikong sa Lerato in Tooseng village Ga-Mphahlele and a detailed description was given in Section 3.2.1. The two solvents acetone and water were used and in all cases equal volumes of solvents were used.

4.2.2 Plant extracts preparation

One hundred grams of powdered leaves were soaked in 500 ml of high analytical grade acetone, which is less lethal to the test organisms (Eloff, 1998). Another plant sample (100 g) was extracted in distilled water. They were left shaking on the orbital shaker for 48 h at 30 ˚C (Stuart Scientific Orbital shaker, UK) and later filtered separately through Whatman no.1 filter paper. Thereafter the acetone extract was evaporated to dryness under reduced pressure at 40 ˚C using a rotary evaporator (Laborator 4000-efficient, Heidolph, Germany). The water extract was freeze-dried using Savant refrigerated vapor Trap, (RVT4104, USA) and stored at 4 ˚C. The percentage yields of acetone and water extracts were 16 and 13 %, respectively. They were stored in air-tight glass bottles before use and later re-dissolved in their respective solvents to give the desired concentrations for the various experiments.

4.2.3 Antibacterial activity assay

The bacteria strains used were those recommended by the National Committee for Clinical Laboratory Standards. The selection of organisms depended on availability and was as follows-: *Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884), *Micrococcus kristinae* (AI5). *Pseudomonas*

aeruginosa ATCC (19582), *Escherichia coli* (ATCC25922), *Enterobacter cloace* (ATCC 13047), *Klebsiela pneumonia* (ATCC 10031) and *Proteus vulgaris* (ATCC 6830). The bacteria were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa. Organisms were chosen based on reports of their human and Livestock pathogenicity.

4.*2.4 Minimum Inhibitory Concentration (MIC)*

The bacteria species were maintained on nutrient agar plates and recovered for testing by sub-culturing in nutrient broth (Oxoid) and incubated at 37˚C for 18 h. Before use, each bacteria culture was diluted 1:100 with fresh sterile nutrient broth (Grierson and Afolayan, 1999). The bacteria were streaked in a radial pattern on the agar plates (Meyer and Afolayan, 1995), which were incubated at 37 ˚C under aerobic conditions and examined after 24 and 48 h. Complete suppression of growth by specific concentration of an extract was required to be declared active (Mathekga *et al.*, 2000). Each extract was tested at a concentration of 5.0, 1.0, 0.5 and 0.1 mg/ml. Streptomycin and chloramphenicol were used as standard (positive) controls with pure solvents (acetone and water) and sample free solutions as blank controls. Each test was replicated three times. Acetone has been reported to be non-toxic to the organism at the concentration used (Meyer and Afolayan, 1995).

4.2.5 Minimum Bactericidal Concentration (MBC)

The Minimum bactericidal concentration (MBC) of the plant extracts was determined by the modified method of Spencer and Spencer (2004). The samples were sub-cultured from MIC plates that showed no growth after 24 h on to a fresh extract-free solid medium and incubated

further for 18-24 h. The highest dilution (least concentration) that yielded no single bacterial colony on a solid medium was taken as MBC. The MBC was not determined for the water extract since it did not exhibit antibacterial activity. It should also be noted that the condition of evaluation for extract effectiveness was similar for all the bacterial and fungal species used.

4.2.6 Antifungal activity assay

The antifungal activity of *M. oleifera* was investigated using four fungal species (*Aspergillus niger* (ATCC 16404), *Aspergillus flavus* (ATCC 9643), *Pennicillium notatum* (LIO) and *Candida albicans* (ATCC10231), which were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa. The selection of fungi used in the study was based on history of being pathogenic to humans and livestock. The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) (Oxid) at 25˚C until they sporulated. Thereafter the fungal spores were harvested by pouring a mixture of sterile glycerol and distilled water to the surface of the plate. Later the spores were scraped with a sterile glass rod. The harvested fungal spores were standardized to an OD_{600nm} of 0.1 before use. The standardized fungal spore suspension (1000µl) was evenly spread on the SDA (Oxoid) using a glass spreader. Wells were bored into the agar media using a sterile 6 mm cork borer and the wells filled with the solution of the extract (0.2 ml) taking care not to allow spillage of the solution to the surface of the agar medium. Acetone and water extract concentrations used were of 0.1, 0.5, 1.0, 5.0 and 10 mg/ml. The plates were allowed to stand on the laboratory bench for 1 hour to allow for proper diffusion of the extract into the media. Plates were incubated at 25 ˚C for 96 h and later observed for zones of inhibition. The effect of the extract on fungal isolates was compared with amphothericin B and miconazole at a concentration of 1 mg/ml (Igbinosa *et al*., 2009).

4.2.7 Statistical analyses

Diameter of fungal growth was measured and expressed as means of percentage growth inhibition of three replicates. They were analysed using General Linear Model procedure of SAS (2003).

4.3 Results

4.3.1 Antibacterial activity

The leaf acetone extract of *M. oleifera* at 5 mg/ml showed antibacterial activities against E. coli (ATCC 25922), *E. cloacae* (ATCC 13047), *P. vulgaris* (ATCC 6830) and *S. aureus* (ATCC 6538) and *M. kristinae* § at 0.5 mg/ml while reference antibiotics streptomycin and chloramphenicol had antibacterial activity at 2 µg/ml (Table 4. 1). As indicated in Table 4. 2, the *M. oleifera* acetone extract was bactericidal on *E. coli* (ATCC 25922) and *M. kristinae*, while it was bacteriostatic on *S. aureus* (ATCC 6538), *E. cloacae* (ATCC 13047) and *P. vulgaries* (ATCC 6830). Although the MBC value for the *M. oleifera* acetone extract against *M. kristinae* was higher (1.0 mg/ml) than its MIC value of 0.5 mg/ml, it is interesting to note that the MIC and MBC values (5 mg/ml) against the inhibited bacteria were the same. The water extract did not show any activity at the highest concentration (5 mg/ml) tested.

4.3.2 Antifungal activity

Both the *M. oleifera* acetone and aqueous extracts did not exhibit antifungal activity against the fungal species *C. albicans* (ATCC10231), *P. notatum* (LIO), *A. flavus* (ATCC 9643) and *A. niger* (ATCC 16404).

Table 4.1: Antibacterial activity of the leaf extracts of *Moringa oleifera*

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) (mg/ml)

Bacteria species	**Gram Reaction**	**Water extract**	**Acetone extract**	**Streptomycin µg/ml**	**Chloramphenicol µg/ml**
Bacillus cereus(ATCC 10702)	+	na	na	< 2	< 2
Bacillus pumilus (ATCC 14884)	+	na	na	< 2	< 2
Staphylococcus aureus (ATCC 6538)	+	na	5 (5)	< 2	< 2
Streptococcus faecalis (ATCC 29212)	+	na	na	< 2	< 2
Micrococcus kristinae §	+		0.5 (1)	< 0.5	< 2
Escherichia coli (ATCC 25922)	-	na	5(5)	< 2	< 2
Pseudomonas aeruginosa (ATCC 19582)	-	na	na	< 5	< 20
Enterobacter cloacae (ATCC 13047)	-	na	5(5)	< 2	< 2
Klebsiella pneumoniae (ATCC 10031)	-	na	na	< 2	< 2
Proteus vulgaris (ATCC 6830)	-	na	5(5)	< 2	< 2

na= not active; MBC values in bracket

§= Environmental strain

Table 4.2: Bacteriostatic and Bactericidal of *Moringa oleifera* acetone extract

Bacterial species	**Gram +/-**	**Bacteriostatic mg/ml**	**Bactericidal mg/ml**
Staphylococcus aureus (ATCC 6538)	+	5.0	na
Micrococcus kristinae §	+	na	1.0
Proteus vulgaris (ATCC 6830)	-	5	na
Escherichia coli (ATCC 25922)	-	na	5
Enterobacter cloacae (ATCC 13047)	-	5	na

na= not active

§= Environmental strain

4.4 Discussion

The susceptibility of some bacteria strains to the extract of *M. oleifera* may be a pointer to its potential as a drug that can be used against these susceptible bacterial strains. Furthermore, antibacterial resistance, especially, among Gram-negative bacteria is an important issue that has created a number of problems in the treatment of infectious diseases and necessitates the search for alternative drugs or natural antibacterial remedies (Khosravi and Behzadi, 2006). The difference in bacterial response was possible due to the nature of the bacterial species. It is noted that the acetone extract of *M. oleifera* leaves exhibited antimicrobial effect against both Gram-positive and Gram-negative bacteria (broad spectrum activities).

The *M. oleifera* acetone extract, however, showed greater anti-bacterial activity against Gram-negative bacteria than Gram-positive bacterial strains. These contrasts with most researchers' findings who reported that most plant extracts have more activity against Gram–positive bacteria (Aiyegoro *et al*., 2008; Boussaada *et al*., 2008; Ashafa and Afolayan, 2009). Noteworthy is the ability of the *M. oleifera* acetone extract to inhibit the growth of *M. kristinae* at 0.5 mg/ml. which is the lowest MIC value in comparison to other bacterial strains. This suggests that *M. kristinae* was more sensitive to *M. oleifera* acetone extract and therefore could be used as an antibacteria against diseases that are caused by *M. kristinae*. This observation can best be explained by the fact that *M. kristinae*, which is an environmental strain, has a low rate of antibiotic resistant genes compared to most clinical bacterial strains; hence its susceptibility to the extract at a lower MIC value compared to clinical strains (Aiyegoro *et al*., 2010).

The non activity of the water extract against microbes investigated in this study is in agreement with previous works, which showed that aqueous extracts of plants generally exhibited little or no anti-microbial activities (Aiyegoro *et al*., 2008; Ashafa *et al*., 2008). Masika and Afolayan (2002) reported that Gram-negative bacteria are more resistant to water extracts. Furthermore, most researchers (Paz *et al.,* 1995; Vlientinck *et al.,* 1995; Martin and Eloff, 1998) have generally reported that water extracts of plants do not have much activity against bacteria. The lesser effectiveness of *M. oleifera* leaf water extracts compared with those of acetone may be due to differences, in polarity of the two solvents, and thus of the extracted constituents (Boussaada *et al*., 2008). It is also suggested that the active principles from plant materials are not readily extractable in water; in this study acetone was a better solvent than water in extracting the active constituents from the leaves of *M. oleifera*.

Compounds like tannins and polyphenol which are found in *M. oleifera* are soluble in acetone (Makkar and Singh, 1992) and have been reported to possess antibacterial activity (Khosravi and Behzadi, 2006). Our findings, however, differ from the study by Dahot (1998) who reported that *M. oleifera* water extracts had antimicrobial activity against *E. coli*, *S. aureus* and *B. subtilis*. The difference could be attributed to variation in the environment where the plant was collected, the season and the physiological stage of the plant when leaves were harvested (Taylor and van Staden, 2001). This affects the chemical composition and the amount of compounds in the plant. In general water extracts are the commonly used and are affordable to resource-limited farmers. The curative advantage is that consumers including animals tend to consume the plant material in large quantities and in high concentrations. This suggests its ability to meet the required physiological levels to inhibit the pathogen growth *in situ*. Yang *et al.* (2006) reported that the

inclusion of *M. oleifera* leaf meal in Broiler feeds reduced the *E. coli* bacteria count in the ileum. In addition, *M. oleifera* leaf water extracts exhibited antimicrobial properties through the inhibition of the growth of *S. aureus* strains isolated from food and animal intestines (Yang *et al*., 2006). This point to the potential of *M. oleifera* as an antimicrobial peptides to replace antibiotics in feeds.

In our study the *M. oleifera* acetone extract had bactericidal properties against *E. coli*, which is mostly known to be multi-resistant Afolayan (2003). The ability of the acetone extract to kill *E. coli* is noteworthy even though it was at the highest concentrations (5.0 mg/ml) tested. Moreover, Gram-negative bacteria have been reported to be resistance to antibiotics (Boussaada *et al*., 2008). According to several authors, these bacteria are generally less sensitive to the activity of plant extracts (Pintore *et al*., 2002; Wilkinson *et al*., 2003; Boussaada *et al*., 2008). Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Adwan *et al*., 1998).

The bactericidal and bacteriostatic activities of the *M. oleifera* acetone extract against *E. coli, M. kristinae, S. aureus, E cloacae* and *P. vulgaris* was established. Compounds like pterygospermin, benzyl glucosinolate and benzyl isothiocynate have, however been isolated from *M. oleifera* leaves and these compounds have been reported to have antimicrobial properties against a wide range of bacteria which could partly explain the observed bacteriostatic and bactericidal activity (Fahey, 2005). It should be noted that for plant materials, there is actually no standard concentration agreed upon as a model measure for determining the antibacterial activity but

according to Aliero and Afolayan (2006); Ashafa *et al*. (2008); Aiyegoro *et al*. (2009), they considered 5 mg/ml as their highest concentration level.

The leaves of *M. oleifera* have also been known to contain a number of phytochemicals such as flavonods, saponins, tannins and other phenolic compounds that have antimicrobial activities (Sato *et al*., 2004; Cushine and Lamb, 2005; Mboto *et al*., 2009). This would suggest that, the antimicrobial activities observed in this study could be attributed to such compounds. The mechanisms of actions of these compounds have been proved to be via cell membranes perturbations (Esimone *et al*., 2006). This coupled with the action of β-lactams on the transpeptidation of the cell wall could lead to an enhanced antimicrobial effect of the combinations (Esimone *et al*., 2006).

According to Dahot (1998), *M. oleifera* leaf extracts contain small peptides which could play an important role in the plant's antimicrobial defense system. The proteins/peptides are believed to be involved in a defense mechanism against phytopathogenic fungi by inhibiting the growth of micro-organisms through diverse molecular modes, such as binding to chitin or increasing the permeability of the fungal membranes or cell wall (Chuang *et al*., 2007). Antimicrobial peptides probably interact with the membranes in two stages. Firstly, cationic amino acids are attracted by negative charges such as phospholipid head groups on the surface. Secondly, hydrophobic and positively charged patches of the peptide interact with the aliphatic fatty acids and the anionic components, respectively (Zasloff, 2002; Koczulla and Bals, 2003). This induces membrane destabilization, and bacteria are thought to be killed by the leakage of cytoplasmic contents, loss of membrane potential, change of membrane permeability and of lipid distribution, the entry of

the peptide and blocking of anionic cell components or the triggering of autolytic enzymes (Zasloff, 2002). Another strategy followed by plants to thwart invaders is based on the localized production of antimicrobial; low molecular weight secondary metabolites known as phytoalexins (Maher *et al*., 1994; Dahot, 1998). The antibacterial activity of *M. oleifera* acetone extract validates some medicinal uses of *M. oleifera* (Fahey, 2005; Fugile, 2005).

In our study none of the extracts showed any antifungal activity. Similar to our results is the report by Dahot (1998), whereby the *M. oleifera* water extracts were found inactive against the growth of *Aspergillus fumigates*, *A. flavus* and *Penicillium expansum* and moderately active against *A. niger* (Dahot, 1998). Variation in the antimicrobial activity of *M. oleifera* water extract could be attributed to the plant's ability to produce a wide range of selective antimicrobial compounds. This could be either in a constitutive or an inducible manner to protect themselves against pathogens (Cammue *et al*., 1992; D´Haeze and Holsters, 2004). Moreover, the synthesis of many presumed defense related compounds are induced when plants are exposed to pathogens (Linthorst, 1991). Antibacterial activity showed by the acetone extracts might justify the reports that *M. oleifera* have medicinal properties. Interestingly the plant's nutritional compounds assist the consumer to enhance their immune system against wide range of pathogens (Oduro *et al*., 2008). Moreover, its ability to inhibit the bacterial growth enables the antibodies generated to destroy the invading pathogens.

4.5 Conclusion

The acetone leaf extracts of *M. oleifera* exhibited some antibacterial activities against some strains such as *E. coli*, *E. cloace*, *P. vulgaris*, *S. aurens* and *M. kristinae*. However, both acetone

and aqueous extracts did not exhibit any fungal activity against fungal species. The ability of acetone extracts to inhibit the growth of some strains of bacteria is an indication of its potential which maybe employed in the management of microbial infections in livestock production, and hence *M. oleifera* leaves has a potentially to be used as a feed supplement due to its high protein and at the same time having the medicinal effect on the animal. It can be expected to have an anthelmintic effect. It is, therefore, of paramount importance to determine the effects of goat supplementation with *M. oleifera* on helminth load.

4.6 References

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Chapter 5 Effects of supplementing cross-bred Xhosa lop eared goats with *Moringa oleifera* Lam on helminth and coocidian-oocyst loads and corresponding body condition score, packed cell volume

(Submitted to *Tropical Animal Health and Production*)

Abstract

The objective of this study was to determine the effect of supplementing indigenous goat with *M. oleifera* leaf on helminth load. A total of 12 castrated 8 months old goats, with a mean body weight of 14.63 ±0.26 kg were randomly allotted to three dietary groups with 4 goats in each. All groups (MOL, SC and GH) were fed on basal diet of grass hay (GH) *ad libitum* and wheat bran (200 g/head/day) for 60 days. In addition, the MOL and sunflower cake (SC) groups were fed 200 g of dried *M. oleifera* leaves and 170 g of SC, respectively. On day 14 the faecal larval count started to decrease significantly, in the MOL and SC supplemented groups until the end of the experiment. Goats supplemented with MOL and SC diets had lower *Haemonchus contortus*, *Trichostrongylus colubriforms* and *Oesophagastum columbianum* worm burdens than those on the GH diet. Goats supplemented with MOL had lower coccidian- oocyst ($P < 0.05$) count of the *Eimeria spp.* The mean BCS and PCV in the GH group started to decline after day 14, while increasing in the MOL and SC groups until the end of the experiment. It can be concluded that supplementing goats with *M. oleifera* improved the BCS, PCV values and suppressed coccidia-oocysts and helminth load.

5.1 Introduction

The productivity of goats is affected by helminths to which goats are highly susceptible (Hoste *et al*., 2008; Hoste and Torres-Acosta, 2011). Internal parasitism can cause major health issues, which have a major effect on the animal's performance and cause great economic loss to the goat farmer (Hoste and Torres-Acosta, 2011). Grazing animals are always exposed to helminths of mixed species. The most common helminths are *Haemonchus contortus*, *Bunostomum trigonocephalum*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Cooperia* species (Hoste *et al*., 2008).

High helminth infestations, coupled with low management levels of goats by resource-limited farmers culminate to considerable deleterious effects and economic losses to goat productivity (Arsenos *et al.*, 2009). Infection with helminths generally reduce feed consumption and utilisation, growth performance, fertility, meat yield and quality (Arsenos *et al*., 2009; Marume, 2010) and result in loss of blood and consequently, death (Hoste *et al*., 2008). The greatest economic losses associated with helminthosis are subclinical (Marume, 2010), as growth rate is compromised and the animals take a long time to reach market weights.

Helminths in small ruminants are generally controlled by use of broad-spectrum synthetic anthelmintics (Hoste and Torres-Acosta, 2011). The effectiveness of synthetic anthelmintics are strongly questioned because of wide spread development of helminth resistance to these compounds (Hoste and Torres-Acosta, 2011). Other effective drugs have long withdrawal period which often make them unsuitable for use in meat and milk goats (Hoste and Torres-Acosta, 2011).

Alternative grazing, nutrition and sanitary management strategies are needed to control helminth infestations in livestock because of the increasing demand for safe food and the development of drug resistance in several helminth species (Hoste and Torres-Acosta, 2011). Among the alternative methods to anthelmintics currently available is the manipulation of the host nutrition. This is done to improve the host resistance and resilience to helminth infection and seems to be one of the promising options (Hoste *et al*., 2008). Protein and energy supplementation are known to improve goat resistance and resilience against parasitic infections (Hoste *et al*., 2008; Xhomfulana *et al*., 2009). Furthermore, recent studies have revealed beneficial effects of some plant secondary compounds such as tannins on the control of gastrointestinal nematode infections (Hoste *et al*., 2008). Supplementation with tannin-containing forage (*Calluna vulgaris, Erica* spp, *Acacia karroo*) has been proven to reduce parasite burden and egg excretion in goats, thereby increasing animal performance (Arsenos *et al*., 2009; Marume, 2010).

Another plant with a potential to reduce internal parasitic load while being used as a feed is *Moringa oleifera* (Mendieta-Araica *et al*., 2011). The nutritional value of the leaves and its antimicrobial activity are discussed in Chapter 3 and 4, respectively. It is presumed its nutritional and medicinal values could have an impact on the helminth load in goats, especially in the communal areas where farmers generally keep the crossbred goats due to indiscriminate crossbreeding (Rumosa Gwaze *et al*., 2009). Bakare and Chimonyo (2011) affirm that goats crossbred with the Xhosa lop-eared are large framed and have a potential to increase meat yield per animal. Communal farmers have also shown renewed interest on the genes of the Xhosa lop-eared goats (Bakare and Chimonyo, 2011). However, the dietary impact of using *M. oleifera* leaves on helminth load of these crossbred Xhosa lop-eared goats is unknown. The objective of

this study was to determine the effect of supplementing goats with *M. oleifera* leaves on helminth load.

5.2 Materials and Methods

5.2.1 Study site

The study was conducted at Honeydale Farm, University of Fort Hare, Alice, South Africa which lies along longitude 32° 78' E and latitude 26° 85' S at an altitude of 450-500 m above sea-level. It is located in the False Thornveld of the Eastern Cape Province which is characterized by mean annual rainfall of 480 mm and mean annual temperature of 18.7°C, respectively (Muchenje *et al*., 2008). Most rain falls in summer during the months of November to March. Goats at the Honeydale farm graze on natural pastures mainly composed of the following grass species; *Aristida congesta, Cymbopogon plurinodis*, *Cynodon dactylon, Digitaria eriantha, Sporobolus africanus, Sporobolus fimbriatus, Themeda triandra* and *Eragrostis* species. *Acacia karroo, Scutia myrtina* and *Maytenus polyacantha* are the dominant tree species (Muchenje *et al*., 2008).

5.2.2 Collection of feeding materials

Harvesting of *M. oleifera* leaves was described in Section 3.2.1. The leaves were air-dried by spreading them on clean plastic under shade for 72 hours and turned several times. The grass hay consisted of mainly *Pennisetum clandestinum*, *Sporobolus africanus* and *Cynodon dactylon* which was mowed (harvested) at an early tender stage from the University of Fort Hare grounds in the month of February to March 2010. The most dominant grass species was *P. clandestinum.* Harvesting of grass was done at a frequency of 14 days, pre-dried in the sun for 24 hours by spreading on a concrete floor and turned several times, thereafter air-dried in a shaded place and

kept until use. Wheat bran was bought from UMtiza Farmers Corp, Alice South Africa, while sunflower cake was bought from Monti Feeds (PTY) LTD, East London South Africa.

5.2.3 Chemical analysis of the feeding diets

The formulated diets were assessed for nutrient composition as described in Section 3.2.2 (Table 5.1).

5.2.4 Management of experimental Animals

Twelve castrated 8 months old crossbred Xhosa lop-eared goats with a mean initial body weight of 14.63 ±0.26 kg bought from the University of Fort Hare's Honeydale farm were used in the study. They were treated against external parasites fortnightly using Drastic Deadline (Flumethrin 1%- Bayer (Pty) Ltd Isando, South Africa). The goats were individually housed in open sided, slatted floor cages (1.5 m X 1.5 m) that complied with welfare standards. The goats were given a 21 day acclimatisation period to feed and housing before commencement of the experiment. The animals were randomly allotted into 3 treatment groups of 4 goats each, balanced in terms of live weight and body condition scores. Then, the three feeding treatments: Sunflower seed cake (SC), the positive control, *M. oleifera* leaf (MOL) and grass hay (GH) negative control were randomly assigned to one of the three groups.

Goats were fed two equal amounts, one in the morning at 08:00 h and the other at 15:00 h in the afternoon. Goats in negative group (GH) were fed on grass hay and 200 g per head/day of wheat bran. The positive control group was fed sunflower cake as a protein source at a level of 170 g/head/day while the group fed on MOL diet was given 200 g per head per day of dried *M.*

oleifera leaves. In addition all the groups were given grass hay *ad libitum* plus 200 g of wheat bran per head/day as an energy source. The experimental (MOL) and positive (SC) diets were formulated at the beginning of the experiment to be isonitrogenous and isocaloric as shown in Table 5.1. Estimation of nutrient requirements was based on Langston University Goat Research for a local goat weighing 17 kg and growing 100 g/day under tropical conditions according to Paul *et al*. (2003). The energy and protein requirements were estimated to be 6.4 MJ ME and 80 g per day to feed the goats for a 60 day period (NRC, 2007). The goats were individually fed in feeding troughs. The quantity for each diet was changed fortnightly according to changes in mean body weights of the goats in each treatment.

5.2.5 Measurements

Goats weights were measured at the beginning of the experiment and then fortnightly in the morning (08: 00 h) using a commercial scale (Ruddscale, Durbanville, South Africa). Concurrently, body condition scores (BCS) were assessed using the 5 point scale (1=very thin to 5=obese) (Aumont *et al*., 1994). Body condition assessment was conducted by one assessor throughout the experimental period for consistency through palpation of the lumbar vertebrae area between the back of the ribs and the front of the pelvic bones.

Table 5.1: Nutritional composition of the experimental supplements (% DM basis)

Component	Grass hay (GH)	Sunflower Meal (SC)	*Moringa oleifera* leaf Meal (MOL)
Dry matter (%)	89.33±0.221	89.00±0.221	88.93±0.221
Crude protein (%)	14.08±0.374[a]	23.27±0.374[b]	23.76±0.374[b]
Acid detergent lignin (%)	4.77±0.295[b]	1.91±0.295[a]	2.06±0.295[a]
Neutral detergent fibre (%)	52.67±0.293[c]	42.04±0.293[b]	34.77±0.293[a]
Acid detergent fibre (%)	24.36±0.756[b]	18.5±0.756[a]	17.15±0.756[a]
Acid detergent cellulose (%)	12.46±0.432[b]	7.04±0.432[a]	7.93±0.432[a]
In-vitro digestibility (%)	61±0.53[a]	67±0.53[b]	70±0.53[b]
Phosphorus (%)	0.50±0.012[a]	0.62±0.012[b]	0.64±0.012[b]
Calcium (%)	1.81±0.074[a]	1.98±0.074[a]	2.78±0.074[b]
Potassium (%)	1.74±0.044[a]	1.92±0.044[b]	2.03±0.044[b]
Sodium (mg)	0.01±0.012[a]	0.01±0.012[a]	0.02±0.012[b]
Zinc (mg)	77.03±3.384[a]	88.92±3.384[b]	89.17±3.384[b]
Copper (mg)	12.67±0.632[a]	19.67±0.632[b]	21.00±0.632[b]
Iron (mg)	286±2.201[a]	325.73±2.201[b]	356±2.201[c]
Polyphenols (%)	0.43±0.023[a]	0.67±0.023[b]	0.77±0.023[b]

[ab] Means in the same row, with different superscript differ significantly ($P < 0.05$)

5.2.6 Packed Cell Volume determination

At the time of weighing, about 3 ml of blood was collected by jugular venipuncture from each of the goats every fortnight into vacutainer tubes containing ethylenediamine tetra-acetic acid (EDTA) anticoagulant for the determination of PCV. Blood was drawn into non-heparinized microhaematocrit capillary tubes (1.40 x 1.60×75mm, Lasec Pty Ltd Cape Town, South Africa) by capillary action, one end of each tube was sealed and centrifuged in a haematocrit centrifuge (MSE, London, Great Britain) for 3 minutes at 12 000 rpm. The PCV was estimated as a percentage using the micro-haematocrit reader (MSE, London, Great Britain).

5.2. 7 Faecal egg count

The goats used in the study were naturally infected with gastro-intestinal worms (mixed infection), during grazing before supplementation. Faecal worm egg counts were taken before the commencement of the experiment, to assist in the grouping of goats according to helminth loads. Subsequently faecal samples were collected fortnightly to estimate coccidian- oocyst levels. However, coccidian-oocyst were not classified into different eimeria species due to technical challenges. The faecal samples were put in labeled screw cap bottles that had 3 % formalin and were filled to capacity to ensure exclusion of air from the container.. The samples were packed and dispatched in a cool box. On arrival at the laboratory, the samples were immediately stored in a refrigerator at 4 ˚C for preservation before laboratory analyses. Faecal worm egg counts were performed using the modified McMaster technique as described by Sloss *et al*. (1994). The obtained values were expressed as eggs per gram of fresh faecal samples, with lower limit detection of 50 eggs per gram. The nematode eggs and coccidian-oocyst were identified using a combination of keys given by Soulsby (1982).

5.2.8 Faecal culture and larval count

Ten grams of faecal samples were collected every fortnight throughout the study period, from the rectum of the goats. The faecal samples were put in the labeled screw cap bottles that were filled to capacity to ensure that air is excluded from the container. On arrival at the laboratory, the samples were immediately stored in a refrigerator at 4 °C for preservation before laboratory analyses. Faecal cultures were prepared for individual goats in each treatment for faecal larval counts (FLC). Due to difficulties associated with distinguishing different helminth eggs, faecal larval counts were used to approximate the levels of helminth infection. The faecal matter was spread into a plastic pan less than 3 cm deep. Thereafter, the faecal matter was covered with peat moss which was autoclaved to avoid cross contamination and misted with tap water to a moist consistency and covered with a lid. Pans were incubated for 7 – 10 days at 27 °C. To reduce the discrepancies the faecal cultures were maintained at the same environment throughout the experiment. The faecal cultures were then placed into a Baermann apparatus to extract larvae according to the Baermann method (Sloss *et al*., 1994). Larvae were counted using a dissection microscope at 7.5 magnification and averaged over three counts to determine the final concentration level.

5.2.9 Worm identification and count at necropsy

At the end of the experiment, goats were humanely slaughtered and dressed at an abattoir. Twenty-four hours before slaughter the goats were deprived of feed and provided with fresh water *ad libitum.* Goats were weighed before slaughter to get their final slaughter weights. Evisceration was conducted immediately after slaughter. The abomasums, small and large

intestines were ligated at their respective junctions to avoid movement of contents from one gastro-intestinal tract organ to another. These organs were individually opened and their contents emptied into a bucket. The mucosa of the respective organs was washed gently with running water. The contents and washings of each organ were made to a total volume of two litres. Then the contents of each bucket were thoroughly mixed. Thereafter, two aliquots of 200 ml were collected from each bucket and the number of worms in each aliquot counted following the procedures by (Hansen and Perry, 1994). The number of worms counted was multiplied by 20 to obtain the total worm burden (Hansen and Perry, 1994).

5.2.10 Ethical issues

The experimental protocol was specifically approved and was in compliance with the University of Fort Hare's Ethics Committee (Clearance number REC-270710-028) on research in animals, and internationally accepted principles for animal use and care. The goats were examined by the local state veterinarian on a weekly basis throughout the entire experimental period to ensure compliance to welfare requirements.

5.2.11 Statistical analyses

The differences in FLC, PCV, BCS, and body weights between the treatment groups were analysed using mixed model procedures for repeated measures of SAS (2003). Faecal larval counts and worm count data were log transformed [ln (χ +10)] and the resulting transformed variables were tested for normality using probability plots, skewness and kurtosis. The transformed data were reported as back-transformed means. Worm count data were analysed using the RANDOM statement in the mixed model procedure. The model used was:

$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + E_{ijk}$

where:

Y_{ijk} = observation (body weight, BCS, PCV, FLC) of each goat;

μ = population mean constant common to all observations;

T_i = fixed effect of diet (MOL, SC, GH);

P_j = fixed effect of week;

$(T \times P)_{ij}$ = diet and infection interaction;

E_{ijk} = random error term, assumed to be normally and independently distributed with mean 0 and variance equal to δ^2. Mean separations were determined using the PDIFF option in SAS (2003). The effect of varying worm counts on PCV of the goats was examined using the quadratic response surface model (SAS, 2003).

5.3 Results

Diet influenced the level of helminth infection (Figure 5.2). The FLC decreased significantly ($P < 0.05$) from day 14 when the sampling was done up to the end of the experiment in the MOL and SC fed groups. In contrast, FLC in the GH treatment group increased continuously throughout the experimental period as shown in Figure 5.2. Lower values ($P < 0.05$) of mean coccidian- oocyst counts were recorded in goats supplemented with MOL diet while higher ($P < 0.05$) values were observed in goats supplemented with GH and SC groups (Figure 5.2). Changes in the mean BCS and PCV are summarized in Figure 5.3 and 5.4 respectively. The BCS in GH group started to decline after day 14; however, in MOL and SC groups it increased in a linear

pattern up to the end of the experiment. The GH group had the lowest ($P < 0.05$) PCV compared to the MOL and SC groups.

Four helminth species namely *Haemonchus contortus*, *Trichostrongylus colubriforms*, *Oesophagostomum columbianum* and *Moniezia* spp were observed. Cestode infection was not evenly distributed within and among the groups. Goats supplemented with MOL and SC diets had lower *T. colubriformis* and *O. columbianum* worm burdens than those on GH (control) diet ($P < 0.05$). *Haemonchus contortus* worm counts were significantly influenced by diet, the group supplemented with MOL had the lowest ($P < 0.05$) count which compared well to that of the SC group (Table 5.2). Overall mean total helminth counts were significantly low for goats which were supplemented with MOL and SC diets compared to those on the GH diet ($P < 0.05$).

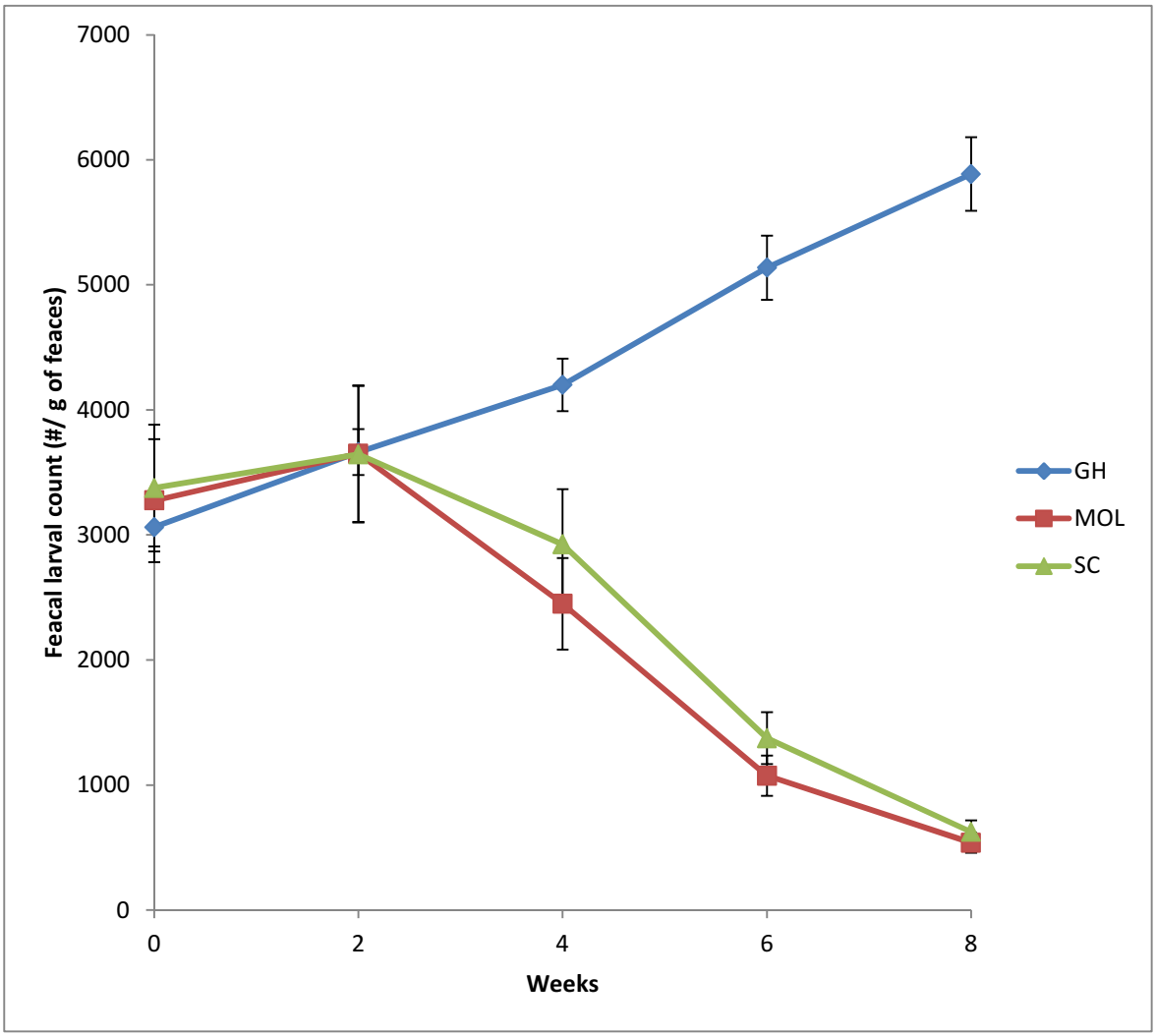

Figure 5. 1: Least square mean changes in Faecal Larval Count (FLC) of goats in different dietary groups (GH, MOL and SC)

GH = Grass hay

MOL = *Moringa oleifera* meal

SC = Sunflower seed cake meal

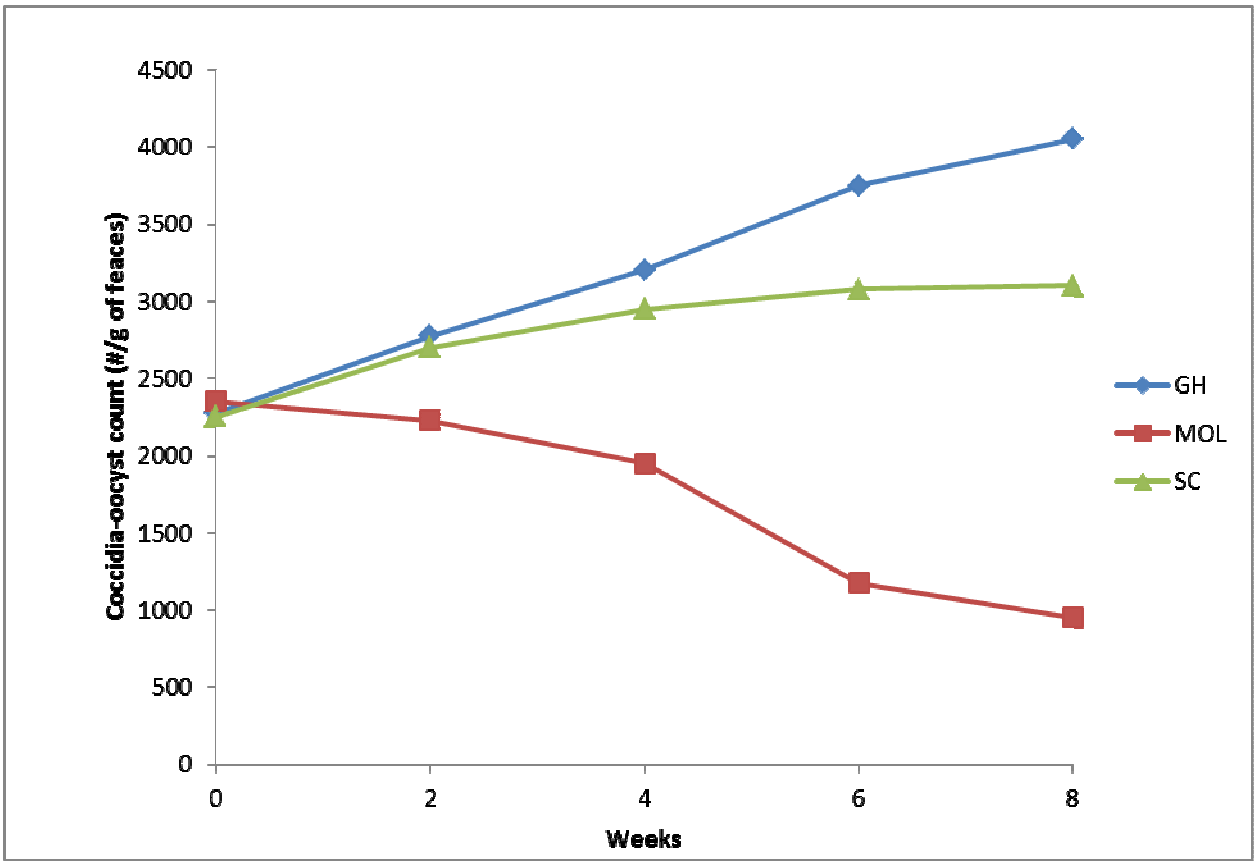

Figure 5. 2: Least square mean changes in coccidia-oocyst count of goats in different dietary groups (GH, MOL and SC)

GH = Grass hay

MOL = *Moringa oleifera* meal

SC = Sunflower seed cake meal

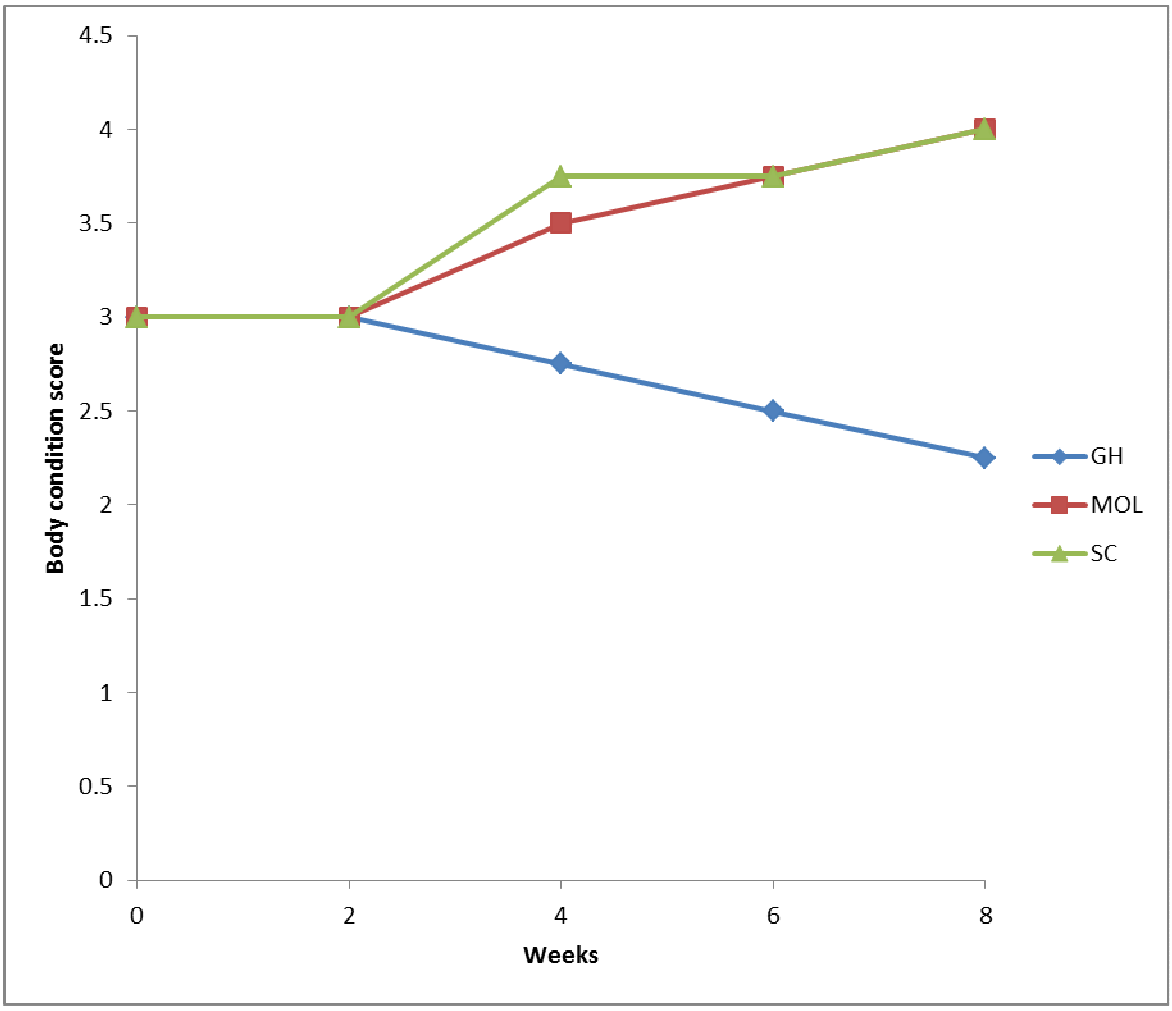

Figure 5. 3: Least square mean changes in body conditions scores (BCS) of goats in different dietary groups (GH, MOL and SC)

GH = Grass hay

MOL = *Moringa oleifera* meal

SC = Sunflower seed cake meal

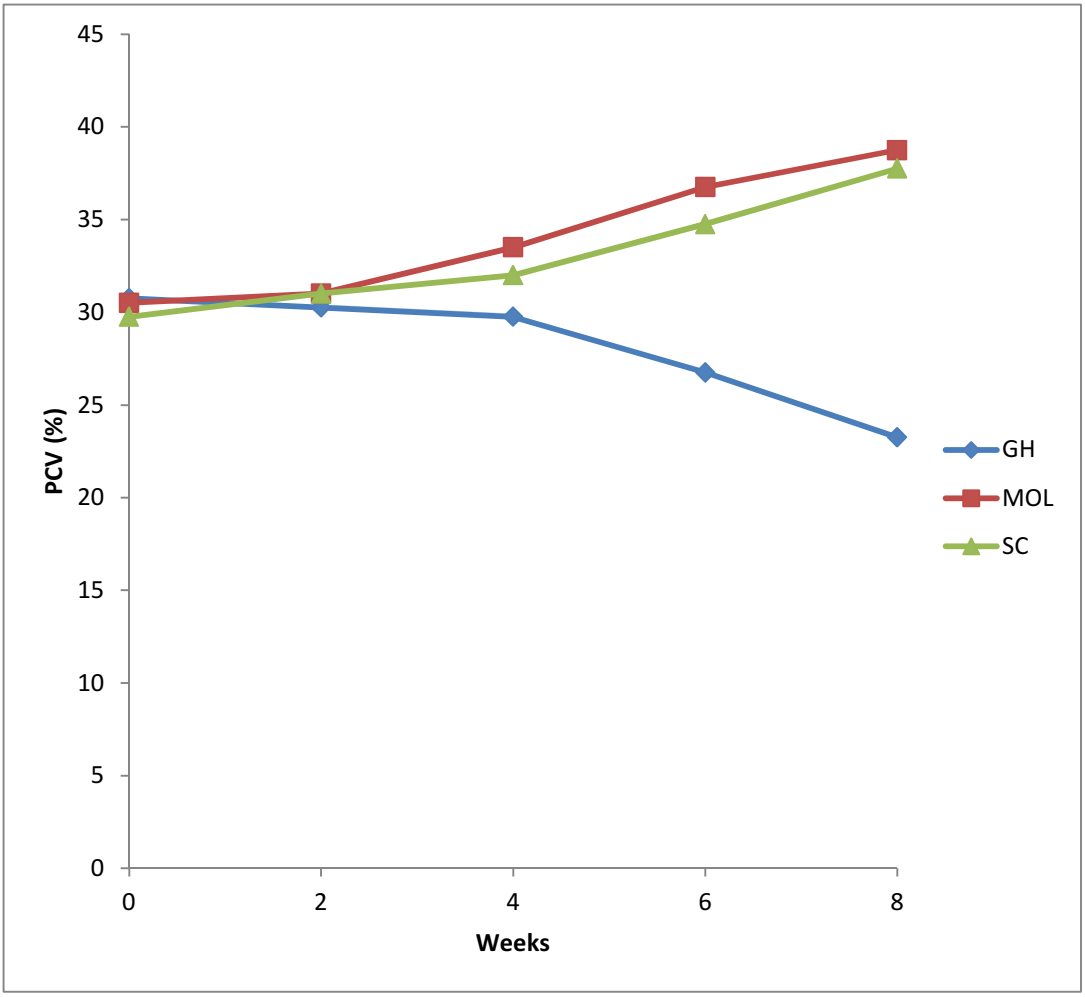

Figure 5. 4: Least square mean changes in PCV of goats in different dietary groups (GH, MOL and SC)

GH = Grass hay

MOL = *Moringa oleifera* meal

SC = Sunflower seed cake meal

Table 5. 2: **Worm counts at necropsy in different dietary groups (GH, MOL and SC)**

Helminth spp	Diet			S.E
	GH	MOL	SC	
Haemonchus contortus	933.75[b]	258.75[a]	288.75[a]	36.93
Trichostrongylus colubriformis	138.75[b]	41.25[a]	45.00[a]	8.48
Oesophagostomum columbianum	157.50[b]	48.75[a]	45.00[a]	9.92

[ab] Means in the same row, with different superscript differ significantly (P< 0.05)

GH = grass hay

MOL = Moringa oleifera leaf meal

SC = Sunflower seed cake

5.4 Discussion

The low helminth infection in goats supplemented with MOL and SC diets suggests that nutrition has an effect on the response of goats to helminth challenge. Arsenos *et al*. (2009) reported that the responses to helminth challenge vary with the nutrition offered to animals. Diets high in protein and energy have been reported to have an impact on the resistance and resilience to helminth infections (Hoste and Torres-Acosta, 2011). This has also been shown in the current work where better FCL and BCS were observed in MOL and SC supplemented goats. Resistance describes the ability of the host to prevent or limit establishment or development of infection while resilience refers to the ability of the host to maintain a reasonable level of production when subjected to helminth challenge (Arsenos *et al*., 2009).

Moringa oliefera decreased helminth infection most likely due to its high crude protein levels. Dietary supplements high in protein have been reported to improve the immune response of animals, which acts to decrease helminth infection (Arsenos *et al*., 2009). Any increase in the intestinal protein supply is known to improve host homeostasis and its immune response against helminths (Rastogi *et al*., 2009). Arsenos *et al*. (2009) suggested that improvement in the dietary protein improves the capacity of infested goats to develop effective immunological response to infection and enhanced the onset of parasite rejection. Furthermore, MOL and SC diets contained considerable amount of protein (23.76 and 23.27 %, respectively) that could have increased availability of protein for digestion and absorption resulting in the improvement of body condition and improving host-resistance to the helminths.

The results of the present study were similar to those reported by Arsenos *et al*. (2009) and Marume (2010) who concluded that supplementary feeding increased the performance as well as the resilience of goats against natural helminth infections. This minimizes any reliance on synthetic anthelmintics. The use of forage plants having anthelmintic effects promote the production of organic meat which is preferred by consumers for health reasons.

The MOL diet contained considerable amount of polyphenolic compounds that may have anthelmintic effect. Polyphenolic compounds have been reported to bind feacal egg proteins and inhibit egg hatching and larval development (Torres-Acosta and Hoste, 2008; Xhomfulana *et al*., 2009). This is supported by lower FLC count in goats fed MOL diet observed in this study, which agrees with Torres-Acosta and Hoste (2008) who found reduced FEC and worm burden in grazing kids supplemented with high energy feed barley. Amaglo *et al*. (2010) reported that dry *M. oleifera* leaves contain 1.4 % condensed tannins while in Chapter 3, 0.3 % of condensed tannins value was reported. The amount of condensed tannins in MOL is negligible; however it could be responsible for its anthelmintic effect. It has been reported that condensed tannins are able to counteract the effects of helminths in ruminants (Xhomfulana *et al*., 2009). It is speculated that its anthelmintic effect is through the improvement of protein supply to the host animal (Xhomfulana *et al*., 2009). It is hypothesized that condensed tannins bind to free protein that could be available to larvae nutrition, thereby depriving the larvae nutrients. This could lead to larvae starvation and death (Watkins, 2003). The cuticle of worm larvae is high in glycoproteins and could bind with condensed tannins causing the death of the larvae (Hoste *et al*., 2008).

Copper oxide wire particles have been reported to reduce *H. contortus* loads in sheep and goats (Watkins, 2003). Watkins (2003) reported that when copper dissolves in the abomasum, it provides an environment not conducive for the survival of the *H. contortus*. As such the higher dietary copper content in MOL diet could have reduced *H. contortus* in the current study, if they have the same antihelmintic effect as copper oxide wire particles? Several mechanisms can be postulated from the presence of high copper concentrations in the abomasums; its ability to alter the reproductive capability of *H. contortus* and the ability to penetrate the cuticle of the *H. contortus* together with lowering of abomasal pH (Watkins, 2003). These could explain the observed effect of the MOL diet in this study of lower FEC, possible reduced ability to feed and overall functions which could lead to its expulsion and/or death, and overall unsuitable habitation for *H. contortus*, respectively. The acidic environment reduces the pathogenic potential of *H. contortus*. Presence of copper in adequate amounts assists in the maintenance of immune system (Watkins, 2003; Soli *et al*., 2010), hence goats supplemented with MOL diet had the ability to mount an enhanced immune response which, we speculate to have caused the reduction of *H. contortus* from the host. However, more research needs to be done to ascertain the mechanism of action.

The goats supplemented with MOL, had a decreased mean coccidian-oocyst count while in other SC and GH the mean coccidian-oocyst count was steadily increasing. Lower values of mean coccidian-oocyst count might indicate that MOL, had an anti-coccidial effect. There are few plants such as *Withania somnifera*, *Artemisia annua* and *Azadirachta indica* that have been reported to possess anti-coccidial effect (Brisibe *et al*., 2008). Further research needs to be

undertaken to determine whether the lower oocyst count was due to improved immunity of the animals or to reduced fecundity of the coccidia.

Contrary to other helminth species, the *Moniezia* spp infestation was not evenly distributed within and among the groups as such the impact of diet on *Moniezia* spp load was difficult to assess. *Moniezia* spp have been reported to be less pathogenic unless in high levels (Hoste *et al*., 2008). In the current study the *Moniezia* spp were low and did not have any significant effect on the various parameters in this study.

Low PCV is indicative of anaemia, haemorrhage, bone marrow failure, leukaemia, malnutrition or specific nutritional deficiency, multiple myeloma and rheumatoid Arthritis (Rumosa Gwaze *et al*, 2009). As such PCV is one of the major indicators of helminth infection especially *H. contortus* (Hoste *et al*., 2008). Changes in these values from normal mainly depend on the severity of helminth infection. In this current study goats fed on MOL and SC had higher PCV values which could be due to high plane of nutrition. Also, the high iron levels in MOL diet could have assisted to reduce anaemic levels in goats, hence high PCV values. The high PCV values in the MOL (38.5) and SC (38.8) groups are also consistent with the observed high body weight and high body condition scores compared to the GH group. It is suggestive that the suppression of helminth infection, elicited by MOL and SC diets means that more protein was portioned towards growth resulting in increased growth rate of the goats. It should be noted that the faecal larval counts were used instead of faecal egg count because the interest was on worm larva that could develop to the next generation, however, it was noted more information could be

obtained if faecal egg count was done and then tested for egg hatchability. Due to the limitations of goats in the farm we used 4 animals per group.

Ethanolic and Methanolic extracts of *M. oleifera* leaves and seeds have been reported to possess anthelmintic activity against the non parasitic Indian earthworm (*Pherima posthuma*) (Rastogi *et al.,* 2009; Girl *et al*., 2011). Amaglo *et al*. (2010) and Girl *et al.* (2011) have documented the anthelmintic activity of *M. oleifera* but its anthelmintic activity was not clarified whether it was through feeding or drenching the extracts. In addition, they did not indicate the helminth species that were affected. Results from feeding trials can be influenced and confounded by the presence, in plants, of other unknown bioactive compounds together with differences in nutritional values. This necessitates the isolation of compounds in diets and individually assessing the anthelmintic property. It should be noted however, that isolation of individual compounds does not always result in bioactive compounds because most times the compounds activity are by synergism effect.

5.5 Conclusion

Results from this study have shown that supplementing cross-bred Xhosa lop-eared goats with MOL diet significantly lowered the FLC, mean coccidial-oocyst count and worm counts at necropsy. In addition, the BCS and PCV values increased in the MOL supplemented goats.

Besides having anthelmintic effects improving goat body condition, *M. oleifera* leaves showed high nutritional content (Chapter 3), it is imperative to evaluate its potential as a protein supplement in goat production and its impact on the carcass characteristics.

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Chapter 6: Effect of supplementing crossbred Xhosa lop-eared goat castrates with *Moringa oleifera* leaves on growth performance, carcass and non-carcass characteristics

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Abstract

The objective of the study was to determine the effect of supplementing *Moringa oleifera* leaves (MOL) on growth performance, carcass and non-carcass characteristics of crossbred Xhosa lop-eared goats. A total of 24 castrated goats aged 8 months, with a mean initial weight of 14.63 ± 0.26 kg were randomly divided into three diet groups with 8 goats. The duration of the trial was 60 days. All goats received a basal diet of grass hay (GH) *ad libitum* and wheat bran (200 g/day each). The MOL and sunflower cake (SC) groups were fed additional 200 g of dried *M. oleifera* leaves and 170 g of SC, respectively. The third group (GH) did not receive any additional ration. The crude protein (CP) of MOL (23.75 %) and SC (23.27 %) were higher ($P < 0.05$) than that of grass hay (GH) diet (14.08 %). The attained average daily weight gain for goats fed MOL, SC and GH were 103.3, 101.3 and 43.3 ± 7.85 g, respectively ($P < 0.05$). Higher ($P < 0.05$) feed intakes observed were in SC (491.5 ± 1.00 g) and MOL (490.8 ± 1.00 g) compared to GH (404.5 ± 1.00 g). The hot carcass weight was higher ($P < 0.05$) for SC (10.5 ± 0.62 kg) and MOL (10.3 ± 0.62 kg) than for the GH group (8.6 ± 0.62 kg). The dressing percentage in SC (55.8 ± 0.63 %) and MOL (55.1 ± 0.63 %) were higher ($P < 0.05$) than that of the GH (52.9 ± 0.63 %). Feeding MOL or SC had a similar effect on growth performance and carcass characteristics of goats and hence *M. oleifera* could be used as an alternative protein supplement in goats.

6.1 Introduction

Goats are among the major economically important livestock species in the Eastern Cape Province of South Africa (Bakare and Chimonyo, 2011). In 2009, there were about 2.3 million goats in the province, which contributes about 37 % of the goat population in the country (Bakare and Chimonyo, 2011). They play a significant role in the livelihoods of resource-limited farmers. Goats are a source of animal protein and income. In addition to provision of tangible products, goats contribute to the livelihoods of the poor through risk mitigation and accumulation of wealth (Rumosa Gwaze *et al.*, 2009; Casey and Webb, 2010). The demand for goat meat is on the rise throughout the world, especially in developing countries due to increased human population, income growth and great need for lean meat (Sanon *et al*., 2008). To meet this demand there is need to improve the productivity of goats, which is relatively low at the present moment, for resource-limited farmers (Solomon *et al*., 2008).

Regardless of their attributes the productivity of goats in many tropical countries is low and has been related to diseases, nutrition, genotype and management (Sanon *et al*., 2008; Simela and Merkel, 2008; Oni *et al*., 2010). The limitations of nutrition could be attributed to seasonal fluctuations in feed quantity and quality (Oni *et al*., 2010). The notable effect of feed scarcity is observed particularly in dry seasons when natural pastures are mature, dry and inadequate (Oni *et al*., 2010), with as low nutritive value as 2 % crude protein (Mendieta-Araica *et al*., 2011). Supplementary feeding with high nutritive feeds could therefore be a prerequisite for viable and sustainable good goat production in such instances.

Supplementing goats with nutritious feed could increase the average daily gain, carcass weight and dressing percentage resulting in the improvement of the meat quality (Safari *et al*., 2009). Usually farmers feed their animals with crop residues and low quality standing hay which are low in nitrogen, high in ligno-cellulose, in short supply of vitamin and mineral content which leads to low digestibility and reduced voluntary feed intake (Gebregiorgis *et al*., 2011). More so, some of the crop residues require expensive inputs such as urea to provide alternative nitrogen. Urea is known to have toxic effects to animals, and there is a high incidence with improperly mixed feed (Antonelli *et al*., 2004). Consequently the energy and nitrogen intake of animals raised on these feeds cannot sustain adequate levels of performance leading to low growth, delayed animal sexual maturity, poor reproductive performance, poor meat quality and low milk yield (Gebregiorgis *et al*., 2011).

Evaluation of alternative protein and energy supplements that could be grown locally with limited resources for sustaining livestock and safe for human consumption are essential. A potential strategy for increasing the quality and availability of feeds for resource-limited livestock farmers in the dry season may be the use of fodder trees and shrub forages. The advantages of most fodder trees and shrub forages are that they are easily propagated and do not require high management input (fertilizer, pesticides, etc) or advanced technology (Mendieta-Araica *et al*., 2011). The trees provide a good and cheap source of protein and micronutrients at the same time alleviating the effect of global warming as they can be used for afforestation and reforestation programmes. In recent years, there has been increased research on alternative protein sources from forage trees and shrubs that can be fed to goats such as *Pterrocarpus lucens*, *Acacia Senegal* (Sanon *et al*., 2008), *Acacia etbaica*, *Dichrostachys cinerea* (Yayneshet

et al., 2008), *Acacia karroo* (Marume, 2010) and *Manihot esculenta* (Oni *et al*., 2010). The merit of using forage trees is that the leaves can be harvested, sun dried and used to compound protein supplements. The replacements of conventional ingredients by trees will make such supplements cheaper than the commercial concentrates (Mendieta-Araica *et al*., 2011).

Another potential nutritious forage plant is *Moringa oleifera* which grows throughout the tropics. It is reported to have nutritious (Chapter 3), therapeutic and prophylactic properties (Chapter 4, and 9; Marcu and Pharm, 2005) with a crude protein range of 23-40 % (Marcu and Pharm, 2005; Mendieta-Araica *et al*., 2011; Chapter 3). In Chapter 5 *M. oleifera* supplementation improved goat body condition scores. This makes it an ideal protein supplement. This is of particular interest to animal nutrition since dietary protein sources are becoming increasingly expensive and difficult to access (Gebregiorgis *et al*., 2011). The leaves can be fed fresh or dry. Dried *M. oleifera* leaves can be stored for longer period without deterioration in nutritive value (Mendieta-Araica *et al*., 2011). As such leaves can be harvested during the periods of high yields and later used for feeding during the dry season when the quality and quantity of feed is low.

Despite, *M. oleifera* leaf's nutritive value, its use as a livestock supplementary feed to improve the growth and carcass characteristics of indigenous goats, such as Xhosa lop-eared goats, in South Africa is unknown and there are a limited number of studies done worldwide (Murro *et al*., 2003; Mendieta-Aracia *et al*., 2011). However, growth performance and carcass characteristics of the crossbred Xhosa lop-eared goats when supplemented with *M. oleifera* are unknown. The objective of the current study was therefore to determine the effect of supplementing crossbred

Xhosa lop-eared goat castrates with *M. oleifera* leaves on growth performance, carcass and non-carcass characteristics.

6.2 Materials and Methods

6.2.1 Management of experiment animals

Twenty-four, 8 months old castrated crossbred Xhosa lop-eared goats with a mean initial body weight of 14.78 ±0.57 kg were used in the study at University of Fort Hare's Honeydale farm from May 2010 to August 2010. The details of the study site where the experiment was conducted, collection of feeding materials and chemical composition of feeding diets were described in Sections 5.2.1, 5.2.2, 3.2.1 and 5.2.3, respectively. The details on animal management, body weights and body condition scores are described in Section 5.2.4 and 5.2.5, respectively.

6.2.3 Chemical analysis of the feeding diets

The formulated diets were assessed for nutrient composition as described in section 3.2.2 (Table 5.1).

6.2.4 Slaughter weights

At the end of the feeding trial, the final live weight (FLW) of each animal was obtained by averaging live weights recorded for two consecutive days, and then the goats were fasted for 16 h. The goats were weighed again prior to slaughter to get slaughter live weights (SLW). They were stunned using the electric stunning method, their jugular veins severed, fully bled and skinned thereafter. The heads were removed at the atlanto-occipital joint and the hooves were cut off at the proximal end of the cannon bones, leaving the carpal and tarsal bones on the carcass.

Gut fill was calculated as the difference between the weights of full and empty digestive tract. Empty body weight (EBW) was computed as the difference between slaughter weight and gut fill. The carcass was split into two halves through the mid-ventral line and the entire alimentary tract removed (with adhering omental and mesenteric fat) after tying the oesophagus and rectum to prevent loss of gut contents. The pluck (respiratory tract, heart and liver) were removed and weighed. The carcasses with kidneys and pelvic fat were immediately weighed after slaughter to determine the hot carcass weight (HCW).

The carcasses were scored for conformation and fatness based on the South African Meat industry conformation scale (SAMIC) (2006). The carcass fatness was graded on a scale of 0 to 6 (0= no visual fat cover, 1 = very lean, 2 = lean, 3 = medium, 4 = fat, 5 = over fat and 6 =excessively over fat). The carcasses were also graded based on dentition using a scale of A: No permanent incisors, AB: one to two (1-2) permanent, B: Three to six (3-6) and C; More than six (> 6) permanent incisors (SAMIC, 2006). The SAMIC (2006) conformation scale of 1 – 5 (with 1= a very flat carcass, 2= a flat carcass, 3= medium carcass, 4= a round carcass, and 5= very round carcass) was used.

After 24 h of refrigeration at 0 °C weights were recorded to obtain cold carcass weights (CCW). These recordings were later used to calculate dressing percentage and chilling losses. Dressing percentage was calculated as the hot carcass weight expressed as a proportion of slaughter live weight. Chilling loss was calculated as the weight lost after chilling the carcass at 0 °C for 24 h.

6.2.5 Statistical analyses

Experimental data were analysed using the General Linear Model Procedure of SAS (2003). Dietary treatments were considered as fixed effects and the residual as the random effect. Each individual served as an experimental unit for all parameters assessed. Pair-wise comparisons of the least square means were performed using the PDIFF procedure of SAS (2003). The statistical model used was

$Y_{ijk} = \mu + T_i + e_{ijk}$

Where Y_{ijk} = dependant variables (Average daily gain, EBW, Dressing %,);

μ= overall mean;

T_i = effect of supplement ($_i$=MOL, SC, GH);

E_{ijk} = Random error.

A chi-square test (SAS, 2003) was used to test whether any association existed between treatment, body condition score, carcass fatness and carcass conformation grades.

6.3 Results

6.3.1 Growth performance

Goats supplemented with MOL and SC consumed the whole daily feed ration, which translated into daily dry matter intake of 490.75 and 491.5 g respectively ($P > 0.05$) as shown in Table 6.1. The GH group had significantly lower dry matter intake compared to that of the treatment group (MOL). The effect of different diets on growth performance is shown in Table 6.1. The two diets, MOL and SC led to similar daily body gain of goats. However, goats on both diets gained more weight ($P<0.05$) than those on the GH diet.

There was an association ($P < 0.001$) between treatment, body condition score, carcass conformation and carcass fatness. The body condition score of goats supplemented with MOL and SC were higher ($P < 0.05$) and had a higher ($P < 0.001$) frequency of body condition score 4 than GH at slaughter (Table 6.2). At the same time the carcass conformation of MOL and SC groups were better ($P < 0.001$) than GH group (Table 6.3). The carcass fatness for MOL and SC groups were classified as 4 and better ($P < 0.001$) than GH (Table 6.4). Goats on SC and MOL diets were heavier at slaughter and consequently had higher empty body weight (EBW), warm carcass weight and dressing percentages than those on the control diet (GH). The dietary treatments significantly influenced the weight and proportion (in %) of EBW of the fresh non-carcass organs such as lung, heart, fat, blood empty digestive tract and liver (Table 6.2).

The effect of diet on the weight of fresh non-carcass organs and their proportions of empty body weight are summarized in Table 6.5.

Table 6. 1: Effect of supplement on growth, carcass and non-carcass measurements

Perfomance (Parameter)	Treatment			SEM
	Grass hay (GH)	Sunflower meal (SC)	*Moringa oleifera* Meal (MOL)	
	n =8	n =8	n =8	
Initial body weight	14.9	14.7	14.4	0.80
Final body weight	17.5[a]	20.8[b]	20.6[b]	1.16
EBW	14.6[a]	19.1[b]	18.4[b]	1.19
HCW	8.5[a]	10.5[b]	10.3[b]	0.62
CCW	8.1[a]	10.1[b]	9.9[b]	0.60
Chilling loss (%)	5.3[a]	4.1[b]	4.3[b]	0.36
Dressing %	44.9[a]	50.8[b]	50.0[b]	0.63
Average daily gain (g)	43.3[a]	101.3[b]	103.3[b]	7.85
DM Feed intake (g)	404.5[a]	491.5[b]	490.75[b]	1.00

[ab] Means in the same row, with different superscript differ significantly (P< 0.05)

EBW- Empty body weight

HCW- Hot carcass weight

CCW- Cold carcase weight

Table 6. 2: Frequency of body condition score (BSC) values in goats supplemented with grass hay (GH), *Moringa oleifera* lesf meal (MOL) and sunflower seed cske (SC)

Supplement	Frequency (%) body condition score			Total	*P*- value
	2	3	4		
GH	20.8 (5)	12.5 (3)	0.0 (0)	33.3 (8)	0.001
MOL	0.0 (0)	0.0 (0)	33.3 (8)	33.3 (8)	
SC	0.0 (0)	0.0 (0)	33.3 (8)	33.3 (8)	
Total	20.8 (5)	12.5 (3)	66.7 (16)	100 (24)	

Values in parentheses indicate the number of cases

Table 6. 3: Frequency of carcass conformation values in goats supplemented with GH, MOL and SC

Supplement	Frequency (%)carcass conformation value			Total	*P*- value
	2	3	4		
GH	16.7 (4)	16.7 (4)	0.0 (0)	33.3 (8)	0.001
MOL	0.0 (0)	0.0 (0)	33.3 (8)	33.3 (8)	
SC	0.0 (0)	0.0 (0)	33.3 (8)	33.3 (8)	
Total	16.7 (4)	16.7 (4)	66.7 (16)	100 (24)	

Values in parentheses indicate the number of cases

Table 6. 4: Frequency of carcass fatness values in goats supplemented with GH, MOL and SC

Supplement	Frequency (%) carcass fatness value			Total	*P*- value
	2	3	4		
GH	25.0 (6)	8.3 (2)	0.0 (0)	33.3 (8)	0.001
MOL	0.0 (0)	0.0 (0)	33.3 (8)	33.3 (8)	
SC	0.0 (0)	0.0 (0)	33.3 (8)	33.3 (8)	
Total	25.0 (6)	8.3 (2)	66.7 (16)	100 (24)	

Values in parentheses indicate the number of cases

Table 6. 5: Effect of diet on weight of fresh non-carcass organs and proportion of empty body weight

Organ weight	GH n =8	SC n =8	MOL n =8	SEM
Lung-trachea-diaphragm (g)	247.4	261	256	5.98
Heart (g)	91.0[a]	96.6[b]	96.9[b]	2.75
Omental and kidney fat (g)	69.5[a]	240.0[b]	243.0[b]	9.15
Liver (g)	259.4[a]	401.0[b]	398.6[b]	24.34
Kidney (g)	41.9[a]	45.4[b]	45.5[b]	0.91
Empty digestive tract (kg)	2.9[b]	1.8[a]	1.9[a]	0.08
Gut content (kg)	3.4[c]	1.6[a]	2.2[b]	0.10
In % of empty body weight (EBW)				
Lung-trachea-diaphragm (%)	1.7	1.4	1.4	0.08
Heart (%)	0.6	0.5	0.5	0.03
Omental and kidney fat (%)	0.5[a]	1.3[b]	1.4[b]	0.08
Liver (%)	1.7[a]	2.1[b]	2.2[b]	0.02
Kidney (%)	0.3	0.3	0.2	0.02
Empty digestive tract (%)	18.8[b]	10.0[a]	10.8[a]	0.92
Gut content (%)	22.3[b]	9.1[a]	12.1[a]	1.29

[ab] Means in the same row, with different superscript differ significantly (P< 0.05)

The proportional weights of the liver of goats fed on SC diet and MOL was almost similar having 2.12 and 2.16 %, respectively ($P > 0.05$). However, they were significantly higher than that of GH diet.

6.4 Discussion

The CP content of grass used in this study was higher than most grass hay which has 9 % (Babayemi, 2009). As such the results from the present study differed from others where goats fed on grass had a decline in body weight (Mushi *et al*., 2009; Safari *et al*., 2009). An increase in body weight could be ascribed to grass having high leaf content depicting higher percentage of minerals, protein and greater energy value (Babayemi, 2009). The high nutritive value was influenced by harvesting grass at early stages of growth. The NDF and ADF in the MOL diet were the lowest suggesting it was highly digestible leading to higher feed intakes compared to other studies (Babayemi, 2009; Mushi *et al*., 2009).

Among various chemical components of a feed, NDF has been proposed as a reliable predictor of voluntary consumption under certain conditions (Gebregiorgis *et al*., 2011). The goats on SC and MOL diets had similar feed intake, however higher ($P< 0.05$) than goats on GH diet. This could be because the SC and MOL diets contained more readily digestible NDF fraction and high amounts of crude protein resulting in high nutrient digestibility (Babayemi, 2009). Feed lower in NDF has higher dry matter intake resulting in higher body gains (Gebregiorgis *et al*., 2011). In addition, low levels of protein in the diet causes reduction in carbohydrate digestion in the rumen leading to reduced feed intake (Babayemi, 2009). The goats on the GH diet had less selective

feeding opportunities as they only fed on grass hay and wheat bran and this could have negatively affected feed intake.

Moringa oleifera leaves have been reported to have high mineral content (Sanchez-Machado *et al*., 2010; Chapter 3) as such it influenced the mineral content of MOL diet, which was significantly higher than the other diets. The amount of calcium (1.5 %), zinc (50 mg/kg), copper (5 mg/kg) and iron (80 mg/kg) in MOL diet were higher than the daily requirements while others were within the range (McDonald *et al*., 2002). No sign of toxicity was observed in the experimental goats, which could be attributed to the availability of minerals to the animal as a number of minerals interact with each other for them to be absorbed or utilized by the animal (Solomon *et al*., 2008). The higher mineral content in the MOL and SC diet is advantageous to the farmers because they would reduce their feed expenditure on buying mineral supplements, which are generally expensive. Adequate minerals, particularly copper, zinc and iron help to maintain the animal in good health, by boosting the animal's immunity system (NRC, 2007). The proportion of phosphorus to calcium (1:3.6) in MOL diet was within the range (1:4). Lower ratios below 1: 1.5 predispose male goats to urinary calculi (McDonald *et al*., 2002).

The EBW, warm carcass weight and dressing percentage depend on FLW at slaughter (Mushi *et al*., 2009) and were consequently affected by treatments. The positive average daily weight gain in the GH diet group in the present study indicates that the grass hay satisfied the maintenance requirements of the goats. This was expected, since the CP (14.08 %) content in GH diet was higher than the maintenance requirement (9.2 %) of goats (NRC, 2007). Van Soest (1994) demonstrated that body weight gain is not impaired if the level of CP in a given diet is more than

8 %. Similarly, rumen function is impaired when nitrogen content of the diet is less than 1.2 % (Solomon *et al.,* 2008). Diet of MOL had the highest polyphenols of 0.77 %, which seemed not to have caused any side effects. It has previously been reported that diets containing less than 4.5 % have no adverse effects on ruminants (Solomon *et al.* 2008). It can be suggested that the low levels of polyphenols in these diets may not be sufficient to affect protein metabolism in the rumen and have effect on growth and carcass performance.

The goats fed on MOL and SC diets had similar average daily weight gain (ADG) and body condition scores, both of which were higher ($P < 0.05$) than those on GH diet. This could be chiefly attributed to high dietary protein and high energy intake observed throughout the experimental period. It has been reported that increment in protein intake increases the feed intake, digestibility and consequently growth rate (Gebregiorgis *et al.,* 2011). In addition, the presence of adequate amino acids, peptides and most macro and micro minerals contained in *M. oleifera* leaves enhances the efficiency of rumen microbial growth and activity (Gebregiorgis *et al.,* 2011). Mendieta-Araica *et al*. (2011) reported that inclusion of *M. oleifera* in the diet significantly increased the feed intake and NDF digestibility of poor quality feed. The lower ADG recorded for goats fed on the GH diet could have been due to lower nutrient intake (Safari *et al*., 2009).

The ADG values (103.3 ± 7.85 g/day) of goats supplemented with MOL diets were higher than those obtained in previous studies in goats fed on different levels of *M. oleifera* leaf diets (Aregheore, 2002; Murro *et al*., 2003). This could be attributed to differences in feed quality and the breed type used in the various studies. The higher ADG and body condition scores of goat on

SC and MOL diets were higher than those on GH diet, could be partly due to the higher fat content in SC and MOL diets. Fats have been reported to boost protein nutrition by coating proteins, thus preventing ruminal microbial degradation, thereby, increasing post-ruminal protein supplies (Safari *et al*., 2009). In addition, the higher ADG observed in goats fed MOL diet could also be partly ascribed to high mineral content which could have increased live-weight gain by improving nutrient utilization efficiency.

The findings, that supplementation with *M. oleifera* increases slaughter and carcass weights of goats were consistent with literature (Yayneshet *et al*., 2008; Safari *et al*., 2009). Since the goats that were supplemented with MOL diet had higher ADGs, they were expected to have greater tissue deposition and consequently high slaughter weights and heavy carcasses (Safari *et al*., 2009). Body condition scores and live weight of goats at the time of slaughter have been reported to influence the carcass quality and yields (Mushi *et al*., 2009). The observed higher empty body weights (EBW) for SC diet than MOL and GH diet, reflects greater gut fill content in goats with higher intake of fibrous feed than higher intake of concentrates (Mushi *et al*., 2009).

Goats fed on GH diet had lower dressing percentage which could be due to higher gut fill content and lower weights which accordingly reduced the dressing percentage. Similar results were reported by Mushi *et al*. (2009) who found gut fill to be 20.1 - 22.3 % of EBW in goats fed grass hay. The relatively higher gut fill content in goats fed hay is associated with their high intake of hay which is bulky in nature leading to both higher gastro-intestinal tract (GIT) development and content (Safari *et al*., 2009). Lower gut fill resulted in higher EBW and hot carcass weights for

SC and MOL diets. Findings from the current study are in agreement with those of Mushi *et al*. (2009) working with pasture, stalled-fed kids.

In the present study, chilling losses decreased with increasing carcass weight; carcasses from GH had higher ($P < 0.05$) chilling losses than of SC and MOL supplemented goats which are comparable. Chilling loss was higher in goats fed on the GH diet probably due to their lower fat content. The amount of fat has a large impact on carcass chilling loss, it is reported that fat acts as an insulation which slows down moisture evaporation (Safari *et al*., 2009). Our findings coincide with those of Mushi *et al*. (2009) who assessed the effect of concentrate levels on carcass attributes. Chilling losses ranging from 2.3 to 8.7 % were reported for different goat genotypes and different feeds (Mushi *et al*., 2009).

The higher carcass conformation scores for goats on MOL and SC diets than GH diets could be associated with higher intakes of DM, energy and protein, which could have led to increased muscle weight (Safari *et al*., 2009). This also suggests that goats respond to nutritional treatment by accretion of more muscle protein and fat (Yayneshet *et al*., 2008). The minimal difference in carcass fatness among dietary groups could be attributed to the unique fattening pattern of goats; they deposit most of the fat around the viscera and less of it in the carcass (Casey and Webb, 2010). Omental and kidney fat were heavier and similar in goats fed on MOL and SC diets compared to those fed on GH diet and this could be attributed to high energy and protein intake from SC and MOL diet. An association between supplementary diet, body condition score, carcass fatness and carcass conformation was expected because as the body condition score increases, it results in the increment of carcass fatness and conformation (Aumont *et al.*, 1994).

Supplementation of goats with nutritive feeds, results in the improvement of body condition score, carcass fatness and carcass conformation.

The organ weights of kidneys, liver, lung-trachea-diaphragm and heart in goats on SC and MOL diets were not significantly different and were heavier ($P < 0.05$) than those from goats fed on GH diet. This was in agreement with results of Sanon *et al*. (2008), who found that such organs were heavier in animals fed high energy and protein diet compared to those fed on low quality diet. Heavier liver weights of goats offered SC and MOL diets could be attributed to high fermentative products such as volatile fatty acids, suggesting inefficient use of dietary nitrogen for rumen microbial protein synthesis and thus a greater amount of nitrogen being metabolized in the liver (Sanon *et al*., 2008). Similar results were also reported by Ouédraogo-Koné *et al*. (2009). On the other hand, the lighter liver weights in goats fed on GH diet would be in accordance with decreasing plane of nutrition, eliciting a reduced metabolic rate and mass of metabolically active tissue such as liver (Wester *et al*., 1995).

6.5 Conclusion

The results of the current study show that *M. oleifera* leaf meal is a potential source of protein to supplement grass hay of low quality. The goats fed on MOL diet had improved growth rate and carcass measurements, which compared well with performance of goats fed with the conventional sunflower seed cake. It is, therefore crucial to determine the effects of MOL supplementation on the quality of chevon from goats supplemented with *M. oleifera*.

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Chapter 7: Physico-chemical characteristics of meat from goats supplemented with *Moringa oleifera* Lam leaves

(Submitted to *South African Journal of Animal Science*)

Abstract

The objective of this study was to determine the physico-chemical characteristics and consumer sensory scores of chevon from cross-bred Xhosa lop-eared goats supplemented with *M. oleifera* leaf meal (MOL). Supplementation influenced initial pH (pH_1) and chevon colour. Higher pH_1 scores were observed in chevon from GH diet. Chevon from MOL and SC supplemented goats had higher values for lightness (L*) 24 hour *post-mortem.* The redness (a*) values of chevon 24 hr *post mortem* were higher in MOL supplemented goats. Warner Bratzler shear force (WBSF) values of chevon from SC (30.1 N) and MOL (29.8 N) were lower than those for meat from GH diet (32.6 N). Chevon from goats fed GH diet (29.5 %) had significantly higher cooking losses than that from MOL (25.4 %) and SC (25.6 %) fed groups. Supplementing crossbred Xhosa lop-eared goats with MOL diet produced chevon with higher physico-chemical characteristics.

7.1 Introduction

Goat meat (chevon) is an important protein source throughout the world, especially in developing countries (Ding *et al*., 2010; Bakare and Chimonyo, 2011). However, undernourished goats tend to produce poor quality meat (Madruga *et al*., 2008; Mushi *et al*., 2009). The differences in meat composition may affect flavour and texture of meat and consequently, consumer preferences (Font i Furnols *et al*., 2009). Chevon has been reported to have lower lightness and higher redness than lamb, mainly due to lower intramuscular fat (Gray *et al*., 1996).

The colour of meat depends upon several individual factors and their interactions. According to Insausti *et al*. (2008) and Muchenje *et al*. (2010), oxidative processes have a negative effect on both colour and flavour, while lipid oxidation products contribute to the development of off-flavours, especially during storage. It has been established that differences in meat colour are associated with variations in intramuscular fat and moisture content (Lawrie, 1991). Higher ultimate pH (pHμ) of muscle is associated with darker or dark red cuts and vice versa (Baron and Anderson, 2002; Muchenje *et al*., 2008).

In addition, ideal pHμ results in meat that is acceptably tender with normal colour (Gray *et al*., 1996) and high pHμ leading to reduced meat shelf life span (Knox *et al*., 2008; Muchenje *et al*., 2009a). This is because high pHμ promotes microorganism growth leading to the development of off-odours. On the other hand, lower pHμ results in pale coloured meat (Baron and Anderson, 2002). Mancini and Hunt (2005) reported that meat colour may be influenced by diet; grass fed animals usually has darker meat with lower intramuscular fat than grain fed animals (Diaz *et al*., 2002; Priolo *et al*., 2002). Furthermore, pasture feeding yields yellow fat due to higher levels of beta-carotene in grass (Muchenje *et al*., 2009b). Supplementing goats with forage trees has been observed to improve body weight and general health of the goats, hence yielding a positive effect on meat quality characteristics (Oni *et al*., 2010).

Supplementation with *M. oleifera* improved the growth rate and carcass measurements (Sarwatt *et al*., 2002; Chapter 6). In addition, *M. oleifera* has high levels of antioxidant compounds (Siddhuraju and Becker, 2003), which have a potential to modulate meat quality (Mielnik *et al*., 2003). Chevon has quality attributes that are concordant with present day consumer demands

(Webb *et al.*, 2005; Simela and Merkel, 2008). In South Africa, over 50 % of the goats are reared under the communal farming system, as described in Chapter 5. The detailed description of the crossbred Xhosa lop-eared goat is in Chapter 5. The effect of supplementing sunflower seed cake on chevon physic-chemical characteristics has been done (Marume, 2010), while the effect of supplementing with *M. oleifera* leaf meal on chevon physico-chemical characteristics has not been studied. Therefore, the objective of the present study was to determine the physico-chemical characteristics of chevon from cross-bred Xhosa lop-eared goats supplemented with *M. oleifera* leaf meal.

7.2 Materials and Methods

7.2.1 Animal, feeding and slaughter management

The study was conducted at university of Fort Hare Farm, Alice in the Eastern Cape province of South Africa. The detailed description of the study site was given in Section 5.2.1. Details of the collection of feeding materials and chemical analysis of the feeding diets were described in Section 5.2.2 and 5.2.3, respectively. Management of experimental and Ethical issues were described in Sections 5.2.4 and 5.2.10, respectively. Body weight and slaughter weight measurements were described in Sections 5.2.5 and 6.2.4, respectively.

7.2.2 Determination of meat quality

The pH measurements and colour were taken from the *M. longissimuss thoracis et lumborum*. Carcass pH was measured 1 hour ($pH_{1)}$ after slaughter and 24 ($pH_{24)}$ hours *postmortem* using a digital pH meter (Crison pH 25 instruments S.A., Alella, Spain) equipped with a penetrating electrode. The pH meter was calibrated using pH 4, pH 7 and pH 9 standard solutions (CRISON

Instruments, SA, Spain) before each measurement was taken. A Minolta colour guide 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-day light, 10º standard observer, was used to measure the muscle colour (L* = Lightness, a* = Redness and b* = Yellowness), 24 hours *postmortem*. Three readings were taken by rotating the Colour Guide 90º between each measurement, in order to obtain a representative average value of the colour. The areas of connective tissue and intramuscular fat were avoided. The colour guide was calibrated before measurements were taken using the green standard.

Blocks of *M. longissimuss thoracis et lumborum* (LTL) muscle measuring approximately 7 x 4 cm long were used for determining cooking loss and shear force values. The muscle was weighed, placed in a water-tight PVC plastic bag and cooked in a water bath at 85˚C for 45 minutes, until an internal temperature of 70˚C was attained. The samples were cooled and reweighed. Cooking loss (CL) was calculated using the following formula: Cooking loss % = [(weight before cooking – weight after cooking) ÷ weight before cooking] × 100
as described by Ding *et al*. (2010). After measurement of cooking loss, cooked samples were used to determine meat Warner Bratzler shear force. Three sub-samples (cut parallel to the muscle fibres with a cross section of 1 x 1 cm and at least 3 cm long) were removed from each cooked muscle. The sub-samples were sheared perpendicular to the fibre direction using an Instron Universal Testing Machine (Moddel 3344, Instron Industrial Products, GC, USA) equipped with a Warner Bratzler (WB) shear force apparatus (cross head speed at 400 mm/min, one shear in the centre of each core). The cooking loss and Warner-bratzler shear force were conducted at the University of Fort Hare, South Africa Meat Science laboratory while colour and pH measurements were conducted at Adelaide abattoir.

7.2.3 Statistical analyses

The PROC GLM procedure of SAS (2003) was used to analyse the effect of diet on slaughter weight, cold dressed weight, dressing percentage, cooking loss, WB shear force, L*, a*, b* and pH values. Mean separations were determined using the PDIFF option in SAS (2003).

The model used was:

$Y_{ijk} = \mu + T_i + E_{ijk}$

Where Y_{ijk} = slaughter weight, cold dress mass, cooking loss, L*, a*, b*, WB shear force values, pH;

μ = overall mean common to all observations;

T_i = effect of dietary supplementation (GH, SC and MOL);

E_{ijk} = random error

7.3 Results

Table 7.1 shows the effect of diet on physico-chemical characteristics of chevon. Goats supplemented with GH had higher ($P < 0.05$) pH at 1 hr *post-mortem* than all other treatments. There was no difference in the effect of dietary supplementation on pH 24 *post-mortem*. Diet had an effect on chevon colour with meat from goats supplemented with MOL and SC having higher ($P < 0.05$) values for lightness (L*) at 24 hour *post-mortem* than the GH group. Chevon from the MOL diet had the highest (a*) values ($P < 0.05$) at 24 hour *post-mortem*. The yellowness (b*) values for meat from goats supplemented with MOL and SC were similar ($P > 0.05$) but higher than those supplemented with the GH diet ($P<0.05$) 24 hour post slaughter. Shear force values of chevon from SC and MOL were higher ($P< 0.05$) than chevon from GH diet (Table 7.1). Chevon from goats supplemented with the GH diet had higher ($P<0.05$) cooking losses than MOL and

SC supplemented groups. The intramuscular fat content of chevon from goats supplemented MOL (2.4 %) and SC (2.4 %) were higher than the GH group (1.1 %).

Table 7. 1: Effect of diet on meat pH, colour, shear force values and cooking loss

Variables	Diets		
	GH (negative control)	MOL	SC (positive control)
Meat pH			
pH_1	6.6±0.08[b]	6.3±0.08[a]	6.4±0.08[a]
pH_{24}	5.7±0.05	5.6±0.05	5.6±0.05
pH_1-$_{24h}$	0.9 ± 0.16[b]	0.7 ± 0.16[a]	0.8 ± 0.18[a]
Colour after 24 h			
L*	41±0.4[a]	45±0.4[b]	43.6±0.4[b]
a*	11±0.2[a]	13±0.2[b]	13±0.2[b]
b*	7.1±0.04[a]	8.4±0.04[b]	7.9±0.04[b]
Shear force (N)	32.6±0.10[b]	29.8±0.10[a]	30.1±0.10[a]
Cooking Loss (%)	29.5±0.48[b]	25.4±0.48[a]	25.6±0.48[a]

[abc] means with different superscripts in a row are different ($P < 0.05$)

7.4 Discussion

The lower decline in pH of chevon from goats supplemented with MOL compared to GH could be attributed to the effect of high polyphenolic compounds in the MOL diet. Anwar *et al*. (2007) reported *M. oleifera* leaves to be a rich source of β-carotene, vitamin A, B and C, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine amino acids, minerals and various phenolics compounds, which have antioxidant properties. Antioxidants are reported to reduce the effect of stress and meat colour, as they reduce the rate of lipid oxidation (Robbins *et al*., 2003; Anwar *et al*., 2007).

The phenolic compounds have enzymatic reactions which enable the conversion of glycogen into lactic acid resulting in decreased pH (Marume, 2010). The ultimate pH was within the acceptable range (5.6 -5.8) reported for goat carcasses (Pratiwi *et al*., 2007; Mushi *et al*., 2009). A high ultimate pH generally reflects depletion of muscle glycogen due to pre-slaughter stress or other factors (Dhanda *et al*., 2003; Mushi *et al*., 2009; Muchenje *et al*., 2009a; 2009b). As such Priolo *et al*. (2002), reported that high energy-diets protect goats from potentially glycogen-depleting stressors. Goats in the present study were exposed to handling when collecting blood and weighing during their management. They had a lairage time of 5 hours. In addition, goats from the three different dietary treatments were transported to the abattoir in the same vehicle, and kept in similar lairage and pre-slaughter conditions, as such, no variation in ante-mortem management among the groups that could influence the results.

In this study castrates were used, which are less prone to stress than entire males (Mushi *et al*., 2009). The observed higher pH_1 for GH goats can, therefore, be associated with low glycogen

reserves due to nutritional deficiency. Generally, the supplemented goats (MOL and SC) had lower pH than the GH goats because GH goats were likely to have less glycogen levels than the supplemented ones. Dhanda *et al*. (2003) and Mushi *et al*. (2009) reported that nutritional stress can result in dehydration, electrolyte imbalances, negative energy balance, glycogen depletion in muscle, and catabolism of protein and fat, ultimately increasing the pHμ. Unfortunately, glycogen concentration was not measured in this study. In future studies it should be measured. Mushi *et al*. (2009) reported that higher pHμ for goats could be associated with low glycogen reserve.

The higher redness (a*) values for goats supplemented with the MOL diet could be attributed to high levels of dietary iron in MOL used in this study. The dietary iron could have influenced the concentration of myoglobin and its chemical state. According to Priolo *et al.* (2002), high iron levels can increase haemoglobin and myoglobin concentrations in meat of grazing animals and also increases meat freshness. The high b* values in goats supplemented with MOL and SC diets could be attributed to β-carotene, which is obtained from plants and *M. oleifera* leaves are reported to be a good source of β-carotene (Anwar *et al*., 2007).

The values for Lightness (L*) and redness (a*) reported in this study were lower than those of Ding *et al*. (2010) for chevon from Guanzhoung Dairy goats. According to Kadim *et al*. (2003), the paleness of chevon could be due to its low concentration of muscle pigments. In the current study, the effect of supplementation on lightness (*L**) value of *M. longissimus dorsi* muscles at 24 hr *post-mortem* were apparent with goats in the MOL group having more pale chevon than the other two groups. High *L** values observed in the MOL group compared to the other groups

could be attributed to the increased marbling as a result of supplementation (Ding *et al*., 2010). The variation or discrepancy in meat colour might be due to diet, age and breed (Kadim *et al*., 2003).

The colour of meat is used to judge the freshness and quality of meat by consumers at the point of purchase (Martínez-Cerezo *et al*., 2005; Ekiz *et al.*, 2010). Meats which are more yellow and dark in colour are usually perceived by consumers as meat obtained from old or sick animals (Ekiz *et al*., 2010), hence consumers prefer to purchase mostly pale to pink meat (Kosum *et al*., 2003). The high L* values in goats supplemented with GH (40.7), MOL (44.9) and SC (43.6) could be due to low pH levels since the pH levels decreases with storage. Meat with higher pHμ usually exhibit lower L* values and have a tendency to yield tougher chevon evidenced by higher WB shear force (Simela, 2005). Chevon from goats supplemented with MOL showed greater a* and b* values, this could be attributed to the influence of polyphenolic compounds which have antioxidant properties. Dietary antioxidant indirectly modifies the chevon colour, probably by decreasing hemoglobin oxidation and activating mechanisms that modify pigment distribution in animal tissues (Simitzis *et al*., 2008). Dietary antioxidants also, minimize rancidity, retard lipid peroxidation, without any damage to sensory or nutritional properties of meat, resulting in maintaining quality and enhancing shelf life (Jang *et al*., 2008). The rate and extent at which muscle pH declines *post-mortem* are both variable and have a greater impact on meat colour and tenderness affecting its physical characteristics.

In this current study, diet influenced the variation of the mean WB shear force values. Goats supplemented with MOL and SC had lower values for WB shear force suggesting they were

tender than chevon from goats supplemented with the GH diet. Generally, the dietary energy intake and consequent carcass fatness affected the tenderness of chevon (Mushi *et al*., 2009). Chevon from MOL and SC supplemented goats had more subcutaneous and intramuscular fat compared to the GH group, which could have prevented the carcasses from drying (Kannan *et al.,* 2006). The WB shear force values (29.8 to 32.6 N) reported in this study, are lower than those (between 29.8 to 35.6 N) for Marume (2010). However, they were within the normal ranges reported elsewhere (Dhanda *et al.*, 2003; Kadim *et al*., 2003). The variation in WB shear force with other results, could be attributed to differences in the cooking temperature and cooking method, ultimate pH, the type of muscle and age of the goats (Dhanda *et al*., 2003; Kadim *et al*., 2003; Webb *et al*., 2005). In general, chevon with WB shear force values exceeding 55 N would be considered as objectionably tough both by trained sensory panel and by consumers (Abdullah and Musallam, 2007; Mushi *et al*., 2009).

Cooking losses ranged from 25.4 to 29.5 %, which is within the normal range for chevon (Webb *et al*., 2005; Kannan *et al*., 2006; Madruga *et al.*, 2008). Cooking losses were lower in chevon from goats supplemented with a high protein diet, such as MOL and SC. The observed lower cooking loss in the MOL and SC are contrary to Mushi *et al*. (2009) and Mapiye *et al.* (2010), who reported that cooking loss increased with increase in protein content. They linked lower cooking loss to higher meat pH scores (Pratiwi *et al*., 2007; Mushi *et al*., 2009). High pH promotes high water binding (low cooking loss) due to higher net charges and greater space between myofilaments (Huff-lonergan and Loneragan, 2005; Mushi *et al*., 2009). Variation in cooking losses are often linked to differences in cooking time and temperature, ultimate pH and muscle fat content (Madruga *et al*., 2008).

7.5 Conclusion

It was concluded that supplementing cross-bred Xhosa lop-eared goats with *M. oleifera* leaf meal produced chevon of comparable quality to sunflower seed cake with higher physico-chemical characteristics. While supplementation of goats with *M. oleifera* has shown to improve physico-chemical charasteritics, further research needs to be done to determine the influence of *M. oleifera* on nutrient, fatty acid profiles and cosumers sensory scores of goat meat.

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Chapter 8: Nutrient composition and consumer sensory scores meat (chevon) from goats supplemented with Moringa (*Moringa oleifera*) leaf meal

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Abstract

The objective of the study was to determine the cholesterol levels, fatty acid, protein and mineral composition and consumer sensory scores of chevon. Twenty-four crossbred Xhosa lop-eared goat castrates supplemented with *Moringa oleifera* leaves (MOL), sunflower seed cake (SC) and grass hay (GH) were used. After slaughter, the *Muscularis longissimus thoracis et lumborum* (LTL) of the right hand side was sampled for cholesterol levels, fatty acid, protein and mineral composition. The chemical composition of chevon differed ($P < 0.05$) among the three diets. Chevon from MOL-supplemented group had higher crude protein (23.57 %), zinc (34.37 mg) and iron (18.57 mg) levels than the other groups. Cholesterol levels were higher in SC (42.84) compared to MOL (38.76) and GH (35.63 mg). Chevon from SC and MOL supplemented group had higher ($P < 0.05$) C18:1c9 than GH group. The values for C16:0 and C18:0 were not significantly different among the three dietary groups. Chevon from GH group had higher ($P < 0.05$) proportions of PUFA (18.93 %), SFA (47.91 %) and *n*-6 (11.35 %) fatty acids than the MOL and SC group. The highest ($P < 0.05$) PUFA/SFA ratio was recorded in GH group (0.40) while the *n*-6/*n*-3 ratio was higher in SC (2.17) and MOL (1.39) than GH group (1.5). Diet also influenced the consumer sensory scores of chevon from goats supplemented with MOL having higher first bite, aroma, flavour and juiceness. The fatty acid cholesterol, protein, mineral compositions and consumer sensory scores varied with diet.

8.1 Introduction

Taste and nutritional composition are important quality attributes of meat for the modern consumers and they affect the characteristics of goat meat (Tshabalala *et al*., 2003). Chevon is an important protein source throughout the world especially in developing countries (Simela and Merkel, 2008; Ding *et al*., 2010). However, chevon composition and quality are known to be influenced by genotype, age, sex, diet and production methods (Tshabalala *et al*., 2003; Todaro *et al*., 2004; Ding *et al*., 2010).

Recently, consumers have increased preference for eating lean meat that does not adversely affect their health (Mapiye *et al*., 2010; Muchenje *et al*., 2010). This has led to the increase in the consumption of goat meat which is leaner and has lower cholesterol content than beef and mutton (Mahgoub *et al*., 2002; Mushi *et al*., 2010). Chevon has low levels of lauric, myristic and palmitic acids, which are associated with the biosynthesis and deposition of cholesterol (Adrizzo, 1999). This has generated increased interest in ways to manipulate the fatty acid and cholesterol composition of meat so as to produce healthier meat with a higher ratio of polyunsaturated to saturated fatty acids and lower cholesterol (Wood *et al*., 2004).

Health conscious consumers use ratios of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) and *n*-6/*n*-3 to evaluate the nutritional value of fat (Alfaia *et al*., 2006; Muchenje *et al*., 2009a; 2009b). Recently the emphasis has shifted from fat quantity to fatty quality due to health concerns (Laaksonen *et al*., 2005; Öhlund *et al*., 2008). Diets with high levels of cholesterol and low ratio of PUFA/SFA have been associated with cardiovascular diseases

(American Heart Association, 2011). In addition, low PUFA/SFA ratios are undesirable as they induce an increase in cholesterolaemia (Wood *et al*., 2004). In contrast, some monosaturated fatty acids (MUFA) and polysaturated fatty acids (PUFA), in particular long-chain *n*-3 PUFA has favourable effects on human health by its ability to reduce arteriosclerosis and thrombotic tendency of blood (Department of Health, 1994; Vasta *et al*., 2009; Mushi *et al*., 2010).

Manipulation of the supplementary feeding results in the change of meat composition which has a potential to improve fatty acid profiles and consequently healthiness of meat (Mapiye *et al*., 2010). In cases where the farmer attempts to shift the fatty acid composition to a more desirable one (increasing the proportion of unsaturated fatty acids), the growth parameters may be influenced negatively (Vasta *et al*., 2009). It has been reported that pasture feeding has a positive effect on fatty acid composition of meat, at the same time having lower growth performance of the animal (Muchenje *et al*., 2009; Vasta *et al*., 2009). Some farmers supplement their goats with multi- purpose forage trees, such as *Moringa oleifera* (Sarwatt *et al*., 2002; Murro *et al*., 2003; Chapter 5 and 6).

In Chapter 6 and 7, it was observed that supplementation of goats with MOL or SC diet influenced the growth rate, carcass measurements and chevon physico-chemical characteristics, respectively. Then, it becomes imperative to determine the effect of MOL supplementation of goats on chevon's nutritional, fatty acid profiles and consumer sensory perceptions. Generally, there is paucity of information on the effects of *M. oleifera* supplementation on protein, mineral, fatty acids and cholesterol composition of chevon and consumer sensory scores. Therefore, it

becomes essential in improving the meat quality and consequently, health of consumers mostly communal farmers. The objective of the current study was therefore to determine the effect of supplementing goats with *M. oleifera* on chevon cholesterol levels, fatty acid, protein, mineral composition and consumer sensory scores.

8.2 Materials and Methods

8.2.1 Study site

The study was conducted at University of Fort Hare farm, Alice, South Africa. The detailed description of the study site was given in Section 5.2.1, while details of the collection of feeding materials are described in Section 3.2.1 and 5.2.2. The experimental diets were described in Section 5.2.3, while the proximate compositions of the diet are given in Table 5.1. Details on animal management are described in Section 5.2.4.

8.2.2 Measurements

The measurements of body weights and BCS were described in Section 5.2.4.

8.2.3 Slaughter procedures

The slaughter procedures were described in Section 6.2.4. The *M. longissimus thoracis et lumborum* (LTL) of the right hand side was sampled, 24 hours after slaughter and vacuum-packaged at 3°C, pending meat chemical and fatty acids analysis.

8.2.4 Chemical composition of meat

A 50 g sample of LTL was ground and freeze dried for the determination of protein, fat, moisture and ash contents, as described by AOAC (2005).

8.2.5 Determination of fat, fatty acid profiles of feed ingredients and meat samples

Total lipid from MOL, SC and GH were extracted according to AOAC (2005) procedures for determination of fatty acids as described by Mapiye *et al*. (2011). Total muscle lipids were quantitatively extracted, according to the method of Folch *et al*. (1957), using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene was added at a concentration of 0.001 % to the chloroform: methanol mixture. A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were dried overnight in a vacuum oven at 50 °C, using phosphorus pentoxide as a moisture adsorbent. Total extractable intramuscular fat was determined gravimetrically from the extracted fat and expressed as percent fat (w/w) per 100 g tissue. The extracted fat from feed and muscle was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at -20 °C pending analyses.

Approximately 10 mg of extracted lipid was transferred into a Teflon-lined screw-top test tube. Fatty acid methyl esters (FAMEs) were prepared for gas chromatography by methylation of the extracted fat, using methanol-BF3 (Christe *et al*., 2001). The FAMEs were quantified using a varian GX 3400 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thickness). Analysis was performed using an initial

isothermic period (40 °C for 2 minutes). Thereafter, temperature was increased at a rate of 4 °C/minute to 230 °C. Finally an isothermic period of 230°C for 10 minutes followed. The FAMEs *n*-hexane (1µ1) were injected into the column using a varian 8200 CX Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250 °C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Vas star Chromatography Software recorded the chromatograms.

Fatty acids methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston manor, Pretoria, South Africa). Conjugated linoleic acid (CLA) standards were obtained from Matreya Inc. (Pleasant Gap, United States). These standards included: *cis*-9, *trans*-11; *cis*-9, *cis*-11, *trans*-9, trans-11 and *trans*-10, *cis*-12-18;2 isomers. All other reagents and solvents were of analytical grade and obtained from Merck chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. The following fatty acid combinations were calculated: omega-3 (*n*-3) fatty acids, omega-6 (*n*-6) fatty acids, total saturated fatty acids (SFA), total monosaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and *n*-6/*n*-3 ratio.

8.2.6 Consumer Sensory assessments

Meat samples that were used for consumer sensory evaluation were obtained from the hind quarters of each carcass and were cut 24 hours after slaughter. The meat samples were cut into cubes (about 2 x 2 cm) which were placed in water-tight PVC plastic bags and cooked in a boiling water bath at a temperature of 85 °C for 45 minutes **(**Babikerm, *et al*., 1990**)**. Salt was added to taste. Fifty-four trained consumer panelists of different gender, age and tribe were drawn from the University of Fort Hare students for the consumer sensory assessment of meat. The panelists were taught how to infer and record scores for each variable tasted. The waiting period between meat sample tasting was 10 minutes. Still water was served to panelists to freshen their mouth between each sub-sample assessment to avoid crossover effects. Eight point descriptive scales were used to evaluate aroma intensity (1= extremely bland to 8= extremely intense), initial impression of juiciness (1 = extremely dry to 8 = extremely juicy), first bite (1 = extremely tough to 8 = extremely tender), sustained impression of juiciness (1 = extremely dry to 8 = extremely juicy), overall tenderness (1 = extremely tough, to 8 = extremely tender), amount of connective tissue (1= extremely abundant to 8 = none), overall flavour intensity (1= extremely bland to 8 = extremely intense), a-typical flavour intensity (1= none to 8 = extremely intense) and off-flavour indicators were also assessed.

8.2.7 Statistical analyses

The nutritive content of the supplementary diets (GH, MOL and SC), were analysed using General Linear Model procedure of SAS (2003). A similar model was used to determine the effect of diet on overall slaughter weight, cholesterol, mineral, protein content, and fatty acid

composition. Differences between least-square means were compared using the PDIFF option in SAS (2003).

The effect of diet on the meat sensory scores was analyzed using the general linear model procedure of SAS (2003). The data was tested for normality and was normal distributed. The model used was as follows:

$Y_{ijkl} = \mu + D_i + G_j + T_k + E_{ijkl}$

Where Y_{ij} = response variable (aroma intensity, initial impression of juiciness, first bite, sustained impression of juiciness, fibre and overall tenderness, amount of connective tissue, overall flavour intensity and relevant a-typical flavour)

μ = overall mean common to all observations;

D_i= effect of dietary supplementation (GH, SC and MOL);

$G_{j=}$ effect of gender on consumer sensory scores;

$T_{k=}$ effect of tribe on consumer sensory scores;

E_{ijkl}= random error.

PDIFF option in SAS (2003) was used for comparison of means.

8.3 Results

The slaughter weights and body condition scores of goats supplemented with GH, MOL and SC were discussed in detail in Chapter 6. The fatty acid compositions of the diet are shown in Table 8.1. The most abound fatty acids in grass hay diet, MOL and SC were C16:0, C18:1c9 and

C18:2c9,12(*n-6*), which accounted for 23.63, 63.4 and 58.0 %, respectively. The highest concentrations of total SFA and *n*-3were observed in the GH diet while MOL had the highest MUFA. Sunflower seed cake had the highest total PUFA, *n*-6, PUFA/SFA ratio and *n*-6/*n*-3 ratio compared to GH and MOL diets.

The chemical composition of chevon differed ($P < 0.05$) among the three diets (Table 8.2) with chevon from MOL having lower moisture content. Also chevon from MOL and SC groups had higher ($P < 0.05$) crude protein, zinc and iron than the GH group. However, cholesterol levels were higher in SC group.

Table 8. 1: Least square means and standard error of means (s.e.m) for fatty acids composition (% total fatty cid) of GH, MOL and SC diet

Fatty acid (% total fatty acid)	GH	MOL	SC
N	4	4	4
C13:0	0.40 ± 0.021^{b}	0.06 ± 0.012^{a}	0.00
C14:0	0.77 ± 0.021^{c}	0.33 ± 0.038^{b}	0.18 ± 0.012^{a}
C15:0	0.31 ± 0.040^{c}	0.02 ± 0.010^{a}	0.10 ± 0.006^{b}
C16:0	23.63 ± 0.271^{c}	8.93 ± 0.309^{a}	17.77 ± 0.417^{b}
C17:0	0.52 ± 0.030^{c}	0.09 ± 0.006^{a}	0.13 ± 0.010^{b}
C18:0	5.22 ± 0.136^{c}	3.71 ± 0.085^{b}	1.87 ± 0.052^{a}
C20:0	3.70 ± 0.139^{c}	2.18 ± 0.065^{b}	0.49 ± 0.036^{a}
C21:0	0.25 ± 0.035^{b}	0.03 ± 0.006^{a}	0.04 ± 0.006^{a}
C22:0	6.26 ± 0.343^{c}	4.47 ± 0.136^{b}	0.67 ± 0.066^{a}
C23:0	0.59 ± 0.049^{b}	0.08 ± 0.000^{a}	0.09 ± 0.012^{a}
Total saturated fatty acids (SFA)	41.65 ± 0.725^{b}	19.90 ± 0.565^{a}	21.34 ± 0.602^{a}
C15:1c10	0.44 ± 0.110^{b}	0.07 ± 0.020^{a}	0.06 ± 0.015^{a}
C16:1c9	0.08 ± 0.026^{a}	0.38 ± 0.586^{b}	0.07 ± 0.021^{a}
C17:1c10	0.00	0.01 ± 0.015^{a}	0.04 ± 0.001^{b}
C18:1c9	9.20 ± 0.609^{a}	63.40 ± 2.196^{c}	18.76 ± 0.125^{b}
C20:1c11	0.16 ± 0.025^{b}	0.05 ± 0.006^{a}	0.00
C24:1c15	0.36 ± 0.036^{c}	0.00 ± 0.000^{a}	0.22 ± 0.006^{b}
Total monounsaturated fatty acids (MUFA)	10.24 ± 0.396^{a}	63.91 ± 0.845^{c}	19.15 ± 0.097^{b}
C18:2c9,12(*n*-6)	23.44 ± 0.480^{b}	11.86 ± 0.555^{a}	58.00 ± 0.557^{c}
C20:2c11,14(n-6)	0.00	0.00	0.08±0.003
C20:3c8,11,14(*n*-6)	0.12 ± 0.015^{b}	0.08 ± 0.010^{a}	0.15 ± 0.015^{b}
Total omega-6 fatty acids (*n*-6)	23.56 ± 0.495^{b}	11.94 ± 0.558^{a}	58.23 ± 0.571^{c}
C18:3c9,12,15(*n*-3)	18.50 ± 0.380^{c}	1.64 ± 0.050^{b}	0.67 ± 0.006^{a}
C20:5c5,8,11,14,17(*n*-3)	5.37 ± 0.240^{c}	1.07 ± 0.055^{b}	0.59 ± 0.057^{a}
Total omega-3 fatty acids (*n*-3)	23.87 ± 0.195^{c}	2.71 ± 0.157^{b}	1.26 ± 0.056^{a}
Total polyunsaturated fatty acids (PUFA)	47.43 ± 0.670^{b}	14.65 ± 1.238^{a}	59.49 ± 0.522^{c}
PUFA/SFA	1.14 ± 0.036^{b}	0.74 ± 0.081^{a}	2.79 ± 0.104^{c}
PUFA/MUFA	4.67^{b}	0.23^{a}	3.11^{b}
n-6/*n*-3	0.99 ± 0.012^{a}	4.41 ± 0.391^{a}	46.21 ± 2.399^{b}

^{abc} Means in the same row, with different superscript differ significantly ($P < 0.05$)

Table 8. 2: Chemical composition and standard errors of *longissimus thoracis et lumborum* muscle of goats supplemented with MOL, SC and GH diets

Nutrients	Diet			
	GH	MOL	SC	S.E
N	8	8	8	
Moisture (%)	76.73[b]	74.33[a]	74.53[a]	0.389
Protein content (%)	21.20[a]	23.57[b]	22.95[b]	0.411
Fat content (%)	1.13[a]	2.39[b]	2.42[b]	0.154
Cholesterol (mg/100g)	35.63[a]	38.76[a]	42.84[b]	2.96
Ash (%)	1.29[a]	1.62[c]	1.38[b]	0.142
Phosphorus (%)	0.20	0.21	0.21	0.007
Calcium (%)	0.01	0.01	0.01	0.008
Iron (mg)	13.93[a]	18.57[b]	14.93[a]	0.484
Copper (mg)	1.08	1.31	1.22	0.06
Zinc (mg)	28.03[a]	34.37[b]	31.95[b]	0.791

[abc] Means in the same row, with different superscript differ significantly ($P < 0.05$)

The least square means of fatty acid compositions of the LTL muscle of goats supplemented with GH, MOL and SC diets are presented in Table 8.3. Chevon from MOL and SC supplemented groups had higher ($P < 0.05$) C18:1c9 than the GH group. The values for C16:0 and C18:0 were not significantly different among the three dietary groups. Chevon from GH group had higher ($P < 0.05$) proportions of PUFA, *n*-6 and *n*-3 fatty acids profiles than MOL and SC groups. The amount of PUFA in GH group was almost double compared to SC group. The SC and MOL groups had higher ($P < 0.05$) amount of MUFA than GH group. The higher ($P < 0.05$) PUFA/SFA ratio was recorded in GH group while *n*-6/*n*-3 ratio was higher in SC group.

The effects of diet on various consumer sensory attributes are shown on Tables 8.4. Diet had an influence ($P < 0.05$) on chevon sensory characteristics. Chevon from goats supplemented with the MOL and SC diets had higher ($P < 0.05$) aroma intensity (A.I) scores than of goats supplemented with GH diet. Other sensory characteristics scores differed ($P < 0.05$) with the diet offered to goats. Female respondents gave higher ($P < 0.05$) scores than male respondents on chevon aroma intensity. Xhosa consumers gave lower ($P < 0.05$) aroma intensity scores than the Ndebele, Shona and the Zulu consumers.

Table 8. 3: Least square means and standard errors of fatty acid composition in percentage fatty acids from the *longissimus thoracis et lumborum* muscle of cross-bred Xhosa lop eared goats given three different diets

Fatty acid (% total fatty acid)	GH	MOL	SC
N	8	8	8
C12:0	0.10±0.021	0.07±0.159	0.06±0.121
C14:0	1.78±0.123[a]	1.80±0.107[a]	2.52±0.107[b]
C15:0	0.62±0.033[b]	0.42±0.028[a]	0.49±0.028[a]
C16:0	21.72±0.545	21.55±0.472	21.64±0.470
C17:0	1.40±0.079	1.28±0.680	1.38±0.068
C18:0	19.21±0.838	18.23±0.726	17.30±0.726
C20:0	0.21±0.007[b]	0.08±0.006[a]	0.06±0.006[a]
C21:0	1.94±0.069[b]	0.64±0.059[a]	0.70±0.059[a]
C22:0	0.93±0.024[b]	0.42±0.021[a]	0.49±0.021[a]
Total saturated fatty acids (SFA)	47.91±1.152	44.49±0.998	44.64±0.998
C14:1c9	0.06±0.008	0.06±0.007	0.07±0.007
C16:1c9	1.67±0.097[a]	1.87±0.084[a]	2.13±0.084[b]
C17:1c10	0.02±0.126	0.23±0.109	0.09±0.009
C18:1c11	0.17±0.016	0.15±0.011	0.18±0.011
C18:1c9	33.45±1.522[a]	41.24±1.319[b]	46.12±1.319[b]
C18:1t11	1.25±0.026	1.16±0.021	1.18±0.021
Total Monounsaturated Fatty Acids (MUFA)	36.62±1.355[a]	44.71±1.174[b]	49.77±1.174[b]
C18:2c9,12(*n*-6)	5.11±0.252[b]	3.52±0.219[a]	3.02±0.219[a]
C18:3c9,12(*n*-6)	0.03±0.004	0.03±0.003	0.04±0.003
C18:2c9t11(*n*-6)(CLA)	0.15±0.007[b]	0.12±0.006[a]	0.11±0.006[a]
C20:2c11,14(*n*-6)	0.55±0.027	0.51±0.024	0.49±0.024
C20:3c8,11,14(*n*-6)	0.03±0.003	0.02±0.002	0.01±0.002
C20:4c5,8,11,14(*n*-6)	5.45±0.212[c]	3.59±0.184[b]	1.65±0.184[a]
C22:2c13,16(*n*-6)	0.03±0.212	0.02±0.01	0.01±0.01
Total omega-6 fatty acids (*n*-6)	11.35±0.926[b]	7.81±0.802[a]	5.33±0.802[a]
C20:3c11,14,17(*n*-3)	0.55±0.021[b]	0.32±0.018[a]	0.23±0.018[a]
C20:5c5,8,11,14,17(*n*-3)	3.13±0.151[b]	2.79±0.131[b]	1.01±0.131[a]
C22:6c4,7,10,13,16,19(*n*-3)	0.62±0.037[b]	0.23±0.032[a]	0.21±0.032[a]
C22:5c7,10,13,16,19(*n*-3)	3.28±0.096[b]	2.29±0.083[b]	1.01±0.083[a]
Total omega-3 fatty acids (*n*-3)	7.58±0.712[b]	5.63±0.617[b]	2.46±0.617[a]
Total polyunsaturated fatty acids (PUFA)	18.93±0.539[c]	13.44±0.463[b]	7.79±0.467[a]
PUFA/SFA	0.41±0.016[b]	0.30±0.014[b]	0.17±0.014[a]
PUFA/MUFA	0.52±0.012[b]	0.30±0.026[b]	0.16±0.014[a]
n-6/*n*-3	1.50±0.092[a]	1.39±0.080[a]	2.17±0.080[b]

[abc] Means in the same row, with different superscript differ significantly ($P < 0.05$)

Table 8. 4: Effect of diet on sensory characteristics

Treatments	Sensory characteristics							
	AI	IJ	FB	SJ	OT	CT	OF	ATF
Control (GH)	4.2±0.12^{a}	4.5±0.11^{a}	5.1±0.11^{a}	4.4±0.17^{a}	4.6±0.11^{a}	4.1±0.01^{a}	4.2±0.01^{a}	2.6±0.18^{b}
Sunflower	4.5±0.12^{b}	4.9±0.11^{b}	5.0±0.11^{a}	4.9±0.11^{b}	6.1±0.11^{c}	4.8±0.09^{b}	4.8±0.10^{b}	1.9±0.18^{a}
M. oleifera	4.7±0.12^{b}	5.1±0.11^{c}	5.4±0.11^{b}	5.5±0.11^{c}	5.7±0.11^{b}	4.7±0.09^{b}	4.7±0.01^{b}	1.8±0.18^{a}

^{abc} values within the same column with different superscripts are significantly different

($P < 0.05$)

Key = AI- Aroma intensity, IJ= Initial juiciness, FB= first bite, SJ= sustain impression of juiciness, OT= overall tenderness, CT= connective tissue, OF= overall flavour, ATF= atypical flavour

8.4 Discussion

It is established that diet and age of the animal can affect meat chemical composition (Ding *et al*., 2010). The lower moisture content in the LTL muscle from goats supplemented with MOL and SC diets could be attributed to higher fat content in muscle in these groups compared to GH group. This is consistent with the report by Lee *et al*. (2008) who also found that chevon with higher fat content had lower water content which is attributed to the amount of space between myofilaments (Omojola, 2007). Goats that were supplemented with higher protein diets (MOL and SC) had higher meat protein values, which is in agreement with French *et al*. (2001) and Geay *et al*. (2001), who reported that higher protein content in meat is related to higher dietary protein. The higher iron and zinc values of meat from goats supplemented with MOL could be attributed to dietary origin (Yalçin *et al*., 2005).

The chevon cholesterol concentrations in this study were lower from goats supplemented with GH and MOL than SC diet. The observed values are considerably lower than 63.8 mg/100 g reported by Casey (1992). Variation of cholesterol levels may be due to the diet offered to goats (Vasta *et al*., 2009). For example, the human consumption of the recommended 200 g per day of meat (Greene and Feldman, 1991) from goats supplemented with GH, MOL and SC represents a cholesterol intake of 71, 78, 86 mg per day, respectively. This would correspond to 24, 26 and 29 %, respectively, of the recommended maximum (300 mg) daily cholesterol intake (American Heart Association, 2011). This makes chevon a health desirable food. Interestingly, the slaughter weight of MOL and SC group were the same, however, the diet MOL lowered the cholesterol level which is of health benefit (Alfaia *et al.*, 2006). Lower levels of cholesterol in GH and MOL group could assist to lower blood cholesterol thereby reducing the risk for atherosclerosis and

coronary heart diseases in consumers. Human body can synthesize its cholesterol as such consumption of diet high in cholesterol result in too much cholesterol circulating in the blood stream leading to the cholesterol deposition in the arteries (Mensink, 2005). This increases risk for blood vessel damage, heart failure, stroke and kidney failures (Greene and Feldman, 1991). The amount of cholesterol in GH and MOL group could have been influenced by the amount of C18:0 (stearic) in the diets (Muchenje *et al*., 2009a). The C18:0 has been shown to exert a neutral or hypocholesterolemic effect on blood cholesterol levels (Mensink, 2005).

The finding that C18:1c9, C16:0 and C18:0 were dominant across different groups is consistent with previous reports (Lee *et al*., 2008; Arsenos *et al*., 2009; Muchenje *et al*., 2009; Marume, 2010). The presence of C18:1c9 having higher values in MOL and SC group is in agreement with Lee *et al*. (2008), who reported high values in protein concentrate diet. The higher SFA level in GH could be attributed to the greater rate of their tissue biosynthesis or production in the rumen from the dietary unsaturated fatty acids (Vasta *et al*., 2009). The diet supplemented to GH group also had higher levels of SFA (41.65 %). The higher forage intake in GH group also contributed to the higher total SFA values. Diaz *et al*. (2002) and Mushi *et al*. (2010) reported that higher forage intake increases rumen activity, consequently increasing the extent of biohydrogenation of dietary PUFA by rumen microbes. The higher SFA in chevon from GH group corroborate with the report of Lee *et al*. (2008) who found that Boer X Spanish intact male goats fed hay alone had higher proportion of SFA. The higher MUFA level content in chevon from MOL group might be partially related to higher dietary fatty acids. This is in agreement with the report by Vasta *et al*. (2009).

The SC group had lower total *n*-3 than MOL and GH groups, which could be attributed to lower levels of C20:3c11,14,17 (*n*-3), C22:6c4,7,10,13,16,19(*n*-3) and C22:5c7,10,13,16,19 (*n*-3). The *n*-6/*n*-3 ratio in this study was lower in MOL and GH groups, however the ratio in all groups was quite lower than the value 4, which is considered as the limit threshold in human diets for preventing cardiovascular diseases (Enser *et al*., 1996; Webb *et al*., 2005, Muchenje *et al*., 2009a). Chevon is a healthy food for human consumption. Apart from improving the slaughter weight of goats, MOL had an added advantage of lowering the *n*-6/*n*-3 ratio. The low *n*-6/*n*-3 ratios are desirable for chevon consumers' health reasons (Department of Health, 1994). The ratios between *n*-6 and *n*-3 fatty acids have important roles in reducing the risk of coronary heart disease (American Heart Association, 2011). Human nutritionists recommend a low intake of saturated *n*-6 and high intake of *n*-3 (Department of Health, 1994).

Mushi *et al*. (2010) reported that animals may have higher proportions of long chain PUFA, which is often associated with membrane phospholipids, attributed to low neutral lipids. Chevon from SC group had low proportion of *n*-3 and PUFA which could be due to dilution of such PUFA by triacylglycerols that increase with carcass fatness (Muchenje *et al*., 2009a; 2009b). It was also observed that chevon from GH group had higher proportions of C18:2c9,12(*n*-6) and C20:4c5,8,11,14 (*n*-6) long chain fatty acid, which might be due to their leaner carcass. In contrast to the observed results from the current study, Mushi *et al*. (2010) found that goats fed on hay alone had higher proportions of C18:3c9,12 (*n*-6) and C15:0 than goats fed on concentrates diet. The ratios of PUFA/SFA were lower in SC group than MOL and GH. These results are similar to those reported by Talpur *et al*. (2008) for longissimus thoracic muscle from pateri goats fed under traditional feeding system. This could be attributed to the hydrogenation of

dietary unsaturated fat in the rumen which is responsible for a decrease in the PUFA to SFA ratio in muscle of all ruminants compared to non ruminants (Enser *et al*., 1996; Talpur *et al*, 2008). The higher PUFA/SFA is desirable (Hoffman and Wiklund, 2006). The PUFA/SFA ratio for all the three groups were below the minimum recommended value of 0.45 (Wood *et al*., 2004) and were also below 0.73 reported by Marume, (2010).

Aroma intensity is related to the amount of fat contained in meat (Calkins and Hodgen, 2007). Chevon from goats supplemented with MOL and SC had higher intramuscular fat content which attributed to high aroma intensity scores. Animal diets have an influence on the organoleptic characteristics of meat. Chevon from goats supplemented with MOL and SC had higher AI scores, which corroborates with findings of Calkins and Hodgen, (2007) that animal diets have a noticeable effect on aroma. Diet influences initial impression of juiciness and sustained impression of juiciness. Findings from this study agree with those of Muchenje *et al*. (2008) that diet influences initial impression of juiciness and sustained impression of juiciness.

Chevon from goats supplemented MOL had higher first bite (FB) scores which is related to tenderness. When the animal is supplemented with energy and protein it accumulates more intra-muscular fat than non-supplemented animals (Xazela *et al*., 2011) which could affect the sensory characteristics. Generally, juiciness of meat is directly related to the intramuscular fat and moisture content (Muchenje *et al*., 2008). Protein and energy supplementation increases the intra-muscular fat thereby increasing marbling (Xazela *et al*., 2011). McMillin and Brock (2005) also observed that high-energy intake increased fat content of chevon and hence, the juiciness, tenderness and texture.

Chevon from goats supplemented with MOL and SC had higher flavour scores which could be attributed to quantity and composition of intramuscular fat, meat pH and antioxidant content. Antioxidants are reported to retard development of oxidation and exert a synergic effect in preventing rancidity (Kanner, 1994). Borton *et al*. (2005) and Priolo *et al.* (2002) also found that flavour was more intense in chevon from animals that were fed concentrates than in chevon from animals that grazed pastures. Tshabalala *et al*. (2003) reported lower flavour intensity in indigenous goat meat which is attributed to lower proportions of unsaturated fatty acids in meat samples of indigenous goats. Generally, meat flavour is affected by a number of factors such as animal age and genotype, feeding regime, carcass fatness level and slaughter weight (French *et al*., 2001; Martinez-Cerezo *et al*., 2005; Muchenje *et al*., 2009a). Flavour can be influenced by the type of diet through the deposition of nutrient components in the fat and animal species. Meat with a desirable flavour tends to have higher levels of intramuscular fat and more intense marbling (McMillin and Brock, 2005). Furthermore, presence of specific flavour compounds or flavour precursors derived from the diet have an effect on flavour (Resconi *et al*., 2009).

Consumer perceptions on the acceptability of meat are linked to socio-cultural factors, especially in the African context. Although chevon and meat products are also of satisfactory eating quality, factors such as gender, tribe and age tend to affect acceptability of chevon from one community to the next (Mahanjana and Cronje, 2000; Dyubele *et al*., 2010). Female consumers in this current study were observed to give higher scores in most sensory attributes, hence finding chevon more acceptable. Similar observations were also made by Simela *et al*. (2008) and Xazela *et al*. (2011). The reason why female consumers gave higher scores is not yet fully understood. Therefore, further research need to be done to find why the female consumers have

high acceptability scores. Also tribe had an influence on chevon acceptability, the Xhosa generally gave low scores in all the sensory attributes compared to the other two tribes. This could be due to the fact that generally Xhosa people prefer mutton over goat meat because of cultural reasons as observed by Xazela *et al*. (2011).

8.5 Conclusion

Chevon from goats that were supplemented with SC and MOL had higher protein, fat and ash levels than the one from those supplemented with GH. The amount of iron and zinc were higher in chevon from MOL supplemented group. Chevon from goats supplemented with GH and MOL diets had desirable fatty acids especially the *n*-6/*n*-3 ratio than goats supplemented with SC diet. Consumer sensory scores were shown to be higher in goats supplemented with *M. oleifera*. Therefore chevon from goats supplemeted with MOL could be a healthy nutritious food from a human nutrition perspective. *Moringa oleifera* leaf diet had better pH, colour and flavour scores and desirable fatty acids which were attributed to the dietary antioxidants. Therefore, it necessitates the determination of the antioxidant activities of *M. oleifera* leaves.

8.6 References

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Chapter 9: Antioxidant activities of *Moringa oleifera* leaf extracts and liver from goats supplemented with *Moringa oleifera* leaves or sunflower seed cake

(Submitted to *Meat science*)

Abstract

The study investigated antioxidant potency of *M. oleifera* leaves. Different *in vitro* systems of standard phytochemical methods were used. The antioxidant effect on the activities of superoxide dismutase (SOD), catalase, lipid peroxidation and reduced glutathione (GSH) were investigated in goats supplemented with *M. oleifera* (MOL) or sunflower seed cake (SC). The acetone extract exhibited higher concentrations of total flavonoids (295.01±1.89 QE/g) followed by flavonols (132.74±0.83 QE/g), phenolics (120.33±0.76 TE/g) and proanthocyanidins (32.59 ±0.50 CE/g) than the aqueous extract. The reducing power of both solvent extracts showed strong antioxidant activity in a concentration dependent manner. The acetone extract depicted higher percentage inhibition against DPPH, ABTS and nitric oxide radicals which were comparable with reference antioxidant (vitamin C and BHT). The MOL increased the antioxidant activity of GSH (186 %), SOD (97.8 %) and catalase (0.177 %). Lipid peroxidation was significantly reduced by MOL followed by SC and hay grass supplements, respectively. The present study suggests *M. oleifera* is a source of compounds with antioxidant activities and its inclusion in the diet was effective in enhancing oxidative stability of goat liver.

9.1 Introduction

Plants and their products are potential sources of phytochemicals that have been found to counteract free radicals due to their antioxidant activity (Khalafalla *et al*., 2010). Free radicals

are molecules that cause oxidative stress as a result of imbalance between the antioxidant defense system and the reactive oxygen species (ROS). The ROS generated induce oxidative damage to essential biomolecules such as proteins, DNA, lipoproteins and lipids (Yazdanparast and Ardestani, 2007). This damage is a crucial etiological factor implicated with various human diseases including aging, cancer, cardiovascular disease, diabetes, stroke, liver cirrhosis, atherosclerosis and Alzheimer's diseases (Hertog *et al*., 1997; Adedapo *et al*., 2008). In meat, lipid peroxidation of fats has been reported to cause chemical spoilage due to generation of ROS such as peroxyl, superoxide anions, singlet oxygen and hydroxyl radicals (Siddhuraju and Becker, 2003). The accumulation of peroxides in the meat of animals may result in the development of rancid flavours and changes in the colour of the meat. This could affect protein solubility and reduced nutritional value of meat (Aqil *et al*., 2006).

There is growing interest in the free radical biology which warrants the usefulness of natural antioxidants for the purpose of improving the quality such as texture, colour, flavour, nutritive value and possibly improving the shelf life of meat. These antioxidants are molecules that prevent uncontrolled formation of free radical and activated species by quenching or chelating their catalytic metal ions (Yazdanparast and Ardestani, 2007). The use of synthetic antioxidants such as butylated hydroxylanisole (BHA) and butylated hydroxyltoluene (BHT) are commercially available but restricted due to their toxicity to some vital organs in the body (Siddhuraju and Becker, 2003).

Nowadays, natural antioxidant agents from plant source are safe and have received a remarkable attention due to their ability to preserve foodstuffs and prevent rancidity caused by oxidation.

Consumption of plant products with antioxidant properties by animals has been reported to pass antioxidant compounds to meat (Middleton *et al*., 2000; Lahucky *et al*., 2010). Example of these compounds include phenols, flavonoids, proanthocyanidins, flavonols, vitamin C, vitamin E, β-carotene, zinc and selenium which have been documented to possess strong antioxidant potential (Cai *et al*., 2004; Okwu, 2004; Aqil *et al*., 2006). Feeding of animals with plants containing these compounds can serve as a route to pass antioxidant activity to their bodies. This has been confirmed in experiments conducted in broilers (Sarraga and Garcia Regueiro, 1999; Kuli-sic *et al*., 2004). Feeding with antioxidant has shown to greatly extend colour shelf-life of meat (Kuli-sic *et al*., 2004).

Moringa oleifera Lam is a highly valued plant in tropic and subtropical countries where it is mostly cultivated (Khalafalla *et al*., 2010). The leaves are highly nutritious (Chapter 3), being a good source of protein, β-carotene, vitamins A, B, C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals and various phenolics compounds (Anwar *et al*., 2007; Khalafalla *et al*., 2010). Besides, its compelling nutritional value it has curative and prophylactic properties (Khalafalla *et al*., 2010). Sunflower seed cake has been used as a livestock feed (Chapter 5 and 6; Mapiye *et al*., 2010; Marume, 2010), however, its antioxidant potential have never been determined.

Research has revealed the potential of *M. oleifera* grown in India and other parts of the world as a source of antioxidant agents (Siddhuraju and Becker, 2003; Khalafalla *et al*., 2010). However, there is little or no data on the polyphenolic contents and antioxidant activity of *M. oleifera* of the South African ecotype and its antioxidant impact when supplemented to goats. Therefore,

there is need to investigate the antioxidant activity of *M. oleifera* in South Africa considering the influence of geographical location and abiotic factor on the phenolics content of this plant (Siddhuraju and Becker, 2003). The present study, therefore investigated the polyphenolic content and antioxidant properties of *M. oleifera* and diet supplemented with *M. oleifera*/sunflower seed cake in Xhosa lop-eared goat crosses.

9.2 Materials and methods

9.2.1 Plant material and extract preparation

The detailed description of *M. oleifer*a leaves collection was given in Section 3.2.1. The extracts preparation was described in detail in Section 4 .2.2.

9.2.2 Management of experimental animals

The detailed description of the study site was given in Section 5.2.1, while details of the collection of feeding materials were described in Section 5.2.2. The experimental diets were described in Section 5.2.2, while the proximate compositions of the diet are given in Table 5.1. Details on animal management and slaughter procedures are described in Section 5.2.4 and 6.24, respectively. A 10 % w/v of liver was cut immediately after slaughter and homogenized in 0.001 M phosphate buffer (pH 7.0).

9.2.3 Polyphenolic antioxidants assays

9.2.3.1 Determination of total phenolics

Total phenolic contents of aqueous and acetone extracts of moringa were determined by the modified method of Wolfe *et al*. (2003) using Folin-Ciocalteu reagent. The extract was mixed

with 5 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml of sodium carbonate (75 g/l). The mixture was vortexed for 15 sec and allowed to stand for 30 minutes at 40˚C for colour development. Absorbance was then measured at 765 nm using the Hewlett Packard UV-VIS spectrophotometer. Extracts were evaluated at a final concentration of 1 mg/ml. Total phenolics content was expressed as mg/g tannic acid equivalent using a derived equation from the calibration curve: Y= 0.1216x, R^2= 0.9365, Where x is the absorbance and Y is the tannic acid equivalent (mg/g).

9.2.3.2 Determination of total flavonols

The total flavonols were estimated according to the method described by Kumaran and Karunakaran (2007). To 2.0 ml of solvents extract was added 2 ml of 2 % $AlCl_3$ prepared in ethanol and 3 ml of sodium acetate solutions (50 g/l). The absorbance of the mixture was measured at 440 nm after incubation at 20 ˚C for 2.5 hrs. Plant extracts were evaluated at a final concentration of 1 mg/ml. Total flavonol content was calculated as quercetin (mg/g) using the following equation derived from the calibration curve: Y = 0.0255x, R^2 =0.9812, Where x is the absorbance and Y is the quercetin equivalent (mg/g).

9.2.3.3 Determination of total proanthocyanidins

The procedure reported by Sun *et al*. (1998) was used to determine total proanthocyanidin content in *M. oleifera*. Aliqouts of 0.5 ml of 1 mg/ml of the extract was mixed with 3 ml of 4 % vanillin-acetone solution and 1.5 ml hydrochloric acid. The absorbance was measured at 500 nm after the mixture was allowed to stand for 15 minutes. The extract was evaluated at a final concentration of 1 mg/ml. Total proanthocyanidin content was expressed as catechin equivalents

(mg/g) using the following equation based on the calibration curve: Y= 0.5825x, R^2 = 0.9277. Where x is the absorbance and Y is the catechin equivalent (mg/g).

9.2.3.4 Determination of total flavonoids

Total flavonoid content was determined using the method described by Ordonez *et al*. (2006). An aliqout of 0.5 ml of 2 % $Alcl_3$ prepared in ethanol solution was added to 0.5 ml of sample solution, followed by measuring the absorbance at 420 nm, after 1 hr of incubation at room temperature. A yellow colour indicates the presence of flavonoids. The extract samples were evaluated at a final concentration of 1 mg/ml. Total flavonoid contents was calculated as quercetin equivalent (mg/g) using the following equation from the calibration curve: Y = 0.025x, R^2 = 0.9812, Where x is the absorbance and Y is the quercetin equivalent (mg/g).

9.2.4 In-vitro antioxidant assays

9.2.4.1 ABTS radical scavenging assay

The method of Re *et al*. (1999) was adopted to determine 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity of *M. oleifera*. The stock solution containing equal volumes of 7 mM ABTS salt and 2.4 mM potassium persulfate was allowed to stand in the dark for 16 hrs at room temperature. The resultant ABTS solution was diluted with methanol until an absorbance of 0.70 ± 0.01 was reached at 734 nm. Varying concentrations of the plant extracts with an aliquot of 1 ml were mixed with 1 ml of ABTS solution and the absorbance was measured at 734 nm after 7 minutes, using the Spectrophotometer (Thermo Spctronic, BioMate 3 Rochester, New York, USA). The ABTS scavenging capacity of the extract was compared with that of BHT and rutin. The percentage inhibition was calculated as:

ABTS radical scavenging activity (%) = $[(Abs_{control} – Abs_{sample})]/ (Abs_{control}) \times 100$

Where $Abs_{control}$ is the absorbance of ABTS radical + acetone extract; Abs_{sample} is the absorbance of ABTS radical + extract/standard.

9.2.5.2 DPPH radical scavenging assay

The effect of extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was estimated using the method described by Liyana-Pathiranan *et al*. (2006). A solution of DPPH (0.135 mM) was prepared and 1 ml of this solution was mixed with 1 ml of acetone or aqueous extract (0.02-0.1 mg). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm using rutin and BHT as reference drugs. The radical scavenging activity was calculated from the equation: Percentage of radical scavenging activity = $(Abs_{control} - Abs_{sample})/ Abs_{control} \times 100$

Where $Abs_{control}$ is the absorbance of DPPH radical + acetone; Abs_{sample} is the absorbance of DPPH radical + sample extract/standard.

9.2.4.3 Determination of reducing power

The method of Yen and Chen (1995) was adopted to determine the reducing power of *M. oleifera.* A volume of 1.0 ml of the extract was prepared in their respective solvents of distilled water and acetone (0.02-0.1 mg/ml) and were mixed individually to the mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_{6)}$ (1 %w/v). The resulting mixture was incubated at 50 °C for 20 minutes, followed by the addition of 2.5 ml of trichloroacetic acid (10 % w/v), which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was pipetted into another test tube containing

2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1 %, w/v). The absorbance was measured at 700 nm against the mixture without the extract (blank sample). Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

9.2.4.4 Scavenging activity of nitric oxide

The nitric oxide (NO) radical scavenging activity of the aqueous and acetone extracts of *M. oleifera* was determined according to the method of Garrat (1964). A volume of 2 ml of 10 mM sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) and later reacted with 0.5 ml of plant extract or BHT or rutin at various concentrations (0.02-0.1 mg/ml). The mixture was incubated at 25 °C. After 150 minutes, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of griess reagent [1.0 ml sulfanilic acid reagent (0.33 % prepared in naphthylenediamine dichloride (0.1 % w/v)]. The mixture was incubated at room temperature for 30 minutes, followed by measurement of the absorbance at 540 nm. The amount of nitric oxide radical inhibited by the extract was calculated using the following equation:

NO radical scavenging activity = $\{(Abs_{control}-Abs_{sample}) / (Abs_{control})\} \times 100$

Where; $Abs_{control}$ is the absorbance of NO radical +methanol; Abs_{sample} is absorbance of NO radical + sample extract or standard.

9.2.5 In-vivo antioxidants assays

9.2.5.1 Determination of catalase activity

Catalase activity was assayed using the catalase assay kit (CAT 100) purchased from Sigma-Aldrich St. Louis MO, USA. The reaction mixture consisted of 0.4 ml of 0.2M H_2O_2, 1 ml of 0.01 M phosphate buffer (pH 7.0) and 0.1 ml of liver homogenate (10 % w/v) was added in a

total volume of 1.5 ml. The reaction was stopped by adding 2 ml of dichromate-acetic acid reagent (5 % $K_2Cr_2O_7$ prepared in glacial acetic acid). Catalase activity was then determined by measuring changes in the absorbance at 620 nm in 1 minute interval for 5 minutes and recorded. Liver was homogenized in 0.01 M phosphate buffer (pH 7.0) and centrifuged at 5000 rpm. Percentage activity was calculated using the equation:

% catalase activity = [(normal activity – inhibited activity)] / (normal activity)] * 100 %.

9.2.5.2 Determination of superoxide dismutase activity

Superoxide dismutase activity was measured using the SOD kit (19160) purchased from Sigma-Aldrich (Chemie GmbH, Germany). The activity was determined by following the manufacturer instruction. Liver homogenate (20 µl) was added to 200 µl of the kit working solution. The mixture was incubated at 37°C for 20 min after gentle shaking and added 20 µl of the kit enzyme working solution. The absorbance of the mixtures was measured spectrophotometrically at 450 nm (Thermo Spctronic, BioMate 3 Rochester, New York, USA) and the SOD activity was calculated using the following equation:

% SOD activity = {[(blank 1 – blank 3) – (sample A – sample A's blank 2)] / (blank 1 – blank 3)} × 100

Where blank 1 was a mixture of the working solution and enzyme working; Blank 2 contained the liver homogenate with working solution and dilution buffer, while water was added to the liver homogenate in the blank 3.

9.2.5.3 Determination of reduced glutathione activity

Reduced glutathione was determined using the Glutathione assay kit catalog number CSO260 purchased from Sigma-Aldrich. (St. Louis, MO, USA). Liver samples were washed twice with PBS immediately after excision and homogenize with liquid nitrogen to fine powder. An aliquot of the powder (0.1 g) was added to 3 ml of 5 % 5-sulfosalicyclic Acid (SSA) solution (0.5 ml) to deproteinize and vortex to remove the precipitated protein. The working mixture of 150 µl of 1X assay buffer (8 ml) was added to 228 µl of the diluted enzyme solution (6 units/ml) and 228 µl of DTNB stock solution (1.5 mg/ml) and re-suspended. After incubating for 5 min at room temperature, 50 µl of the diluted NADPH solution was added and then mixed to generate a yellow colour. The absorbance of reduced glutathione was measured at 412 nm and calculated by subtracting the final absorbance from the blank. The values of the glutathione standard solutions were used to determine the standard curve and the ΔA412/min equivalent was calculated to 1 nmole of reduced glutathione per well. The activity of the enzyme was expressed as percentage (%).

9.2.5.4 Estimation of lipid peroxidation

Lipid peroxidation in the liver was estimated colourimetrically by Thiobarbituric acid reactive substances (TBARS) using malondialdehyde (MDA) as a standard. In brief, 0.1 ml of liver homogenate (10 % w/v) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37 %, 15 % trichloroacetic acid and 0.25 N HCl). All the tubes were placed in a boiling water bath for 30 min, and allowed to cool. The amount of malondialdehyde formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 535 nm using a spectrophotometer at 1 minute intervals for 5 minutes against reference blank. Percentage

activity was calculated using the equation: % inhibition of lipid peroxide = $\{A_o - A_1\}/A_o \times 100$, where A_o is the absorbance of the control and A_1 is the absorbance of the sample extract.

9.2.6 Statistical analyses

All data were expressed as mean ± SD. The data were analyzed using General linear Model Procedure of SAS (2003). Pair-wise comparison of the least square means was performed using the PDIFF test.

The model used was

$Y_{ij} = \mu + T_i + E_{ij}$

Where Y_{ij} = antioxidant parameters;

μ= is the overall mean;

T_i= is the effect of dietary supplementation or extract;

E_{ij}= is the random error.

In the model the data was tested for normality and was normally distributed.

9.3 Results

9.3.1 Total polyphenolic contents

The amount of flavonoids, flavonols, phenols and proanthocyanidins in acetone extract were found to be 295.01 QE/g, 132.74 QE/g, 120.33 TE/g, and 32.50 CE/g, respectively (Table 9.1) which are greater than the aqueous extract.

9.3.2 ABTS radical scavenging activity

The acetone extract of *M. oleifera* leaves was fast and effective scavengers of the ABTS radical and its activity compared well with BHT, but different as compared with the aqueous extract as shown in Figure 9.1. The percentage inhibition of acetone and aqueous extracts, BHT and rutin were 95.27, 72.89, 98.47 and 73.8 %, respectively at a concentration of 1.0 mg/ml. The IC_{50} values of acetone and aqueous extracts were 0.192 and 0.665 mg/ml, respectively.

9.3.3 DPPH radical scavenging activity

The scavenging effects of acetone and aqueous extracts on the DPPH radical are illustrated in Figure 9.2. Acetone extract of *M. oleifera* leaves significantly reduced the DPPH radical in concentration dependent manner with higher activity than the aqueous extract. In comparison, acetone and aqueous extracts had percentage inhibition of 98.24 and 83.56 %, respectively while BHT (98.62) used as positive control showed similar activity with acetone extract at 1 mg/ml (Figure 9.2). The activity was concentration dependent.

9.3.4 Reducing power

The reducing power of *M. oleifera* extracts increased with an increase in concentration. Antioxidant activity of acetone extract was found to be strongly effective on reducing power when compared with the aqueous extract but significantly lower than vitamin C and BHT used as reference drugs at 0.5 mg/ml (Figure 9.3).

Table 9. 1: Total polyphenolic contents of the leaf extracts of *M. oleifera* (n =3)

Solvent extracts	Phenolics (TE/g)	Flavonoids (QE/g)	Flavonols (QE/g)	Proanthocyanidin (CE/g)
Acetone MOL	120.33 ± 0.76^{b}	295.01 ± 1.99^{b}	132.74 ± 0.83^{b}	32.59 ± 0.50^{b}
Aqueous MOL	40.27 ± 0.99^{a}	45.1 ± 0.47^{a}	18.10 ± 0.18^{a}	16.91 ± 0.87^{a}

^{a,b} Means with different superscript in the same column differ significantly ($P < 0.05$)

QE = Quercetin equivalent

TE = Tannic acid equivalent

CE = Catechin equivalent

9.3.5 Nitric oxide radical scavenging activity

The effect of both solvent extracts of *M. oleifera* leaves against nitric oxide radical was evaluated. Both solvent extracts appreciably reduced the release of nitric oxide radicals, but significantly lower as compared with BHT the positive control, in a concentration dependent manner (Figure 9.4). The aqueous extract moderately scavenged the formation of nitric oxide radical. At 1 mg/ml, the percentage inhibitions of NO radical by BHT, acetone and aqueous extract were 98.47, 65.77 and 59.4, respectively.

9.3.6 In-vivo antioxidant

The effect of MOL, SC and GH supplement on the activities of antioxidant enzymes assays in this present study are shown in Table 9.2. Diet supplemented with MOL and SC significantly increased the activity of GSH as compared with the GH group. In comparison, the activities of CAT and SOD of diet supplemented with MOL and SC were increased appreciably than the goats fed with ordinary GH. However, the activities of the GSH, CAT and SOD of the goats supplemented with MOL showed higher activity. MOL and SC inhibited the amount of MDA generated in liver homogenate of goats while better activity was observed in group supplemented with MOL as presented in Table 9.2.

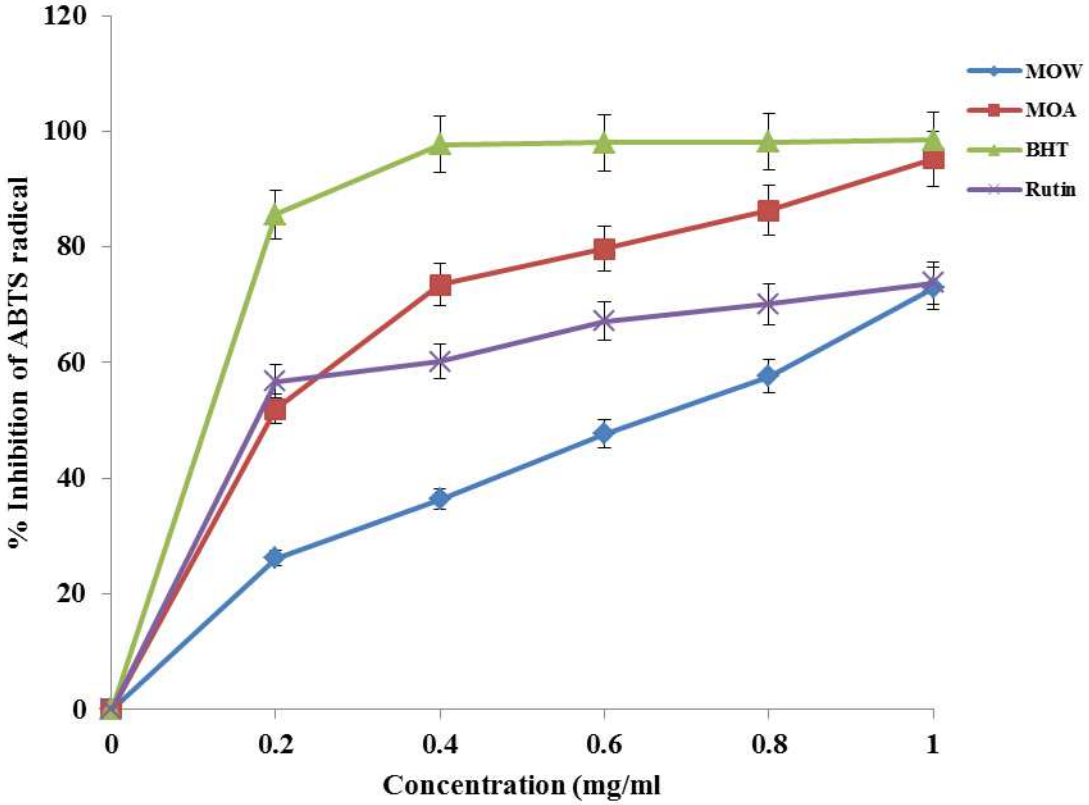

Figure 9. 1: ABTS radical scavenging activities of the acetone and aqueous *M. oleifera* leaf extracts

BHT = 2,2-azinobis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt,

MOW = *M. oleifera* water extract,

MOA = *M. oleifera* acetone extract,

RTN = Rutin

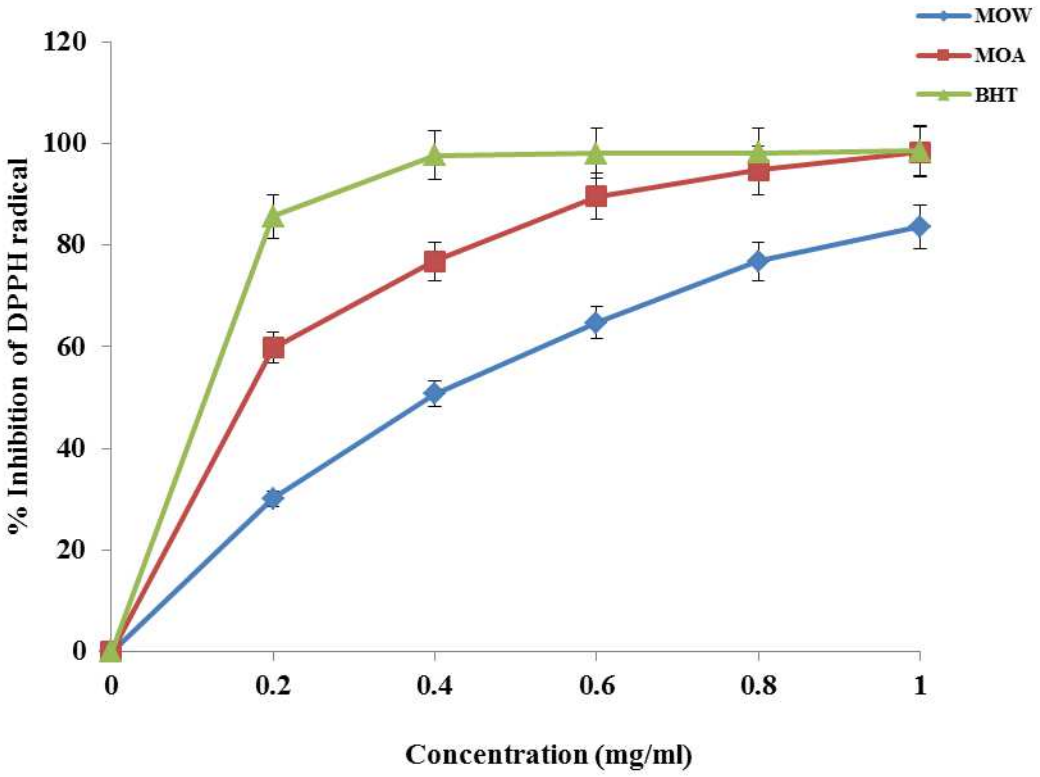

Figure 9. 2: DPPH radical scavenging activity of acetone and aqueous leaf extract of *M. oleifera*

BHT = 2,2-azinobis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt

MOW = *M. oleifera* water extract

MOA = *M. oleifera* acetone extract

RTN = Rutin

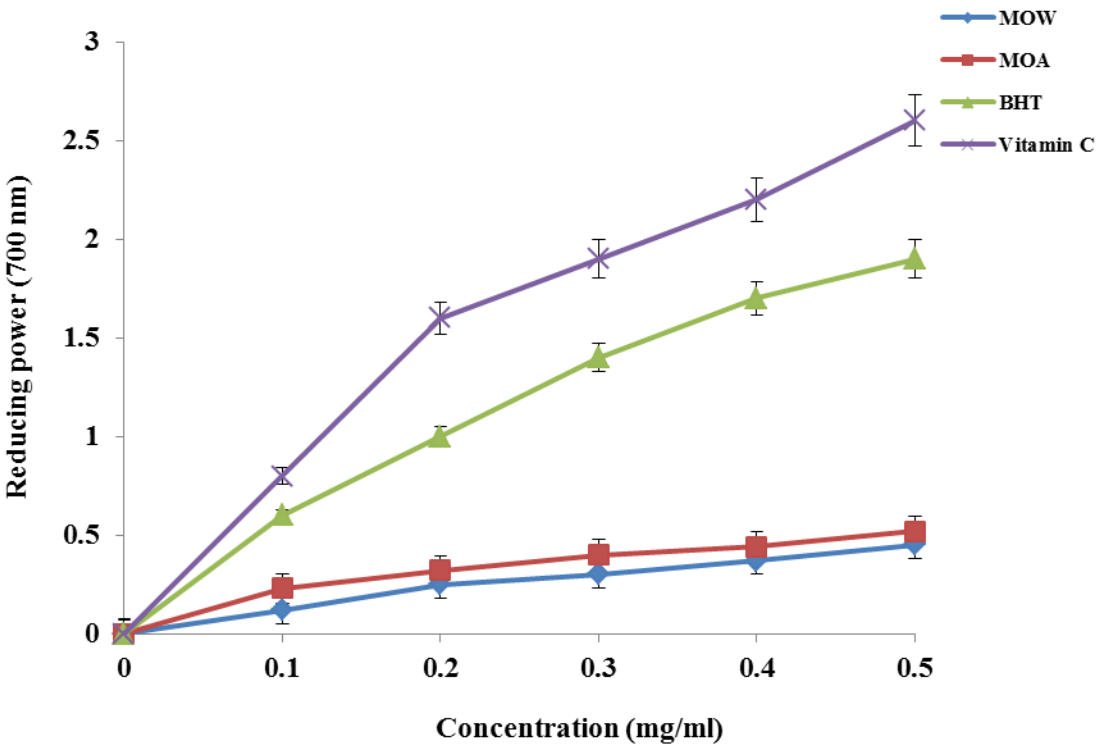

Figure 9. 3: Reducing power of acetone and aqueous leaf extract of *M. oleifera*

BHT = 2,2-azinobis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt

MOW = *M. oleifera* water extract

MOA = *M. oleifera* acetone extract

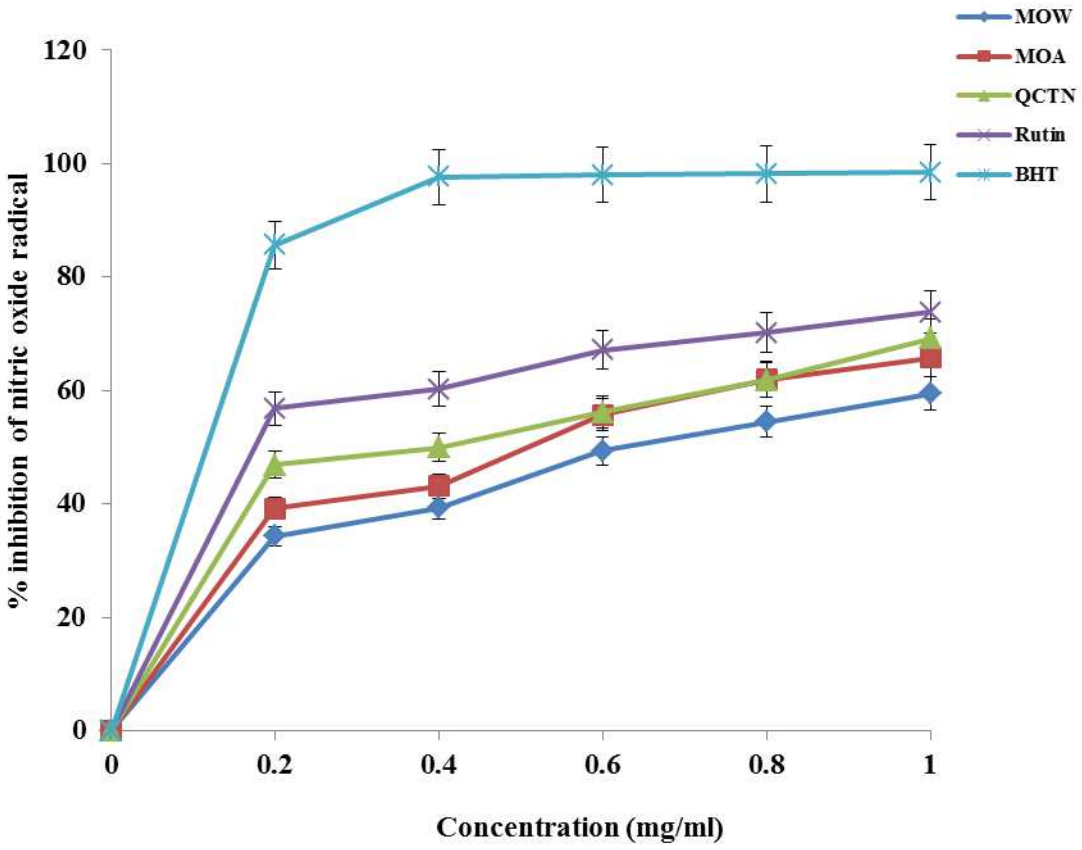

Figure 9. 4: The nitric oxide radiical scavenging activity of acetone and aqueous leaf extract of *M. oleifera.*

BHT = 2,2-azinobis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt,

MOW = *M. oleifera* water extract

MOA = *M. oleifera* acetone extract

RTN = Rutin

QCTN = Quercetin

Table 9. 2: Effect of diet (MOL, SC and GH) on LPO, antioxidant and GSH in goat liver

Treatment	GSH (%) n=8	CAT (%) n=8	SOD (%) n=8	TBARS (%) n=8
M. oleifera	186.0 ± 1.0^{c}	0.177 ± 0.006^{c}	97.80 ± 1.21^{c}	81.33 ± 0.99^{c}
Sunflower	135.0 ± 2^{b}	0.145 ± 0.009^{b}	89.15 ± 1.55^{b}	38.76 ± 0.45^{b}
Grass hay (control)	119.0 ± 8.39^{a}	0.027 ± 0.02^{a}	74.5 ± 1.43^{a}	1.99 ± 0.02^{a}

[ab] Means with different superscripts in the same column differ significantly ($P < 0.05$)

GSH = Reduced glutathione activity

CAT = catalase activity

SOD = Superoxide dismutase activity

TBARS = Thiobarbiuric acid reactive substances

9.4 Discussion

Polyphenolic compounds exist widely in the plant kingdom and are common in leaves, flowering tissues and pollens (Siddhuraju and Becker, 2003). These compounds are used to modulate lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis due to their antioxidant activity and anti-inflammatory action (Frankel and Meyer, 2000; Siddhuraju and Becker, 2003). The present study revealed higher values of phenols, flavonoids, flavonols and proanthocyanidins in acetone extract of *M. oleifera* leaves than the aqueous extract. The observed result could be due to different degree of polarity of the solvents used for the extraction of polyphenolic compounds and thus could contribute significantly to the antioxidant and free radical scavenging activity. Similar observation was demonstrated by Siddhuraju and Becker (2003) who reported antioxidant activity of methanolic leaf extract of *M. oleifera* from three different agro-climatic origins due to high phenolic and flavonoid content.

The phenolic content of *M. oleifera* leaves observed in this study corroborated with the findings of Frum and Viljoen, (2006) and Sreelatha and Padma (2009) on different fractions of this plant. The synergistic effect of phenolic compounds may contribute significantly to the ability of the extracts to adsorb and neutralize free radicals or decompose peroxides (Adedapo *et al*., 2008). Their ability as free radical scavengers could be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Atawodi *et al*., 2010; Oyedemi *et al*., 2010).

The reaction of antioxidant compounds present in *M. oleifera* leaves with DPPH radical discolourized the visible deep purple colour by measuring the changes in absorbance at 517 nm.

The degree of discolouration indicates the scavenging potential of the extract due to hydrogen proton donation (Verma *et al*., 2009). In this study, both solvent extracts scavenged DPPH radicals in a concentration dependent manner while higher activity was observed in the acetone extract (Figure 9.2). The acetone extracts could serve as free radical inhibitors acting possibly as primary antioxidants. Lu and Foo (2000) reported a high correlation between DPPH scavenging potential and total phenolic content of plant extracts. Sreelatha and Padma (2009) demonstrated that methanol extract of *M. oleifera* leaves significantly reduced DPPH radicals though lower than our observed results. Variation could be due to differences in polarity of the solvents and geographical location of the plant (Sreelatha and Padma, 2009).

The acetone extract had strong activity to quench $ABTS^{+}$ which may be ascribed to the presence of phenolic compounds with hydroxyl group attached to the aromatic ring structures (Vinson *et al*., 1998). The findings in this study was comparable to that of DPPH radical but contradictory to the findings of Oyedemi *et al*. (2010) who found that scavenging ability of *S. henningsii* extract against DPPH was greater than ABTS radicals. This observation opposes the fact that different test and solvent system could affect comparison of antioxidant activity based on different assays methods ((Frankel and Meyer, 2000). The observed result could be attributed to the high phenolic contents especially flavonoids in the acetone extract. The present study reported the ABTS radical scavenging activity of *M. oleifera* leaves in acetone for the first time despite various antioxidant assays carried out on this plant.

Nitric oxide is a key signaling in the physiological and pathological conditions and when reacting with macromolecules may induce inflammatory activities. It has been reported to play

an important role in various inflammatory processes such as carcinomas, muscle sclerosis, arthritis and ulcerative colitis (Hazra *et al*., 2009). The percentage inhibition displayed by both acetone and aqueous extracts showed a potent scavenger of nitric oxide and thus confirmed the use of this plant for the treatment of anti-inflammatory diseases caused by nitric oxide formation. In this study, acetone extract of *M. oleifera* leaves depicted a significant inhibitory effect against nitric oxide generation which is comparable to quercetin but lower to BHT and rutin used as reference drugs.

Siddhuraju and Becker (2003) reported that the reducing power of bioactive compounds was associated with antioxidant activity. As a result, it is imperative to determine the reducing power of this plant in order to elucidate the relationship between their antioxidant effect and the ability to transform Fe^{+3} to Fe^{+2}. The reducing power of the plant extracts was found to be concentration dependent. The antioxidant activity of both solvent extracts is correlated with their total polyphenolic contents. Several studies have shown the correlations between reducing power and polyphenolic contents in plant extracts (Pourmorad *et al*., 2006; Thirugnanasampandan *et al*., 2008). The findings obtained from this study agreed with Siddhuraju and Becker (2003) who showed that antioxidant properties were concomitant with the development of reducing power. Therefore, phenolic compounds present in *M. oleifera* leaf extracts are good electron donors and could terminate the radical chain reaction by converting free radicals to stable products.

The consumption of *M. oleifera* leaves by both humans and animals have been shown to possess high nutritive value and antioxidant compounds (Atawodi *et al*., 2010; Khalafalla *et al*., 2010; Mendieta-Araica *et al*., 2011). Ingestion of this plant as animal diet exhibited the medical and

therapeutic activities in the body (Fahey, 2005). Superoxide dismutase has been reported as one of the most important antioxidant defense enzymes that scavenge superoxide anion in order to lessen toxic effects caused by this radical (Curtis *et al*., 1972; Liyana-Pathiranan *et al*., 2006). The present study revealed the high percentage inhibition of superoxide anion in the goats fed on MOL diet than the GH diet. This observation implies an efficient protective mechanism of *M. oleifera* leaves against scavenging superoxide anion which may be attributed to the high concentration of phenolics and flavonoids contents (Robak and Glyglewski, 1998).

Reduced glutathione (GSH) is a non enzymatic biological antioxidant present in the liver. It protects cellular proteins against reactive oxygen species in the body (Arivazhagan *et al*., 2000). The activity of GSH was significantly increased in goats supplemented with MOL diet which is associated with a decrease in the level of lipid peroxidation. Results on plant materials have been observed in rats that were given plant extracts in a dose dependent manner though not fed (Badami *et al*., 2005; Choi *et al*., 2010; Oyedemi *et al*., 2010). Generally, high phenolic contents of the acetone extract correspond with antioxidant activity due to the combined effect of these compounds.

Catalase is another antioxidant enzyme widely distributed in the animal tissues (Oyedemi *et al*., 2010). The enzyme is reported to protect the system from highly reactive hydroxyl radicals through hydrogen peroxide decomposition (Chance *et al*., 1952). Reduction of this enzyme activity may promote the cellular damage caused by the assimilation of superoxide and hydrogen peroxide. However, in this study the ingestion of *M. oleifera* leaves by the goats had a higher inhibition of catalase activity than SC and GH diet. This indicates the hepato-protective ability of

this plant against liver damage. The study showed that the antioxidants present in the leaves had the ability to be transferred to the animal. They had a role in modifying the concentration of antioxidants, pro-oxidants in muscles. Dietary *M. oleifera* could have assisted to counteract the action of pro-oxidants in muscle tissues both in living goats and also after slaughter. In Chapter 7 it was observed *M. oleifera* diet influenced the colour and flavour of meat. Presence of antioxidants in meat assists in the preservation of meat. Further research needs to be carried to find the antioxidant potential of meat from goats supplemented with *M. oleifera* leaves.

9.5 Conclusions

The present study suggested that the aqueous and acetone extracts of *M. oleifera* leaves, have potent antioxidant activities but at different degree. The antioxidant potential may be attributed to the presence of polyphenolic compounds. Thus consumption of diet supplemented with *M. oleifera* leaves could protect the animals against diseases induced by oxidative stress and also assist in the preservation of meat. The protective effect of *M. oleifera* may explain its extensive use in life and possible health benefits.

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Chapter 10: General Discussion, Conclusion and Recommendation

10.1 General discussion

Goats play an important role to resource-limited farmers by providing animal protein, income and are essential for socio-cultural purposes, but their production and productivity in developing countries is constrained by many challenges. The major challenge being the inadequacy of feed especially protein, during the winter or drought periods. The presence of helminths, also brings in a lot of challenges to the goat production system (Campbell, 2003). Inadequacy of feed slows goat growth as well as predisposes goats to challenges of helminthes. Increasing goat productivity requires the provision of diets rich in proteins (Sarwatt *et al*., 2002). However, commercial protein supplements are expensive and unavailable to the resource-limited farmers. This calls for need to look for alternative feeds such as forage plants, which have nutritional and medicinal properties, which could be offered to goats so as to improve goat productivity for resource limited farmers. The objective of this study was to determine the effect of *M. oleifera* leaf meal supplementation on goat performance, carcass and meat quality, as well as evaluate its medicinal properties.

In Chapter 3, the nutritional value of *M. oleifera* leaves was determined. From the study, it was found that the dried moringa leaves had 30.3 % crude protein and 19 amino acids. This is in agreement with Sanchez-Machado *et al.* (2010) and Fadiyimu *et al*. (2010), who reported high crude protein content of *M. oleifera* leaves that varied from 17.9 to 29.68 %, which is one of the highest found in forage plants. *Moringa oleifera* leaves had high values of minerals especially iron and selenium. It was imperative to initially assess the nutritional value of *M. oleifera* leaves,

which could assist in formulating diet for goats using the *M. oleifera* leaves. *Moringa oleifera*'s nutritional properties encouraged investigation of its medicinal properties and use as an alternative protein supplement in animal production. In Chapter 4 and 9 the *M. olefera* extracts exhibited some antibacterial and antioxidant activities, respectively. This is in agreement with Sreelatha and Padma (2009) who reported that *M. oleifera* have some medicinal properties. The antioxidant and antibacterial activities of *M. oleifera* could be beneficial to animals as it could improve their health and overall productivity. Besides *M. oleifera*'s antibacterial and antioxidant properties, supplementation with MOL suppressed the internal parasite load on goats (Chapter 5).

In Chapter 6 it was hypothesized that supplementation of goats with *M. oleifera* leaf meal has similar growth performance, carcass and non-carcass characteristics with those supplemented with Sunflower seed cake or Grass hay. Goats supplemented with the MOL diet had higher ADG, BCS, slaughter weight, warm carcass weight, and carcass conformation scores than those that received the GH diet (Chapter 6). The goats supplemented with MOL compared well with those supplemented with SC diet. Similarly, Muro *et al*. (2003), reported that sheep supplemented with *M. oleifera* had higher growth performance, suggesting that *M. oleifera* could be used as an alternative protein source (Mendieta-Araica *et al*., 2011) and therefore, can be used as supplementary feed to improve growth performance and carcass characteristics of goat. It could be an ideal feed supplement for resource-limited farmers, who in most cases have inadequate financial resources to buy conventional supplementary feeds.

Results from Chapter 7 showed that chevon from MOL and SC supplemented goats had higher values for lightness (L*) 24 hour *post-mortem* which could be due to the dietary antioxidant properties (Sreelatha and Padma, 2009). The redness (a*) values of chevon 24 hr *post mortem* were higher in MOL supplemented goats. This could be attributed to high dietary antioxidant and iron consumed by the goats on the MOL diet, which could have increased haemoglobin and myoglobin concentrations (Priolo *et al*., 2001; Sreelatha and Padma, 2009). Warner Bratzler shear force values of chevon from SC were lower than those for meat from GH diet which could be ascribed to the high amount of intramuscular fat (Talpur *et al*., 2008). The observation that chevon from goats supplemented with MOL or SC had higher protein content than those on GH diet was attributed to higher dietary protein intake. Supplementing with *M. oleifera* leaf meal produced chevon of comparable quality to SC but with desirable fatty acid composition (Chapter 8). The *n*-6/*n*-3 PUFA ratio was lower in chevon from goats that received MOL and GH and this was related to dietary fatty acid composition. Wood *et al*. (2004) reported that the quality and composition of fatty acids in chevon are related to the presence of some of their precursors in the diet.

The antioxidant and antibacterial activities of *M. oleifera* could be beneficial to animals as it could improve their health and overall productivity.

10.2 Conclusion

Findings from the study showed that *M. oleifera* leaves have high nutritive values, especially crude protein, minerals and desirable fatty acids. The high nutritive value makes *M. oleifera* an ideal alternative protein supplement. The *M. oleifera* leaf meal (MOL) improved the dry matter

intake, growth performance, and the carcass characteristics of goats. Furthermore, MOL diet improved the physico-chemical characteristics of meat. It was noted that MOL produced similar economic benefits as sunflower seed cake meal. *Moringa oleifera* leaf meal also produced chevon of good quality which had higher protein content. Interestingly, it reduced *n*-6/*n*-3 fatty acid ratio and increased PUFA/SFA ratio in chevon comparatively to the goats fed on grass hay, which is of health benefit to the human consumer. Supplementing goats with *M. oleifera* leaf meal reduced the cholesterol levels in chevon, which is desirable to healthy conscious consumers. It also reduced helmintic load of goats. It is speculated that it impacted the resistance and resilience of the goats. *Moringa oleifera* leaves are of therapeutic and medicinal value since they exhibited antimicrobial and antioxidant properties. It was therefore, concluded that *M. oleifera* has got multiple beneficial effects. Its use as a protein supplement brought a lot of desirable characteristics of improving goat performance, meat quality and at the same time suppressing the effect of helminth infection. Finally, *M. oleifera* meal could be used as an alternative protein supplement with a potential to substitute the conventional protein supplements, notwithstanding the present high cost of *M. oleifera* meal. If the cultivation is done at a wide scale, its price might be cheaper.

10.3 Recommendations

Although findings from the study showed that *M. oleifera* leaves have nutritive and medicinal properties and can be used to improve goat growth performance and meat quality, its potential use as a feed supplement is affected by current high price (Dry *M. oleifera* leaves cost R25.00 per kg which is equivalent to US $ 3.5). The feed industry is yet to explore the potential of *M. oleifera* leaves and similarly few farmers are aware of its benefit as a livestock feed supplement.

Secondly, it is found in few localities in South Africa making it a scarce resource, which results in its high prices.

It is recommended that:

1. Farmers should be made aware of the properties of *M. oleifera* and encourage them to plant it at large scale. This could enable farmers to produce the meal at lower cost for economic use in animal supplementation.
2. Further research should be done to determine the effect of feeding *M. oleifera* leaf meal to monogastric animals, such as pigs and chickens on their meat fatty acids.
3. There is also need to determine the effect of feeding *M. oleifera* seed cake on livestock performance and meat quality characteristics.
4. Further research is also recommended to determine if feeding with *M. oleifera* leaf meal affects the hatchability of worm eggs.

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Appendix 1: Meat sensory evaluation form

Sensory analysis of chevon

Name:…….. **Date:……..**

Panelist Number:……

Please evaluate the following samples of chevon (goat meat) for the designed characteristics.

	Characteristics	Rating scale	1	2	3
1	**Aroma intensity** Take a few short sniffs as soon as you receive the piece of meat. Typical chevon aroma	1= Extremely bland 2= Very bland 3=Fairly bland 4= Slightly bland 5= Slightly intense 6= Fairly intense 7= Very intense 8=Extremely intense			
2	**Initial impression of juiceness** The amount of fluid exuded on the cut surface when pressed between the thumb and the forefinger	1= Extremely dry 2= Very dry 3= Fairly dry 4= Slightly dry 5= slightly juicy 6= Fairly juicy 7= Very juicy			

		8= Extremely juicy			
3	**First bite** The impression that you form on the first bite	1= Extremely tough 2= Very tough 3= Fairly tough 4= Slightly tough 5= Slightly tender 6= Fairly tender 7= Very tender 8= Extremely tender			
4	**Sustained impression of juiceness** The impression of juiceness that you form as you start chewing	1= Extremely dry 2= Very dry 3= Fairly dry 4= Slightly dry 5= Slightly juicy 6= Fairly juicy 7= Very juicy 8= Extremely juicy			
5	**Muscle fibre and overall tenderness** Chew sample with a light chewing action	1= Extremely tough 2= Very tough 3= Fairly tough 4= Slightly tough 5= Slightly tender			

		6= Fairly tender 7= Very tender 8= Extremely tender			
6	**Amount of connective tissue (Residue)** The chewiness of the meat	1= Extremely abundant 2= Very abundant 3= excessive amount 4= Moderate 5= Slightly 6= Traces 7= Practically none 8= None			
7	**Overall flavour intensity** This is the combination of taste while chewing and swallowing referring to the typical chicken flavour	1= Extremelybland 2= Very bland 3= Fairly bland 4= Slightly bland 5= Slightly intense 6= Fairly intense 7= Veryintense 8= Extremely intense			
8	**A- Typical flavour intensity**	1= None 2= Practically one 3= Traces			

		4= Moderate 5= Slightly intense 6= Fairly intense 7= Veryintense 8= Extremely intense			

TICK RELEVANT A-TYPICAL FLAVOUR/S					
1	Liver/bloody		**5**	Metallic	
2	Cooked vegetable		**6**	Sour	
3	Pasture/grassy		**7**	Unpleasant	
4	Animal like/ kraal (manure)		**8**	other	