

**EFFECTS OF *MORINGA OLEIFERA* ON HAEMATOLOGY AND GROWTH
PERFORMANCE OF BLANTHRAX VACCINATED CASTRATED BAPEDI GOATS**

by

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EFFECTS OF MORINGA OLEIFERA ON HAEMATOLOGY AND GROWTH PERFORMANCE OF BLANTHRAX VACCINATED CASTRATED BAPEDI GOATS

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

I further declare that I submitted the dissertation to originality checking software and that it falls within the accepted requirements for originality.

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ABSTRACT

BaPedi goats play a substantial role in the economy of the Limpopo province and contribute to food security in smallholder farming communities. However, livestock diseases are a major problem in hot provinces where droughts and harsh environmental conditions often trigger soil-borne diseases such as anthrax. Livestock farmers conduct vaccination programs that are essential for the health of their animals. However, factors such as malnutrition may cause a poor immune response in animals, thus, the vaccine may fail to achieve the desired outcome. The study aimed to ascertain the immunomodulatory effects of *M. oleifera* leaves supplemented to the diets of castrated BaPedi goats following vaccination with Blanthrax vaccine. The study also evaluated the effect of different drying methods on the nutritional content of *M. oleifera* leaves. The present study revealed that *M. oleifera* leaves are rich in minerals and crude protein. Significant differences in means ($p < 0.05$) were observed for most elements subjected to different drying methods. No significant differences ($p > 0.05$) were observed amongst means for haemoglobin (Hb), red blood cells (RBC), haematocrit, mean corpuscular (MCV), mean corpuscular haemoglobin (MCH), red cell width (RCDW), white cell count (WCC), lymphocytes, and eosinophils. Out of the parameters that were evaluated, only the means for platelet and monocyte counts showed significant differences ($p < 0.05$). There were no significant differences ($p > 0.05$) amongst growth performance and feed efficiency parameters. Results of the present study indicate that *M. oleifera* leaves can be used as a feed supplement without having any adverse effects on the blood parameters of BaPedi goats.

Keywords: Vaccination, Dietary supplementation, Growth performance, Haematology, Indigenous goats.

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DEDICATION

I dedicate this dissertation to my mom Mrs Zandile C. Gumede and my late father Mr Thandokwakhe F. Gumede, my sister Sindisiwe I. Gumede, my son, niece and nephew (Usiphile Gumede, Esona, and Eyona Mbokazi). Finally, I dedicate it to my supervisors Prof. Khanyisile R. Mbatha and Dr Teedzai Chitura

ABBREVIATIONS

Ca	Calcium
Cu	Copper
Fe	Iron
Hb	Haemoglobin
MCV	Mean corpuscular volume
Mg	Magnesium
P	Phosphorus
RBC	Red blood cells
WCC	White cell count

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CHAPTER I: GENERAL INTRODUCTION

1.1. BACKGROUND AND JUSTIFICATION

Small ruminant production plays an integral role in South African communal agriculture (Schoeman, Cloete & Olivier, 2010). According to Hove *et al.* (2001); Gwanzura, Ng'ambi & Norris (2011), the successful production of indigenous breeds is affected by several factors inclusive of malnutrition-related issues. Forage diets need to be supplemented to meet the daily nutritional intake requirements for these animals. Trees and shrubs have been used as a source of nutrients and medicinal benefits for livestock; however, most of these plant sources do not contain all the components required for optimal performance (Nouman *et al.*, 2014). Smallholder farmers have used herbal medicines as therapeutics in livestock for centuries. Herbal medicines and natural forages occur naturally, require minimal or no industrial processing, and are often used to treat illnesses and malnutrition. Oriental herbs can be divided into two categories, which are nutritional and medicinal (Mirzaei-Aghsaghal, 2012). Documentation of the use of herbal and nutritional plants is essential particularly given the escalating costs of drugs and feed as well as the escalating resistance of pathogens to drugs (Zhang & Ma, 2018).

In South Africa, the success of communal livestock production is affected by the unavailability of highly nutritional plant sources for grazing, and this often leads to a frail immune system, thus, the animal might not be able to withstand pathogens (Hoste, Torres-acosta & Aguilar-caballero, 2008). Anthrax and blackleg are among common and important diseases in South Africa because their presence hinders the trade of livestock or animal products, either within the country or internationally (Bath, van Wyk & Pettey, 2005; Musemwa *et al.*, 2008; Brown, 2011). Cases of anthrax were reported with major economic losses, especially in humid and dry areas of the country. Ruminants are the most vulnerable group among all mammals and all age groups are susceptible to anthrax (de Garine-Wichatitsky *et al.*, 2013; Tsegaye, Belay & Haile, 2013). Several studies reported the occurrence of disease in animals following vaccination (Lange, Neubauer & Seyboldt, 2010; Chakraborty *et al.* 2012; Ayele, Tigre & Deressa, 2016; Abreu *et al.*, 2017). According to Musemwa *et al.* (2008) and Michel & Bengis (2012), uncontrolled movement of livestock can pose a big threat to communal farming closer to national parks and wildlife ranches since the diseases can be transmitted from wild animals to domestic animals through contaminated soil and running water. The spore-forming bacterium, *Bacillus anthracis* (*B. anthracis*) can

survive for many years in the environment without a host. Therefore, this poses a need to look for alternatives to boost the immune response of communal animals for them to be able to withstand such threats.

Moringa oleifera (*M. oleifera*) is indigenous to north-western India and Pakistan (Patel *et al.*, 2010). Over the past years, interest has developed in this tree because of its fast growth, higher nutritional traits, medicinal properties, and utilization as a livestock fodder crop (Verma *et al.*, 2009; Nouman *et al.*, 2014). *Moringa oleifera* thrives best in the tropics and subtropical areas such as eastern and southern Africa (Moyo, Masika & Muchenje 2012; Nouman *et al.*, 2014;). According to several studies, levels of substances such as tannins and phytates are very low, and therefore it is recommended that *M. oleifera* can be used for its health benefits in livestock (Udom & Idiong, 2011; Nouman *et al.*, 2014). Several studies explored the nutritional and medicinal benefits of *M. oleifera* as a supplement in livestock feeds (Ogbe & Affiku, 2011; Moyo, Masika & Muchenje, 2012; Nouman *et al.*, 2014). Moyo *et al.* (2011), reported that dried leaves of *M. oleifera* have average crude protein (CP) levels of 30.3 % and the tree is also rich in vitamins and minerals. Trace minerals are needed by the animal body to create immune cells for a strong defence system (Chandra, 1997). *Moringa oleifera* is reported to contain antioxidants such as polyphenols that are part of the chief groups of phytochemicals described by the presence of either phenolic acids or flavonoids in their chemical structure. These phytochemicals may aid in boosting the immune profile of the animal and combating intestinal parasites (Nakale, 2018; Ma *et al.*, 2018). du Toit, Sithole & Vorster (2020), indicated that quantities of polyphenols found in *M. oleifera* leaves are within an acceptable range for animal consumption without causing any adverse effects.

Forage resources are not readily available throughout the year for animal feeding, especially in drought-prone areas. Therefore, drying forage is one of the strategies used for feed preservation by preventing the growth of bacteria during storage. However, if the drying process is not done correctly, unwanted biochemical reactions may occur, thus leading to the loss of colour, fragrance and nutrients (Babu *et al.*, 2018; Tetteha *et al.*, 2019). Sun drying and shade drying are mostly practiced especially in communal areas due to low-cost implications that do not require the addition of preservatives (Aremu & Akintola, 2014; Ademiluyi *et al.*, 2018; Managa *et al.*, 2020). The stage of forage during the harvesting is crucial and should be planned before the harvest. However, natural drying methods have limitations such as; more

exposure to environmental contaminations and increased drying time because climate, drying temperature and drying time influence the final quality of forage (Tetteha *et al.*, 2019; Managa *et al.*, 2020). Therefore, appropriate technologies for preserving animal forages by drying could benefit communal farmers by decreasing feed shortages thus increasing animal productivity through the consumption of high-quality fodder (Balehegn *et al.*, 2020).

1.2. PROBLEM STATEMENT

BaPedi goats play a significant role in the economy of the Limpopo province and contribute to food security in smallholder farming communities (Motubatse *et al.*, 2008). However, livestock diseases are a major problem in hot provinces where droughts and harsh environmental conditions often trigger soil-borne diseases such as anthrax (Musemwa *et al.*, 2008; Van den Bossche & Coetzer, 2008). Livestock farmers conduct vaccination programs, which are essential for the health of their animals. Vaccination activates the immune response thus, protecting the animals from contagious diseases.

To prevent outbreaks of anthrax and blackleg, local communal farmers often use the blanthrax vaccine which is a combined vaccine that contains strains of (Sterne 34F2) of *B. anthracis* in alum-precipitated *Clostridium chauvoei* (*C. chauvoei*) vaccine. However, on some occasions, the vaccine fails to achieve the desired outcome due to the poor immune response of the vaccinated animals. One of the factors identified to cause a poor immune response in vaccinated animals is malnutrition, especially in drought-prone areas (Taylor & Roeder, 2007; Abreu *et al.*, 2017). Goats in African savannas depend on the utilization of browse species for their source of nutrients and medicinal benefits (van Wyk *et al.*, 2020). However, most of the common woody species do not contain all the components required for optimal performance of livestock (Hejcman, Hejcmanová & Pavlů, 2016). *Moringa oleifera* is reported to contain antioxidants that may aid in boosting the immune profile of livestock (Sreelatha & Padma, 2009). There is no evidence of a similar study on the effects of *M. oleifera* on growth performance and haematological status in BaPedi goats. Therefore, this study aims to determine the effects of dietary supplementation of *M. oleifera* on the immunity and growth performance of BaPedi goats.

OBJECTIVES

The main objective of the study was to determine:

Ascertain the effect of dietary supplementation with *Moringa oleifera* on growth performance and haematology of castrated BaPedi goats following vaccination with Blanthrax vaccine.

Specific objectives

- (i) The mineral content of *M. oleifera* leaves exposed to air and oven drying
- (ii) The effects of dietary supplementation of *M. oleifera* on the immune response of castrated BaPedi goats following vaccination with Blanthrax
- (iii) The effects of *M. oleifera* supplementation on growth performance of Blanthrax vaccinated and castrated BaPedi goats.

1.3. HYPOTHESES

- (i) There is no significant difference in mineral composition between air dried and oven dried *M. oleifera* leaves.
- (ii) *Moringa oleifera* dietary supplementation has no effects on the immune response of Blanthrax vaccinated castrated BaPedi goats
- (iii) *Moringa oleifera* supplementation has no effects on the growth performance of Blanthrax vaccinated and castrated BaPedi goats

1.4. STUDY LAYOUT

This dissertation contains six chapters. Chapter one consists of a background to the study, the aim, and the objectives for undertaking this type of research. This chapter also includes the problem statement and justification of the study. Chapter two discusses the literature review relative to indigenous goat production systems, growth and health requirements of indigenous goats, and physiological effects of vaccination in animals. This chapter also deliberates the nutritional contents and the benefits of feeding livestock with *Moringa oleifera* (*M. oleifera*). Chapter three narrates the material and methods in addressing the aim of the study. Chapter four presents the research findings for the three objectives of this study. Chapter five discusses results on the mineral composition of *M. oleifera* leaves and the effect of drying methods on the nutritional composition of *M. oleifera* leaves. The effects of varying inclusion levels

of *M. oleifera* leaves as a feed supplement following vaccination on haematological parameters of BaPedi goats and the effects of varying inclusion levels of *M. oleifera* leaves as a feed supplement following vaccination on the growth performance of BaPedi goats are discussed. Chapter six presents the conclusions and recommendations from the undertaken study.

CHAPTER 2: LITERATURE REVIEW

2.1. INTRODUCTION

Livestock production exists throughout South Africa, with variations according to the production systems and vegetation type. Most livestock in communal areas is comprised of indigenous breeds raised under an extensive system and animals graze and/or browse on natural pastures throughout the year due to the financial constraints of the farmers (Mohlatlole, Dzomba & Muchadeyi, 2015). Despite the hardness of the indigenous breeds, successful production is often affected by unfavourable environmental and physiological challenges. Therefore, communal farmers often use natural herbs as forages and herbal remedies to combat such challenges (Qwele *et al.*, 2013). Nonetheless, some natural forages that are available may not satisfy the optimal nutritional and health needs of livestock. The aim is to provide an insight into the use of *M. oleifera* as fodder and herbal remedy in BaPedi goat production systems. The physiological changes in goats following vaccination are also discussed.

2.2. THE ROLE OF LIVESTOCK FARMING IN THE GLOBAL ECONOMY

The livestock industry is rapidly emerging in the agricultural economy. It subsidizes approximately 40% of the world value of agricultural output and supports the livelihoods of over one billion human beings worldwide (Food and Agriculture Organisation (FAO), 2019). In South Africa, in the year 2017, the total value of livestock products was estimated to be 50.6% (Department of Agricultural Forestry and Fisheries (DAFF), 2018). As of 2013, 70% of the arable land in South Africa was utilized for cattle, small stock, poultry, pigs, ostriches, and game production with the largest contributor being poultry and small stock production (Meissner, Scholtz & Palmer, 2013; Goni, Skenjana & Nyangiwe, 2018).

Small ruminant production plays an integral role in most countries, especially in rural areas (Fikru & Omer, 2015). This may be due to the belief that small ruminants adapt easily to different types of environments, they grow faster and require fewer inputs for their production (Webb, 2014; Yune & Abdela, 2017). Nonetheless, South Africa contributes only 3% of the African goat population with Limpopo province as one of the highest producing provinces (Louw, 2018; van Wyk *et al.*, 2020). The South African goat industry focuses on meat (chevon), milk, and fibre. The production systems are classified as commercial and smallholder/emerging (Webb, Casey & Simela, 2005).

2.3. INDIGENOUS GOAT BREED PRODUCTION SYSTEMS IN SOUTH AFRICA

Three basic systems of goat production exist in South Africa, namely, semi-intensive, intensive, and extensive production systems. These systems are also practised in other parts of the world and they play a huge role in animal health management (Mataveia *et al.*, 2018). The choice of a production system is determined by primary factors such as economic status, availability of resources, population dynamics, degree of urbanization, and cultural background (Otaru & Iyiola-Tunji, 2015; Rust, 2019).

2.3.1. Extensive production system

Extensive farming requires ample land for productivity than intensive production (Ferraz & de Felício, 2010). In extensive systems, goats are permitted to browse on areas of land that are more inclined to nature; where population densities are low, and land is inexpensive. In this type of system, the existence of the animals is more important rather than productivity because animals are commonly not provided with shelter, they rest where they browse, and they fully depend on the rangeland or pasture throughout the year (Ozcan *et al.*, 2014). Extensive farming in communal areas is largely dominated by natural pastures as sources of feed for livestock because farmers rarely purchase commercial feeds (Zvinorova *et al.*, 2017). Hence, forage diets need to be supplemented to meet the daily nutritional intake requirements for these animals (Gwanzura, Ng'ambi & Norris, 2011).

For the healthcare of animals in this system, farmers depend on government veterinary services as few can afford to purchase commercial drugs or consult private veterinarians. Where government veterinary services are not accessible, some farmers do not apply any disease control methods to treat their animals or use traditional medicines (Goni, Skenjana & Nyangiwe, 2018; Forabosco & Negrini, 2019). Trees and shrubs have been used as sources of nutrients and medicinal benefits for livestock, however, some of these plant sources do not contain all the components required for optimal performance of livestock (Hove *et al.*, 2001; Nouman *et al.*, 2014).

2.3.2. Semi-intensive production system

A production system may combine both extensive and intensive components, depending on the production stage of the animals. In the semi-intensive system, goats are unconfined from browsing and they scavenge on waste (Hodgkinson, Matus & Lopez, 2013). During the planting season, the movement of livestock is restricted to prevent damage to crops as they are fed on cut grasses and are occasionally

supplemented with household edible wastes or commercial concentrates. This system is commonly practised by the smallholder and emerging farmers in the communal areas in most parts of Africa and animals are returned in the evening to their homesteads (Hodgkinson, López & Navarrete, 2009). This production system is characterized by low inputs with inadequate, poor-quality feeding resources and poor management practices because minimal care is given to the animals in terms of shelter, feeding, and health care (Salami, Kamara & Brixiova, 2010). Both extensive and semi-intensive systems comprise of indigenous goats which have small body frames and low meat yield (Mohlatlole, Dzomba & Muchadeyi 2015). Goats are commonly kept as multipurpose breeds, although these breeds are mainly used for meat production.

2.3.3. Intensive production system

In this system, animals are confined and are not permitted to graze or browse. Throughput gains are the basis of commercialization (Irz, Lin & Wiggins, 2001). This system is accompanied by high monetary inputs because it involves the use of sophisticated technology such as equipment and gadgets to maximize production and increase profits. The intensive system is commonly practised by commercial farmers (Fraser, 2001). Biosecurity measures such as restricted farm access by vehicles and humans are practised. However, following biosecurity measures within the farm can sometimes become a challenge because animals are close to each other and pathogen build-up may be probable unless hygienic measures are correctly adhered to (Alarcón, Allepuz and Mateu, 2021). The challenges for animal health management are to maintain a management plan and to ensure that the level of biosecurity is effective to prevent diseases (Gunn *et al.*, 2008; Robertson, 2020).

2.4. CHALLENGES FACED BY COMMUNAL GOAT FARMERS

Globally, goat meat demand is less than beef. However, in some emerging countries, goats are the main source of red meat for humans (Madruga & Bressan, 2011). Indigenous goats are usually characterised by small body size, slow growth rate, low milk and carcass yield (Otaru & Iyiola-Tunji, 2015). They are mainly multi-coloured, bearded, horned, with medium to broad lopped ears and they have short hair. Most differences detected in phenotypic classification studies were mostly because of environmental factors which result in different ecotypes. These variations have caused some inconsistencies regarding breed and ecotype identification (Monau *et al.*, 2020).

Indigenous goats are commonly perceived as less productive than exotic breeds since communal farmers are confronted with challenges at various levels of the goat production value chain such as inaccessibility to input supplies like medicines and feed supplementation to alleviate the effects of diseases and seasonal variations in feed quality and quantity, respectively (Monau *et al.*, 2020).

2.4.1. Feed supply shortages

According to Brown (2011), goats dominate the underprivileged parts of the world as the main livestock species because they utilize minimal forage and survive in harsh environments which makes them an asset to communal farmers. The communal sector tends to be more reliant on communal rangelands for livestock grazing. Farmers often lack the financial stability to supplement their animals with commercial feeds for a more balanced diet (Nabukenya *et al.*, 2014; Tavirimirwa *et al.*, 2019). An imbalance in diet may reduce the performance of an animal, thus, resulting in increased susceptibility to diseases and a low reproductive performance (Ngongoni *et al.*, 2007; Kawas, Andrade-Montemayor & Lu, 2010). Gebeyehu *et al.* (2013) revealed that feed shortage is becoming prevalent due to premature and unpredictable rainfall patterns. Shortages of highly nutritional feed sources are the most prominent goat production limitation. Furthermore, unpredictable and untimely climate patterns that happen mostly during drier seasons of the year disturb the growth of vegetation that livestock depends on as a feed source (Upadhyay, 2020).

2.4.2. High prevalence of diseases

The grazing of livestock in the communal lands may bring about health challenges to animals. Hurtado & Giraldo-Ríos (2018) stated that communal grazing promotes a build-up of pathogens that may affect the production of livestock. Furthermore, there is a lack of control over access to the land by people and the movement of animals since the land is shared and not defined. In cases where livestock is reared close to nature reserves, contact with wildlife is probable (Brown, 2011). According to Sikhweni & Hassan (2013), farmers in the Limpopo province who are situated close to national parks estimated that 23% of livestock deaths were related to disease transmissions from wildlife to livestock. The health issues are the main constraint in communal goat production systems where vaccinations are not a common practice (Mdladla, Dzomba & Muchadeyi, 2017).

Anthrax is a bacterial disease caused by a bacterium called *Bacillus anthracis*. It is an important zoonotic disease affecting both humans and animals. Several animal

species are susceptible to this disease including ruminants (Brown, 2011). Anthrax is spread through grazing in contaminated areas or drinking contaminated water or inhaling spores. Flies that feed on the body fluids and muscle of infected carcasses are also culprits in the transmission cycle because they infect nearby vegetation through infectious vomit and faecal droplets that might be grazed by livestock (Bengis & Frean, 2014). Humans can be infected by anthrax by consuming or handling meat from contaminated animals. Biting flies are also responsible for the transmission of spores to humans (Gunn *et al.*, 2008; Fasanella *et al.*, 2013). In African communal farming systems, anthrax might be unavoidable because animals that die due to anthrax are commonly incorrectly disposed, leading to environmental contamination and further transmission to healthy animals (Moyo *et al.*, 2014). Hence, education on animal health and zoonotic diseases is very important to alter such cultural practices (Bengis & Frean, 2014).

Blackleg is a bacterial disease that is caused by the bacterium *Clostridium chauvoei* which is prevalent in large ruminants and rare in small ruminants (Useh, Nok & Esievo, 2006; Moyo *et al.*, 2014). Most cases of blackleg occur during the humid months or high rainfall months as this promotes the exposure and activation of the bacteria spores in the soil. Animals ingest the spores whilst grazing (Ziech *et al.*, 2018). In South Africa, vaccination against blackleg started in 1923 and booster vaccinations were recommended in acute cases (Scheuber, 1928). Araujo *et al.* (2019), revealed that booster vaccinations significantly increase immune response. However, vaccine failure has been reported and associated with practices such as inappropriate management practices like inconsistent vaccinations and the reuse of needles by farmers, especially in communal areas (Useh, Nok & Esievo, 2006). Moyo *et al.* (2014) stated that most farmers in communal areas do not vaccinate their livestock against blackleg due to lack of affordability, resulting in disease outbreaks.

2.4.3. Poor reproduction performance

Approximately 60% of goats in South Africa are kept under communal systems where there are various socio-economic constraints such as land issues, lack of financial support, and poor market access. The poor care and management of indigenous goats lead to poor genetic improvement and a low reproduction rate (van Marle-Köster & Visser, 2018). Farmers in communal areas depend on extension services for support even though these services are not sufficient in other parts of the country (Kosgey *et al.*, 2011). Reproduction performance in communal does is characterised by high kid

mortalities, low kidding rate and low twinning rate partly due to malnutrition and inbreeding (Belay *et al.*, 2014, Slayi *et al.*, 2014). The does and bucks run together throughout the year and few bucks remain in the herd for up to five years thus, triggering inbreeding and low kidding rates (Webb & Mamabolo, 2004). Therefore, research and development efforts to enhance the production of communal goats and the livelihood of communal farmers are crucial (Rotter & Van de Geijn, 1999; Belay *et al.*, 2014). Furthermore, different approaches are essential to ensure that communal farmers have access to more genetic material for genetic improvement (Mdladla, Dzomba & Muchadeyi, 2017).

2.4.4. Theft and predation

Livestock predation and theft are some of the major challenges faced by communal farmers in Limpopo province. Occurrences of livestock predation by the wildlife from national parks have been reported in parts of Africa (Kgathi *et al.*, 2012). Therefore, to prevent this problem, farmers should provide shelter to keep their livestock locked at night (Mdladla, Dzomba & Muchadeyi, 2017).

2.5. PHYSIOLOGICAL EFFECTS OF VACCINES IN GOATS

Animal health is one of the most important pillars in livestock farming because livestock is highly susceptible to pathogens such as viruses and bacteria which cause diseases (Morton, 2007). Poor animal management compromises the immune system leading to the development of clinical diseases and low production efficiency. Therefore, regular vaccinations aid in boosting the immune response of the livestock (Kaasschieter *et al.*, 1992; Roth, 2011). However, several animal factors influence physiological responses to vaccination thus, farmers should acknowledge that animal welfare directly influences the level of immune resistance of animals (Morton, 2007; Taylor & Roeder, 2007; Caroprese *et al.*, 2010; Abreu *et al.*, 2017).

2.5.1. Effects on haematological parameters

Haematological elements reflect the physiological responsiveness of the animal to its internal and external environments (Tibbo *et al.*, 2004; Gwaze, Chimonyo & Dzama, 2010). Binta *et al.* (1996) and Tibbo *et al.* (2004) revealed that red blood cells (RBC), haemoglobin (Hb), lymphocytes, and neutrophils of the indigenous goat breeds were different from the exotic goat breeds. The naming of indigenous goats is based on different ethnic groups and geographical environments but most of them have similar

phenotypic and genotypic characteristics (Monau *et al.*, 2020; Mdladla, Dzomba & Muchadeyi, 2017).

Immunoglobulins also called antibodies are globulins found in blood serum and they play a crucial part in the immune response of the animal (Keyt *et al.*, 2020). Immunoglobins identify and attach themselves to antigens, such as bacteria, thus, assisting in their destruction. Immune responses are different with each antigen level presented to the immune system (Alberghina *et al.*, 2010). This determines the specific antibody levels that need to be produced to destroy the antigen presented (Angulo-Valadez *et al.*, 2010). Madhanmohan *et al.* (2011) reported that vaccination protects the immune system from diseases even if the pathogen loads are low. Hence, vaccination can assist the immune system in either preventing or reducing the multiplication of bacteria.

Immunoglobulin M (IgM) plays an imperative role in the immune response of the animal because it is characterized as the first class of antibodies produced after the primary introduction to a foreign antigen (Keyt *et al.*, 2020). Both IgM and immunoglobulin G (IgG) agglutinins can appear in the blood from day 12 to 47 following vaccination (Taher & Ewais, 1997; Aboshady *et al.*, 2020). Ndumnego *et al.* (2016) evaluated and compared the humoral immune response in goats following single and double vaccinations against *B. anthracis*. The results showed that double vaccination act as a booster and brings an improved and sustained immune response with significantly higher levels of IgM and IgG.

2.5.2. Effects on growth performance

Jo *et al.* (2014) reported a reduced growth performance and feed intake in vaccinated animals when compared to non-vaccinated animals. A study on beef steers revealed a relationship between vaccination and animal performance parameters such as average daily gain and feed efficiency, however, in most instances feed intake is not affected by vaccination (Arthington *et al.*, 2013). de Miguel *et al.* (2021), reported reduced feed intake and decreased growth rate following vaccination. Since vaccination may bring about mild inflammation, it is hypothesised that this response increases nutrient demand to support the immune system rather than the growth rate, thus reducing overall growth performance (Rakhshandeh & de Lange, 2012).

2.6. NUTRITIONAL REQUIREMENTS OF INDIGENOUS GOATS

Rural communities commonly rear indigenous goats under the extensive system, browsing natural pastures throughout the year (Nabukenya *et al.*, 2014; Mataveia *et al.*, 2018). Genetic and environmental factors such as nutrition directly affect the growth rate and health status of goats (Simões *et al.*, 2021). The animals that are appropriately nourished tend to be more efficient in converting the nutrients consumed in feed (Webb, Casey & Simela, 2005; Goetsch, Merkel & Gipson, 2011). Casey & Webb (2010) concluded that understanding diet selections and appropriate administration of physiological responses through nutrition and limiting stressful environments and situations favours optimum growth rates, health status, and production in indigenous goats. Papachristou & Nastis (1996) reported that goats prefer deciduous woody species to evergreen species. However, Dziba *et al.* (2003) found that both deciduous and evergreen species are favoured. Goats prefer to browse to attain high levels of nutrient intake (Lu, 1988).

Protein supplementation into the diet of animals is vital to attain optimum animal production and health. Proteins form an essential part of the blood and antibody formation. Inadequate dietary proteins will lead to a compromised immune system (Luo *et al.*, 2004). Although commercial concentrates may be used as protein supplements, the costs are often beyond the affordability of most smallholder farmers. Brown *et al.* (2016), studied haematological parameters of BaPedi goats supplemented with *Vachellia karoo* which revealed no adverse effects in blood parameters and growth performance was improved. Thus, farmers may use high protein forages as protein supplements for their livestock to enhance productivity (Jayanegara, Ardani & Sukria, 2019).

Energy requirements for growth and maintenance in animals depend on factors such as the sex of the animal, age, body size, and environmental conditions such as temperature (National Research Council (NRC), 2007). Souza *et al.* (2014) discovered that factors such as the body size of the goat, the castration status and the environment may also contribute to a lower energy requirement because the animals do not require more energy to maintain normal body functions. Dziba *et al.* (2003) confirmed that body size may also influence energy intake in goats.

Deficiencies of trace elements lead to disorientation of the biochemical functions in the body, reduced reproductive efficiency, high mortality rates, declined weight gains and declined Hb (Braun, Trumel & Bezille, 2010). Complementing diets with trace elements

is vital for optimum nutrition of small ruminants (Dove, 2010). Some elements may even shift the sex ratio of unborn kids towards fewer male kids. Mineral requirements fluctuate across different animal species due to their diverse requirement needs (Suttle, 2010). Therefore, extrapolation from one species to another or within species that are of different purposes and ages should be avoided (Araújo *et al.*, 2010). Although feeding minerals is essential, excessive supplementing can bring about toxicity to an animal or prevent other elements from being absorbed by the body of the animal, for example, excessively feeding sulphur may bring about copper deficiency (Haenleina & Anke, 2011). This may tend to be dissimilar with vitamins as they can be expelled by the body if they are in surplus. Therefore, it is vital to formulate mineral supplements for each species of animal (Araújo *et al.*, 2010).

Vitamins are essential for the maintenance of natural body processes such as the growth and health of the animal. Vitamins need to be ingested from the diet to a greater or lesser degree because goats are usually unable to synthesize vitamins sufficiently (Hart, 2009). Signs of deficiency include poor growth rates, reduced resistance to infections, and poor reproduction efficiency. Any insufficiency in vitamins may slow or block the metabolic process in which that vitamin facilitates, leading to poor performance (Araújo *et al.*, 2010). Vitamin A enhances the immune status of goats, Vitamin E works as an antioxidant in concurrence with selenium. Deficiencies may lead to poor growth. Vitamin C promotes the formation of cells that make up the immune system (Kawas, Andrade-Montemayor & Lu, 2010). Table 2.1. displays the mineral requirements of castrated indigenous goats.

Unlimited access to water is crucial for healthy and productive goats. Water intake depends on the moisture content of the feed, age, environmental temperatures, and stage of production of the goats, however, on average, grazing goats require 10% of live body weight of water per day. During hot weather, most animals may have elevated water intake. It is also important to ensure that the water is clean, this is particularly vital for kids (Hart, 2009).

Table 2.1 Estimated daily mineral requirements for indigenous castrated goats (Souza *et al.*, 2014; Hart, 2020).

Micronutrients (%)		Macro nutrients (ppm)	
Ca	0.3- 0.8	Fe	50- 100
P	0.25- 0.4	Cu	10- 80
Na	0.2	Co	0.1- 10
K	0.8- 2.0	Zn	20- 500
Cl	0.2	Mn	0.1-3
S	0.2- 0.32	Se	0.1-3
Mg	0.18- 0.4	Mo	0.1 -3
		I	0.5- 50

Calcium-Ca, Phosphorus- P, Sodium- Na, Potassium- K, Chloride- Cl, Sulphur- S, Magnesium- Mg

2.7. UTILISATION OF *M. OLEIFERA* FOR COMMUNAL GOAT FEEDING

Research has been carried out on ways to enhance the quality and availability of feed sources through supplementation to reduce nutritional challenges in indigenous goats. Planted forages, forage conservation, and the use of multi-purpose trees such as *M. oleifera* have been cited as alternatives to improve the quality of diets as equated to purchased concentrates or agro-industrial by-products.

2.7.1. Description of *M. oleifera*

Moringa oleifera is also known as *Moringa pterygosperma Gaertm.* It is the most widely distributed species of a monogeneric family of Moringaceae (Anwar *et al.*, 2007; Pandey *et al.*, 2019). This cultivar is native to north western India and Pakistan but now it is widely grown in other countries of the world such as South Africa (Nouman *et al.*, 2014). There are over 13 designations for this plant that have been found in different parts of the world (Singh *et al.*, 2020). *Moringa oleifera* is widely distributed in the tropical and subtropical areas of the world and even in areas with less fertile soil because it can tolerate temperatures of up to a maximum of 48°C, with less moisture and low rainfall of approximately 300-500 mm per annum. The tree cultivates well in dry sandy soil and prefers soil pH that is slightly acidic to slightly alkaline (Ma *et al.*, 2018).

Moringa oleifera twigs are soft and green in colour and the leaves are blunt pointed. The plant has white flowers and the seeds are contained in the pods of the tree. Ten to fifty seeds can be found in each pod (Chuang *et al.*, 2007). Fresh and raw *M. oleifera* seeds are soft but as soon as they get dried, they become hard and resemble small

beans because of their shape and colour. The bark of the tree is soft and gummy (Leone *et al.*, 2016). *Moringa oleifera* is also called 'the miracle plant' or 'the tree of life' because of the diverse properties it possesses which can be used to improve nutrition and health in both animals and humans. The plant is entirely edible from the leaves to the roots and is used as fodder and herbal medicine because it is highly potent (Bolarinwa, Aruna & Raji, 2019; Garcí *et al.*, 2019; Silva *et al.*, 2019).

2.7.2. Chemical composition of *M. oleifera*

2.7.2.1. *Moringa oleifera* seeds

Moringa oleifera seeds are widely used in medicine, cosmetics, and food supplements because they are rich in proteins, Ca, Fe, vitamin C, and carbohydrates (Trigo *et al.*, 2021). The seeds of this plant contain oils that have physical and chemical properties that contain antifungal and anti-inflammatory properties (Singh *et al.*, 2020). It has properties equivalent to the properties of olive oil (Chuang *et al.*, 2007). In some countries, dry seeds have been used as a coagulant for the treatment of water. One of the active mechanisms presented in the seeds is the protein identified as a polyelectrolyte, with the potential to promote water coagulation (Nwaiwu, Ibrahim & Raufu, 2012). Few studies were carried out on the utilization of the seed meal in animal production because *M. oleifera* seeds have higher levels of fibre as well as higher amounts of anti-nutritional factors compared to the leaves (Stevens *et al.*, 2015; EL-Hedainy, EL-Wakeel & Rashad, 2020). EL-Hedainy, EL-Wakeel & Rashad (2020) however, observed improved performance in Barki sheep fed varying levels of *M. oleifera* seed meal compared to the control.

2.7.2.2. *Moringa oleifera* leaves

Kakengi *et al.* (2003) observed that *M. oleifera* comprises approximately 1-23 g of tannin in every 1 kg of leaves. Tannins restrict the utilization of proteins, as well as make carbohydrates and lipids less available for absorption in the animal's body. Trypsin and amylase inhibitors, lectins, cyanogenic glucosides, and glucosinolates are not found in the leaves (de Garine-Wichatitsky *et al.*, 2009). Tannins, saponins, cyanogenic glucosides, and glucosinolates are present in twigs and stems. However, since their concentrations are insignificant, it is endorsed that *M. oleifera* can be used for health and nutritional benefits in livestock (Nouman *et al.*, 2014). The presence of saponins in the leaves does not display any hemolytic action (Makkar & Becker, 1997). Foidl & Paull (2008) reported that the protein content of *M. oleifera* leaves is approximately 20–35%, which is high on a dry weight basis and contains substantial

quantities of all the essential amino acids required by the animals. The *M. oleifera* leaves contain approximately 27.51% of crude protein (CP), 19.25% of crude fibre, 2.23% of crude fat, 7.13% of ash, 76.53% of moisture, 43.88% of carbohydrates, and the energy value was 1296 KJ/g (Oduro, Ellis & Owusu, 2008). In addition, the leaves are nutritious because they contain quantities of Vitamin A, B and C, Ca, Fe, P, and proteins, which, are essential for growth (Murro, Muhikambele & Sarwatt 2002). However, despite the high nutrient content of *M. oleifera* leaf meals, there are limited trials that explored its feeding to indigenous Bapedi goats.

2.7.3. Nutritional benefits of *M. oleifera* supplementation in livestock

Moringa oleifera is considered a potential forage whether in a dried or wet form and studies have documented the nutritional content of this tree (Jayanegara, Ardani & Sukria, 2019; Mataveia *et al.*, 2019). *Moringa oleifera* can be utilized as a protein enhancement to a basal diet consisting of a low-quality forage because it can increase the dry matter content (Garci´ *et al.*, 2019). Various factors may affect the nutritional composition of the plant such as the morphological parts, the age, the harvest season, the frequency of harvest, and the environment in which the trees were grown (Edwards *et al.*, 2012). Babiker *et al.* (2017) compared the nutritional compositions of *M. oleifera* and Lucerne meal and reported a higher mineral content and a lower protein and fibre content in *M. oleifera* compared to Lucerne meal. Therefore, *M. oleifera* may be considered as an alternative mineral supplement in livestock.

2.7.4. Health benefits of supplementing *M. oleifera* in livestock

The immune system protects and defeats infections in the body (Banji, Banji & Kavitha, 2012). The immune system can become vulnerable to conditions that may impair its capability to overcome all hazards which can result in immunodeficiency. The immune system could be boosted with the use of immunostimulants (Mirzaei-Aghsaghali, 2012; Ramírez-Acosta *et al.*, 2018). In less developed countries, livestock are often given natural herbs as medicine for the improvement of the immune system (Banji, Banji & Kavitha, 2012). Olugbemi, Mutayoba & Lekule (2010), noted the potential of including *M. oleifera* in poultry diets as it increases levels of RBC which are responsible for the transportation of oxygen and carbon dioxide in the blood as well as to manufacture Hb, hence improving the health of the animal. Banji, Banji, and Kavita (2012) showed that the production of WCC such as leucocytes and neutrophils was elevated in mice due to extracts of *M. oleifera* leaves. Su & Chen (2020), reported that supplementing between 25% to 75% of *M. oleifera* leaves can increase haematological profiles and

blood globulins, while the cholesterol levels in the blood of small ruminants are decreased. Furthermore, Ramírez-Acosta *et al.* (2018) revealed that *M. oleifera* contains some inflammatory properties that are required by the body to block pathogen build-up causing diseases in response to inflammation. Feeding supplements with antioxidant properties improve stress tolerance in ruminant animals. Therefore, since *M. oleifera* is perceived to be high in antioxidants, it may also aid in reducing inflammation in goats brought by vaccination (Jo *et al.*, 2014).

CHAPTER 3: MATERIALS AND METHODS

3.1. DETERMINATION OF THE MINERAL COMPOSITION OF *M. OLEIFERA* LEAVES FOLLOWING DIFFERENT DRYING METHODS

3.1.1. Site description

The study was conducted at the University of Limpopo experimental farm at latitude 27.55 °S and longitude 24.77 °E (Thompson, Matamale & Kharidza, 2012; Brown *et al.*, 2016). The farm is situated in the tropic of Capricorn zone, in the southern region of Limpopo province. The average temperature ranges from 20 to 36 °C during the wet season (November to January) and from 5 to 25 °C during the dry season (May to July). It receives a mean annual rainfall of approximately 400 mm (Brown *et al.*, 2016). The topsoil is of acceptable quality and deep soils do not contain stones within a 1.5 diameter depth, which shows suitability for horticulture use (Moshia *et al.*, 2008). Mabapa, Ayisi & Mariga (2017), reported on the physical and chemical properties of soils at 0-60 cm depth. The study showed that clay percentage was 32, Nitrogen content was 0.1%, phosphorus and potassium content was 12.5 and 318 mg/L.

3.1.2. Data collection

3.1.2.1. Air dehydration

Fresh *M. oleifera* leaves were harvested at the University of Limpopo farm, in the summer season of 2020. Moisture content from a representative sample was determined by using a LASEC© moisture balance. A portion of leaves was air dried on a concrete floor at room temperature for 96 hours until they were crispy while retaining their greenish colour. Representative samples were ground using a laboratory grinder and stored in glass containers pending chemical analyses.

3.1.2.2. Oven dehydration

The other portion of fresh harvested *M. oleifera* leaves were then oven dried for 72 hours at 60°C. and were ground using a laboratory grinder. The ground sample was stored in glass bottles and stored for chemical analyses.

3.1.3. Mineral analysis of *M. oleifera* leaves

Moisture content was determined by subtracting the weight of the dried sample (DS) from the fresh sample (FS) weight.

$$\% \text{Moisture} = \frac{FS - DS}{FS} * 100$$

Total ash content was determined by incinerating the samples in a Muffle furnace at 550°C overnight (AOAC, 1990). Molybdenum, sulphur, silver, barium, bismuth, calcium, cadmium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, and zinc was determined at the Limpopo Agro-Food Technology Station (LATS) Laboratory, University of Limpopo as described by ISO 11885 (2007).

3.1.4. Statistical analyses

Means, standard deviations, and standard errors were calculated using Procedure of means (Proc mean) of Statistical Analysis Software (SAS) version 9.4 (2019). Data on the mineral content of *M. oleifera* leaves were analysed using analyses of variance (ANOVA) The model $Y_{ij} = \mu + T_i + e_{ij}$ was applied where Y_{ij} = minerals, μ = the overall mean, T_i = drying methods and e_{ij} = random error. For any significant differences observed ($p < 0.05$), Fisher least significant difference (LSD) was used (SAS, 2019).

3.2. DETERMINATION OF THE EFFECTS OF DIETARY SUPPLEMENTATION OF *M. OLEIFERA* ON THE IMMUNE RESPONSE OF BAPEDI GOATS FOLLOWING VACCINATION WITH BLANTHRAX VACCINE

3.2.1. Site description

The study was conducted at the University of Limpopo experimental farm as described in section 3.1.1. Steel, individual well-ventilated pens, with an area of 3.75 m² were installed at the site for the experiment. The pens were equipped with a feeder and a drinker. The experimental site was thoroughly cleaned and disinfected daily to avoid a build-up of infections.

3.2.2. Experimental animals

Twelve clinically healthy BaPedi goats with average ages of twelve months and body weight of 19 ± 1.47 kg were randomly selected from the same flock (Lee *et al.*, 2014; Brown *et al.*, 2016b). Male goats were used in this study because studies by Binta *et al.* (1996) and Tibbo *et al.* (2004) revealed that sex can affect haematological values in farm animals. Health assessments using farm records and clinical observations

(body temperature, respiratory rate, pulse, heart rate, signs of gastrointestinal malfunction such as diarrhoea/ constipation, examination of mucous membranes of the conjunctiva to evaluate the circulatory system and signs of anaemia) were done daily throughout data collection to ensure only healthy goats were used. All procedures used in this experiment followed the ethical standards of the University of South Africa (UNISA) and the University of Limpopo (UL) Animal Ethics Committees. The experiment proceeded after ethical clearance (2019_CAES_AREC_131; AREC/10/2019:1R). The goats were randomly divided and allocated a treatment diet in a completely randomized design. From the first week until the fourth week of the trial, the goats were fed a basal diet comprising of 90.77% Lucerne (*Medicago sativa*) and 9.23% Buffel grass (*Cenchrus ciliaris*) as shown in Tables 3.1 and 3.2 for acclimatization of their rumen microbes (Odenyo *et al.*, 1997). In the fifth and sixth weeks, the goats were fed diets comprising of varying levels of *M. oleifera* as shown in Tables 3.1 and 3.2. The animals had *ad-libitum* access to freshwater per day throughout the data collection period. At the end of the first week of the trial, all the experimental goats were vaccinated with 2 millilitres of blanthrax vaccine per goat via the subcutaneous route as prescribed by the vaccine manufacturer (Intervet South Africa, (Pty) Ltd). One of the goats was removed following the recommendations that were given by the attending veterinarian. Thus, the number of experimental units used for the data analysis was eleven.

Table 3.1 Formulation of dietary treatments used for the study

Treatment code	Treatment Description
T ₁ C _{9.23} L _{90.77} M ₀	Three bucks were fed a basal diet, 9.23 % <i>C. ciliaris</i> grass hay, and 90.77% Lucerne hay without <i>M. oleifera</i> .
T ₂ C ₆₄ L ₁₆ M ₂₀	Four bucks were fed 64 % percent <i>C. ciliaris</i> grass hay and 16 % percent Lucerne hay containing 20 % of <i>M. oleifera</i> .
T ₃ C ₅₀ L ₀ M ₅₀	Four bucks were fed ad-libitum 50 % percent <i>C. ciliaris</i> grass hay and 0 % percent Lucerne hay containing 50 % of <i>M. oleifera</i> .

T: Treatment, C: *C. ciliaris*, L: Lucerne, M: *M. oleifera*

Table 3.2 Nutrient composition of experimental diets used in the study

Nutrients	T ₁ C _{9.23} L _{90.77} M ₀	T ₂ C ₆₄ L ₁₆ M ₂₀	T ₃ C ₅₀ L ₀ M ₅₀
CP %	11.70	10.48	15.00
NDF %	52.53	50.36	39.00
Ash %	8.41	8.860	8.50
Zn (mg/kg)	11.81	2.25	4.25
Fe (mg/kg)	449.07	605.6	434.90
Mn (mg/kg)	69.57	129.21	98.78
Cu (mg/kg)	10.39	10.88	10.21
Mg (g/kg)	1.26	0.83	166.95
Na (g/kg)	1.22	0.52	23.55
P (g/kg)	2.87	2.06	73.40
Ca (g/kg)	8.48	3.41	523.45
K (g/kg)	13.82	13.35	550.20

T: Treatment, C: *C. ciliaris*, L: Lucerne, M: *M. oleifera*, CP: Crude protein, NDF: neutral detergent fibre, Zn: Zinc, Fe: Iron, Mn: Manganese, Cu: Copper, Mg: Magnesium, Na: Sodium, P: Phosphorus, Ca: Calcium, K: Potassium

3.2.3. Experimental diets

Thirty kilograms of fresh *M. oleifera* leaves and Lucerne was harvested and air dried on a concrete floor at room temperature for 96 hours and turned several times until they were crispy in touch, while retaining their greenish colouration. The dried leaves of *M. oleifera* and lucerne were then stored at room temperature until needed for inclusion in the experimental diets. Buffel grass which is one of the palatable grass species in the Limpopo province was used in this study (Smit, 2005). It contains an average of 6.1 % CP. Lucerne hay was used to supplement the protein content of Buffel grass. It contains an average of 11.4% CP. Three experimental diets were formulated by replacing a conventional supplement of lucerne with *M. oleifera*. The inclusion levels varied from 0% to 50% as presented in Table 3.1 (Aregheore, 2002). The experimental diets were offered at 4% of the animal's body weight daily.

Analyses of CP, ash and NDF in dietary treatments were done in triplicates at the Animal Nutrition Laboratory, University of Limpopo as described by AOAC (1990). The mineral composition was determined at the Limpopo Agro-Food Technology Station (LATS) Laboratory, University of Limpopo as described by ISO 11885 (2007) and shown in Table 3. 2.

3.2.4. Data collection procedures

The experiment was conducted in three phases for 42 days. The first phase was the adaptation phase which lasted for 7 days. The second phase was the vaccination phase, which lasted for 21 days. The duration of the second phase depended on the establishment of immunization in goats which according to the manufacturer takes approximately 14 days. The third phase was the inclusion phase of varying levels of *M. oleifera*. This phase lasted for 14 days.

Blood was collected from each animal on day 0 and at the end of each phase. This was done to evaluate changes in the haematological response of the goats. Five millilitres of blood samples were collected by the veterinarian from each goat via the external jugular vein for haematological analyses, and a vacutainer tube containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant was used (Daramola *et al.*, 2005; Aikhuomobhogbe & Orheruata, 2006; Brown *et al.*, 2016). Blood samples for haematological analysis were stored in a cooler box containing ice packs and they were immediately transported to the University of Pretoria, Department of Companion Animal Clinical Studies, Gauteng province for white blood cell count (WCC), lymphocyte count, monocytes count, eosinophil count, red blood cell (RBC) count, mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet counts and haemoglobin (Hb). All blood parameters were referenced according to Jackson & Cockcroft (2002), as guidelines for reference values.

3.2.5. Statistical analyses

Descriptive statistics such as the mean, standard deviation, and standard error were calculated using Procedure of means (Proc mean) of Statistical Analysis Software (SAS) version 9.4 (2019). Data on blood parameters were analyzed using a general linear model (GLM) procedure of statistical ANOVA (SAS, 2019). The model $Y_{ij} = \mu + T_i + e_{ij}$ was applied where Y_{ij} = blood profiles, μ = the overall mean, T_i = dietary treatments and e_{ij} = random error. A completely randomized design was used using an individual goat as a replicate (Steel & Torrie, 1980). Duncan's multiple range test was used for any significant differences ($p < 0.05$) amongst treatment means (Duncan, 1955).

3.3. DETERMINATION OF THE EFFECTS OF *M. OLEIFERA* SUPPLEMENTATION ON GROWTH PERFORMANCE OF VACCINATED BAPEDI GOATS

3.3.1. Site description

The study was conducted at the University of Limpopo experimental farm, as described in sections 3.1.1 and 3.2.1.

3.3.2. Experimental animals

Twelve clinically healthy BaPedi goats with average ages of twelve months (19 ± 1.47 kg BW) were randomly selected from the same flock, as described in section 3.2.2.

3.3.3. Experimental diets

Moringa oleifera leaves, Buffelgrass and lucerne were used in this study, as described in section 3.2.3.

3.3.4. Data collection procedures

The experiment was conducted in three phases for 42 days, as described in section 3.2.4. Bodyweight data were collected on day 0 from each goat and every seven days in the morning before feeding. The feed offered and refusals were weighed using an electronic scale each day from day 7 until day 42. Feed intake (FI) was calculated as the difference between feed offered and feed refused. Live body weights were used to calculate growth rate (GR) and feed conversion ratio (FCR). The metabolic weight gain (MWG) is expressed as the bodyweight gain (BWG) in grams (g) to the exponential of 0.75. The FCR was obtained by dividing feed intake by weight gain. The formulae used were:

$$\text{I. } \text{FCR (Kg)} = \frac{\text{FI}}{\text{BWG}}$$

$$\text{II. } \text{MWG (g)} = \text{BWG}^{0.75}$$

$$\text{III. } \text{GR} = \frac{\text{Final body weight} - \text{initial body weight}}{\text{number of days}}$$

3.3.5. Statistical analyses

Means, standard deviations, and standard errors were calculated using the procedure of means (Proc mean) of Statistical Analysis Software (SAS) version 9.4 (2019). Data on growth performance were analyzed using a general linear model (GLM) procedure of statistical ANOVA (SAS, 2019). The model $Y_{ij} = \mu + T_i + e_{ij}$ was applied where Y_{ij} = growth performance, μ = the overall mean, T_i = dietary treatments and e_{ij} = random error. A completely randomized design was used, using an individual goat as a

replicate (Steel & Torrie, 1980). Duncan's multiple range test was used for any significant differences ($p < 0.05$) amongst treatment means (Duncan, 1955).

CHAPTER 4: RESULTS

4.1. MINERAL COMPOSITION OF *M. OLEIFERA* LEAVES FOLLOWING DIFFERENT DRYING METHODS

Nutritional analyses for *M. oleifera* leaves that have been subjected to oven and air drying methods showed significant variations in the mineral content. Air dried samples showed significantly higher means ($p < 0.05$) for most of the elements analyzed compared to the oven dried- samples (Table 4.1). However, Mg and K minerals were significantly higher ($p < 0.05$) in oven dried as compared to air dried samples. The *M. oleifera* leaves in this study had on average the highest level of K (54.17 mg/L) followed by Ca (52.21 mg/L), S (49.62 mg/L), Mg (16.67 mg/L), and P (7.22 mg/L).

Table 4.1 Mineral composition of essential and non-essential minerals found in *M. oleifera* leaves and the effect of the drying method on mineral retention

MINERAL Mg/L	Drying methods	
	OVEN DRIED	OVEN DRIED (Mean±SEM) [#]
Mo	0.94±0.02 ^a	0.79±0.04 ^b
S	52.30±0.85 ^a	46.93±1.90 ^a
Ag	0.56±0.00 ^a	0.55±0.00 ^b
Ba	3.43±0.06 ^a	3.08±0.09 ^b
Bi	6.06±0.11 ^a	5.33±0.18 ^b
Ca	57.80±0.15 ^a	46.63±0.47 ^b
Cd	5.76±0.08 ^a	4.95±0.19 ^b
Co	0.42±0.01 ^a	0.38±0.01 ^b
Cr	4.92±0.07 ^a	4.33±0.16 ^b
Cu	0.43±0.00 ^a	0.41±0.00 ^b
Fe	1.42±0.02 ^a	1.25±0.03 ^b
K	52.90±0.15 ^a	55.43±0.47 ^b
Mg	15.90±0.10 ^a	17.43±0.27 ^b
Mn	0.82±0.01 ^a	0.51±0.01 ^b
Na	2.38±0 ^a	2.28±0.03 ^b
P	7.34±0.10	7.09±0.24
Zn	0.41±0.01 ^a	0.35±0.02 ^b
Ash %	0.0078±0.0002 ^a	0.0073±0 ^a
Moisture %	10.07±0.00 ^a	9.35±0.00 ^b

^{a, b} Means with different superscripts on the same row differ significantly ($p < 0.05$)

[#]SEM- Standard error of the means

Mo: Molybdenum, S: Sulphur, Ag: Silver, Ba: Barium, Bi: Bismuth, Ca: Calcium, Cd: Cadmium, Co: Cobalt, Cr: Chromium, Cu: Copper, Fe: Iron, K: Potassium, Mg: Magnesium, Mn: Manganese, Na: Sodium, P: Phosphorus, Zn: Zinc

4.2. THE EFFECTS OF DIETARY SUPPLEMENTATION OF *M. OLEIFERA* ON THE IMMUNE RESPONSE OF BAPEDI GOATS FOLLOWING VACCINATION WITH BLANTHRAX VACCINE

A comparison of haematological profiles across phases of BaPedi goats fed varying levels of *M. oleifera* following vaccination.

Measurements on blood parameters before and after vaccination were carried out during the study. There were no significant differences ((Table 4.2) ($p > 0.05$)) amongst means for the parameters except for MCV, platelet and monocyte count which were significantly different ($p < 0.05$; Figure 4.1; 4.2. and 4.3). There were no significant differences ($p > 0.05$) between means of haematological parameters under different inclusion levels of *M. oleifera* leaves in the diets.

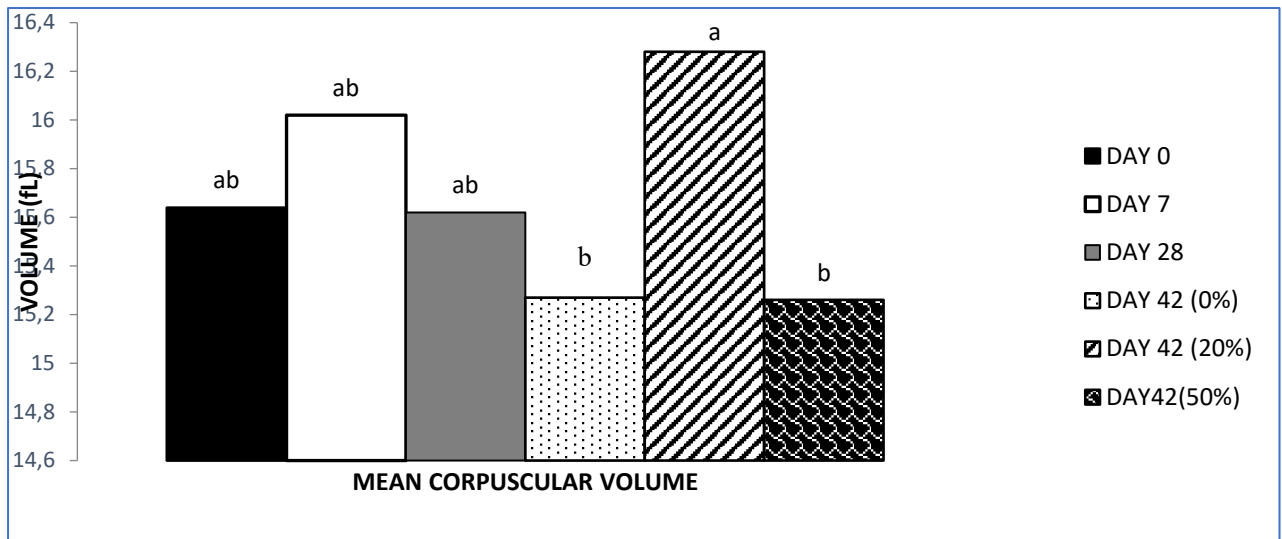


Figure 4.1 A comparison of blood MCV profile across phases from day 0 to day 42

Day 0: initial values, Day 7: phase 1, Day 28: phase 2, Day 42 (0%): phase 3 Vaccination+ 0% *M. oleifera* inclusion, Day 42 (20%): phase 3 Vaccination+ 20% *M. oleifera* inclusion, Day 42 (50%): phase 3 Vaccination+ 50% *M. oleifera* inclusion

*Significantly different ($p < 0.05$) in comparison to the initial value

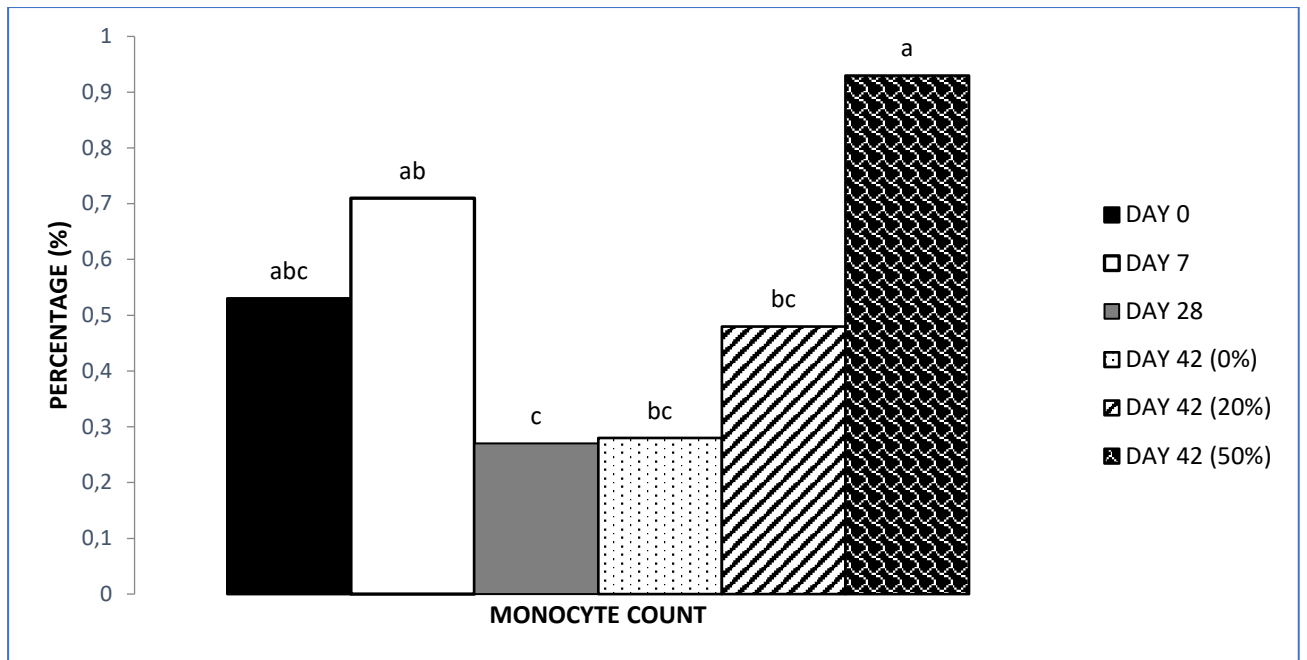


Figure 4.2 A comparison of blood monocyte profile across phases from day 0 to day 42

Day 0: initial values, Day 7: phase 1, Day 28: phase 2, Day 42 (0%): phase 3 Vaccination+ 0% *M. oleifera* inclusion, Day 42 (20%): phase 3 Vaccination+ 20% *M. oleifera* inclusion, Day 42 (50%): phase 3 Vaccination+ 50% *M. oleifera* inclusion

*Significantly different ($p < 0.05$) in comparison to initial values

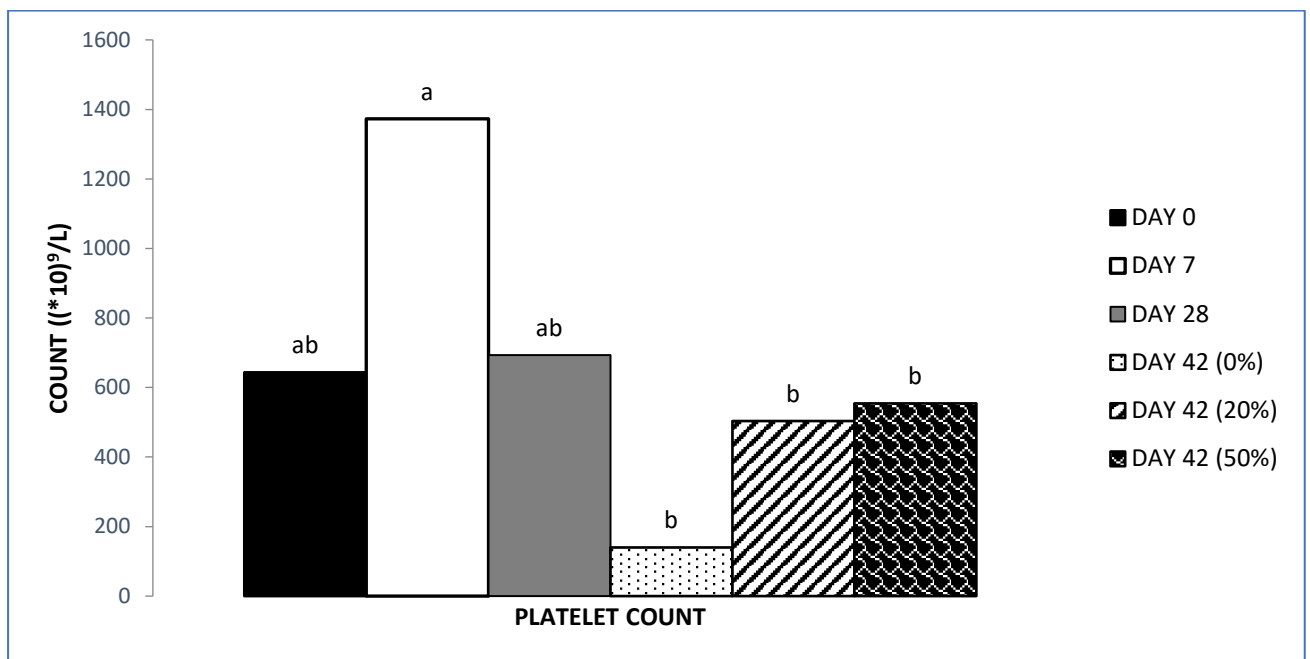


Figure 4.3 A comparison of blood platelet profile across phases from day 0 to day 42

Day 0: initial values, Day 7: phase 1, Day 28: phase 2, Day 42 (0%): phase 3 Vaccination+ 0% *M. oleifera* inclusion, Day 42 (20%): phase 3 Vaccination+ 20% *M. oleifera* inclusion, Day 42 (50%): phase 3 Vaccination+ 50% *M. oleifera* inclusion

*Significantly different ($p < 0.05$) in comparison to initial values

Table 4.2 Blood parameters across all phases from day 0- 42 and varying *M. oleifera* inclusion levels from day 29-42

Parameters	Adaptation phase		Vaccination phase	Vaccination + <i>M. oleifera</i> phase		
	DAY 0	DAY 7	DAY 28	VMO0	VMO20	VMO50
	(Mean±SEM) [#]	(Mean±SEM) [#]	(Mean±SEM) [#]	DAY 42		
	n=12	n=11	n=11	n=3	n=4	n=4
Hb (g/dL)	92.91±3.30	92.67±5.16	84.64±4.08	79.33±2.40	90.00±4.45	83.50±8.38
RBC (([*] 10) ⁶ μL)	17.83±0.70	17.42±0.85	16.40±0.89	15.57±0.88	16.92±0.68	16.41±1.88
HCT (L/L)	0.28±0.01	0.28±0.02	0.26±0.01	0.23±0.01	0.28±0.02	0.25±0.03
MCH (pg)	5.19±0.10	5.70±0.29	5.32±0.17	5.10±0.15	5.23±0.13	5.10±0.07
MCHC (g/dL)	33.43±0.35	33.14±0.26	33.15±0.19	33.47±0.27	32.65±0.37	33.53±0.51
RCDW (%)	21.79±0.34	21.23±0.30	21.84±0.31	22.47±0.88	21.93±0.34	21.68±0.24
WCC (([*] 10) ³ μL)	16.68±1.07	20.03±2.10	16.96±1.32	14.50±1.28	16.29±2.38	17.55±1.31
Lymphocyte (%)	10.87±0.98	13.54±1.56	13.74±1.07	11.64±1.10	10.36±2.04	10.32±0.80
Eosinophil (%)	0.20±0.07	0.42±0.10	0.40±0.09	0.48±0.17	0.27±0.06	0.35±0.16

^{a,b,c}Means with different superscripts on same row differ significantly (p < 0.05)

[#]SEM, Standard error of the means

WCC: white blood cell count, RBC: blood erythrocytes count, HCT: Haematocrit, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, Hb: haemoglobin, RCDW: red cell distribution width

Day 0: initial values, Day 7: phase 1, Day 28: phase 2, Day 42 (VMO₀): phase 3 Vaccination+ 0% *M. oleifera* inclusion, Day 42 (VMO₂₀): phase 3 Vaccination+ 20% *M. oleifera* inclusion, Day 42 (VMO₅₀): phase 3 Vaccination+ 50% *M. oleifera* inclusion

4.3. THE EFFECTS OF *M. OLEIFERA* SUPPLEMENTATION ON GROWTH PERFORMANCE OF VACCINATED BAPEDI GOATS

Comparison of growth performance across phases

There were significant differences ($p < 0.05$) amongst growth performance parameters and no differences were observed ($p > 0.05$) for feed efficiency parameters (Table 4.4). The VMO₅₀ displayed higher mean values on BWG, GR, and MWG ($p < 0.05$) and there were no significant differences ($p > 0.05$) in FI and FCR for VMO₀, VMO₂₀ and VMO₅₀ in the *M. oleifera* supplementation phases (Table 4). VMO₀ had 0 GR and BWG.

Table 4.4 Comparison of growth performance parameters across phases from day 0 – 42

Variable	Adaptation phase	Vaccination Phase	Vaccination+ <i>M. oleifera</i> phase (Day 29-42)		
	Day 1-6 (Mean±SEM) [#] n=12	DAY 7-28 (Mean±SEM) [#] n=11	VMO ₀ (Mean±SEM) [#] n=3	VMO ₂₀ (Mean±SEM) [#] n=4	VMO ₅₀ (Mean±SEM) [#] n=4
BW (Kg)	19.27±1.47 ^b	20.24±7.51 ^b	25.17±1.7 ^a	19.63±0.73 ^b	22.25±1.28 ^{ab}
BWG	0.55±0.25 ^{ab}	0.52±0.27 ^{ab}	0±0.26 ^b	1±0.27 ^{ab}	1.38±0.32 ^a
GR	0.08±0.04 ^{ab}	0.07±0.04 ^{ab}	0±0.04 ^b	0.14±0.04 ^{ab}	0.20±0.05 ^a
MWG	0.70±0.18 ^{ab}	1.13±0.13 ^a	0.33±0.21 ^b	0.92±0.23 ^{ab}	1.21±0.24 ^a
FI		7.13±2.05	7.42±3.57	6.57±4.88	6.59±3.93
FCR		0.04±0.00	0.03±0.00	0.03±0.00	0.03±0.00

^{a,b}Means with different superscripts on the same row differ significantly ($p < 0.05$)

[#]SEM: Standard error of means

CHAPTER 5: DISCUSSION

5.1. MINERAL COMPOSITION OF *M. OLEIFERA* LEAVES FOLLOWING DIFFERENT DRYING METHODS

Green feed sources are not readily available throughout the year for animal feeding, especially in drought-prone areas. Therefore, drying feedstuffs is one of the strategies for feed preservation by preventing the growth of bacteria during storage. However, if the drying process is not done correctly, unwanted biochemical reactions may occur, leading to the loss of colour, fragrance and nutrients (Babu *et al.*, 2018; Tetteha *et al.*, 2019). In general, correct drying of feedstuffs is reported to enhance the nutrient density and lower anti-nutrients compared to fresh feed such as leaves (Navale *et al.*, 2014, Babu *et al.*, 2018). Different plant parts of *M. oleifera* contain different levels of nutrients and phytochemicals. Therefore, it is essential to use appropriate techniques to achieve maximum nutrient retention (Tetteha *et al.*, 2019).

In the animal industry, practices such as sun-drying and shade drying are mostly practised especially in communal areas due to lower cost implications (Aremu & Akintola, 2014). However, natural drying methods have limitations such as more exposure to environmental contaminations and increased drying time because factors such as climate, drying temperature and drying time influence the final quality of feed as compared to mechanical dryers such as ovens (Aremu & Akintola, 2014; Tetteha *et al.*, 2019).

According to the present study, *M. oleifera* leaves are a rich source of macronutrients such as Ca, K, and S. These elements are essential for a wide range of functions in the body of the animal, such as the blood plasma, muscles, and nerve impulses (Araujo *et al.*, 2019). This agrees with Moyo *et al.* (2011) and Mulyaningsih & Yusuf (2018) who reported Ca as the most abundant element in *M. oleifera* leaves. However, according to the literature, the levels of the essential elements were below the recommended daily intake (RDA) for goat nutrition for this study, which implies that *M. oleifera* can be used as a supplement to form a complete diet (see table 2.1, Souza *et al.*, 2014; Hart, 2020). Elements such as Se and I were not detected in the present analyses, the leaves could be deficient in this element. The absence of Se in the leaves of *M. oleifera* utilized in this study slightly differs from the findings of Moyo *et al.* (2011) who reported the presence of Se in the leaves. Gupta & Gupta (2017) stated that this observation might be due to the soil type or stage of development of the tree

at harvest, however, more research may be required on this topic. The Zn, Fe, Cu, Co, Mn, Cr, and Mo are some of the micro elements that are needed for growth, metabolic functions, haematology, and a stronger immune system (Dove, 2010). The mineral content of *M. oleifera* leaves in this study slightly varied from levels reported in tropical regions (García *et al.*, 2019). These variations may be due to factors such as the different geographic locations, soil type, stage of growth of the tree, the season of harvesting, and storage conditions before analyses (Mulyaningsih & Yusuf, 2018). The presence of non-nutritive elements found in *M. oleifera* leaves used in this study, such as Ba (308 mg/L), Cd (4.95 mg/L), and Ag (0.55mg/L) could have arisen from environmental contaminations, however, their presence in low concentrations may not cause toxicity in the animal (Pakade, Cukrowska & Chimuka, 2013; Biel, Jaroszewska & Łysoń, 2016).

The present study revealed significantly higher means for most elements in air dried samples than in oven dried samples (Table 4.1). This indicates that drying methods can influence mineral retention. The results agree with studies done by Mbah, Eme & Paul (2012) and Emelike & Ebere (2016) indicating how different elements either increase, decrease, or remain the same due to the method of dehydration. Oluwalana *et al.* (2011) reported higher concentrations of Mg and K elements in oven dried samples as compared to air dried samples which agrees with the present study. Therefore, as the temperature increases, the concentrations of these elements also increase. In contrast, results of heavy metals found in this study show that the levels of these elements were lower in oven dried samples compared to air dried samples, suggestive that drying may destroy anti-nutritional elements in leaves.

In conclusion, the results of this study show that *M. oleifera* leaves are rich in minerals and can be used in animal feed for various purposes, which include boosting the immune system and improving growth performance. Therefore, it is vital to consider that different drying methods do affect nutrient retention in leaves. According to the findings of this study and other related studies, we can conclude that *M. oleifera* has the potential to be used as a supplement in the diets of animals.

5.2. THE EFFECTS OF DIETARY SUPPLEMENTATION OF *M. OLEIFERA* ON THE IMMUNE RESPONSE OF BAPEDI GOATS FOLLOWING VACCINATION WITH BLANTHRAX VACCINE

Haematological profiles may be utilised to assess the immunological status of goats (Al-Seaf & Al-Harbi, 2012). However, factors such as age, nutrition, stress, management and environmental factors are known to have an effect on blood profiles in small ruminants (Mohammed *et al.*, 2016). In comparison across the three phases, there were no significant differences in total WCC throughout the study. This varies from the findings of Jo *et al.* (2014), who observed an increase in total WCC following vaccination of indigenous goats. However, the current results agree with Kumar *et al.* (2017), who found no significant differences in total WCC but observed significant differences in monocyte counts. Furthermore, monocyte counts were significantly reduced following vaccination, this could be due to a mild inflammation following vaccination. Useh *et al.* (2006), reported a significant decrease in monocyte count following blackleg infection, however, there was no significant difference in total WCC. Therefore, it may be useful to observe the specific leukocytes rather than WCC in combination.

The results of the current study also revealed a significant increase in monocyte counts on VMO₅₀ compared to monocyte counts following the vaccination phase. The significant differences in monocyte counts observed in the literature and the present study show that monocytes act as first-line mediators that prepare the immune system for defence. Inclusion of *M. oleifera* commenced on day 22 post-vaccination to allow sufficient time for the animals to develop immunologic protection (Hoebe, Janssen & Beutler, 2004). Therefore, the significant increase in monocyte count for VMO₅₀ suggests an enhanced immune response to vaccination which could have been caused by 50% *M. oleifera* inclusion compared to 0% and 20% of *M. oleifera* inclusion levels. This observation could also be associated with the higher content of heavy metals such as Ag and Cd in the experimental diet due to supplementation with 50% *M. oleifera* as reported by Kar *et al.* (2015). However, Shen, Chi & Xiong (2019) stated that toxicity from heavy metals such as Cd affects the bones, thus, causing anaemia in the animals. However, there was no evidence of anaemia and MCH levels were within the range of healthy goats in animals fed a diet containing 50% *M. oleifera* although MCV and RBC levels were lower compared to VMO₂₀.

The recommended platelet count interval for healthy goats is 300- 600 ($\times 10^9$)/L, however, platelet counts for day 0 were out of range (Jackson & Cockcroft, 2002). Higher levels of platelet count outside the reference intervals as seen on day 0 are not of pathological importance, especially in young animals but occasionally may be brought about by an infection or anaemia (Eclinpath, 2021). According to Kumar *et al.* (2017), platelet count does not play any role in immune response following vaccination. However, in the present study, mean platelet counts increased during the adaptation period and significantly reduced following vaccination towards the normal ranges of healthy goats as recommended by Jackson & Cockcroft (2002). Although there were no significant differences in mean platelet counts during the *M. oleifera* supplementation phase amongst all treatments, 0% *M. oleifera* supplementation decreased platelets counts to below the recommended reference interval and supplementation with 20% and 50% *M. oleifera* leaves decreased the platelet counts to within the recommended reference interval of healthy goats (Table 4.3). This could be due to the *M. oleifera* immune-boosting effects as reported by Adouko *et al.* (2020). Metcalf-Pate *et al.* (2013) and Ali, Wuescher & Worth (2015), observed that platelets may play a role in the immune response in animals which agrees with the results of the present study. Low levels of Hb and RBC may also be an indication of anaemia, however, Hb values were within 80-120 g/Dl and RBC values were within 17-20 ($\times 10^6$) μ L which are values for healthy goats as recommended by Jackson & Crockcroft, 2002; Brown *et al.*, 2016.

Mean corpuscular volume (MCV) is a measurement of the average size of RBC. However, in the present study, MCV levels on day 0 were slightly below the reference range of 16-25 fL for healthy goats (Jackson & Cockcroft, 2002). Mohammed *et al.* (2016) observed similar findings in different goat breeds. The causes of these discrepancies are not clear, since the goats did not show signs of anaemia. A significant increase to within the reference range of healthy goats in MCV for VMO₂₀ was expected due to higher levels of 11mg Cu/kg and 606mg Fe/kg minerals which aid in red blood cell formation compared to treatment diets for VMO₀ and VMO₅₀. The goats in VMO₂₀ were observed to be healthy which indicates that the higher Fe content than the recommended requirement for indigenous goats of 50-100mg Fe/kg did not cause any toxicity (Souza *et al.*, 2014; Alfaro *et al.*, 2021). The results of the present study are similar to the findings of Jiwuba *et al.* (2017), who observed a significant difference in MCV values for *M. oleifera* inclusion levels and MCV levels were

maintained within the range of healthy goats, suggesting that *M. oleifera* may be used as a supplement without compromising the nutritional quality of the feed.

Balanced nutrition is vital in maintaining a healthy immune status in animals (Brown *et al.*, 2016). Insignificant differences in most of the blood parameters among VMO₀, VMO₂₀, and VMO₅₀ observed in this study agree with Moyo, Masika & Muchenje (2012) and Osman, Shayoub & Babiker (2012) who reported insignificant differences in blood parameters of animals fed *M. oleifera* as a supplement. Results of this study show that most of the blood parameters were within the range of healthy indigenous goats (Jackson & Cockcroft, 2002; Brown *et al.*, 2016; Daramola *et al.*, 2005). However, VMO₀ had the lowest mean platelet and monocyte counts while VMO₅₀ had the highest counts. This could be caused by a high content of Mg in *M. oleifera* leaves supporting the formation of platelets, thus alleviating the occurrence of anaemia (Rishi *et al.*, 1990). According to Ali, Wuescher & Worth (2015), there is a positive relationship between platelets and monocyte counts because platelets influence the immune response by activating immune cells such as monocytes. This is evident in the present study because as the level of *M. oleifera* inclusion increased, the means of platelet and monocyte counts increased as well. With this in mind, it can be concluded that the presence of heavy metals, the marginal mineral content of Cu and the high content of Fe in the diets for VMO₂₀ and VMO₅₀ in the present study did not bring about any toxicity or anaemia to the animals. Therefore, the possibility of anaemia in the present study can be ruled out and accept that supplementing with 20% to 50% of *M. oleifera* may assist in boosting the immune system of the animals.

The high mineral content of *M. oleifera* leaves acts as an innate immune booster following vaccination. Results in the present study reveal that 50% *M. oleifera* can be used as a supplement without having any adverse effects on haematological parameters in indigenous goats.

5.3. THE EFFECTS OF *M. OLEIFERA* SUPPLEMENTATION ON GROWTH PERFORMANCE OF VACCINATED BAPEDI GOATS

Results from the present study showed that the growth performance parameters were influenced significantly by vaccination and varying *M. oleifera* inclusion levels. The mean values obtained for body weight gain, growth rate, and metabolic weight gain for VMO₅₀ were significantly higher than those in the control (VMO₀) but similar to VMO₂₀. The higher values for VMO₅₀ and VMO₂₀ on BWG, GR, and MWG could have

been influenced by the lower levels of NDF and higher protein content in the diet, making it to be more digestible and nutritious ensuing higher growth rates (Moyo, Masika & Muchenje (2012). The significant decrease in BWG and GR following vaccination agrees with Jo *et al.* (2014), who reported a significant decrease in growth performance following vaccination of goats, which could be due to some of the side effects such as inflammation that may arise from vaccination. However, the introduction of varying levels of *M. oleifera* to the diets of the goats, increased BWG, GR, and MWG, suggesting that *M. oleifera* due to its composition of elements such as antioxidants may aid in reducing inflammation and thus improving growth performance.

This study agrees also with Mahfuz & Piao (2019) and Aregheore (2002), who reported an improved growth performance in indigenous goats after supplementing with varying levels of *M. oleifera*. However, BWG and GR for VMO₀ were zero without affecting the FI, this agrees with the findings of Jo *et al.* (2014), who reported that vaccination decreases BWG without affecting FI. It could also be due to the significantly higher bodyweight of the goats in VMO₀ due to random grouping. The current findings disagree with the results of Yusuf, Mlambo & Iposu (2018), who reported insignificant differences in growth performance of indigenous goats fed 5 to 10% *M. oleifera* supplementation, therefore, it can be assumed that the minimum levels of *M. oleifera* that can be used as a supplement are 10% for improved growth performance in indigenous goats.

It may be concluded from the results of this study that *M. oleifera* can be used in feeding indigenous goats without causing adverse effects on growth performance. Inclusion levels of 50% *M. oleifera* yielded the highest results in terms of GR, MWG, and BWG.

CHAPTER 6: GENERAL SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1. GENERAL SUMMARY

Limpopo province has the highest number of households that practice goat farming; however, most of them own less than 10 goats (Statistics South Africa, 2016). Mdladla, Dzomba & Muchadeyi (2017) identified several production constraints that are faced by communal farmers in this province. It is recommended that farmers need to be equipped with good management practices to enhance production such as strategic feeding during less favourable periods, and health care. One of the ways to minimize costs and promote optimal health and nutrition in animals is using natural feed additives. The *M. oleifera* contains high levels of nutrients that aid in immunomodulation and the reduction of malnutrition. It grows well in tropical and sub-tropical areas such as Limpopo province (Mabapa *et al.*, 2017).

This study examined the effect of air and oven drying methods on the mineral retention of *M. oleifera* leaves (objective 1). It also evaluated the effect of *M. oleifera* supplementation on growth performance and health status in vaccinated indigenous goats (objectives 2 & 3). Results in the present study revealed an improved immune response and growth performance in goats supplemented with *M. oleifera* leaves following vaccination. However, the nutritional composition may be affected by the type of drying methods used. This study showed that oven drying reduces the nutritional composition of some minerals, including the levels of heavy metals that might be present in the leaves. Therefore, both oven and air-drying methods have their limitations and strengths. Further investigations using a larger sample size using both males and females and increasing the duration of data collection has the potential to yield more significant results. The study also explored the oven drying technique using one temperature level. Further studies might investigate nutrient retention in oven dried samples using different temperature levels and durations of the drying period.

6.2. CONCLUSION AND RECOMMENDATIONS

Moringa oleifera leaves has been shown to exhibit several elements that attribute to enhanced health and performance in domestic animals. Supplementing with *M. oleifera* leaves in indigenous goat diets had a positive effect on growth performance and immunological response in goats following vaccination. Twenty percent and 50%

M. oleifera supplementation yielded improved results compared to the control diet following vaccination.

Currently, in South Africa, the cost of purchasing *M. oleifera* is expensive as compared to other protein sources. The high cost is influenced by the perceived health and nutritional benefits of this plant. However, the advantage of Limpopo province is, that it is in the tropical zone, therefore, the growth and prosperity of this tree may be favoured without large monetary inputs. Therefore, *M. oleifera* may be produced by communal farmers in their homes in Limpopo province to overcome feed shortages, combat malnutrition and poor immune response in livestock. However, more research-based knowledge transfer is required for awareness to farmers especially in communal areas to cultivate *M. oleifera* in abundance for a more affordable protein and mineral source for their livestock.

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