

**Bone, liver and muscle mineral composition of Bontebok, Blesbok and Impala species
harvested from different regions**

By

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Declaration

I, Papama Mpangele declare that this dissertation has never been submitted to any University. Everything written in this project contains my original work, which I conducted under the supervision of Professor L.C Hoffman. All the assistance that I have received towards making this project a success has been credited in the acknowledgements.

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Prof. L.C Hoffman (Supervisor)

Abstract

The aim of the study was to investigate the mineral concentration of different game species, harvested in different regions. Bone, liver and muscle (*Longissimus thoracis et lumborum*) mineral composition of game animals (bontebok, *Damaliscus pygargus pygargus*; blesbok, *Damaliscus pygargus phillipsi*; and impala, *Aepyceros melampus*) were investigated. The muscle of all three animals were also analysed for their proximate (moisture, protein, lipid, ash) chemical composition. Impala animals were harvested in three different production systems (intensive, semi-extensive and extensive production systems) and in different regions (Castle de Wildt in Limpopo, South Africa; Mount Etjo in Otjozondjupa, Namibia; and Bredasdorp in Western Cape, South Africa) whilst bontebok and blesbok were harvested in the Western Cape from two different extensive farms. The proximate results for all species ranged between 73.68 – 75.72% moisture, 21.41 – 24.15% protein, 1.44 – 2.38% fat and 1.14 – 1.35% ash.

The mineral results showed significant concentrations of Boron (B), Aluminium (Al), Vanadium (V), Antimony (Sb), Chromium (Cr), Nickel (Ni), Strontium (Sr), Barium (Ba), Lead (Pb), and Silicon (Si) micro minerals in the rib of all three (bontebok, blesbok, and impala) species. Whilst the liver had high concentrations of Manganese (Mn), Cobalt (Co), Copper (Cu), Selenium (Se), Molybdenum (Mo), and Mercury (Hg) for all species. Iron (Fe), Zinc (Zn), Arsenic (As), and Cadmium (Cd) concentrations differed in species and animal samples. Iron (Fe) and Zinc (Zn) were high in concentrations for all bontebok animal samples and were very low in the blesbok animal samples. However, impala had high levels of Fe and Zn in the rib. The results also showed that mineral concentrations differed in impala harvested in different production systems. Extensively reared impala from the Western Cape had high concentrations of Cr, Fe, Co, Zn, As,

Se, Sr, Mo, Hg, and Si, whereas impala from an extensive system in Namibia had high concentrations of Al, V, Cr, Mn, As, Mo, Hg, and Pb. Impala reared under intensive conditions from Limpopo had high concentrations of V, Mn, Fe, Co, Cu, Zn, As, and Ba, whereas the semi-extensively reared impala from Limpopo had high concentrations of Mn, Fe, Zn, and Ba. The macro minerals from impala from Namibia were found to be very low concentrations as compared to those from Limpopo and the Western Cape. It can therefore, region/production system type does have an effect on the concentration of minerals in these animal samples.

Keywords: game animals; harvesting site; mineral profile; proximate composition; production systems

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List of abbreviations

Al	Aluminium
ANOVA	Analysis of variance
As	Arsenic
B	Boron
Ba	Barium
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
Fe	Iron
HCL	Hydrochloric acid
Hg	Mercury
ICP-MS	Inductively coupled plasma mass spectrometry
ISTD	Internal standard solution
K	Potassium
LOD	Limit of detection
LSMeans	Least Square mean
LTL	<i>Longissimus thoracis et lumborum</i> muscle

Mg	Magnesium
ml	Millilitre
Mn	Manganese
Mo	Molybdenum
Na	Sodium
Ni	Nickel
NIST	National Institute of Standards and Technology
P	Phosphorus
Pb	Lead
Sb	Antimony
Se	Selenium
SEM	Standard Error of Means
Si	Silicon
Sr	Strontium
USA	United States of America
V	Vanadium
XLSTAT	Statistical Software for Excel
Zn	Zinc

Chapter 1: Background

1.1. Introduction

The commercial utilisation of game in South Africa has shown extensive growth over the last 20 years, and consequently, game farmers now play a key role in the conservation of many game species (Otieno and Muchapondwa, 2016). Game farming has also been introduced to the agricultural industry and it is well known and developing in South Africa. Many South African cattle farms are being converted to game farms and the conversion rate has increased from 2 to 2.5% per annum (Chiyangwa, 2018). Additionally, Pasmans and Hebinck (2017) reported that since 1996, 12% of the land for livestock in the Eastern Cape province of South Africa has been converted into game ranches. According to Saayman *et al.* (2018), the estimated gross income from the game industry across South Africa is R1.96 billion. Taylor *et al.* (2016) also reported that the estimated total revenue of live sales from all South African wildlife ranches (including those that are privately owned) sums up to R4.328 billion. Dekker and Van Der Waal (2000) noted 22 years ago that game ranches occupied approximately 3.6 million ha in the Limpopo province of South Africa, and they covered 26% of the province's surface area. Although data is lacking all indications are that this percentage is much higher today.

Similar to livestock, game animals also require minerals for efficient performance, growth, and reproduction. Twenty-two minerals are known to be essential for the members of the animal kingdom (Underwood, 1976). These comprise seven macro minerals: Calcium (Ca); Phosphorus (P); Sodium (Na); Potassium (K); Chlorine (Cl); Magnesium (Mg); and Sulphur (S) and fifteen micro minerals: Chromium (Cr); Copper (Cu); Manganese (Mn); Zinc (Zn); Selenium (Se); Tin (Sn); Iodine (I); Cobalt (Co); Vanadium (V); Fluorine (F); Folybdenum (Mo); Silicon (Si); Iron

(Fe); Nickel (Ni); and Arsenic (As). However, Laaka (2014) included Boron (B), Cadmium (Cd), Barium (Ba), Aluminium (Al), Lead (Pb) and Mercury (Hg) as essential micro minerals. All these mineral elements exist in the cells and tissues of the animal in specific concentrations, which is essential for the normal growth and health and productivity of the animal (López-Alonso, 2012; Stasiak, 2017).

Minerals are important for three major functions (Smart *et al.*, 1981). Firstly, as a structural component of the body, animal samples, and tissues. Secondly, they play a role as constituents of body fluid and tissues as electrolytes that are essential for maintenance of osmotic pressure, acid-base balance, membrane permeability and tissue irritability (Miller, 2017). Thirdly, they act as catalysts in enzyme and hormonal systems as an integral and specific component of the structure of metallic-enzymes or as activators within those systems (Underwood, 1981). Essential mineral deficiency results in delayed growth and puberty in livestock. Body micro mineral profile, immunity and reproductive functions are directly related, where Copper, Cobalt, Zinc, Iodine and Manganese are likely to influence these functions. In most cases, mineral deficiency limits production (McDowell and Conrad, 1977) and can also negatively affect both animal and human health, as minerals can be source of micro and macro minerals in human diets.

Several studies have been conducted on nutritional aspects of game animals, such as the game meat quality (fat and lean) and meat palatability (colour, pH, juiciness, flavour, etc), however, little has been said about the mineral profile of game animals. According to Wassenaar *et al.* (2019), the production of game meat is important for the development of the game industry because it contributes significantly to the game rancher's economy and is also seen as a healthy protein food source for humans. However, animal health plays an important role in animal and

meat production, hence it is importance to understand the mineral status of animals. As already mentioned, mineral deficiencies are a major problem that could limit animal production (López-Alonso, 2012; Stasiak, 2017) and should be corrected for by providing mineral licks to animals. However, this cannot be achieved without having base-line information on the mineral status of game animals. Thus, the main aim of this research is to investigate the mineral concentration of game animals using samples from the bone, liver, and the muscle so as to provide some base-line information on some of the more commonly ranched species.

1.2. Problem statement

Nutritional disorders, including mineral deficiencies, toxicities and mineral imbalance severely inhibit grazing animals in developing countries and have more significant consequences than infectious disease (Loosli and McDowell, 1985). These deficiencies impair the animal's immune response and increase its susceptibility to disease, in particular contagious diseases (e.g., diarrhoea, respiratory disorders). In young ruminants, macro and micro mineral deficiencies contribute to metabolic disorders and clinical changes in individuals and therefore in the herd. The role of micro minerals, particularly Copper and Zinc in maintaining reproductive rhythm is well documented, as they are specific activators of enzymes in the reproductive system (McDowell *et al.*, 1984). Infertility has been regularly recorded where there are deficiencies in Copper and Manganese deficiencies, resulting in depressed or delayed oestrous and poor conception rate (Underwood, 2012). Similarly, Zinc has been shown to be important for gonadal growth in lambs and is responsible for infertility in ewes (Underwood, 1981).

Mineral deficiencies in different types of vegetation and soils of Africa may result in low mineral intake by animals. However, before a feed is classified to have a mineral deficiency, baseline

data is needed to know and understand what a normal mineral concentration would be. Even though this information is available for domesticated animals, there is limited to no information for wild species farmed in Southern Africa.

1.3. Justification

Of all the nutrients required by an animal, minerals play a very important role as they are responsible for all life processes, including growth and reproduction (López-Alonso, 2012). However, animal mineral concentrations are dependent on both soil and plant associations. Mineral contents of natural vegetation are primarily determined by the mineral availability and mineral composition of the soil (Smart *et al.*, 1981). Soil mineral concentrations influence the concentrations within the plants and consequently, the mineral concentrations in the muscle tissue of the animals feeding on the plants (McDowell and Conrad, 1977; Zomborszky *et al.*, 1996). The extent and pattern of mineral deficiencies or excess in plants vary in different agro-climatic conditions as available mineral content in green vegetation depends on physical and chemical properties of soil, soil erosion, cropping pattern, fertilizer/chemical application, species and genetic variation in plants, stage of maturation as well as the presence of other minerals (McDowell *et al.*, 1983).

Mineral imbalances in soil and forages are found to be responsible for low production and reproduction among ruminants. Certain minerals can be included in animal diets after the shortage of a specific mineral has been identified. Providing animals with a proper diet becomes easy if the animal's feeding status at a given time is known. Therefore, studies on the soil-plant-animal relationship in respect to minerals are important and have great implications on animal production (Biswas and Samanta, 2002). However, there is insufficient information about the

mineral profile of game animals; hence this study aims to investigate the mineral concentration of the bone, muscle and liver from different game species reared in different regions under different production systems.

1.4. Aim and Objectives

1. The overall aim of the study was to determine the mineral concentration in bone, liver and muscle of bontebok (*Damaliscus pygargus pygargus*), blesbok (*Damaliscus pygargus philipsi*) and impala (*Aepyceros melampus*) species harvested in different regions.
2. The specific objectives are:
 - To investigate the bone, liver and muscle mineral concentration of impala species harvested in Limpopo (intensive and semi-extensive production system), Namibia (extensive production system) and Western Cape (extensive production system).
 - To investigate bone liver and muscle mineral concentration of bontebok and blesbok species harvested in the Western Cape, in an extensive production system.

1.5. Null Hypothesis

- There is no significant difference in mineral concentration of bone, liver and muscle of impala harvested in different regions.
- There is no significant difference in the mineral concentration of bone, liver and muscle of bontebok and blesbok harvested in different regions.

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Chapter 2: Literature review

Production of game meat has become an important facet of the agricultural industry in South Africa. According to Hoffman *et al.* (2005), the production of meat plays a considerable role in increasing the economy of game farms. Berry (1986) also reported that game meat production had high income yields as compared to other activities (such as trophy hunting and live sales) practiced on game farms. Game meat has been reported to have a low-fat content of <3% (Hoffman, 2007) and it also contains a higher proportion of polyunsaturated fatty acids (Hoffman and Wiklund, 2006), whereas red meat from farmed livestock has a fat content >5% depending on the leanness of the meat (Williamson *et al.*, 2005). High fat content meat is a risk to human health in that it may lead to high cholesterol which could lead to heart disease, obesity, strokes and hypertension (Hoffman *et al.*, 2005). Due to these health problems, there has been an increasing demand for game meat world-wide (Neethling *et al.*, 2016). Game meat can be considered to have a high nutritional value as it is not only a good source of protein but also serves as a source of vitamins and minerals (Costa *et al.*, 2016).

Minerals are elements that form part of the ash from which impurities have been removed. More than 60 mineral elements, both macro and micro elements, have been detected in the ash of the organs and tissues of animals (Ashton, 2016). These mineral elements occur in the cells and tissues of the animal in specific concentrations and are essential for the normal growth, health, and productivity of the animal (Ranjith and Pandey, 2013). The concentration of minerals also varies according to species. For example, Hoffman *et al.* (2005) reported that, when compared to beef, some game meat species such as impala and duiker (*Sylvicapra grimmia*) have higher Phosphorus (P), Potassium (K) and Calcium (Ca) concentrations and lower levels of Sodium (Na) in their meat. Even though the majority of the naturally occurring minerals originate in

animal tissues and organs (kidney, liver, heart, rib, etc), many are thought to be present simply because they are constituents of the animal's food and may not have an important function in the animal's metabolism (McDonald *et al.*, 2002).

In order for an element to be categorised as important, evidence showing that pure diets lacking the element causes deficiency symptoms in the animals should be provided and this deficiency can be prevented by including the element in the diets (McDonald *et al.*, 2002). Such minerals include Ca, Na, Magnesium (Mg), K, Iron (Fe), Copper (Cu), Manganese (Mn) and Zinc (Zn) (Kunachowicz *et al.*, 2005). These minerals are essential in the animal's body and have different functions. For instance, Zn is responsible for the proper growth and development of an organism (including the biosynthesis of the proteins) and it is necessary for the functioning of the respiratory, reproductive, and immune systems (Biesalski, 2005). Other functions of minerals include transportation of respiratory gases (Blicharski *et al.*, 2013), oxidation-reduction processes and iron transportation (Gortat, 2013) as well as absorption of nutrients. Exposure of animals to low levels of required minerals may cause reproductive impairment, physiological abnormalities, and behavioural modifications (Nriagu *et al.*, 2009). There is, therefore, in cases of mineral deficiencies, a need to supplement animal diets with minerals as this is not only a health threat to animals but also to human health. Humans cannot synthesis these minerals; as some depend primarily on meat and organs/by-products of animals for their mineral intake.

The importance of mineral concentration in bones and organs/by-products has been overlooked in the meat industry. Like the meat, organs and bones also play a major role in meat production as they are the main storage environments of most minerals. However, as reported by Umaraw *et al.* (2015), there is a significant difference in mineral concentrations between muscles and

organs. Umaraw *et al.* (2015) showed a high concentration of Na in the kidney, whilst Cu was found to be high in the liver (6.97 mg/100 g), Fe was high in the spleen (31.16 mg/100 g) and Zn was high in the muscle (4.15 mg/100 g) of young growing goats.

The liver is the major organ typically used to determine if an animal has any mineral deficiencies. In this case, supplements are provided to increase the concentration of the deficient minerals. On the other hand, the mineral status of game animals remains unknown as most conclusions have been made based on what the mineral content of livestock is. Using the bontebok and its close relative, blesbok (both grazers) and impala (a mixed browser/grazer) would provide a baseline of what could be expected for the mineral content of game animals and whether these antelopes differ in mineral composition. The aim of this literature is to review the mineral concentration in bone, liver and muscle of game animals; these animal samples types were selected as they are the main storage areas of most minerals.

2.1. Mineral classification

Understanding the functions and transformation of minerals in living organisms has been improved by the contributions made in molecular biology. This has contributed to the knowledge base on the complex mechanism that is responsible for transporting trace minerals in cell membranes (Mayland and Shewmaker, 2001). Macro and micro minerals have four essential roles in the functioning of the body; namely: structural, physiological, catalytic and regulatory functions (Suttle, 2010; Radwinska and Zarczynska, 2014). The structural function involves elements that build organ and tissue structures (e.g., Ca, Mg, P, Si in bones and teeth, Phosphorus, and Sulphur in muscle proteins) (Radwinska and Zarczynska, 2014).

Physiological functions are responsible for supplying electrolytes to the body fluid and tissues. Electrolytes are important for the maintenance of osmotic pressure, acid-base balance, membrane permeability and tissue irritability (Ranjith and Pandey, 2013). Macro and micro elements operate as catalysts in enzyme and endocrine systems and can constitute integral and specific structural elements of metalloenzymes and hormones (Underwood, 1981).

The grouping of essential minerals into macro (major) and minor (trace) categories depends upon their concentration in the animal or quantities required in the diet (McDonald *et al.*, 2002). Macro elements are required by livestock in high concentrations; normally >50 mg/kg body weight (BW), whilst micro elements are required in very small quantities, typically <50 mg/kg BW (Radwinska and Zarczynska, 2014). Table 2.1 summarises essential micro and macro minerals and their required concentrations in an animal's body.

Table 2.1: Minimal concentration of important macro and micro minerals typically found in an animal's body.

	Minerals	Mineral concentration mg/kg
Macro minerals	Calcium (Ca)	15000
	Phosphorus (P)	10000
	Potassium (K)	2000
	Sodium (Na)	1600
	Chlorine (Cl)	1100
	Sulphur (S)	1500
	Magnesium (Mg)	400
Micro minerals	Iron (Fe)	20 – 80
	Zinc (Zn)	10 – 50
	Copper (Cu)	1 – 5
	Molybdenum (Mb)	1 – 4
	Selenium (Se)	1 – 2
	Iodine (I)	0.3 - 0.6
	Manganese (Mn)	0.2 - 0.5
	Cobalt (Co)	0.02 - 0.1

Source: McDonald *et al.*, 2002

2.1.1 Macro minerals

Macro minerals are minerals that are required in large quantities by the animal's body (McDonald *et al.*, 2002). Like trace minerals, macro minerals are essential for the animal's growth, health, and reproduction. Overfeeding macro minerals causes high manure excretion rates which after many years of manure application leads to salt overload in soils (Norell and Chahine, 2014). Salt build-up results in plant toxicities, closes soil air surfaces, promotes water stress, and decreases crop production. In addition, imbalances of Ca, or K, or Mg ions in the soil can cause a deficiency of these minerals due to cation competition in the plant material consumed by animals (Norell and Chahine, 2014). Table 2.3 summarises macro minerals and their deficiencies in animals.

Table 2.2: Macro minerals and mineral deficiencies in animals

Micro minerals	Deficiencies
Calcium and Phosphorus	Development of rickets in young animals and osteomalacia in adult animals. Animals chewing foreign objects (e.g, bones and wood), weak muscles and stiffness in joints are added symptoms to phosphorus deficiency.
Magnesium	In calves, conditions such as tetany, depleted bone magnesium and low serum magnesium levels are seen. Hypomagnesaemia is common to adult ruminants.
Potassium	Severe paralysis, retarded growth, weakness, tetany, and death
Sodium	Low osmotic pressure which leads to the body being dehydrated.

Source: McDonald *et al.*, 2002

2.1.2 Micro minerals

Trace minerals, for example, Co, Cu, Fe, I, Mn, Mo, Se, and Zn have been recognised as being a basic requirement for normal functioning of all biological processes in the body (López-Alonso, 2012). The deficiency of micro mineral weakens productivity in livestock. Body micro mineral profile, immunity, and reproductive functions are directly related, where Cu, Co, Zn, I and Mn are likely to influence these functions (Shahjalal *et al.*, 2008). Trace minerals must be supplied to animals in optimal concentrations and according to their requirements as these requirements change during the rapid growth of the animal and the reproduction cycle (López-Alonso, 2012). Traditionally and even to date, adding organic salts in animal diets has been the norm to accomplish trace mineral requirements for animals (Cortinhas *et al.*, 2012).

As illustrated in Figure 2.1, a declining trace mineral concentration will influence an animal's performance. Clinical signs arise with a decrease in the animal's immunity and enzyme functions, optimum and normal growth, and fertility. According to Larson (2005), subclinical signs are more dangerous to animals than clinical signs. As they are not physically evident, the animal continues to reproduce and grow at a slower rate. On the other hand, clinical signs bring attention to the producer as they quickly become visible, thereby making it easier for the producer to act in terms of supplementing the mineral deficiency. Table 2.2 shows a summary of some essential micro minerals and their deficiencies in animals.

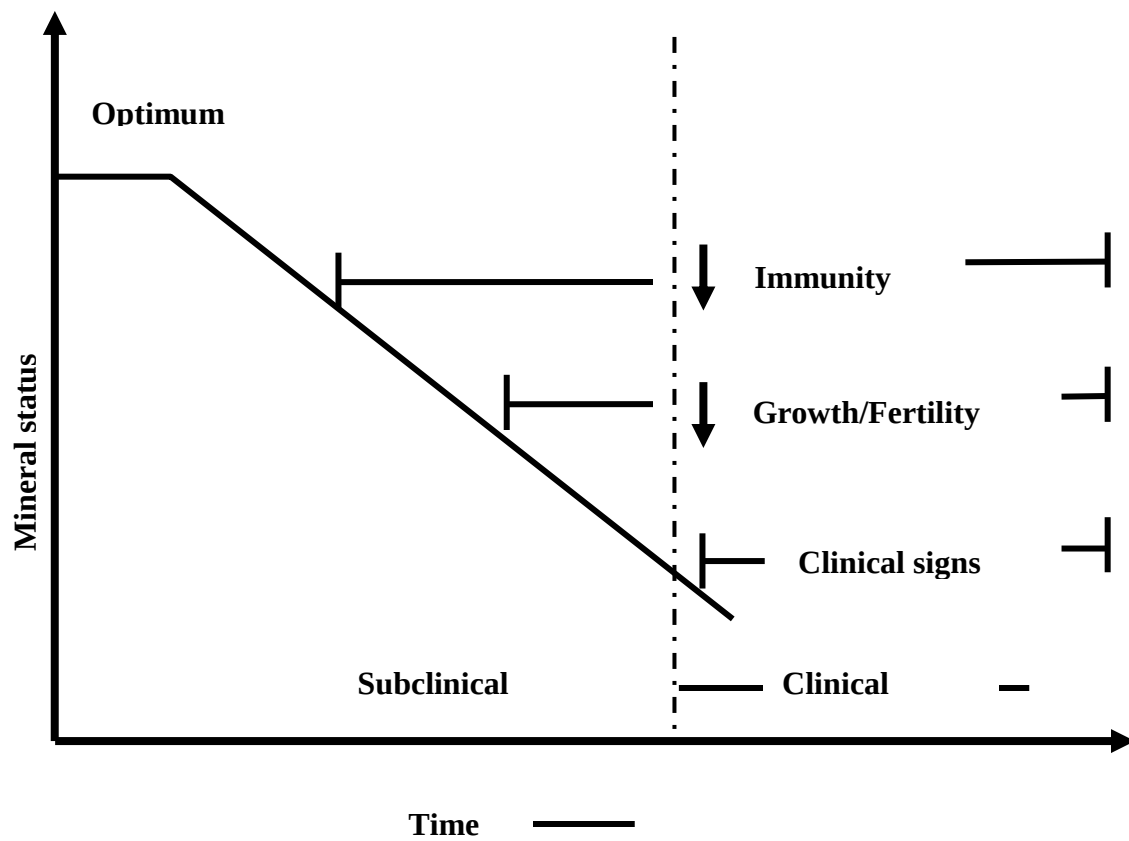


Figure 2.1: Effect of decreasing trace mineral status on animal performance (Wikse, 1992)

Table 2.3: Micro minerals and mineral deficiencies in animals

Micro minerals	Mineral deficiencies
² Selenium	Nutritional muscular dystrophy or commonly known as the White muscle disease
¹ Iron	Anaemia
¹ Copper	Falling disease in calves, inhibited growth and development, bone mineralisation, bone abnormalities, stiffness, anaemia, scouring, infertility, depigmentation of hair and wool, gastrointestinal disturbances, and lesions in the brain stem and spinal cord.
² Zinc	Skin peeling, scabbing, itching and hair loss
¹ Manganese	Lack of muscle control in new-born, abnormal skeletal system, retarded growth and failing reproduction.
¹ Vanadium	Impaired growth and reproduction and disturbed lipid metabolism

¹McDonald *et al.*, 2002; ²Radwinska and Zarczynska, 2014

2.2. Effect of mineral content in animal tissues

Bone is a vital tissue in the animal skeleton, with the structural function being the most important. This includes organ protection, locomotion, muscle activity, and load-bearing functions. Bone also serves as a reservoir for minerals. Nutrition is the main factor affecting the composition of bone. According to Bleich (1990), French *et al.* (1956) and Robbins (1993), bone breakability is highly associated with mineral imbalances as bones become weak and porous when there is a shortage of minerals. This can have an impact on the bone size and degree of mineralization, which in turn can affect mechanical performance (Burr, 2002; Olguin *et al.*, 2013). Mineral levels in animal diets can also contribute towards the mechanical performance of bones. This can range from macro minerals, Ca and P to micro minerals, including Mg, Mn, Cu, S and Zn (Corah, 1996). Deficiencies of these minerals can lead to weak bones and result in abnormalities such as poor bone mineralization, rickets, bone fracture and osteoporosis (Olguin *et al.*, 2013).

Of the body tissues, liver and bone have proved especially useful in anticipating mineral disorders in livestock since they are storage locations for certain minerals (Underwood and Suttle, 1999). The liver is a very important organ that is involved in a variety of functions such as metabolism of fat, carbohydrate, protein, vitamins, and minerals, excretion of waste products and detoxification (Zaefarian, 2019). It is a major storage location for fat-soluble vitamins (A, D, K, and E) as well as vitamin B12 and glycogen. It is also involved in the activation of vitamin D. Amongst these many functions, the liver also acts as a storage site for numerous minerals and is an important indicator of chronic mineral deficiencies and toxicities (Johnson *et al.*, 2007). According to Smit (2004), game meat consists of several mineral elements, which include P, Mg, Zn, Fe, Na, Cu, and Ca. The liver, kidney, heart, lean meat, fish, and poultry are considered a

good source of iron; lack of which causes anaemia in humans and is the most common of all nutritional deficiencies (Smit, 2004).

Mineral deficiencies can also occur in muscles, and these deficiencies can cause diseases in animals. The shortage of Selenium (Se) which leads to nutritional muscular dystrophy (white muscle disease) is an example. According to Beytut *et al.* (2002), white muscle disease involves hyaline production of skeletal muscles in different parts of the animal's body, heart muscle, tongue, and the diaphragm. This disease is mostly seen in domestic animals younger than six months.

2.3. Factors affecting mineral concentration.

2.3.1 Species

An important aspect of adaptability is concerned with local breeds and species that, through the centuries, have accustomed themselves to a certain dietary and management regime. Any sudden change, such as increased body size, will result in a higher maintenance and production requirement. This gives rise to a need for more nutrients, which, if not supplied, will cause a general upset in the animal's metabolism as it attempts to meet these demands (McDowell, 1985). For instance, it is common for cattle introduced into a new area to exhibit signs of mineral deficiency, while indigenous breeds that are usually slow-growing and late-maturing do not show the deficiencies to a similar degree. In addition, the possibility that non-adapted cattle to certain climates that sweat copiously in high temperatures and lose saliva and mucus from the mouth may lose substantial quantities of minerals, particularly in dry areas (McDowell, 1985). Table 2.4 is a summary of how different domesticated and game animals differ in muscle mineral concentration.

Table 2.4: Comparison of mineral concentration in muscles of game and domesticated animals

Minerals (mg/kg)	Species			
	Pigs ³	Impala ¹	Cattle ²	Barbari kids ⁴
Iron (Fe)	0.02	112.22	37.00	37.70
Potassium (K)	3668.41	7399.00	1470.00	344.90
Sodium (Na)	329.35	643.75	460.00	700.30
Zinc (Zn)	16.13	82.20	-	41.50

¹Hoffman *et al.*, 2005; ²Srinivasan *et al.*, 1998; ³Stasiak *et al.*, 2017; ⁴Umaraw *et al.*, 2015

2.3.2 Age

Animal age is considered an important factor that determine mineral requirements. Increasing age may result in a decline in dietary requirements as the main requirement for growth for a given live weight gain often remains constant, while appetite increases in proportion to body size. These growth changes result in the need for the trace elements Fe and Zn being greatest since their requirements for growth are particularly high (Cunha and McDowell, 2012). Likewise, Se and I deficiencies occur in the offspring of clinically normal-appearing dams. Thus, when other factors (particularly absorption) are constant, the young ruminant is more vulnerable to trace-element deficiencies (Cunha and McDowell, 2012). The older animal also has the advantage of being more resistant to mineral excesses.

Young animals have the advantage of being more efficient in digesting specific nutrients as compared to mature animals, particularly pertaining to absorption efficiency (McDowell, 1985) and the mobilisation of minerals between organs within the body. As example, Rook and Storry (1962) found that approximately 30% of skeletal Mg in young ruminants can be mobilized under conditions of Mg deficiency while in adult animals, only 2% of bone Mg can be used for physiological needs. This is most apparent in that older cattle are much more susceptible to grass tetany.

2.3.3 Physiological status of the animals

Mineral requirements are highly dependent on the level of productivity and physiological state of the animal (McDowell, 1985). For instance, an in-calf heifer in her first lactation would have significantly higher mineral requirements than a mature dry cow. Cows that produce high yields of milk would naturally need more dietary Ca and P than low yielding cows because of the

richness of these elements in milk (Cunha and McDowell, 2012). Improved management practices that lead to improved milk production and growth rates for grazing ruminants will necessitate more attention to mineral nutrition (McDowell, 1985).

2.4.4 Sex

Female ruminants normally mature earlier than the males (Ledger, 1963) and deposit more intramuscular fat, particularly during the gestation period. This, therefore, increases the dietary requirement for pregnant animals. In game animals, males can lose substantial amounts of body fat during the rut, due to reduced feed intake and the energy spent in fighting for dominance and maintaining the harem (Hoffman, 2000). According to Ortega-Barrales and Fernández-de Córdova (2015), sex does not seem to have a major influence on the concentrations of minerals in muscle/meat. Iron is contained in the myoglobin protein and according to Hoffman *et al.* (2005) high myoglobin concentration in muscles is due to systemic exercises. This would, therefore, mean that an increase in myoglobin results in an increase in iron concentration; such would mostly be seen in mature bull animals due to their frequent exposure to physical exercises, particularly during the rut.

2.3.5 Nutrition

Nutrition is by far the most important factor that affects mineral intake and mineral requirements. Providing animals with adequate forage is advisable to ensure that all mineral requirements are met. Forage intake can be reduced by factors such as the amount of protein (<7.0%) and increased degree of lignification in the plants which then results in a decrease in the entire amount of minerals consumed. According to McDowell (1985), the mineral concentration of animals during the dry season is lower when compared to the rainy season as tropical forages

contain fewer minerals during the dry season. However, McDowell *et al.* (1984) reported that minerals are mostly limiting during the rainy season in countries such as Kenya, Brazil, and South Africa.

Nutrition not only includes diets that are given to animals directly but also focuses on the naturally occurring vegetation where animals graze and/or browse freely. South Africa is characterised by nine different biomes namely. Neethling *et al.* (2016) note that wild animal species in South Africa are primarily dependant on the natural vegetation for feed, however, South African vegetation varies in mineral nutrition and concentration. For example, mineral concentration in the grassland biome cannot be the same as that in the Savanna biome as these two biomes have different vegetation and soil types. Grassland consists of grasses only, whilst the Savanna has a combination of a grassy ground layer and an upper layer with woody plants (Huntley, 1984).

Furthermore, because game farming is increasing in South Africa and is mainly practiced for breeding purposes, trophy hunting, and meat production, producers need to pay more attention to nutrition as it not only affects mineral concentration within the organs but also the breeding performance and health of the animals. Formulated feed diets should contain all the necessary nutrients required to maintain growth, good health, and reproduction. If any mineral deficiencies occur, the applicable levels of mineral supplements in the feed should be revisited.

2.3.6 Water availability

Water is by far the most important component after oxygen, to maintain life processes. The Earth's surface is covered by about 95% of water but only 2.5% is available for consumption (FAO, 2006). Agriculture accounts for 70% of the global water withdrawals (FAO, 2012) and animal production comprises 29% of the water used in agriculture (Mekonnen and Hoekstra, 2012). An inadequate supply of water can be a danger to the health of an animal and can also cause a reduction in the animal's performance. Animal water supplies typically have a very low concentration of macro minerals and provide only a few grams of each mineral per day under normal water consumption rates (Norell and Chahine, 2014). Nonetheless, water can provide 50% or more of the animal's daily requirements for some of the minerals (Norell and Chahine, 2014). In addition, ration adjustments may be required if the animal's water supply has high mineral contents.

2.4 Soil-plant-animal relationship

The mineral concentration in animals is dependent on the soil and plant associations. The mineral content of natural vegetation is primarily determined by the mineral availability and composition of the soil (Smart *et al.*, 1981). Mineral concentrations of soils influence the concentrations within the plants and as a result, it affects the mineral concentrations in the muscle tissue of the animals grazing on the plants (McDowell and Conrad, 1977; Zomborszky *et al.*, 1996). Extent and pattern of mineral deficiencies/excess in plants vary in different agro-climatic conditions as available mineral content in green vegetation depends on physical and chemical properties of soil, soil erosion, cropping pattern, fertilizer/chemical application, species and genetic variation in plants, stage of maturation as well as the presence of other minerals

(McDowell *et al.*, 1983). Studies on the soil-plant-animal relationships with respect to minerals are important and can have significant implications in animal production (Biswas and Samanta, 2002).

2.5 Effect of production systems on the mineral content of game species.

Similar to livestock animals, game animals are also maintained and reared in different production systems (intensive, semi-extensive, and extensive production systems) depending on the production end goal. Intensive farming involves keeping animals in small paddocks, thereby restricting their movement. It aims for high production on the smallest possible land area frequently making use of strategic supplementary feeding and breeding programs (Taylor *et al.*, 2016). The feed is supplemented according to the body's requirement or a certain deficiency. This production system is predominantly practiced in areas that are compactly populated and where grazing land is limited. Animal feed can either be purchased or grown on the farm (Mubiru, 2013). The intensive production system aims to maximise animal production and thus increase the nutritional value of the animal and the volume of meat. From a nutritional perspective, the addition of mineral supplements in animal diets is of great importance to avoid animal losses due to these deficiencies.

A semi-extensive production system involves the utilization of both intensive and extensive production systems. The systems allow for grazing in larger camps but also provide supplementation when necessary (Van Heerden, 2018). For example, in times of drought and when grazing land is limited, additional feed is supplemented. Moreover, when the animals are unhealthy due to mineral deficiencies, that particular mineral can be supplemented by means of salt licks or mixing supplements with the fed diets.

In an extensive production system, animals are allowed to graze freely on natural vegetation with no supplementation provided (Van Heerden, 2018). Animals walk long distances in search of water and feed. Losses of animals can result due to exposure to diseases because of reduced management practices, which results in poor meat quality. Animals are highly exposed to diseases such as milk fever, hypocalcaemia, grass tetany, ketosis, etc. The mineral concentration is expected to be lower for animals reared in this production system when compared to animals in the intensive and semi-extensive systems as they are not provided with supplements.

2.6 Supplementing to correct mineral imbalances.

In cases where shortages/deficiencies of a certain mineral occur, supplementation of that particular mineral would need to be provided to the animals. Mineral deficiencies can be corrected by supplementary feeding which can be achieved by means of a mineral or salt lick. As animals cannot synthesize their own minerals, plant and plant products are the main sources of these minerals, hence plant composition has an impact on the animal's mineral intake (McDonald *et al.*, 2002). All the factors that influence the mineral concentration in plants are therefore important (Underwood and Suttle, 1999). Minerals can be provided directly and/or indirectly to animals.

The direct administering of mineral includes oral drenching, salt licks, free-choice supplementation, the inclusion of deficient minerals in diets, injection, etc. Indirect supplementation focuses mainly on the soil and plants, this involves the inclusion of fertilisers, foliar sprays, actions to change the pH of the soil and availability of forages that have a high mineral content. Usually, diets for animals contain a mineral element supplement and, on occasions, it is necessary to include extra supplies of some minerals, such as Ca for laying hens.

Common sources of minerals used in mineral supplements are limestone for Ca, dicalcium phosphate (CaHPO_4) for P, common salt for Na, and calcined magnesite for Mg (McDonald *et al.*, 2002). Trace elements are usually supplied in a salt form, e.g. Se as sodium selenite (McDonald *et al.*, 2002).

There is insufficient information regarding the mineral requirement for most game farmed species such as the bontebok, blesbok, and impala in South Africa. There is limited information on their base-line concentration of macro and micro minerals.

2.7 Species description

2.7.1 Bontebok (*Damaliscus pygargus pygargus*)

Historically, the bontebok species were thought to be extinct, and only 27 individuals were in fact saved by Cape farmers in the Bredasdorp area in 1837 (Skinner and Chimimba, 2005). Corwell and Birss (2017) report that the statistics of the management of this species within the Natural Distribution Range (and Extended Distribution Range) in the Western Cape is approximately 1650 animals. Furthermore, Corwell and Birss (2017) also noted that about 7500 of these species were found in properties outside the native range of the species throughout South Africa. The bontebok species are currently used for trophy hunting (Furstenburg, 2016). Bontebok are tolerant of human activities and adapts reasonably well to changes in the environment.

Translocation of bontebok from Bontebok National Park has formed the nucleus of reintroduced populations in other protected areas such as provincial and local authority nature reserves. The bontebok is endemic to the Western Cape province of South Africa, although the introduction of the species has been made in other provinces. Bontebok were originally confined to the coastal plain (60–200 m) east of the Kogelberg in the Western Cape where they are believed to have concentrated on the renosterveld areas (Boshoff and Kerley 2001). Skinner and Chimimba (2005) mentioned that this antelope is a medium-sized species, with the females weighing slightly less than the males. It can be difficult to differentiate the female from the male bontebok in the field since they are similar in colour, with the males being slightly darker. The horns of the females are slenderer and hardly thicken up at the base (Skinner and Chimimba, 2005).

The Bontebok species is known to be a complete grazer (Beukes, 1984) and has a high preference for newly burnt and short grasses (Kraaij and Novellie, 2010). Water is an essential habitat requirement as they stay within 1.5 km from surface water during the dry season (Luyt 2005). Within their natural range on the coastal lowlands, grassy areas are predominantly found in the renosterveld areas but bontebok also makes use of small grassy microhabitats or recently burnt fynbos and strand-veld habitats (Radloff, 2008).

Due to the translocation of this species outside its natural range, anecdotal information seems to indicate that problems are being experienced with potential mineral deficiencies. One such problem is where the pelt becomes light (Suttle, 1992), even having a coppery hue to it which is thought to be caused by a copper deficiency. However, there are no baseline data for this species on what the concentration range of normal meat, liver and bone minerals should be. An ideal

area to evaluate what this could be is to analyse these samples in bontebok found in their natural range, the Renosterveld.

2.7.2 Blesbok (*Damaliscus pygargus*)

Blesbok's existence was first reported in plateau grasslands of the Highveld district in the 17th century; possibly due to the easy accessibility of water in that region (Skinner and Chimimba, 2005). This species is popular for meat production and trophy hunting. The distribution of blesbok included parts of western and north-western KwaZulu-Natal, the northern Karoo in the Northern and Eastern Cape, as well as the Highveld of the Free State and Gauteng (Lloyd and David, 2008). Blesbok are now found widespread throughout southern Africa (Skinner and Chimimba, 2005). They abundantly occur and in summer rainfall areas where farm ranches are privately owned. These regions include the Grassland Biome of the Free State, North West, Eastern Cape, Gauteng, Mpumalanga province and the western edge regions of Kwazulu-Natal province. However, Chase and Meadows, (2007) and Watson *et al.* (2011) reported that these species can also be found in coastal areas and in regions with no seasonal rainfall.

The bontebok (*Damaliscus pygargus pygargus*) and blesbok (*Damaliscus pygargus*) are closely related and can interbreed with viable hybrid offspring.

According to Smit (2004), the blesbok is mostly mistaken for the bontebok as they appear similar, colour, and structure even though the bontebok colours are more striking than that of the bontebok. Another characteristic that can be used to differentiate these two antelopes is the bald spot in the middle of the forehead on the blesbok species that the bontebok does not exhibit (Smit, 2004). Blesbok are exclusively grazers, feeding only on grass (Heyns, 2011). There seems

to be no evidence of any preference for certain plant communities, but the blesbok grazes less eagerly on unburnt areas (Du Plessis, 1972). Blesbok are similar to sheep in that they graze very close to the ground by gathering feed using its lips (Smit, 2004). Similar to bontebok, blesbok also show preference for newly burnt grasses during the early summer when the sprouts are not yet developed (Du Plessis, 1972). After the first heavy frost, usually, in May, blesbok enters a period of minimal activity, which lasts until the spring when the grass starts to sprout (Smit, 2004). Just like the bontebok, there is no baseline information about the mineral concentrations in the meat, bone or liver in this species.

2.7.3 Impala (*Aepyceros melampus*)

Impala are a pronghorn species with males (60-65 kg) relatively larger than females (40-45 kg) in terms of body size. The males are distinctively different from females as they have strong rigid lyre-shaped horns whilst the females have no horns (Jarman and Jarman, 1973; Lewis *et al.*, 1997). Skinner and Smithers (1990) and Hoffman (2000) had similar findings on the body weight of this species, where they reported that mature males weigh ± 50 kg whilst mature females weigh ± 40 kg. This antelope is reddish-brown in colour with a light brown patch on the upper body and has an untainted white colour underneath, at the belly section (Skinner and Smithers, 1990).

Impala occurs throughout the wooded grassland and open woodland biomes of Eastern, Central, and Southern Africa, and are mixed grazers/browsers (Jarman and Jarman, 1973; Cooper, 1982; Lewis *et al.*, 1997). They occur from northern Kenya south to northern Kwa-Zulu Natal in South Africa and extending westwards towards southern Angola (Skinner and Smithers, 1990). Despite their limited mobility, impala respond to the climatic variability of semi-arid

environments by grazing during wet seasons and browsing during drier periods (Laaka, 2014). In the northern parts of the sub-region, they are associated particularly with acacia and mopane, *Colophospermum mopane*, where the cover and the availability of surface water are essential habitat requirements (Laaka, 2014). Impala are intermediate mixed feeders.

2.8 Summary

Meat is fundamental to human health for proteins, vitamins, and minerals. Thus, it is essential to provide animals with balanced diets as failure to feed diets with adequate nutrition and required minerals can be a threat to animal and human health by increasing the chances of diseases. Mineral nutrition is of paramount importance to animals in maintaining a normal life process, normal growth, health, and reproduction. In cases where deficiencies of certain minerals arise, it is advisable to add supplements in diets or provide a mineral lick before the animal starts showing clinical symptoms. Selecting and using the correct mineral supplements can save the farmer from animal losses and can contribute to increased animal production. However, over supplementation can lead to unnecessary mineral excretion, or even poisoning, hence it is important to provide a proportion that is according to the animal's mineral requirement.

Before any mineral can be supplemented, knowledge is required to determine which mineral is limited. However, very little information exists on what is the normal range of the minerals for the three species mentioned. Each of the species plays an important role in the South African game ranching industry. The bontebok is a grazer that was historically found in the Rensoterveld region in the Cape Province whilst the closely related blesbok, also a grazer was found to be abundant in the grassland regions. The impala is one of the most widely distributed and hunted species in South Africa and is a mixed feeder grazing grass and browsing. It also has the ability

to adapt its specific eating habits to what is available. Yet, little information exists on the mineral range found within its body.

The muscle, liver, and bone all play an important role in the mineral concentration within an animal's body and thus an analysis of the mineral profile of these animal samples in these three species will be of value in providing base-line information on what is a normal range for these species.

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Chapter 3: Bone, liver, and muscle mineral concentration of impala (*Aepyceros melampus*) reared in different regions/production systems.

Abstract

The effect of region/production system on the mineral concentration in liver, muscle and rib samples of impala was investigated. Impala from intensive (INT) and semi-extensive (SE) production systems in Limpopo, an extensive production system from the Western Cape (EXT (W)) and an extensive production system in Namibia (EXT (N)) were compared. Proximate analysis was conducted on the muscle whilst mineral analyses were conducted on the bone, liver, and muscle. Differences were detected for the moisture, protein and fat contents between the regions/production systems. Muscle from the INT and SE regions/production systems had a higher ($P \leq 0.05$) moisture content to the muscle from EXT (W) and EXT (N), while protein content in the muscle from the EXT (N) region/production system was higher ($P \leq 0.05$) than from the other regions/production systems. Fat content was higher ($P \leq 0.05$) in the EXT (W) and SE production systems. There were no differences ($P > 0.05$) in the ash percentage for all region/production systems. Micro mineral (Boron (B), Aluminium (Al), Iron (Fe) and Cobalt (Co)) concentrations in the liver did not differ ($P > 0.05$) between region/production systems. Conversely, liver in the EXT (W) region/production system had higher ($P \leq 0.05$) concentrations of Se, Sr, Mo, Pb, and Si, whilst EXT (N) liver had higher concentrations of Vanadium (V), Chromium (Cr), Nickel (Ni), Cadmium (Cd), and Mercury (Hg). Higher concentrations of Manganese (Mn), Copper (Cu), Zinc (Zn), and Arsenic (As) in the liver were observed in the INT production system compared to livers from other region/production system. Liver in the SE production system had a high concentration of Barium (Ba). Macro minerals in the liver were

found to be high in the EXT (W) region/production system. On the other hand, concentrations of Cobalt (Co), Nickel (Ni), Strontium (Sr), Cadmium (Cd), and Silicon (Si) in the muscle did not differ ($P>0.05$) between regions/production systems. However, higher micro minerals (Al, V, Cr, Mn, Cu, Zn, As, Mo, Ba, and Pb) were detected in muscle from the EXT (N) region/production system whilst EXT (W) muscle had higher Fe and Se levels. The results showed no difference ($P>0.05$) for concentrations of Cr, Mn, Fe, Co, Cu, As, Se, Cd, and Sb in the rib throughout all the regions/production systems. The rib had very high concentrations of Fe, Zn, Sr, and Si micro minerals. Higher concentrations of all macro minerals in the rib were recorded for the EXT (W), INT, and SE regions/production systems. The results showed that region/production system does have an effect on the concentration of minerals in these animal samples.

Keywords: Game animals; production systems; animal samples; proximate composition; mineral profile

3.1 Introduction

Game farming has developed into an established agricultural industry in South Africa (Hoffman *et al.*, 2005a). This has resulted in an increase in the production of game meat; Taylor *et al.* (2016) reported a total mass of 21.22 million kg of game meat produced from wildlife ranches in 2014 through culling and trophy hunting. Such contributions reflect positively on the economy. According to Child *et al.* (2012), since there has been substantial growth in game farming in southern Africa, a large proportion of land used for livestock has been converted to accommodate game animals. Impala traits enable it to survive in different habitats (Skinner and Smithers, 1990) resulting in a large dispersal of this species in southern Africa. According to Bourgarel *et al.* (2002) and Hoffman (2000) the large dispersal of the impala, together with their relative abundance as a species, has resulted in the impala being well suited to maintain yearly cropping regimes. Impala are categorised as intermediate, mixed feeders amongst wild ruminants (Laaka, 2014). Fairall (1983) reported that the browsing habits of the impala make them suitable to the South African bush-veld as they browse on trees and shrubs.

The impala is a selective grazer that prefers feeding on fresh grass shoots (during the wet season), herbs and shrubs (Laaka, 2014). According to Jarman and Jarman (1973), impala has an increased feed intake in arid seasons as compared to wet seasons and the female impala reach their asymptotic mass of 43.2 kg at the age of ± 3 years whilst at the age of 4.5-5 years, males weigh 56.6 kg (Van den Berg, 2009). The Serengeti area in Tanzania has been reported as having larger and heavier impala in all body measurements than impala from southern Africa (Fairall and Braack, 1976). Many factors such as season, habitat, nutrition (including water availability), and age might be contributing factors for these differences; nutrition being the most important factor as it includes mineral nutrition which plays a significant role in animal growth. Minerals

are all the elements that are needed by animals for normal life processes such as animal health, growth, and reproduction (Mirzaei, 2012). Minerals are categorised into micro and macro minerals and all are said to be essential to animals (McDonald *et al.*, 2002). Many of the micro minerals (Zn, Fe, B, As, Cr, Mn, and V, amongst others) are stored in the internal organs with the liver being the main storage location for most of these minerals (Johnson *et al.*, 2007). Macro minerals (Ca, Mg, K, P, S, and Na) are mainly found in the skeletal system. According to McDonald *et al.* (2002), minerals can either be needed in large or small quantities depending on the animal's requirements. They can be obtained from feedstuff (through grazing/browsing or through supplied diets and/or by supplementation), from water and from the soil. Very little to no research has been conducted on the mineral requirements of game animals. Mineral concentration in animals differs with anatomical location (Laaka, 2014). Findings have shown high micro mineral concentrations in the liver and bones whilst macro minerals were mostly reported to be high in bones. However, no research has been conducted on the effect of production systems on mineral concentrations in game animals particularly the impala species.

There is, therefore, a need to carry out further research in this area so as to have a better understanding of the mineral status of game animals. The purpose of this study is to determine the mineral composition in the liver, muscle and rib of impala reared in different regions/production systems so as to provide base line data for animal nutritionists and veterinarian practitioners.

3.2 Methods and materials

3.2.1 Study site

Impala were harvested from three different regions and three production systems. The regions were Castle de Wildt in Limpopo, Mount Etjo game reserve in Namibia and Bredasdorp, Western Cape. The impalas from Limpopo were hunted in both an intensive (INT) and semi-extensive (SE) production systems which were located in the Savanna biome. The intensive production system had small camps and supplementary feed was provided to the animals whilst the semi-extensive production system had large camps and the animals depended primarily on natural vegetation with minimal supplementary feed. Bredasdorp is a region situated in the Fynbos biome of the Western Cape, and the animals grazed extensively (EXT W) on natural vegetation with no supplementation. Mount Etjo game farms in Namibia practice an extensive production system (EXT N) with large camps and only natural vegetation as feed. This farm is located in the North of Namibia, in the Otjozondjupa region which comprises of thorn-bush vegetation, with mainly acacia trees.

Six ram animals of similar age ($\pm$ 15 months old) were harvested from each production system. Ethical clearance was approved at the University of Fort Hare (MUC081SMPA01), Eastern Cape province of South Africa.

3.2.2 Experimental procedure

The harvesting of animals was according to South African standard procedures (Hoffman, 2000). All animals were hunted during the day with light calibre rifles. These animals were shot in the head and the jugular vein was severed for exsanguination after the animal had dropped.

Thereafter they were taken to a pre-prepared slaughter area for carcass dressing which involves removal of internal organs and animal hides.

3.2.3 Sampling procedure

All internal organs were removed from the carcass immediately after slaughter. The liver and *Longissimus thoracis et lumborum* (LTL) muscle were vacuum packed and stored in a -20°C freezer until analysed. The last rib was cut from each animal, cleaned of excessive meat and stored in plastic bags in a -20°C freezer until defatting and analysis.

3.2.4 Defatting of bone for analysis

The rib bone samples were thawed overnight at 4°C prior to defatting. The porcelain crucibles for all samples were heated to 100°C, then were left in a desiccator for half an hour to cool prior to weighing. Bone samples were weighed and dried at 100°C for 24 hours. After 24 hours, samples were taken out of the oven and cooled in the desiccator for half an hour. Sample weights were then recorded. The dried bones were then defatted by being placed in an 800 ml Erlenmeyer flask where 10 ml of petroleum ether was poured into the flask. After 24 hours, the bone fat layer was removed, and the flask was again topped up with the petroleum ether. After 48 hours, the petroleum ether was discarded once more, and samples were allowed to rest for 2 hours before being dried at 100°C for 24 hours and bone samples were weighed again after the second drying. Bone samples were then placed in a furnace for 24 hours at 600°C. Samples were taken out of the furnace and allowed to cool in a desiccator for 40-60 minutes. Sample weights were taken. The samples were then crushed using a mortar and pestle and the ash was weighed. The samples were then kept in a drying oven at 60°C to prevent them from absorbing moisture until they were analysed for mineral analysis.

3.2.5 Chemical analysis

3.2.5.1 Acid digestion

After all the meat and liver samples were homogenised and the bone samples ashed, samples were digested at the Soil Science Department at Stellenbosch University. One g of the sample was weighed into appropriate vessels whereafter 1ml of concentrated peroxide and 6ml of concentrated nitric acid was added and the sample left to predigest for 20min before sealing the vessels for microwave digestion in a MARS microwave digester (model MARS 5) (CEM cooperation, 3100 Smith farm road, Matthews, NC 28104). The power level of the microwave was 1600W at 100%. The samples were heated for 25 minutes from 20 to 210°C at a pressure of 800 psi. Samples were then diluted 10x with Deionised water into acid cleaned sample tubes. Nine ml of concentrated hydrochloric (HCL) acid was measured into 20ml centrifuge tubes, 1ml of the digested solution was added into the tubes. The samples were then ready for mineral analysis.

3.2.5.2 Macro mineral analysis

Macro elements (Ca, K, Mg, Na, and P) were analysed on a Thermo ICap 6200 ICP-AES (Thermo Fisher Scientific Inc 168 Third Avenue in Waltham, Massachusetts). The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards purchased from Inorganic Ventures (Inorganic Ventures, 300 Technology Drive, Christiansburg, VA 24073) to quantify selected elements. Throughout the analysis and prior to sample analysis, a NIST-traceable quality control standard (De Bruyn Spectroscopic Solutions, Bryanston, South Africa) was analysed to verify the accuracy of the calibration so as to monitor drift.

3.2.5.3 Micro mineral analysis

An Agilent 7900 quadrupole ICP-MS was used to analyse micro minerals (B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sb, Ba, Hg, Pb, and Si). This was achieved by first calibrating the instrument, using NIST traceable standards from Inorganic Ventures (Inorganic Ventures, 300 Technology Drive, Christiansburg, VA 24073) to quantify selected elements. A NIST-traceable quality control standards at high and low concentration levels (De Bruyn Spectroscopic Solutions, Bryanston, South Africa) were analysed to verify the accuracy of the calibration before sample analysis, as well as after every 12 samples to monitor drift.

Samples were introduced via a 0.4ml/min micro mist nebulizer into a peltier-cooled spray-chamber at a temperature of 2°C. The instrument was optimised for analysis in HMI mode, where all samples and standards are diluted with argon gas to minimise matrix load to the analyser. Oxide formation was less than 0.3%. Three replicate measurements with appropriate dwell times were carried out on each analyte. All elements were measured in the He-collision mode. An internal standard (ISTD) solution containing Sc, Y, Ge, Rh, and In was introduced online to monitor instrument drift and correct for matrix differences between samples and standards. Appropriate ISTD's were matched to analytes according to their proximity in mass and ionisation potential. During the course of the analysis, ISTD recovery was between 90 and 110% for all samples, and recovery for drift monitor standards between 95 and 105%. Table 3.1 indicates the limit of detection (LOD) for the specific elements.

Table 3.1: Micro and macro mineral's limit of detection (LOD)

Micro minerals		Macro minerals	
(ug/kg)	LOD value	(ug/kg)	LOD value
B	966.5	Ca	7.5
Al	65.7	K	7.5
V	3.6	Mg	7.5
Cr	19.8	Na	7.5
Mn	9.6	P	7.5
Fe	25.0		
Co	3.9		
Ni	5.9		
Cu	9.0		
Zn	7.2		
As	5.6		
Se	3.3		
Sr	16.1		
Mo	22.9		
Cd	3.5		
Sb	4.5		
Ba	4.1		
Hg	1.2		
Pb	3.4		
Si	7.5		

B – Boron; Al – Aluminium; V – Vanadium; Cr – Chromium; Mn – Manganese; Fe – Iron; Co – Cobalt; Ni – Nickel; Cu – Copper; Zn – Zinc; As – Arsenic; Se – Selenium; Sr – Strontium; Mo – Molybdenum; Cd – Cadmium; Sb – Antimony; Ba – Barium; Hg – Mercury; Pb – Lead; and Si – Silicon.

3.2.6 Proximate analysis

The proximate analysis was only done on the *Longissimus thoracis et lumborum (LTL)* muscle samples. The samples were analysed to determine the moisture (Method 934.01) and ash (Method 942.05) content according to the AOAC (2002). The protein content was measured using the AOAC (1992) procedure 992.15, whereas the fat was determined using the chloroform/methanol (2:1) fat extraction method according to Lee *et al.* (1996). All analyses were performed in duplicate.

3.3 Statistical analysis

The experimental data was analysed using XLSTAT version 19.2 statistical program. Due to physiological differences between the sample types, production systems were compared using one way analysis of variance (ANOVA) for individual sample types of liver, muscle and rib. Bonferroni post-hoc test was used to identify the statistical significance at the 5% level with all results $P \leq 0.05$ considered to be statistically significant.

The following statistical model was used:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

Where: Y_{ij} is the response variable (minerals)

μ is the overall mean

α_i is the effect due to treatment (extensive system Namibia, extensive system Western Cape, intensive system Limpopo and semi-extensive system Limpopo)

ϵ_{ijk} is the error

3.4 Results

3.4.1 Proximate composition

Results in Table 3.2 show the effect of region/production system on proximate composition of the *Longissimus thoracis et lumborum* (LTL) of impala. The impala muscle from INT and SE regions/production systems had high moisture contents (75.4% and 75.5%, respectively) followed by impala muscle in the EXT (W) region/production system (74.7%) whilst the EXT (N) region/production system had the lowest moisture content (73.7%). The EXT (N) region/production system had the highest protein content (24.2%) that differed significantly ($P \leq 0.05$) from the protein content of other regions/production systems. There was no difference ($P > 0.05$) in protein content between the EXT (W) region/production system and the INT region/production system, and EXT (W) and SE regions/production systems. The fat content was highest in the SE region/production system (2.2%) and did not differ from the EXT (W) region/production system (1.9%). Fat was lowest in the INT region/production system (1.4%) and similar to the EXT (N) region/production system. There was no significant difference in the ash percentage for all regions/production systems ($P > 0.05$).

Table 3.2: Proximate composition ‘as is-basis’ (LSMeans $\pm$ SEM) of the impala *Longissimus thoracis et lumborum* muscle from different regions/production systems.

Proximate (g/100g)	chemical	Production system				P-values
		EXT (W)	EXT (N)	INT	SE	
Moisture		74.7 ^b $\pm$ 0.08	73.7 ^c $\pm$ 0.17	75.4 ^a $\pm$ 0.12	75.5 ^a $\pm$ 0.24	< 0.0001
Protein		22.4 ^{bc} $\pm$ 0.19	24.2 ^a $\pm$ 0.13	22.8 ^b $\pm$ 0.12	22.0 ^c $\pm$ 0.22	< 0.0001
Fat		1.9 ^{ab} $\pm$ 0.16	1.7 ^{bc} $\pm$ 0.06	1.5 ^c $\pm$ 0.05	2.4 ^a $\pm$ 0.06	0.000
Ash		1.2 $\pm$ 0.03	1.2 $\pm$ 0.01	1.2 $\pm$ 0.01	1.2 $\pm$ 0.02	0.873

^{a,b,c} Means with different superscripts in the same row differ significantly from each other ($P \leq 0.05$). EXT – extensive production system Western Cape; EXT (N) – extensive production system Namibia; INT – intensive production system Limpopo; SE – semi-extensive production system Limpopo; SEM – Standard error of mean

3.4.2 Macro mineral concentrations in impala liver, muscle and rib from different regions/production systems.

Results from Table 3.3 show the effect of region/production system on macro mineral concentration in impala liver, muscle and rib. The findings showed significant ($P \leq 0.05$) concentrations of Ca and Na in the liver from the EXT (W), INT and SE regions/production systems, whilst the Ca concentration in the liver from SE was similar ($P > 0.05$) to that in the EXT (N) region/production system. A significant concentration of K was detected in the liver from EXT (W), EXT (N) and SE regions/production systems; however, the liver from the SE region/production system was similar ($P > 0.05$) to the liver in the INT region/production system. Mg was high ($P \leq 0.05$) in the liver from EXT (W), EXT (N) and INT regions/production systems, and the liver from EXT (N) and INT were similar ($P > 0.05$) to that in the SE region/production system. The liver from EXT (W) and EXT (N) regions/production systems had high ($P \leq 0.05$) P concentration, although EXT (N) was similar ($P > 0.05$) to INT and SE regions/production systems. Additionally, the liver from the EXT (W) production system had numerically high macro mineral concentrations than all the other production systems. The region/production system did not affect ($P > 0.05$) any of the macro mineral concentrations in the muscle. K was the most dominant muscle mineral followed by P. The rib in EXT (W), INT and SE regions/production systems all had significantly higher ($P \leq 0.05$) macro mineral concentrations compared to the rib in EXT (N) region/production system.

Table 3.3: Macro mineral concentrations (g/100g sample as is) (LSMeans $\pm$ SEM) in impala liver, muscle and rib from extensive production system Western Cape [EXT (W)], extensive production system Namibia [EXT (N)], intensive production system Limpopo (INT) and semi-extensive production system Limpopo (SE).

Production system					
	EXT (W)	EXT (N)	INT	SE	P - value
Liver					
Ca	0.008 ^a ± 0.000	0.005 ^b ± 0.000	0.007 ^a ± 0.001	0.006 ^{ab} ± 0.001	0.002
K	0.410 ^a ± 0.028	0.407 ^a ± 0.011	0.322 ^b ± 0.025	0.328 ^{ab} ± 0.013	0.006
Mg	0.024 ^a ± 0.002	0.021 ^{ab} ± 0.000	0.021 ^{ab} ± 0.001	0.019 ^b ± 0.001	0.020
Na	0.168 ^a ± 0.014	0.096 ^b ± 0.001	0.154 ^a ± 0.017	0.122 ^{ab} ± 0.008	0.001
P	0.561 ^a ± 0.032	0.492 ^{ab} ± 0.004	0.444 ^b ± 0.030	0.423 ^b ± 0.011	0.002
Muscle					
Ca	0.004 ± 0.000	0.005 ± 0.000	0.004 ± 0.001	0.004 ± 0.000	0.135
K	0.451 ± 0.010	0.460 ± 0.005	0.450 ± 0.006	0.445 ± 0.014	0.710

Mg	0.033 ± 0.001	0.032 ± 0.000	0.031 ± 0.001	0.031 ± 0.001	0.127
Na	0.042 ± 0.001	0.041 ± 0.001	0.041 ± 0.001	0.044 ± 0.001	0.365
P	0.279 ± 0.008	0.281 ± 0.003	0.268 ± 0.004	0.268 ± 0.007	0.234
Rib					
Ca	292.45 ^{ab} ± 8.937	191.270 ^c ± 15.534	330.375 ^a ± 120.59	246.429 ^b ± 8.827	< 0.0001
K	41.610 ^a ± 1.752	24.128 ^b ± 1.244	51.893 ^a ± 3.816	43.783 ^a ± 3.433	< 0.0001
Mg	75.240 ^a ± 1.947	40.121 ^c ± 2.613	75.344 ^a ± 1.579	54.159 ^b ± 0.841	< 0.0001
Na	90.101 ^a ± 1.769	53.339 ^c ± 3.318	86.748 ^a ± 2.618	69.424 ^b ± 2.434	< 0.0001
P	135.059 ^{ab} ± 3.764	96.636 ^c ± 7.482	155.379 ^a ± 6.725	114.265 ^{bc} ± 4.608	< 0.0001

^{a,b,c} Means with different superscripts in the same row differ significantly from each other ($P \leq 0.05$). EXT – extensive production system Western Cape; EXT (N) – extensive production system Namibia; INT – intensive production system Limpopo; SE – semi-extensive production system Limpopo; SEM – Standard error of means; Ca – Calcium; K – Potassium; Mg – Magnesium; Na – Sodium; P – Phosphorus;

3.4.3 Micro mineral concentrations in impala liver, muscle and rib from different regions/production systems.

Results from Table 3.4 show the effect of region/production system on the micro mineral concentration in impala liver, muscle and rib. The concentration of B, Al, Fe, and Co in the liver was not influenced by region/production system. However, the liver from the EXT (N) region/production system had high ($P \leq 0.05$) V, Cr, and Cd concentrations. Mn concentrations were high ($P \leq 0.05$) in the liver from EXT (N), INT and SE regions/production systems. Ni concentration was high ($P \leq 0.05$) in the liver from EXT (W) and EXT (N) production systems, however, the liver from EXT (W) was not significantly different from that in the INT production system and liver from the INT production system was similar to that in the SE production system. Cu was high ($P \leq 0.05$) in the liver from the INT region/production system. The concentration of Zn was highest ($P \leq 0.05$) in the liver from INT and EXT (N) regions/production systems followed by EXT (W) and SE production systems. A high ($P \leq 0.05$) concentration of As was found in the liver from the INT, EXT (W) and EXT (N) regions/production systems though the liver from EXT (W) and EXT (N) regions/production systems had similar levels to that in the SE region/production system. Mo was higher ($P \leq 0.05$) in the liver from EXT (W) and EXT (N) regions/production systems whilst liver from EXT (N) had a similar concentration to the liver in the INT region/production system whilst that in the SE region/production system differed from those in other regions/production systems. Ba was highest ($P \leq 0.05$) in the liver from the SE region/production system. High ($P \leq 0.05$) concentrations of Hg were detected in the liver from the EXT (W) and EXT (N) regions/production systems. A high ($P \leq 0.05$) concentration of Si was found in the liver from the EXT (W), INT and SE regions/production systems; however, liver from the INT and SE regions/production systems did not differ from the liver in the EXT (N) region/production system.

Concentrations of Co, Ni, Sr, Cd, and Si in the muscle did not differ ($P>0.05$) between production systems. On the other hand, Al, V, Cr, Mn, Mo, Ba, and Pb concentrations were higher ($P\leq 0.05$) in the muscle from the EXT (N) region/production system compared to the other production systems. Fe concentration was high ($P\leq 0.05$) in the muscle from EXT (W) and EXT (N) regions/production systems, and muscle from the EXT (N) did not differ ($P>0.05$) from the muscle in the INT and SE regions/production systems. High ($P\leq 0.05$) concentrations of Cu were found in muscle from EXT (N) and EXT (W) regions/production systems, whilst the muscle from the EXT (W) region/production system was similar ($P>0.05$) to that in the SE region/production system which did not differ ($P>0.05$) from muscle in the INT region/production system. Zn was higher ($P\leq 0.05$) in the muscle from the EXT (N), EXT (W) and SE regions/production systems and was low in muscle from the INT region/production system. The concentration of As differed significantly in muscle from the EXT (N) and INT regions/production systems. The concentration of Se in the muscle varied in all regions/production systems with muscle from the EXT (W) having the highest ($P\leq 0.05$) concentration.

The results showed no difference ($P>0.05$) for Co, Ni, Cu, Se, Cd, and Sb concentrations between all the regions/production systems for the rib bone. However, B had a high ($P\leq 0.05$) concentration in the rib from EXT (W) and EXT (N) regions/production systems and was low ($P>0.05$) in the rib from INT and SE regions/production systems. Al, Mo and Pb had high ($P\leq 0.05$) concentrations in the rib from EXT (N) region/production system. High concentrations for V were detected in the rib from the INT and EXT (N) region/production system, with the exception that EXT (N) did not differ from SE and SE did not differ from EXT (W). Ribs from EXT (W), EXT (N) and INT regions/production systems had high concentrations of Cr; however, EXT (N) and INT were similar to SE. Mn was highest ($P\leq 0.05$) in the rib from EXT (N), INT and EXT (W) regions/production systems, however,

EXT (W) and INT did not differ ($P>0.05$) from SE. Significant ($P\leq 0.05$) differing Fe concentrations were recorded for the ribs from the EXT (W), INT and SE regions/production systems. Zn was high ($P\leq 0.05$) in the ribs from INT and EXT (W) regions/production systems, although, EXT (W) did not differ from SE ($P>0.05$) and SE was similar to EXT (N) ($P>0.05$). High concentration of As was recorded in the ribs from the EXT (W), EXT (N) and INT regions/production systems but EXT (W) and INT regions/production systems did not differ ($P>0.05$) from SE region/production system. Sr and Si had high concentrations in the rib from the EXT (W) region/production system. Ribs from the INT and SE regions/production systems had significant concentration of Ba.

Table 3.4: Micro mineral concentrations (mg/100g sample as is) (LSMeans $\pm$ SEM) in impala liver, muscle and rib from extensive production system Western Cape [EXT (W)], extensive production system Namibia [EXT (N)], intensive production system Limpopo (INT).

Micro minerals	Production system				P-value
	EXT (W)	EXT (N)	INT	SE	
	Liver				
B	5.12 ± 5.12	<LOD	<LOD	5.98 ± 5.980	0.580
Al	10.83 ± 4.3	8.1 ± 1.93	7.16 ± 1.38	3.19 ± 1.4	0.236
V	<LOD ^c	0.11 ^a ± 0.01	0.04 ^{bc} ± 0.01	0.05 ^b ± 0.01	< 0.0001
Cr	0.44 ^b ± 0.15	2.21 ^a ± 0.28	0.07 ^b ± 0.05	<LOD ^b	< 0.0001
Mn	26.78 ^b ± 0.97	43.13 ^a ± 0.84	44.85 ^a ± 4.33	39.41 ^a ± 1.73	0.000
Fe	1468.82 ± 162.33	1171.75 ± 121.07	1115.49 ± 183.1	873.66 ± 141.74	0.086
Co	1.04 ± 0.09	0.96 ± 0.04	1.74 ± 0.36	0.93 ± 0.15	0.030
Ni	0.24 ^{ab} ± 0.04	0.29 ^a ± 0.04	0.12 ^{bc} ± 0.04	<LOD ^c	< 0.0001
Cu	237.23 ^b ± 66.98	429.84 ^b ± 147.11	2562.73 ^a ± 920.2	368.07 ^b ± 102.94	0.009
Zn	363.78 ^b ± 23.5	377.49 ^{ab} ± 12.99	464.96 ^a ± 37.21	312.33 ^b ± 11.51	0.002
As	0.11 ^{ab} ± 0.01	0.13 ^{ab} ± 0.02	0.19 ^a ± 0.06	0.01 ^b ± 0.01	0.007
Se	9.79 ^a ± 0.69	6.01 ^b ± 0.49	4.94 ^b ± 1.39	1.46 ^c ± 0.17	< 0.0001
Sr	0.85 ^a ± 0.05	0.53 ^b ± 0.08	0.45 ^b ± 0.04	0.043 ^b ± 0.002	< 0.0001
Mo	17.29 ^a ± 1.21	13.23 ^{ab} ± 1.07	11.62 ^b ± 1.12	7.47 ^c ± 0.31	< 0.0001
Cd	0.08 ^b ± 0.01	0.26 ^a ± 0.03	0.04 ^b ± 0.01	0.07 ^b ± 0.01	< 0.0001

Sb	<LOD	<LOD	<LOD	<LOD	-
Ba	0.4 ^c ± 0.05	0.71 ^{bc} ± 0.06	0.88 ^b ± 0.12	1.93 ^a ± 0.18	< 0.0001
Hg	0.03 ^a ± 0.01	0.04 ^a ± 0.001	<LOD ^b	<LOD ^b	< 0.0001
Pb	1.05 ^a ± 0.11	0.45 ^b ± 0.08	0.31 ^b ± 0.05	0.17 ^b ± 0.08	< 0.0001
Si	88.82 ^a ± 31.03	<LOD ^b	71.81 ^{ab} ± 22.9	17.12 ^{ab} ± 17.12	0.021

Micro minerals	EXT (W)	EXT (N)	INT	SE	P-value
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Liver

B	<LOD	<LOD	<LOD	<LOD	-
Al	1.38 ^b ± 0.92	1.36 ^a ± 2.1	1.31 ^b ± 1.31	0.79 ^b ± 0.52	0.000
V	<LOD ^b	0.08 ^a ± 0.00	<LOD ^b	<LOD ^b	< 0.0001
Cr	<LOD ^b	2.31 ^a ± 0.31	0.49 ^b ± 0.32	0.38 ^b ± 0.31	< 0.0001
Mn	1.13 ^b ± 0.07	1.59 ^a ± 0.1	1.2 ^b ± 0.03	1.31 ^b ± 0.04	0.000
Fe	291.68 ^a ± 9.03	275.34 ^{ab} ± 9.25	247.12 ^b ± 7.6	240.96 ^b ± 1.00	0.002

Co	0.01 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.836
Ni	0.51 ± 0.21	1.3 ± 0.51	0.32 ± 0.16	0.33 ± 0.19	0.100
Cu	16.64 ^{ab} ± 0.87	19.06 ^a ± 0.8	12.95 ^c ± 0.38	13.85 ^{bc} ± 0.54	< 0.0001
Zn	123.94 ^{ab} ± 5.12	142.51 ^a ± 4.77	108.06 ^b ± 3.41	123.82 ^{ab} ± 11.56	0.020
As	<LOD ^b	0.05 ^a ± 0.01	0.03 ^a ± 0.02	<LOD ^b	0.000
Se	2.2 ^a ± 0.07	1.54 ^b ± 0.1	1.03 ^c ± 0.02	0.7 ^d ± 0.06	< 0.0001
Sr	0.25 ± 0.02	0.42 ± 0.08	0.17 ± 0.17	0.27 ± 0.02	0.297
Mo	<LOD ^b	0.32 ^a ± 0.05	<LOD ^b	0.05 ^b ± 0.05	< 0.0001
Sb	<LOD	<LOD	<LOD	<LOD	-
Cd	0.02 ± 0.01	0.02 ± 0.00	<LOD	<LOD	0.052
Ba	0.22 ^b ± 0.02	0.72 ^a ± 0.07	0.18 ^b ± 0.08	0.29 ^b ± 0.03	< 0.0001

Hg	<LOD	<LOD	<LOD	<LOD	-
Pb	$0.17^b \pm 0.02$	$0.26^a \pm 0.03$	$0.13^b \pm 0.04$	$0.26^b \pm 0.07$	0.125
Si	59.37 ± 18.79	<LOD	63.55 ± 20.13	67.27 ± 21.28	0.039

Micro minerals	EXT (W)	EXT (N)	INT	SE	P-value
Rib					
B	79.522 ^a ± 11.191	80.40 ^a ± 5.74	44.787 ^b ± 8.373	26.895 ^b ± 5.185	0.000
Al	22.904 ^b ± 5.211	66.95 ^a ± 1.347	19.182 ^b ± 2.019	14.938 ^b ± 2.684	< 0.0001
V	0.034 ^c ± 0.009	0.144 ^{ab} ± 0.013	0.169 ^a ± 0.026	0.095 ^{bc} ± 0.011	0.000
Cr	3.409 ^a ± 0.783	2.770 ^{ab} ± 0.153	2.233 ^{ab} ± 0.658	0.628 ^b ± 0.138	0.016
Fe	1372.964 ^a ± 135.351	667.202 ^b ± 51.046	1528.885 ^a ± 131.936	1314.011 ^a ± 89.401	< 0.0001
Co	0.191 ± 0.053	0.120 ± 0.013	0.161 ± 0.018	0.124 ± 0.028	0.369
Ni	4.074 ± 1.436	5.757 ± 1.306	2.77 ± 0.701	1.317 ± 0.260	0.061
Cu	10.688 ± 2.759	9.666 ± 0.483	6.980 ± 0.294	5.673 ± 0.548	0.094
Zn	613.007 ^{ab} ± 30.644	431.349 ^c ± 25.032	647.767 ^a ± 42.065	495.561 ^{bc} ± 25.896	0.000

As	$0.087^{ab} \pm 0.020$	$0.107^a \pm 0.006$	$0.075^{ab} \pm 0.009$	$0.035^b \pm 0.011$	0.007
Se	1.806 ± 0.708	0.927 ± 0.150	0.075 ± 0.623	0.186 ± 0.033	0.149
Sr	$3018.305^a \pm 165.619$	$1264.954^b \pm 172.209$	$1882.413^b \pm 170.731$	$1518.928^b \pm 122.757$	<0.0001
Mo	$0.161^b \pm 0.054$	$0.363^a \pm 0.016$	$0.101^b \pm 0.071$	$0.094^b \pm 0.030$	0.002
Cd	0.162 ± 0.071	0.120 ± 0.021	0.054 ± 0.023	0.056 ± 0.022	0.149
Sb	0.099 ± 0.045	0.065 ± 0.007	0.091 ± 0.065	0.019 ± 0.008	0.497
Ba	$232.125^b \pm 9.024$	$415.412^b \pm 42.953$	$1748.219^a \pm 266.100$	$1813.016^a \pm 366.597$	0.000
Hg	<LOD	<LOD	<LOD	<LOD	—
Pb	$2.826^b \pm 0.299$	$9.428^a \pm 1.737$	$1.738^b \pm 0.174$	$1.034^b \pm 0.203$	< 0.0001
Si	$654.520^a \pm 156.231$	$224.759^b \pm 31.695$	$313.076^{ab} \pm 45.549$	$245.954^b \pm 46.040$	0.007

^{a,b,c} Means with different superscripts in the same row differ significantly from each other ($P \leq 0.05$). EXT – extensive production system Western Cape; EXT (N) – extensive production system Namibia; INT – intensive production system Limpopo; SE – semi-extensive production system Limpopo; SEM – Standard error of means; LOD – limit of detection (**Table 3.1**); B – Boron; Al – Aluminium; V – Vanadium; Cr – Chromium; Mn – Manganese; Fe – Iron; Co – Cobalt; Ni – Nickel; Cu – Copper; Zn – Zinc; As – Arsenic; Se – Selenium; Sr – Strontium; Mo – Molybdenum; Cd – Cadmium; Sb – Antimony; Ba – Barium; Hg – Mercury; Pb – Lead; and Si – Silicon.

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3.5 Discussion

3.5.1 Proximate composition

Game meat has been reported as being healthy as it contains high protein content and has very low fat levels (Hoffman, 2007). Consumer preferences have changed from purchasing and eating only the meat of domesticated animals to eating game meat due to the latter's health advantages (Niewiadomska *et al.*, 2020). According to Hoffman *et al.* (2005b), proximate composition can differ depending on the species, type of muscle, age of the animal, and sex. Additionally, Hocquette *et al.* (2010) noted that proximate composition differs between muscle types because of differences in muscle structure, muscle function and muscle location in the carcass. Nutrition is also considered to effect proximate composition as it can influence the fat and protein contents.

In the current study, impala muscle from different production systems was analysed. INT and SE region/production systems had high moisture content and low protein content, whilst the protein content was significantly high in the EXT (N) production system

Needham *et al.* (2020) reported comparable results for the moisture and protein contents of impala muscle to that of the current study, where the semi-extensive production was high in moisture and low in protein content whilst the opposite was seen for the extensive production system. In this investigation, the fat content was significantly higher for the SE production system followed by the EXT (W) production system, EXT (N) production system and lastly the INT production system. This is possibly influenced by the fact that the impala animals in the SE production system were allowed to graze freely on the vegetation with no limitation and were

also provided with supplementary feed when needed. Results in Table 3.1 showed no significant difference in the ash content across all regions/production systems.

3.5.2 Macro mineral concentration in impala liver, muscle and rib from different regions/production systems.

Macro minerals in the liver were found in different concentrations from the impala rams sampled from the different production systems. However, the impala liver from EXT (W) showed higher macro mineral concentrations than any other production system. Results of the high macro minerals in the EXT (W) region/production system might be due to the fact that the EXT (W) is in the Fynbos biome which consists of Renosterveld vegetation. The Renosterveld is a relative clay-based veld type. Clayey soil types are all soils that develop from shale, mudstone, dolomite and igneous rocks. These soil types are rich in nutrients, are very fertile and produce palatable vegetation for animals (Van Rooyen, 2010). Nutrient-rich soils influence mineral absorption by plants, and thus by animals.

There was no significant difference in the concentrations of macro minerals in the muscles in all regions/production systems. However, even though no macro mineral concentrations differences were detected between these regions/production systems, the muscle from the EXT (N) region/production system had numerically higher concentrations of Ca, K and P, whilst the muscle from the EXT (W) region/production system had high concentration of Mg and the SE region/production system had a high concentration of Na. High macro minerals were only observed in the extensive regions/production systems and semi-extensive region/production system whereas the intensive region/production system had low concentrations. This suggests that since the EXT (W), EXT (N) and SE had higher concentrations of macro minerals than the

INT production system; supplementary feed containing macro minerals would not be needed as a requirement to animals reared in these production systems, as sufficient minerals seem to be available to the animals during their grazing/browsing. Interesting, although the INT system's impala received feed, the possibility exists that the feed fed had insufficient minerals and a mineral lick might be required.

Impala ribs from the extensive production system from the Western Cape [EXT (W)], intensive production system from Limpopo (INT) and from the semi-extensive production system from Limpopo (SE) had higher macro mineral concentrations than impala rib in the extensive production system from Namibia [EXT (N)]. Regional factors such as the season in which the animals were hunted, the soil condition, soil pH and the type of vegetation in the different regions could have had an effect on these results. López-Alonso (2012) and Underwood (1999), reported that some minerals become more concentrated in soils with a pH that is higher than 6.5, whereas others become less concentrated. The mineral nutritional value of forage plants for grazing animals is greatly influenced by the condition of the soil which is also dependent on the amount of water it absorbs.

Climate conditions may also have had an effect on these results, considering the fact that the animals in question were hunted during different seasons which was March (end of summer) for EXT (W), INT, and SE and during the dry winter of July for EXT (N). Besides the fact that the Western Cape Province has winter rainfall in the fynbos biome (Rutherford *et al.*, 2006), mineral concentrations were still higher than those in Namibia. Namibia is known to be a very hot and dry country (Lushetile, 2009) with no rainfall at all during winter. These climatic conditions might have contributed to the low concentrations of macro minerals seen in the EXT (N)

region/production system since the soils would not be as fertile as soils receiving more water. High mineral concentrations were expected in the INT and SE production system as these regions/production systems are located in the Limpopo Province which has summer rainfall (Rutherford *et al.*, 2006) as well as the fact that the animals were given supplementary feed containing a mineral pre-mix.

Vegetation plays an important role in the mineral concentration of plant-animal pathways since some minerals are obtained through plants. According to Neethling *et al.* (2016), wild species in South Africa are primarily dependant on the natural vegetation for feed. However, these plants have different mineral concentrations as they grow in different soils and receive different amounts of precipitation. The type of vegetation occupying a region depends on the biome in that particular region (Neethling *et al.*, 2016). For instance, in the current study, the INT and SE production systems in the Limpopo region are located in the Savanna biome. This biome is covered by a mixture of a grassy ground layer and an upper layer with woody plants (Huntley, 1984). The EXT (W) region/production system is located in the Fynbos biome which consists of evergreen low and tall shrubs, grass-like plants and sedges (Huntley, 1984), whilst EXT (N) comprised mainly of thorn-bush vegetation (mostly acacia trees). According to Lushetile (2009), Namibia has varying soil types; however, most of the soils are poor, making it very difficult for plants to grow. This could in turn influence the animal's nutrition and mineral concentration since animals are primarily dependent on plants.

3.5.3 Micro mineral concentration in impala liver, muscle and rib from different regions/production systems.

The regions/production systems had an influence on the micro mineral concentration of the impala liver. High concentrations of Se, Sr, Mo, Pb, and Si were detected in liver from the EXT (W) region/production system, V, Cr, Ni, Cd, and Hg were highest in liver from the EXT (N) region/production system whilst the liver from the INT region/production system had high concentrations of Mn, Cu, Zn, and As, and lastly, Ba was high in the liver from the SE region/production system. Biologically, the liver from an intensive production system would be expected to have high mineral concentrations due to the extra supplementary feed animals receive as the latter typically includes a vitamin-mineral premix.

The variation of mineral concentration in these regions/production systems may have been due to nutritional and regional factors. These factors include soil type and soil parent material in a region. According to Singh and Schulze (2015), soil type plays an important role in mineral absorption by plants, and thus, by animals. Plants need ~17 minerals in order to complete their normal life processes (Singh and Schulze, 2015) and these minerals are derived directly from the soil or indirectly, through fertilisers and manure (Parikh and James, 2012). In order to understand the soil type, it is essential to know soil parent material as it directly affects the nutrient mineral content in the soil (Singh and Schulze, 2015). However, this influence is mostly noted in young soil and decreases with increasing soil age and soil weathering. A better understating of the effect of soil parent material on soil mineral composition would require a review of the mineral composition of common rocks that build up the soil parent material. This will then make it easy to know the type of minerals existing in a certain type of soil. For instance, the feldspars which is

59.5% igneous rock contains macro minerals (K, Ca and Na) and micro minerals (Cu and Mn), whilst micas, a 3.8% igneous rock contains macro minerals (K, Ca, Na, and Mg) and micro minerals (Fe, Ni, Mn, Co, Zn and Cu) (Klein and Hurlbut, 1993). The concentrations of these minerals in the rocks/soil then affect the uptake of these minerals by plants and by animals as they depend primarily on plants for feed. This would most likely influence impala liver from EXT (W), EXT (N) and semi-extensive production systems since these animals graze directly on plants grown in the soil. The liver had very high concentrations of Cu (2562.73 mg/kg) and Zn (464.96 mg/kg) in the INT production system; most likely, due to the supplements the animals were fed on. The liver is known to be the main storage of Cu and Zn, according to McDonald *et al.* (2002), Cu is known to exist in all body cells, but it is more concentrated in the liver than in any other organs or tissue. Similar results were reported by Umaraw *et al.* (2015), where the liver showed a higher concentration of Cu than the muscle and other by-products.

Micro mineral concentrations in the muscle were mostly detected in the EXT (N) production system and had high concentrations of Al, V, Cr, Mn, Cu, Zn, As, Mo, Ba, and Pb, whilst EXT (W) had high concentrations of Fe and Se. These high mineral concentrations could have been as a result of the different soil types in these regions/production systems.

Some minerals were below the limit of detection (<LOD) in the muscles and include B which was <LOD in all regions/production systems, V <LOD in EXT, INT and SE, Cr <LOD in EXT (W), As <LOD in EXT (W) and SE, Mo <LOD in EXT (W) and INT, Sb and Hg <LOD in all regions/production systems and Si <LOD in EXT (W).

Fe had high concentrations compared to other minerals in the EXT (W) and EXT (N) regions/production systems. A high Fe concentration is often associated with high myoglobin

levels which are mostly found in animals that are active. Deborah and Alan (2013) mentioned that iron is contained in the myoglobin protein and according to Hoffman *et al.* (2005a), high myoglobin concentration in muscles is due to systemic exercises. This would, therefore, mean that an increase in myoglobin results in an increase in Fe concentration. The results of the current study concur with Hoffman *et al.* (2005a) due to the fact that high Fe concentrations were only observed in the extensive regions/production systems where the animals would have had more exercise.

Besides the liver, bones have high mineral absorption capabilities that are important for bone formation. The level of mineral concentration in ribs differed across the production systems. EXT (W) was found to have high Cr, Sr, and Si concentrations, EXT (N) had the highest concentrations of B, Al, Mn, As, Mo and Pb minerals, V, Fe and Zn were high in the INT production system and SE showed high concentrations of Ba. Of these minerals, Fe (1528.885mg/100g), Zn (647.767mg/100g), Sr (3018.305mg/100g), and Si (654.520mg/100g) had very high concentrations.

Providing animals with these minerals is of paramount importance in ensuring a strong animal skeletal system. Bleich (1990) and Robbins (1993) noted that mineral imbalances in bone results in porous and weak bones and therefore bone breakage. Browning and Cwieson (2013) reported that Sr is 99% concentrated in the bones. Pagano *et al.* (2007) reported that supplementing Sr through feed improves the metatarsal and femur strength, mineral content, and mineral density of bones. Browning and Cwieson, (2013) reported that supplementing adult goats with Sr increases the naturally occurring anabolic hormone insulin-like growth factor which is relevant in animal production.

3.6 Conclusion

The aim of this study was to provide baseline data about the mineral status of impala liver, muscle, and rib harvested from different regions/production systems. The regions/production systems do affect both micro and macro minerals of the investigated animal samples. Normally, animals in an intensive production system would be expected to have significant amounts of minerals due to the supplementary feed they receive. However, this was not the only factor contributing to the deposition of minerals in the investigated animal samples; regional factors such as seasonal changes and soil types also need to be considered. The concentration of micro minerals was found to vary in the animal samples according to the production systems.

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Chapter 4

Bone, liver and muscle mineral concentration of the bontebok and blesbok harvested from the Western Cape

Abstract

The effect of species on the mineral concentration in animal samples (liver, *Longissimus thoracis et lumborum* muscle and rib) of the Bontebok (*Damaliscus pygargus*) and blesbok (*Damaliscus pygargus phillipsi*) was investigated. Both these species were harvested in the Western Cape and were extensively kept. The muscles of both species were analysed for the moisture, protein, fat and ash contents. There was significant difference detected in the moisture content between the species, however, the protein content was high ($P \leq 0.05$) in the bontebok muscle whilst the blesbok was reported to have a high ($P \leq 0.05$) fat and ash contents. The blesbok liver and muscle had high ($P \leq 0.05$) concentration of macro minerals, excluding Na which did not differ ($P > 0.05$) between the two species. There was no significant difference in the concentration of macro minerals in the rib of both species, excluding K which was found to be highest in the blesbok. The bontebok liver was found to have a high concentration of Fe, Zn, Se, and Cd whilst the blesbok had high concentration of the Al, Cr, Mn, Co, Ni and Mo. For micro mineral concentrations, the bontebok liver had the highest concentration of Fe, Zn, Se and Cd whilst the blesbok liver had the highest concentration of Al, Cr, Mn, Co, Ni and Mo. On the other hand, the bontebok muscle had a significant concentration of B, Fe, Ni, Zn, Sb and Hg and the blesbok muscle had a high concentration of Mn, Cu, Se and Ba concentrations. In addition, the bontebok rib was recorded to have a significant concentration of Fe, Zn, Sr and Ba and the blesbok rib had

a high concentration of Cu. The research shows that species does have an effect on mineral composition.

4.1 Introduction

Game farming in South Africa has been categorised as the 6th largest agricultural sector (Neethling, 2016) that provides employment to over a hundred thousand individuals (Cloete *et al.*, 2015). Game farming focuses on trophy hunting, live sales, eco-tourism, and game meat production (Neethling, 2016). Blesbok is one of the species that is harvested for meat production and trophy hunting in South Africa whilst the scarce bontebok is typically hunted by trophy hunters. This species is widely distributed and is abundant on privately owned ranches. Blesbok is sometimes mistaken for bontebok because of similar characteristics (Smit, 2004). Both species are known to be complete grazers (Skinner and Chimimba, 2005) and both the bontebok and blesbok have a high preference for short grasses and newly burnt veld (Kraaij and Novellie, 2010).

The increasing demand for game meat as a healthier protein source has resulted in an increase in the production of game animals (Dahlan and Norfarizan Hanoon, 2008). Game meat has been classified as having high nutritional value (Hofmeyr, 2014; Costa *et al.*, 2016) when compared to domesticated ruminants (cattle and sheep), but its mineral nutritional content has been overlooked. Animal mineral status plays a major role in animal production as it provides baseline data on whether mineral supplements are required or not. Failure to meet the mineral requirements of animals can be a problem for the animals as it slows production, leads to poor health, and delays growth. Mineral licks are often provided to animals to supplement the deficient mineral(s) in cases where there are mineral shortages. However, there is insufficient information to provide the baseline data of what the mineral status of game animals should be. Thus, the aim of this study was to investigate the mineral concentration of the bone, liver, and muscle from blesbok and bontebok species found in the Western Cape province of South Africa.

4.2 Methods and materials

4.2.1 Study site

The bontebok and blesbok were both harvested in the Western Cape Province (Wellington (33.6405° S, 19.0097° E) and Witsand (34.3964° S, 20.8508° E), respectively). Both these farms are located in the Fynbos biome, although the bontebok was from the Renosterveld vegetation and the blesbok was from the fynbos vegetation. All these Animals depended primarily on natural feed with no supplementary feed. Six rams of ± 15 months old were harvested from each farm.

4.2.2 Experimental procedure

The details of this section are as described in section 3.2.2

4.2.3 Sampling procedure

The details of this section are as described in section 3.2.3

4.2.4 Defatting of bones for analysis

The details of this section are as described in section 3.2.4

4.2.5 Chemical analysis

4.2.5.1 Acid digestion

The details of this section are as described in section 3.2.5.1

4.2.5.2 Macro element analysis

The details of this section are as described in section 3.2.5.2

4.2.5.3 Micro analysis

The details of this section are as described in section 3.2.5.3

4.2.6 Proximate analysis

The details of this section are as described in section 3.2.6

4.3 Statistical analysis

The experimental data was analysed using XLSTAT version 19.2. Due to physiological differences between the sample types these were not compared, instead the species were compared using one way analysis of variance (ANOVA) for individual sample types of liver, muscle, and rib. Bonferroni post-hoc test was used to identify the statistical significance at the 5% level with all results $P \leq 0.05$ considered to be statistically significant.

The following statistical model was used:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

Where: Y_{ij} is the response variable (minerals)

μ is the overall mean

α_i is the effect due to treatment (bontebok and blesbok species)

ϵ_{ijk} is the error

4.4 Results

4.4.1 Proximate composition

Results in Table 4.2 show the proximate composition in the *Longissimus thoracis et lomborum* (LTL) of bontebok and blesbok species. There was no significant difference detected between the moisture of these two species. However, the bontebok had a higher protein content ($P \leq 0.05$) than the blesbok, whereas the fat and ash content were found to be higher in the blesbok ($P \leq 0.05$).

Table 4.2: Proximate composition ‘as is-basis’ (LSMeans $\pm$ SEM) of the bontebok and blesbok *Longissimus thoracis et lumborum* muscle.

Proximate (g/100g)	Species		
	Bontebok	Blesbok	P-value
Moisture	75.71 $\pm$ 0.329	75.72 $\pm$ 0.144	0.987
Protein	22.42 $\pm$ 0.323	21.41 $\pm$ 0.141	0.010
Fat	1.66 $\pm$ 0.024	2.33 $\pm$ 0.097	<0.0001
Ash	1.17 $\pm$ 0.070	1.35 $\pm$ 0.049	0.003

4.4.2 Macro mineral concentration in bontebok and blesbok liver, muscle and rib.

Results from the Table 4.3 show the effect of species on the macro mineral concentration in bontebok and blesbok liver, muscle and rib. All the macro mineral concentrations (except Na) differed significantly between the livers of the two species, with blesbok liver having the higher ($P \leq 0.05$) concentration of all the minerals than bontebok. There was a significant difference in all macro minerals between the two species with the blesbok showing the higher ($P \leq 0.05$) concentrations for all macro minerals for the muscle. There was no significant difference in all macro mineral concentrations in the ribs of the two species, except on K which was significantly high for the rib from the blesbok.

Table 4.3: Macro mineral concentrations ‘g/100g as is’ (LSMeans $\pm$ SEM) in bontebok and blesbok liver, muscle and rib.

Liver			
Macro minerals	Bontebok	Blesbok	P-value
Ca	0.588 $\pm$ 0.054	0.821 $\pm$ 0.043	0.007
K	26.951 $\pm$ 1.175	35.982 $\pm$ 2.028	0.003
Mg	2.024 $\pm$ 0.066	2.554 $\pm$ 0.105	0.002
Na	13.548 $\pm$ 1.585	15.552 $\pm$ 1.666	0.404
P	41.817 $\pm$ 2.042	50.428 $\pm$ 2.197	0.017
Muscle			
Ca	0.320 $\pm$ 0.018	0.696 $\pm$ 0.104	0.005
K	40.300 $\pm$ 1.797	57.135 $\pm$ 4.042	0.003
Mg	2.458 $\pm$ 0.123	3.846 $\pm$ 0.304	0.002
Na	3.878 $\pm$ 0.203	7.595 $\pm$ 0.411	<0.0001
P	22.540 $\pm$ 0.981	34.987 $\pm$ 2.629	0.001
Rib			
Ca	205.322 $\pm$ 31.208	196.209 $\pm$ 16.755	0.802
K	1.525 $\pm$ 0.293	3.388 $\pm$ 0.181	0.000
Mg	4.076 $\pm$ 0.642	2.957 $\pm$ 0.150	0.121
Na	6.701 $\pm$ 1.138	5.948 $\pm$ 0.340	0.540

P	92.369 ± 14.119	87.059 ± 7.132	0.744
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SEM – Standard error of means; Ca – Calcium; K – Potassium; Mg – Magnesium; Na – Sodium;
P – Phosphorus;

4.4.3 Micro mineral concentration in bontebok and blesbok liver, muscle and rib.

Results from Table 4.4 show the effect of species on the micro mineral concentration in bontebok and blesbok liver, muscle and rib. There was no significant difference in the concentration of V, Cu, As, Sr, Ba, Hg, Pb and Si. A significant concentration of Al, Cr, Mn, Co, Ni and Mo was detected in the blesbok liver whilst the bontebok liver had a significant concentration of Fe, Zn, Se and Cd. No significant difference was detected in the concentration of Al, V, Cr, Co, As, Sr, Mo, Cd, Pb and Si in the muscle. The bontebok muscle had higher ($P \leq 0.05$) concentrations of B, Fe, Ni, Zn, Sb and Hg whilst the blesbok muscle had higher ($P \leq 0.05$) concentrations of Mn, Cu, Se and Ba. There were no significant differences in the concentration of B, Al, Cr, Mn, Co, Ni, As, Se, Cd, Sb, Pb and Si between the two species for the rib. The bontebok rib had higher ($P \leq 0.05$) concentration of Fe, Zn, Sr and Ba, whilst the blesbok rib had a higher ($P \leq 0.05$) concentration of Cu.

Table 4.4: Micro mineral concentrations ‘mg/100g as is’ (LSMeans $\pm$ SEM) in bontebok and blesbok liver, muscle and rib.

Liver			
Micro minerals	Bontebok	Blesbok	P-value
B	<LOD	<LOD	—
Al	3.725 $\pm$ 1.289	10.909 $\pm$ 1.112	0.002
V	0.015 $\pm$ 0.010	0.027 $\pm$ 0.002	0.239
Cr	0.000 $\pm$ 0.000	0.319 $\pm$ 0.024	<0.0001
Mn	32.685 $\pm$ 2.380	50.140 $\pm$ 2.449	0.000
Fe	3134.365 $\pm$ 718.236	1.395 $\pm$ 0.105	0.001
Co	0.881 $\pm$ 0.038	1.266 $\pm$ 0.047	<0.0001
Ni	0.000 $\pm$ 0.000	0.228 $\pm$ 0.041	0.000
Cu	666.710 $\pm$ 379.653	816.726 $\pm$ 173.392	0.727

Liver			
Micro minerals	Bontebok	Blesbok	P-value
Zn	442.612 ± 71.216	0.518± 0.014	0.000
As	0.205 ± 0.046	0.178 ± 0.014	0.589
Se	4.664 ± 0.436	3.531 ± 0.200	0.040
Sr	1.213 ± 0.319	0.636 ± 0.038	0.102
Mo	5.153 ± 1.671	16.990 ± 0.829	<0.0001
Cd	0.951 ± 0.173	0.299 ± 0.056	0.005
Sb	<LOD	<LOD	—
Ba	0.844 ± 0.121	1.087 ± 0.063	0.107
Hg	0.076± 0.017	0.353 ± 0.132	0.064
Pb	0.808 ± 0.271	0.262 ± 0.023	0.072

Si	42.083 ± 27.164	0.000 ± 0.000	0.152
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Muscle

Micro minerals	Bontebok	Blesbok	P-value
B	2.627± 0.380	0.000 ± 0.000	< 0.0001
Al	7.553 ± 1.804	9.056 ± 1.119	0.495
V	0.046± 0.003	0.044 ± 0.030	0.940
Cr	0.820 ± 0.122	4.330 ± 3.297	0.312
Mn	1.987 ± 0.074	3.630± 0.405	0.003
Fe	433.184 ^a ± 35.014	0.608 ± 0.049	< 0.0001
Co	0.242 ± 0.118	0.105 ± 0.039	0.295
Ni	1.722 ± 0.543	0.442 ± 0.081	0.042
Cu	19.064 ± 1.312	37.996 ± 3.644	0.001

Muscle			
Micro minerals	Bontebok	Blesbok	P-value
Zn	312.924 ± 17.567	0.379 ± 0.023	< 0.0001
As	0.097± 0.013	0.120 ± 0.013	0.256
Se	1.064± 0.056	1.456 ± 0.102	0.007
Sr	0.230± 0.074	0.406 ± 0.069	0.112
Mo	0.170± 0.044	0.187± 0.187	0.930
Cd	0.003± 0.003	0.002 ± 0.002	0.783
Sb	0.083± 0.014	0.000 ± 0.000	0.000
Ba	0.284± 0.030	0.917± 0.085	< 0.0001
Hg	0.071± 0.007	0.000 ± 0.000	< 0.0001
Pb	1.166 ± 1.003	0.095 ± 0.016	0.311

Si	30.410 ± 19.253	0.000 ± 0.000	0.145
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Rib

Micro minerals	Bontebok	Blesbok	P-value
B	1.675 ± 0.460	1.351 ± 0.182	0.528
Al	1.904 ± 0.569	4.037 ± 0.867	0.067
V	0.004 ± 0.001	0.007 ± 0.001	0.072
Cr	0.22 ± 0.064	0.323 ± 0.058	0.300
Mn	0.348 ± 0.062	0.414 ± 0.033	0.372
Fe	120.788± 48.525	0.167 ± 0.019	0.032
Co	0.010 ± 0.002	0.018 ± 0.003	0.062
Ni	0.467 ± 0.140	0.703 ± 0.181	0.325
Cu	0.843 ± 0.163	1.718 ± 0.184	0.005

Rib			
Micro minerals	Bontebok	Blesbok	P-value
Zn	52.432 ± 9.232	0.061 ± 0.003	0.000
As	0.007 ± 0.002	0.010 ± 0.001	0.379
Se	0.100 ± 0.053	0.044 ± 0.004	0.317
Sr	438.611 ± 95.383	128.176 ± 12.505	0.009
Mo	<LOD	<LOD	
Cd	0.008 ± 0.004	0.028 ± 0.009	0.066
Sb	0.015 ± 0.009	0.002 ± 0.002	0.184
Ba	262.136± 49.663	96.374 ± 8.852	0.008
Hg	<LOD	<LOD	
Pb	0.377 ± 0.111	0.262 ± 0.027	0.339

Si	16.775 ± 3.348	40.184 ± 13.188	0.116
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LOD – limit of detection (**Table 4.1**); B – Boron; Al – Aluminium; V – Vanadium; Cr – Chromium; Mn – Manganese; Fe – Iron; Co – Cobalt; Ni – Nickel; Cu – Copper; Zn – Zinc; As – Arsenic; Se – Selenium; Sr – Strontium; Mo – Molybdenum; Cd – Cadmium; Sb – Antimony; Ba – Barium; Hg – Mercury; Pb – Lead; and Si – Silicon.

4.5 Discussion

4.5.1 Proximate composition

Game meat is generally known for having a high nutritional value because of its low-fat content and for being a good source of protein. For this reason, game meat demand is increasing (Costa *et al.*, 2016). Game meat is associated with low risks to diseases such as cholesterol, obesity, heart disease, strokes, and hypertension (Hoffman *et al.*, 2005). Costa *et al.* (2016) reported that the fat content of game species is considered to be low as it is generally below 5%, whereas the protein content was reported to range between 20% and 25%. In the current study, the bontebok and blesbok had a protein and fat content below 25% and 5% respectively. The proximate component contents for the blesbok in this study were similar to the ranges reported by Costa *et al.* (2016) for the proximate composition of blesbok for different *Longissimus dorsi* muscle cuts, which were 68.20 – 75.33% for moisture, 19.30 – 22.43% protein, 0.21 – 6.8% fat and 1.24 – 4.2% ash. Hoffman and Wiklund (2006) showed similar findings where the blesbok had a moisture content of 75.7%, protein content of 23.5% and fat content of 1.7%.

No significant difference was detected for the moisture content between bontebok and blesbok. However, the protein content (22.42%) of the bontebok differed significantly from that of blesbok (21.41%). Although, according to Hoffman *et al.* (2009), protein content of game meat differs according to species, this difference might be caused by the known negative correlation between protein and lipid contents. In support of the latter, the fat and ash contents were higher in the blesbok (2.33% and 1.35%, respectively) than the bontebok (1.66% and 1.17%, respectively).

4.5.2 Liver, muscle, and rib macro mineral concentration of the bontebok and blesbok.

The bontebok and blesbok are considered to be similar and were both harvested in the Fynbos biome, these two species had different mineral composition in their samples. According to Richards (1993), the Fynbos biome is categorised into two vegetations, which are the fynbos vegetation and the Renosterveld vegetation. As such in this study, the bontebok species originated from the Renosterveld vegetation while the blesbok species was from the fynbos vegetation. The fynbos vegetation is well-known for consisting of acidic sandy soils that are low in nutrients (Richards, 1993). These soils are largely deficient in essential nutrients for plant growth (Bothma and Du Toit, 2010). Whilst, the Renosterveld vegetation consists of more fertile clay soils thus producing nutritious plants (Bonsma and Du Toit, 2010). This can significantly affect the deposition of minerals by animals since animals depend primarily on plants for nutrients.

For all the three sample types (bone, liver and muscle), the blesbok had higher macro mineral concentrations as compared to the bontebok. Based on literature, the blesbok would have shown lower macro mineral concentrations compared to the bontebok. However, the climatic conditions during harvesting of the species could also contribute to the macro mineral concentrations in different species as plant growth is affected by climate. The bontebok was harvested during summer, whilst the blesbok was harvested in winter; the Fynbos biome has winter rains, and this could have partly attributed to the high macro mineral concentrations of the blesbok.

4.5.3 Liver, muscle and rib micro mineral concentration of the bontebok and blesbok.

The micro mineral in the liver, muscle and the rib between these species occurred in different concentrations, with some minerals being higher than others. For instance, the bontebok liver had very high concentrations of Fe, Zn, Se and Cd, whereas the blesbok liver had significant

concentrations of Al, Cr, Mn, Co, Ni and Mo. These differences might have been due to factors such as the soil type and the vegetation these species were grazing on. Even though the concentration of these minerals differed between species and animal samples, the concentration of Fe and Zn remained high for all the bontebok samples. It could be that the clay soils in the Renosterveld are a good source of Fe and Zn minerals.

High concentrations of Fe, Zn, Sr and Ba were detected in the bontebok rib; meanwhile the blesbok rib had significant concentration of the Cu. Of these micro minerals, the Sr (438.611 mg/100g) and Ba (262.136 mg/100g) concentrations in the bontebok rib were very high. These two micro minerals are known to be essential for bone formation. According to Browning and Cowieson (2013) an animal's body has 99% of strontium which is found in the bones.

4.6 Conclusion

The results from the study show that species does have an effect in the mineral concentrations from the bone, liver and muscle. However, this may have been influenced by the fact that the Fynbos biome in which these species were harvested has different vegetation types. This might then suggest different soil types influencing mineral absorption by animals, via the plants.

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Chapter 5: General discussion, conclusion and recommendations

5.1 General discussion

Previous research has shown that game meat is healthy due to its low fat (Hoffman, 2007) and high protein contents. Hoffman (2007) reported that beef had the lowest protein (19.2%) content and high-fat content (14.2%), whereas game animals (springbok, nyala, blesbok, and impala) had high protein contents (ranging between 20 – 23.8%) and low-fat contents (0.8 – 2.45%). According to Neethling *et al.* (2016), the high protein and low-fat contents in game meat have resulted in a growth in its demand amongst discerning consumers. Costa *et al.* (2016) reported the proximate composition of different game animals, where moisture ranged between 68.20 and 76.90%, protein being between 17.4 and 25.71%, fat ranged between (.21 and 6.8% and ash was between 1.03 and 4.6%. The proximate composition results for the current study also fell between these ranges.

However, there is limited information about the mineral status of game animals. Researchers have reported on the importance of minerals in domesticated animals but little has been said about what are the expected mineral concentrations in game animals. Nonetheless, the consequences of mineral deficiencies in animals are well known, these include delayed growth, poor health, reduced production rate, etc. Preventative measurements such as the inclusion of the deficient mineral in diets and providing salt licks have been practiced correcting mineral shortages in animals.

Additionally, factors such as age, sex, nutrition, type of species and the physiological status of animals have been reported as factors that affect the mineral concentration of animals. Nutrition is of greater importance in determining mineral concentrations in animals, this is

due to the fact that animals depend mainly on the feed they consume for minerals in their body.

5.2 Conclusion

It can be concluded that game meat is a good source of protein when compared to meat from domesticated animals. Also, minerals are essential to animals as they are important for normal life processes. They are also important because humans depend primarily on meat for protein and minerals as they cannot synthesis their own minerals. The liver and rib have shown to be the better mineral storages whilst the muscle has lower mineral levels. Mineral deficiencies in animals can be corrected by providing mineral supplements in diets and salt licks. However, there is still a need to conduct further research about the mineral profile of other game species so as to have baseline data of their mineral concentration.

5.3 Recommendations

Since there is no base-line data about the mineral status of game animals, there is a need to conduct further research on this aspect. Factors such as vegetation, soil, water and climate should be taken into consideration when conducting this research about mineral status in animals. Knowing the type of soil and the type of minerals found in these soils would be of great significance in determining and understanding the mineral concentrations in animals. Also, it would be of importance to analyse the minerals found in the vegetation that these animals feed on. Given that some game species are selective grazers, others are browsers and others mixed feeders, and there is a variety of vegetation (grasses, trees and shrubs) a general overview of what the mineral concentration is expected to be is essential.

5.4 Future Research

From this study, the listed areas can be identified for future research. These include:

- Soil samples should be taken and analysed for mineral composition since it is also a source of minerals.
- Balancing production system per region as to give a solid comparative analysis.
- Production system and region should be treated independently of each other and should be treated differently for the statistical analysis, with enough replicates to allow ANOVA.
- ANOVA should be performed on whether different hunting season has an effect on mineral composition.

5.5 References

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Abstract

The aim of the study was to investigate the mineral concentration of different game species, harvested in different regions. Bone, liver and muscle mineral composition of game animals (Bontebok, blesbok and impala) were investigated. The muscle of all three animals were also analysed for their proximate (moisture, protein, lipid, ash) chemical composition. Impala animals were harvested in three different production systems (intensive, semi-extensive and extensive production systems) and in different regions (Limpopo, Namibia, and Western Cape) whilst bontebok and blesbok were harvested in the Western Cape from two different extensive farms. The proximate results for all species ranged between 73.68 – 75.72% moisture, 21.41 – 24.15% protein, 1.44 – 2.38% fat and 1.14 – 1.35% ash.

The mineral results showed significant concentrations of B, Al, V, Sb, Cr, Ni, Sr, Ba, Pb, and Si micro minerals in the rib of all three (bontebok, blesbok, and impala) species. Whilst the liver was high in Mn, Co, Cu, Se, Mo, and Hg for all species. Fe, Zn, As, and Cd concentrations differed in species and organs. Fe and Zn had high concentrations in all bontebok organs and were very low in the blesbok organs. However, impala had high levels of Fe and Zn in the rib. The results also showed that mineral concentrations differed in impala harvested in different production systems. Extensive reared impala from the Western Cape had high concentrations of Cr, Fe, Co, Zn, As, Se, Sr, Mo, Hg, and Si, whilst impala from an extensive system in Namibia had high concentrations of Al, V, Cr, Mn, As, Mo, Hg, and Pb, compared to the impala reared under intensive conditions from Limpopo who had high concentrations of V, Mn, Fe, Co, Cu, Zn, As, and Ba, whilst the semi-extensive reared impala from Limpopo had high concentrations of Mn, Fe, Zn, and Ba. The macro minerals in the impala from Namibia were found in very low

concentrations as compared to those from Limpopo and the Western Cape. Therefore, region/production system does have an effect on the concentration of minerals in these organs.

Keywords: game animals; organs; mineral profile; proximate composition; production systems

Chapter 1: Background

1

Introduction

The commercial utilisation of game in South Africa has shown extensive growth over the last 20 years, and consequently, game farmers now play a key role in the conservation of many game species (Otieno and Muchapondwa, 2016). Game farming has also been introduced to the agricultural industry and it is well known and developing in South Africa. Many South African cattle farms are being converted to game farms and the conversion rate has relatively increased from 2 to 2.5% per annum (Chiyangwa, 2018). Additionally, Pasmans and Hebinck (2017) reported that since 1996, 12% of the land for livestock in the Eastern Cape has been converted into game ranches. According to Saayman *et al.* (2018), the estimated gross income from the game industry across South Africa is R1.96 billion. Taylor *et al.* (2016) also reported that the estimated total revenue of live sales from all South African wildlife ranches (including those that are privately owned) sums up to R4.328 billion. Dekker and Van Der Waal (2000) noted 22 years ago that game ranches occupied approximately 3.6 million ha in the Limpopo Province and they covered 26% of the province's surface area; although data is lacking all indications are that this percentage is much higher today.

Similar to livestock, game animals also require minerals for efficient performance, growth and reproduction. Twenty-two minerals are known to be essential for the members of the animal kingdom (Underwood, 1976). These comprise seven macro-minerals: Calcium (Ca), Phosphorus (P), Sodium (Na), Potassium (K), Chlorine (Cl), Magnesium (Mg) and Sulphur (S) and fifteen micro minerals: chromium (Cr), copper (Cu), manganese (Mn), zinc (Zn), selenium (Se), tin (Sn), iodine (I), cobalt (Co), vanadium (V), fluorine (F), molybdenum (Mo), silicon (Si), iron (Fe), nickel (Ni) and arsenic (As). However, Laaka (2014) included boron (B), cadmium (Cd),

barium (Ba), aluminium (Al), Lead (Pb) and Mercury (Hg) as essential ³ micro minerals. All these mineral elements exist in the cells and tissues of the animal in specific concentrations, which is essential for the normal growth and health and productivity of the animal (López-Alonso, 2012; Stasiak, 2017).

Minerals are important for three major functions (Smart *et al.*, 1981). Firstly, as a structural component of the body, organs, and tissues. They also play a role as ³ constituents of body fluid and tissues as electrolytes ¹⁴ that are essential for maintenance of osmotic pressure, acid-base balance, membrane permeability and tissue irritability. Lastly, they act as catalysts in enzyme and hormonal systems as an integral and specific component of the structure of metallic-enzymes or as activators within those systems (Underwood, 1981). Essential mineral deficiency results in delayed growth and puberty in ² livestock. Body micro mineral profile, immunity and reproductive functions are directly related, where copper, cobalt, zinc, iodine and manganese are likely to influence these functions. In most cases, mineral deficiencies limit production (McDowell and Conrad, 1977) and can also negatively affect both animal and human health, as humans depend primarily on animal products for some of their minerals.

Several studies have been conducted on nutritional aspects of game animals, such as the game meat quality (fat and lean) and also meat palatability (colour, pH, juiciness, flavour, etc) although little has been said about the mineral profile of game animals. According to Wassenaar *et al.* (2019), the ⁴⁵ production of game meat is important for the development of the game industry because it contributes significantly to the game rancher's economy and is also seen as a healthy protein food source for humans. However, animal health ¹¹ plays an important role in animal and meat production, thus the importance of understanding the mineral status of animals. As mentioned, mineral deficiencies are a major problem that could limit animal production (López-

Alonso, 2012; Stasiak, 2017) and should be corrected for by providing mineral licks to animals. However, this cannot be achieved without having base-line information on what the mineral status of game animals is expected to be. Thus, the main aim of this research is to investigate the mineral concentration of game animals using samples from the bone, liver and the muscle so as to provide some base-line information on some of the more commonly ranched species.

Problem statement

Nutritional disorders, including mineral deficiencies, toxicities and mineral imbalance are severely inhibiting grazing animals in developing countries and have more significant consequences than infectious disease (Loosli and McDowell, 1985). These deficiencies impair the animal's immune response and increase its susceptibility to disease, in particular contagious diseases (e.g., diarrhoea, respiratory disorders). In young ruminants, macro and micro mineral deficiencies contribute to metabolic disorders and clinical changes in individuals and therefore in the herd. The role of trace minerals, particularly copper and zinc in maintaining reproductive rhythm is well documented, as they are specific activators of enzymes in the reproductive system (McDowell *et al.*, 1984). Infertility has regularly been recorded in copper and manganese deficiencies resulting in depressed or delayed oestrous and poor conception rate (Underwood, 2012). Similarly, zinc has been shown to be important for gonadal growth in lambs and is responsible for infertility in ewes (Underwood, 1981).

Mineral deficiencies in different vegetations of Africa may result in low mineral intake by animals. However, before a feed is classified to have a mineral deficiency, baseline data is needed to know and understand what a normal mineral concentration would be. Even though this information is available for farm animals, there is limited to no information for wild species farmed in Southern Africa.

Justification

Of all the nutrients required by an animal, minerals play a very important role as they are responsible for all life processes, including growth and reproduction. However, animal mineral concentrations are dependent on both soil and plant associations. Mineral contents of natural vegetation are primarily determined by the mineral availability and mineral composition of the soil (Smart *et al.*, 1981). ¹ Soil mineral concentrations influence the concentrations within the plants and consequently, the mineral concentrations in the muscle tissue of the animals feeding on the plants (Zomborszky *et al.*, 1996; McDowell and Conrad, 1977). ² The extent and pattern of mineral deficiencies or excess in plants vary in different agro-climatic conditions as available mineral content in green vegetation depends on physical and chemical properties of soil, soil erosion, cropping pattern, fertilizer/chemical application, species and genetic variation in plants, stage of maturation as well as the presence of other minerals (McDowell *et al.*, 1983).

² Mineral imbalances in soil and forages are found to be responsible for low production and reproduction among ruminants. There is therefore a need to supplement animals with mineral licks in cases where mineral deficiencies occur. Also, certain minerals can be included in animal diets after the shortage of a specific mineral has been identified. Providing animals with a proper diet becomes easy if the animal's feeding status at a given time is known. ² Therefore, studies on the soil-plant-animal relationship in respect to minerals are important and have great implications on animal production (Biswas and Samanta, 2002). However, there is insufficient information about the mineral profile of game animals; hence this study aims to investigate the mineral concentration of the bone, muscle and liver from different game species.

Chapter 2: literature review

Production of game meat has become an important facet of the agricultural industry in South Africa. According to Hoffman *et al.* (2005), the production of meat plays a considerable role in increasing the economy of game farms. Berry (1986) also reported that game meat production had high income yields as compared to other activities (such as trophy hunting and live sales) practiced on game farms. Game meat has been reported to have a low fat content of <3% (Hoffman, 2007) and it also contains a higher proportion of polyunsaturated fatty acids (Hoffman and Wiklund, 2006), whereas red meat from farmed livestock has a fat content >5% depending on the leanness of the meat (Williamson *et al.*, 2005). High fat content meat is a risk to human health in that it may lead to high cholesterol which could lead to heart disease, obesity, strokes and hypertension (Hoffman *et al.*, 2005). Due to these health problems, there has been an increasing demand for game meat world-wide (Neethling *et al.*, 2016). Game meat can be considered to have a high nutritional value as it is not only a good source of protein but also serves as a source of vitamins and minerals (Costa *et al.*, 2016).

Minerals are elements that form part of the ash from which impurities have been removed. More than 60 mineral elements, both macro and micro elements, have been detected in the ash of the organs and tissues of higher animals (Ashton, 2016). ³ These mineral elements occur in the cells and tissues of the animal in specific concentrations and are essential for the normal growth, health, and productivity of the animal (Ranjith and Pandey, 2013). The concentration of minerals also varies according to species. For example, Hoffman *et al.* (2005) reported that, when compared to beef, some game meat species such as impala and duiker have higher phosphorus (P), potassium (K) and calcium (Ca) concentrations and lower levels of sodium (Na) in their meat. Even though the majority of the naturally occurring minerals originate in animal tissues

and organs (kidney, liver, heart, rib, etc), many are thought to be present simply because they are constituents of the animal's food and may not have an important function in the animal's metabolism (McDonald *et al.*, 2002).

In order for an element to be categorised as important, evidence showing that pure diets lacking the element causes deficiency symptoms in the animals should be provided and this deficiency can be prevented by including the element in the diets (McDonald *et al.*, 2002). Such minerals include Ca, Na, magnesium (Mg), K, iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) (Kunachowicz *et al.*, 2005). These minerals are essential in the animal's body and have different functions. For instance, Zn is responsible for the proper growth and development of an organism (including the biosynthesis of the proteins) and it is necessary for the functioning of the respiratory, reproductive, and immune systems (Biesalski, 2005). Other functions of minerals include transportation of respiratory gases (Blicharski *et al.*, 2013), oxidation-reduction processes and iron transportation (Gortat, 2013) as well as absorption of nutrients. Exposure of animals to low levels of required minerals may cause reproductive impairment, physiological abnormalities, and behavioural modifications (Nriagu *et al.*, 2009). There is, therefore, in cases of mineral deficiencies, a need to supplement animal diets with minerals as this is not only a health threat to animals but also to human health. Humans cannot synthesise these minerals; they depend primarily on meat and organs/by-products of animals for their mineral intake.

The importance of mineral concentration in bones and organs/by-products has been overlooked in the meat industry. Like the meat, organs and bones also play a major role in meat production as they are the main storage environments of most minerals. However, as reported by Umaraw *et al.* (2015), there is a significant difference in mineral concentrations between muscles and

organs. Umaraw *et al.* (2015) showed a high concentration of sodium (Na) in the kidney, whilst copper (Cu) was found to be high in the liver (6.97 mg/100 g), iron (Fe) was high in the spleen (31.16 mg/100 g) and zinc (Zn) was high in the muscle (4.15 mg/100 g) of goat kids.

The liver is the major organ typically used to determine if an animal has any mineral deficiencies. In this case, supplements are provided to increase the concentration of the deficient minerals. On the other hand, the mineral status of game animals remains unknown as most conclusions have been made based on what the mineral content of livestock is. Using the bontebok and its close relative, blesbok (both grazers) and impala (a mixed browser/grazer) would provide a base-line of what could be expected for the mineral content of game animals and whether these antelopes differ in mineral composition. The aim of this study is to review the mineral concentration in bone, liver and muscle of game animals; these organ types were selected as they are the main storage areas of most minerals.

2.1. Mineral classification

Understanding the functions and transformation of minerals in living organisms has been improved by the contributions made in molecular biology. This has contributed to the knowledge base on the complex mechanism that is responsible for transporting trace minerals in cell membranes (Mayland and Shewmaker, 2001). Macro and micro minerals have four essential roles in the functioning of the body; namely: structural, physiological, catalytic and regulatory functions (Radwinska and Zarczynska, 2014; Suttle, 2010). The structural function involves elements that build organ and tissue structures (e.g., calcium, magnesium, phosphorus, silicon in bones and teeth, phosphorus and sulphur in muscle proteins) (Radwinska and Zarczynska, 2014).

Physiological functions are responsible for supplying electrolytes to the body fluid and tissues. Electrolytes are important ³⁶ for the maintenance of osmotic pressure, acid-base balance, membrane permeability and tissue irritability (Ranjith and Pandey, 2013). Macro and micro elements ¹⁴ operate as catalysts in enzyme and endocrine systems and can constitute integral and specific structural elements of metalloenzymes and hormones (Underwood, 1981).

⁴ The grouping of essential minerals into macro (major) and minor (trace) categories depends upon their concentration in the animal or quantities required in the diet (McDonald *et al.*, 2002). Macro elements are elements required by the animal in high concentrations; normally >50 mg/kg body weight (BW), whilst micro elements are required in very small quantities, typically <50 mg/kg BW (Radwinska and Zarczynska, 2014). Table 2.1 summarises essential micro and macro minerals and their required concentrations in an animal's body.

2.1.1 Macro minerals

Macro minerals are minerals that are required in large quantities by the animal's body (McDonald *et al.*, 2002). Similar to trace minerals, macro minerals are essential for the animal's growth, health, and reproduction. Overfeeding macro minerals causes high manure excretion rates which after many years of manure application leads to salt overload in soils (Norell and Chahine, 2014). Salt build-up results in plant toxicities, closes soil air surfaces, promotes water stress and decreases crop production. In addition, imbalances of Ca, or K, or Mg ions in the soil can cause a deficiency of these minerals due to cation competition in the plant material consumed by animals (Norell and Chahine, 2014). Table 2.3 summarises macro minerals and their deficiencies in animals.

2.1.2 Micro minerals

Trace minerals, for example, Co, ²⁰Cu, Fe, I, Mn, Mo, Se, and Zn have been recognised as being a basic requirement for normal functioning of all biological processes in the body (López-Alonso, 2012). The deficiency of micro mineral weakens ²productivity in livestock. Body micro mineral profile, immunity, and reproductive functions are directly related, where Cu, Co, Zn, I and Mn ³⁷are likely to influence these functions (Shahjalal *et al.*, 2008). Trace minerals must be supplied to animals in optimal concentrations and according to their requirements as these requirements change during the rapid growth of the animal and the reproduction cycle (López-Alonso, 2012). Traditionally and even to date, adding organic salts in animal diets has been the norm so as to accomplish all trace mineral requirements for animals (Cortinhas *et al.*, 2012).

As illustrated in Figure 2.1, a declining trace mineral concentration will influence an animal's performance. Clinical signs arise with a decrease in the animal's immunity and enzyme functions, optimum and normal growth, and fertility. According to Larson (2005), subclinical signs are more dangerous to animals than clinical signs. As they are not physically evident, the animal continues to reproduce and grow at a slower rate. On the other hand, clinical signs bring attention to the producer as they quickly become visible, thereby making it easier for the producer to act in terms of supplementing the mineral deficiency. Table 2.2 shows a summary of some essential micro minerals and their deficiencies in animals.

2.2. Effect of mineral content in animal tissues

Bone is a vital tissue in the animal skeleton, with the structural function being the most important. This includes ³⁴organ protection, locomotion, muscle activity, and load-bearing functions. Bone also serves as a reservoir for minerals. ³⁴Nutrition is the main factor affecting the composition of bone. According to Bleich (1990), French *et al.* (1956) and Robbins (1993), bone

breakability is highly associated with mineral imbalances as bones become weak and porous when there is a shortage of minerals. This can have an impact on the bone size and degree of mineralization, which in turn can affect mechanical performance (Olguin *et al.*, 2013; Burr, 2002). Mineral levels in animal diets can also contribute towards the mechanical performance of bones. This can range from major minerals, Ca and P, to micro minerals, including Mg, Mn, Cu, S and Zn (Corah, 1996). Deficiencies of these minerals can lead to weak bones and result in abnormalities such as poor bone mineralization, rickets, bone fracture and osteoporosis (Olguin *et al.*, 2013).

Of the body tissues, liver and bone have proved especially useful in anticipating mineral disorders in livestock since they are storage locations for certain minerals (Underwood and Suttle, 1999). The ¹¹liver is a very important organ that is involved in a variety of functions such as metabolism of fat, carbohydrate, protein, vitamins, and minerals, excretion of waste products and detoxification (Zaefarian, 2019). It is a major storage location for fat-soluble vitamins (A, D, K, and E) as well as vitamin B12 and ¹¹glycogen. It is also involved in the activation of vitamin D. Amongst these many functions, the liver also acts as a storage site for numerous minerals and is an important indicator of chronic mineral deficiencies and toxicities (Johnson *et al.*, 2007). According to Smit (2004), game meat consists of several mineral elements, which include P, Mg, Zn, Fe, Na, Cu, and Ca. The liver, kidney, heart, lean meat, fish and poultry are considered a good source of iron; lack of which causes anaemia in humans and is the most common of all nutritional deficiencies (Smit, 2004).

Mineral deficiencies can also occur in muscles, and these deficiencies can cause diseases in animals. The shortage of Selenium (Se) which leads to nutritional muscular dystrophy, commonly known as white muscle disease, is an example. According to Beytut et al. (2002),

white muscle disease involves hyaline production of skeletal muscles in different parts of the animal's body, heart muscle, tongue and the diaphragm. This disease is mostly seen in domestic animals younger than 6 months.

2.3. Factors affecting mineral concentration.

2.3.1 Species

An important aspect of adaptability is concerned with local breeds and species that, through the centuries, have accustomed themselves to a certain dietary and management regime. Any sudden change, such as increased body size, will result in a higher maintenance and production requirement. This gives rise to a need for more nutrients, which, if not supplied, will cause a general upset in the animal's metabolism as it attempts to meet these demands (McDowell, 1985). For instance, it is common for cattle introduced into a new area to exhibit signs of mineral deficiency, while the indigenous breeds that are usually slow-growing and late-maturing do not show the deficiencies to a similar degree. In addition, the possibility that non-adapted cattle to certain climates that sweat copiously in high temperatures and lose saliva and mucus from the mouth may lose substantial quantities of minerals, particularly in dry areas (McDowell, 1985). Table 2.2 is a summary of how different domesticated and game animals differ in muscle mineral concentration.

2.3.2 Age

Animal age is considered an important factor for mineral requirements. Increasing age may result in a decline in dietary requirements as the main requirement for growth for a given live weight gain often remains constant, while appetite increases in proportion to body size. These growth changes result in the need for the trace elements Fe and Zn being greatest since their requirements for growth are particularly high (Cunha and McDowell, 2012). Likewise, Se and I

deficiencies occur in the offspring of clinically normal-appearing mothers. Thus, when other factors (particularly absorption) are constant, the young ruminant is more vulnerable to trace-element deficiencies (Cunha and McDowell, 2012). The older animal also has the advantage of being more resistant to mineral excesses.

Young animals have the advantage of being more efficient in digesting specific nutrients as compared to mature animals, particularly pertaining to absorption efficiency (McDowell, 1985) and the mobilisation of minerals between organs within the body. As example, Rook and Storry (1962) found that approximately 30% Mg in young ruminants' skeletal structures can be mobilized under conditions of Mg deprivation while in adult animals, only 2% of bone Mg can be used for physiological needs. This is most apparent in that older cattle are much more susceptible to grass tetany.

2.3.3 Physiological status of the animals

Mineral requirements are highly dependent on the level of productivity and physiological state of the animal (McDowell, 1985). For instance, a pregnant immature beef cow in her first lactation would have significantly higher mineral requirements than a mature dry cow. Cows that produce high yields of milk would naturally need more dietary Ca and P than low yielding cows because of the richness of these elements in milk (Cunha and McDowell, 2012). Improved management practices that lead to improved milk production and growth rates for grazing ruminants will necessitate more attention to mineral nutrition (McDowell, 1985).

2.4.4 Sex

Female ruminants normally mature earlier than the males (Ledger, 1963) and deposit more intramuscular fat, particularly during the gestation period. This, therefore, increases the dietary

requirement for pregnant animals. In game animals, males can lose substantial amounts of body fat during the rut, due to reduced feed intake and the energy spent in fighting for dominance and maintaining the harem (Hoffman, 2000). According to Ortega-Barrales and Fernández-de Córdova (2015), sex does not seem to have a major influence on the concentrations of minerals in muscle/meat. Iron is contained in the myoglobin protein and according to Hoffman *et al.* (2005) high myoglobin concentration in muscles is due to systemic exercises. This would, therefore, mean that an increase in myoglobin results in an increase in iron concentration; such would mostly be seen in mature bull animals due to their frequent exposure to physical exercises, particularly during the rut.

2.3.5 Nutrition

Nutrition is by far the most important factor that affects mineral intake and mineral requirements. Providing animals with adequate forage diets is advisable to ensure that all mineral requirements are met. Forage intake can be reduced by factors such as the amount of protein (<7.0%) and increased degree of lignification in the plants which then results in a decrease in the entire amount of minerals consumed. According to McDowell (1985), the mineral concentration of animals during the dry season is lower when compared to the rainy season as tropical forages contain fewer minerals during the dry season. However, McDowell *et al.* (1984) reported that minerals are mostly found deficient during the rainy season in countries such as Kenya, Brazil, and South Africa.

Nutrition not only includes diets that are given to animals directly but also focuses on the naturally occurring vegetation where animals graze and/or browse freely. South Africa is characterised by nine different biomes namely: Thicket, Savanna, Grassland, Nama karoo, Succulent karoo, Indian Ocean coastal belt, Fynbos, Desert and Forest (Mucina and Rutherford,

2006). The Savanna biome is the largest biome that covers approximately 399571 km² of South Africa followed by the Grassland biome, covering 31986 km² (Mucina and Rutherford, 2006). The smallest biome is the Forest which is estimated to be less than 2000 km² (Booyesen and Tainto, 2012). All these biomes occur in different regions, with different vegetation types (Neethling *et al.*, 2016). Figure 2.2 shows all nine biomes and their different occupational spaces across South Africa. Neethling *et al.* (2016) note that wild species in South Africa are primarily dependant on the natural vegetation for feed, however, South African vegetation varies in mineral nutrition and concentration. For example, mineral concentration in the grassland biome cannot be the same as that in the Savanna biome as these two biomes have different vegetation and soil types. Grassland consists of grasses only, whilst the Savanna has a combination of a grassy ground layer and an upper layer with woody plants (Huntley, 1984).

Furthermore, because game farming is increasing in South Africa and is mainly practiced for breeding purposes, trophy hunting, and meat production, producers need to pay more attention to nutrition as it not only affects mineral concentration within the organs but also the breeding performance and health of the animals. Artificially formulated feed diets should contain all the necessary nutrients required to maintain growth, good health, and reproduction. If any mineral deficiencies occur, the applicable levels of mineral supplements in the feed should be revisited.

2.3.6 Water availability

Water is by far the most important component after oxygen, to maintain life processes. The Earth's surface is covered by about 95% of water but only 2.5% is available for consumption (FAO, 2006). Agriculture accounts for 70% of the global water withdrawals (FAO, 2012) and animal production comprises 29% of the water used in agriculture (Mekonnen and Hoekstra, 2012). An inadequate supply of water can be a danger to the health of an animal and can also

cause a reduction in the animal's performance. Animal water supplies typically have a very low concentration of macro minerals and provide only a few grams of each mineral per day under normal water consumption rates (Norell and Chahine, 2014). Nonetheless, water can provide 50% or more of the animal's daily requirements for some of the minerals (Norell and Chahine, 2014). In addition, ration adjustments may be required if the animal's water supply has high mineral contents.

2.4 Soil-plant-animal relationship

The mineral concentration in animals is dependent on the soil and plant associations. The mineral content of natural vegetation is primarily determined by the mineral availability and mineral composition of the soil (Smart *et al.*, 1981). Mineral concentrations of soils influence the concentrations within the plants and as a result, it affects the mineral concentrations in the muscle tissue of the animals grazing on the plants (Zomborszky *et al.*, 1996; McDowell and Conrad, 1977). Extent and pattern of mineral deficiencies/excess in plants vary in different agro-climatic conditions as available mineral content in green vegetation depends on physical and chemical properties of soil, soil erosion, cropping pattern, fertilizer/chemical application, species and genetic variation in plants, stage of maturation as well as the presence of other minerals (McDowell *et al.*, 1983). Studies on the soil-plant-animal relationships with respect to minerals are important and can have significant implications in animal production (Biswas and Samanta, 2002).

2.5 Effect of production systems on the mineral content of game species.

Just like livestock, game animals are also maintained and reared in different production systems (intensive, semi-extensive, and extensive production systems) depending on the production end goal. Intensive farming involves keeping animals in small paddocks, thereby restricting their

movement. It aims for high production on the smallest possible land area frequently making use of strategic supplementary feeding and breeding programs (Taylor *et al.*, 2016). The feed is supplemented according to the body's requirement or according to a certain deficiency. This production system is predominantly practiced in areas that are compactly populated and where grazing land is limited. Animal feed can either be purchased or grown on the farm (Mubiru, 2013). The intensive production system aims to maximise animal production and thus increase the nutritional value of the animal and the volume of meat. From a nutrition perspective, the addition of mineral supplements in animal diets is of great importance to avoid animal losses due to these deficiencies.

A semi-extensive production system involves the utilization of both intensive and extensive production systems. The systems allow for grazing in larger camps but also provide supplementation when necessary (Van Heerden, 2018). For example, in times of drought and when grazing land is limited, additional feed is supplemented. Secondly, when the animals are unhealthy due to mineral deficiencies, that particular mineral can be supplemented by means of salt licks or mixing supplements with the fed diets.

In an extensive production system, animals are allowed to graze freely on natural vegetation with no supplementation provided (Van Heerden, 2018). Animals walk long distances in search of water and feed. Losses of animals can result due to exposure to diseases because of reduced management practices, which results in poor meat quality. Animals are highly exposed to diseases such as bloat, hypocalcaemia, grass tetany, ketosis, etc. The mineral concentration is expected to be lower for animals reared in this production system when compared to animals in the intensive and semi-extensive systems as they are not provided with supplements.

2.6 Supplementing to correct mineral imbalances.

In cases where shortages/deficiencies of a certain mineral occur, supplementation of that particular mineral would need to be provided to the animals. Mineral deficiencies can be corrected by supplementary feeding which can be achieved by means of a mineral or salt lick. As animals cannot synthesize their own minerals, plant and plant products are the main sources of these minerals, hence plant composition has an impact on the animal's mineral intake (McDonald *et al.*, 2002). All the factors that influence the mineral concentration in plants are therefore important (Underwood and Suttle, 1999). Minerals can be provided directly and/or indirectly to animals.

The direct administering of mineral includes oral drenching, salt licks, free-choice supplementation, the inclusion of deficient minerals in diets, injection, etc. Indirect supplementation focuses mainly on the soil and plants, this involves the inclusion of fertilisers, foliar sprays, actions to change the pH of the soil and availability of forages that are known to have a high mineral content. Usually, diets for animals contain a mineral element supplement and, on occasions, it is necessary to include extra supplies of some minerals, e.g., Ca for laying hens. Common sources of minerals used in mineral supplements are limestone for Ca, dicalcium phosphate (CaHPO_4) for P, common salt for Na, and calcined magnesite for Mg (McDonald *et al.*, 2002). Trace elements are usually supplied in a salt form, e.g. Se as sodium selenite (McDonald *et al.*, 2002).

There is insufficient information regarding the mineral requirement for most game farmed species such as the bontebok, blesbok, and impala in South Africa. There is limited information on their base-line concentration of macro- and micro-minerals.

2.7 Species description

2.7.1 Bontebok (*Damaliscus pygargus pygargus*)

Historically, the bontebok species were thought to be extinct, and only 27 individuals were in fact ¹² saved by Cape farmers in the Bredasdorp area in 1837 (Skinner and Chimimba, 2005). Presently, Corwell and Birss (2017) report that the statistics of the management of this ¹² species within the Natural Distribution Range (and Extended Distribution Range) in the Western Cape is approximately 1650 animals. Furthermore, Corwell and Birss (2017) also noted that about 7500 of these species were found in ¹² properties outside the native range of the species throughout South Africa. The bontebok species are currently used for trophy hunting (Furstenburg, 2016). ¹² Bontebok are tolerant of human activities and adapts reasonably well to changes in the environment.

¹⁷ Translocation of bontebok from Bontebok National Park has formed the nucleus of reintroduced populations in other protected areas such as provincial and local authority nature reserves. The ¹⁷ bontebok is endemic to the Western Cape, South Africa, although the introduction of the species has been made in most provinces. Bontebok were originally ⁹ confined to the coastal plain (60–200 m) east of the Kogelberg in the Western Cape where they are believed to have concentrated on the renosterveld areas (Boshoff and Kerley 2001). Skinner and Chimimba (2005) mentioned that this antelope is a medium-sized species, with the females weighing slightly less than the males. It can be very difficult to differentiate the female from the male bontebok in the field since they are similar in colour, with the males being slightly darker. The ¹² horns of the females are more slender and hardly thicken up at the base (Skinner and Chimimba, 2005).

The Bontebok species is known to be a virtually complete grazer (Beukes, 1984) and has a high preference for newly burnt and short grasses (⁹ Kraaij and Novellie, 2010). Water is an essential

habitat requirement as they stay within 1.5 km from surface water during the dry season (Luyt 2005). Within their natural range on the coastal lowlands, grassy areas are predominantly found in the renosterveld areas but bontebok also makes use of small grassy microhabitats or recently burnt fynbos and strand-veld habitats (Radloff, 2008).

Due to the translocation of this species outside its natural range, anecdotal information seems to indicate that problems are being experienced with potential mineral deficiencies. One such problem is where the pelt becomes light (Suttle, 1992), even having a coppery hue to it which is thought to be caused by a copper deficiency. However, there are no baseline data for this species on what the concentration range of normal meat, liver and bone minerals should be. An ideal area to evaluate what this could be is to analyse these organs in bontebok found in their natural range, the Renosterveld.

2.7.2 Blesbok (*Damaliscus pygargus*)

Blesbok's existence was first reported in plateau grasslands of the Highveld district in the 17th century; possibly due to the easy accessibility of water in that region (Skinner and Chimimba, 2005). This species is popular for meat production and trophy hunting. The ¹ distribution of blesbok included parts of western and north-western KwaZulu-Natal, the northern Karoo in the Northern and Eastern Cape, as well as the Highveld of the Free State and Gauteng (Lloyd and David, 2008). Blesbok are now found widespread throughout southern Africa (Skinner and Chimimba, 2005). They mostly occur and are abundant in summer rainfall areas where farm ranches are privately owned. ¹ These regions include the Grassland Biome of the Free State, North West, Eastern Cape, Gauteng, Mpumalanga and the western edge of Kwazulu-Natal provinces. However, Chase and Meadows, (2007) and Watson *et al.* (2011) reported that these species can also be found in coastal areas and in regions with no seasonal rainfall.

The bontebok (*Damaliscus pygargus pygargus*) and blesbok (*Damaliscus pygargus*) are closely related and can interbreed with viable hybrid offspring.

According to Smit (2004), the blesbok is mostly mistaken for the bontebok as they appear to be the same in shape, colour, and structure even though the bontebok colours are more striking than that of the bontebok. Another characteristic that can be used to differentiate these two antelopes is the bald spot in the middle of the forehead on the blesbok species that the bontebok does not exhibit (Smit, 2004). Blesbok are exclusively grazers, feeding only on grass. There seems to be no evidence of any preference for certain plant communities, but the blesbok grazes less eagerly on unburnt areas (Du Plessis, 1972). Blesbok are similar to sheep in that they graze very close to the ground by gathering feed using its lips (Smit, 2004). Similar to bontebok, blesbok also show preference for newly burnt grasses during the early summer when the sprouts are not yet developed (Du Plessis, 1972). After the first heavy frost, usually, in May, blesbok enters a period of minimal activity, which lasts until the spring when the grass starts to sprout (Smit, 2004). Just like the bontebok, there is no baseline information about the mineral concentrations in the meat, bone or liver in this species.

2.7.3 Impala (*Aepyceros melampus*)

Impala are a pronghorn species with males (60-65kg) relatively larger than females (40-45kg) in terms of body size. The males are distinctively different from females as they have strong rigid lyre-shaped horns whilst the females have no horns (Jarman and Jarman, 1973; Lewis *et al.*, 1997). Skinner and Smithers (1990) and Hoffman (2000) had similar findings on the body weight of this species, where they reported that mature males weigh ± 50 kg whilst mature females weigh ± 40 kg. This antelope is reddish-brown in colour with a light brown patch on the

upper body and has an untainted white colour underneath, at the belly section (Skinner and Smithers, 1990).

Impala occurs throughout the wooded grassland and open woodland biomes of Eastern, Central, and Southern Africa, and are mixed grazers/browsers (Jarman and Jarman, 1973; Lewis *et al.*, 1997; Cooper, 1982). They occur from northern Kenya south to northern Kwa-Zulu Natal in South Africa and extending westwards towards southern Angola (Skinner and Smithers, 1990). Despite their limited mobility, impala respond to the climatic variability of semi-arid environments by grazing during wet seasons and browsing during drier periods (Laaka, 2014). In the northern parts of the sub-region, they are associated particularly with acacia and mopane, *Colophospermum mopane*, where the cover and the availability of surface water are essential habitat requirements (Laaka, 2014). Impala are intermediate mixed feeders.

2.8 Summary

Meat is fundamental to human health for proteins, vitamins, and minerals. Thus, it is essential to provide animals with balanced diets as failure to feed diets with adequate nutrition and required minerals can be a threat to animal and human health by increasing the chances of diseases. Mineral nutrition is of paramount importance to animals in maintaining a normal life process, normal growth, health, and reproduction. In cases where deficiencies of certain minerals arise, it is advisable to add supplements in diets or provide a mineral lick before the animal starts showing clinical symptoms. Selecting and using the correct mineral supplements can save the farmer from animal losses and can contribute to increased animal production. However, over supplementation can lead to unnecessary mineral excretion, or even poisoning, hence it is important to provide a proportion that is according to the animal's mineral requirement.

Before any mineral can be supplemented, knowledge is required to determine which mineral is limited. However, very little information exists on what is the normal range of the minerals for the three species mentioned. Each of the species plays an important role in the South African game ranching industry. The bontebok is a grazer that was historically found in the Rensoterveld region in the Cape Province whilst the closely related blesbok, also a grazer was found to be abundant in the grassland regions. The impala is one of the most widely distributed and hunted species in South Africa and is a mixed feeder grazing grass and browsing. It also has the ability to adapt its specific eating habits to what is available. Yet, little information exists on the mineral range found within its body.

The muscle, liver, and bone all play an important role in the mineral concentration within an animal's body and thus an analysis of the mineral profile of these organs in these three species will be of value in providing base-line information on what is a normal range for these species.

Chapter 3: Bone, liver, and muscle mineral concentration of impala (*Aepyceros melampus*) reared in different regions and production systems.

Abstract

The effect of region/production system on the mineral concentration in organs (liver, *Longissimus thoracis et lumborum* muscle and rib) of impala was investigated. Impala from intensive (INT) and semi-extensive (SE) production systems in Limpopo, an extensive production system from the Western Cape (EXT (W)) and an extensive production system in Namibia (EXT (N)) were compared. Proximate analysis was conducted on the muscle whilst mineral analyses were conducted on the bone, liver, and muscle. Differences were detected for the moisture, protein and fat contents between the regions/production systems. Muscle from the INT and SE regions/production systems had a higher ($P \leq 0.05$) moisture content to the muscle from EXT (W) and EXT (N), while protein content in the muscle from the EXT (N) region/production system was higher ($P \leq 0.05$) than from the other regions/production systems. Fat content was higher ($P \leq 0.05$) in the EXT (W) and SE production systems. There were no differences in the ash percentage for all region/production systems ($P > 0.05$). Micro mineral (B, Al, Fe and Co) concentrations in the liver did not differ ($P > 0.05$) between region/production systems. Conversely, liver in the EXT (W) region/production system had higher ($P \leq 0.05$) concentrations of Se, Sr, Mo, Pb, and Si, whilst EXT (N) liver had higher concentrations of V, Cr, Ni, Cd, and Hg. Higher concentrations of Mn, Cu, Zn, and As in the liver were observed in the INT production system whilst the liver in the SE production system had a high concentration of Ba. Macro minerals in the liver were found to be high in the EXT (W) region/production system. On the other hand, concentrations of Co, Ni, Sr, Cd, and Si in the muscle did not differ

($P>0.05$) between regions/production systems. However, higher micro minerals (Al, V, Cr, Mn, Cu, Zn, As, Mo, Ba, and Pb) were detected in muscle from the EXT (N) region/production system whilst EXT (W) muscle had higher Fe and Se levels. The results showed no difference ($P>0.05$) for concentrations of Cr, Mn, Fe, Co, Cu, As, Se, Cd, and Sb in the rib throughout all the regions/production systems. The rib had very high concentrations of Fe, Zn, Sr, and Si micro minerals. Higher concentrations of all macro minerals in the rib were recorded for the EXT (W), INT, and SE regions/production systems. The results showed that region/production system does have an effect on the concentration of minerals in these organs.

Keywords: Game animals; production systems; organs; proximate composition; mineral profile

3.1 Introduction

Game farming has developed into an established agricultural industry in South Africa (Hoffman *et al.*, 2005a). This has resulted in an increase in the production of game meat; Taylor *et al.* (2016) reported a total mass of 21.22 million kg of game meat produced from wildlife ranches in 2014 through culling and trophy hunting. Such contributions reflect positively on the economy. According to Child *et al.* (2012), since there has been substantial growth in game farming in southern Africa, a large proportion of land used for livestock has been converted to accommodate game animals. Impala traits enable it to survive in different habitats (Skinner and Smithers, 1990) resulting in a large dispersal of this species in southern Africa. According to Bourgarel *et al.* (2002) and Hoffman (2000) the large dispersal of the impala, together with their relative abundance as a species, has resulted in the impala being well suited to maintain yearly cropping regimes. ⁵ Impala are categorised as intermediate, mixed feeders amongst wild ruminants (Laaka, 2014). Fairall (1983) reported that the browsing habits of the impala make them suitable to the South African bush-veld as they browse on trees and shrubs.

The impala is a selective grazer that prefers feeding on fresh ⁵ grass shoots (during the wet season), herbs and shrubs (Laaka, 2014). According to Jarman and Jarman (1973), impala has an increased feed intake in arid seasons as compared to wet seasons and the female impala reach ⁴² their asymptotic mass of 43.2 kg at the age of ± 3 years whilst at the age of 4.5-5 years, males weigh 56.6 kg (Van den Berg, 2009). The Serengeti area in Tanzania has been reported as having larger and heavier impala in all body measurements than impala from southern Africa (Fairall and Braack, 1976). Many factors such as season, habitat, nutrition (including water availability), and age might be contributing factors for these differences; nutrition being the most important factor as it includes mineral nutrition which plays a significant role in animal growth. Minerals

are all the elements that are needed by animals for normal life processes such as animal health, growth, and reproduction (Mirzaei, 2012). Minerals are categorised into micro and macro minerals and all are said to be essential to animals (McDonald *et al.*, 2002). Many of the micro minerals (Zn, Fe, B, As, Cr, Mn, and V, amongst others) are stored in the internal organs with the liver being the main storage location for most of these minerals (Johnson *et al.*, 2007). Macro minerals (Ca, Mg, K, P, S, and Na) are mainly found in the skeletal system. According to McDonald *et al.* (2002), minerals can either be needed in large or small quantities depending on the animal's requirements. They can be obtained from feedstuff (through grazing/browsing or through supplied diets and/or by supplementation), from water and from the soil. Very little to no research has been conducted on the mineral requirements of game animals. Mineral concentration in animals differs with anatomical location (Laaka, 2014). Findings have shown high micro mineral concentrations in the liver and bones whilst macro minerals were mostly reported to be high in bones. However, no research has been conducted on the effect of production systems on mineral concentrations in game animals.

There is, therefore, a need to carry out further research in this area so as to have a better understanding of the mineral status of game animals. The purpose of this study is to determine the mineral composition in the liver, muscle and rib of impala reared in different regions/production systems so as to provide base line data for animal nutritionists and veterinarian practitioners.

3.2 Methods and materials

3.2.1 Study site

Impala were harvested from three different regions and three production systems. The regions were Castle de Wildt in Limpopo, Mount Etjo game reserve in Namibia and Bredasdorp, Western Cape. The impalas from Limpopo were hunted in both an intensive (INT) and semi-extensive (SE) production systems which were located in the Savanna biome. The intensive production system had small camps and supplementary feed was provided to the animals whilst the semi-extensive production system had large camps and the animals depended primarily on natural vegetation with minimal supplementary feed. Bredasdorp is a region situated ¹⁵ in the Fynbos biome of the Western Cape, and the animals grazed extensively (EXT W) on natural vegetation with no supplementation. Mount Etjo game farms in Namibia practice an extensive production system (EXT N) with large camps and only natural vegetation as feed. This farm is located in the North of Namibia, in the Otjozondjupa region which comprises of thorn-bush vegetation, with mainly acacia trees.

Six ram animals of similar age (± 15 months old) were harvested from each production system. Ethical clearance was approved at the University of Fort Hare (MUC081SMPA01).

3.2.2 Experimental procedure

The harvesting of animals was according to South African standard procedures (Hoffman, 2000). All animals were hunted during the day with light calibre rifles. These animals were shot in the head and the jugular vein was severed for exsanguination after the animal had dropped. Thereafter they were taken to a pre-prepared slaughter area for carcass dressing which involves removal of internal organs and animal hides.

3.2.3 Sampling procedure

All internal organs were removed from the carcass immediately after slaughter. The liver and *Longissimus thoracis et lumborum* (LTL) muscle ²² were vacuum packed and stored in a -20°C freezer until analysed. The last rib was cut from each animal, cleaned of excessive meat and stored in plastic bags in a -20°C freezer until defatting and analysis.

3.2.4 Defatting of bone for analysis

The rib bone samples were thawed overnight at 4°C prior to defatting. The porcelain crucibles for all samples were heated to 100°C, then were left in a desiccator for half an hour to cool prior to weighing. Bone samples were weighed and dried at 100°C for 24 hours. After 24 hours, samples were taken out of the oven and cooled in the desiccator for half an hour. Sample weights were then recorded. The dried bones were then defatted by being placed in an 800 ml Erlenmeyer flask where 10 ml of petroleum ether was poured into the flask. After 24 hours, the bone fat layer was removed, and the flask was again topped up with the petroleum ether. After 48 hours, the petroleum ether was discarded once more, and samples were allowed to rest for 2 hours before being dried at 100°C for 24 hours and bone samples were weighed again after the second drying. Bone samples were then placed in a furnace for 24 hours at 600°C. Samples were taken out of the furnace and allowed to cool in a desiccator for 40-60 minutes. Sample weights were taken. The samples were then crushed using a mortar and pestle and the ash was weighed. The samples were then kept in a drying oven at 60°C to prevent them from absorbing moisture until they were analysed for mineral analysis.

3.2.5 Chemical analysis

3.2.5.1 Acid digestion

After all the meat and liver samples were homogenised and the bone samples ashed, samples were digested at the Soil Science Department at Stellenbosch University. One g of the sample was weighed into appropriate vessels whereafter 1ml of concentrated peroxide and 6ml of concentrated nitric acid was added and the sample left to predigest for 20min before sealing the vessels for microwave digestion in a MARS microwave digester (model MARS 5) (CEM cooperation, 3100 Smith farm road, Matthews, NC 28104). The power level of the microwave was 1600W at 100%. The samples were heated for 25 minutes from 20 to 210°C at a pressure of 800 psi. Samples were then diluted 10x with Deionised water into acid cleaned sample tubes. Nine ml of concentrated hydrochloric (HCL) acid was measured into 20ml centrifuge tubes, 1ml of the digested solution was added into the tubes. The samples were then ready for mineral analysis.

3.2.5.2 Macro element analysis

Macro elements (Ca, K, Mg, Na, and P) were analysed on a Thermo ICap 6200 ICP-AES (Thermo Fisher Scientific Inc 168 Third Avenue in Waltham, Massachusetts). The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards purchased from Inorganic Ventures (Inorganic Ventures, 300 Technology Drive, Christiansburg, VA 24073) to quantify selected elements. Throughout the analysis and prior to sample analysis, a NIST-traceable quality control standard (De Bruyn Spectroscopic Solutions, Bryanston, South Africa) was analysed to verify the accuracy of the calibration so as to monitor drift.

3.2.5.3 Micro element analysis

¹⁰ An Agilent 7900 quadrupole ICP-MS was used to analyse micro elements ²⁷ (B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sb, Ba, Hg, Pb, and Si). This was achieved by first calibrating the instrument, ¹⁰ using NIST traceable standards from Inorganic Ventures (Inorganic Ventures, 300 Technology Drive, Christiansburg, VA 24073) to quantify selected elements. A NIST-traceable quality control standards at high and low concentration levels (De Bruyn Spectroscopic Solutions, Bryanston, South Africa) were analysed to verify the accuracy of the calibration before sample analysis, as well as after every 12 samples to monitor drift.

⁶ Samples were introduced via a 0.4ml/min micro mist nebulizer into a peltier-cooled spray-chamber at a temperature of 2°C. The instrument was optimised for analysis in HMI mode, where all samples and standards are diluted with argon gas to minimise matrix load to the analyser. Oxide formation was less than 0.3%. Three replicate measurements with appropriate dwell times were carried out on each analyte. All elements were measured in the He-collision mode. An internal standard (ISTD) solution containing Sc, Y, Ge, Rh, and In was introduced online to monitor instrument drift and correct for matrix differences between samples and standards. Appropriate ISTD's were matched to analytes according to their proximity in mass and ionisation potential. During the ¹³ course of the analysis, ISTD recovery was between 90 and 110% for all samples, and recovery for drift monitor standards between 95 and 105%. Table 3.1 indicates the limit of detection (LOD) for the specific elements.

3.2.6 Proximate analysis

The proximate analysis was only done on the *Longissimus thoracis et lumborum (LTL)* muscle samples. The samples were analysed to determine the moisture (Method 934.01) and ash (Method 942.05) content according to the AOAC (2002). The protein content was measured using the AOAC (1992) procedure 992.15, whereas the fat was determined using the chloroform/methanol (2:1) fat extraction method according to Lee *et al.* (1996). All analyses were performed in duplicate.

3.3 Statistical analysis

The experimental data was analysed using XLSTAT version 19.2 statistical program. Due to physiological differences between the sample types, production systems were compared using one way analysis of variance (ANOVA) for individual sample types of liver, muscle and rib. Bonferroni post-hoc test was used to identify the statistical significance at the 5% level with all results $P \leq 0.05$ considered to be statistically significant.

The following statistical model was used:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where: Y_{ij} is the response variable (minerals)

μ is the overall mean

α_i is the effect due to treatment (extensive system Namibia, extensive system Western Cape, intensive system Limpopo and semi-extensive system Limpopo)

ε_{ijk} is the error

3.4 Results

3.4.1 Proximate composition

Results in Table 3.2 show the effect of region/production system on proximate composition of the *Longissimus thoracis et lumborum* (LTL) of impala. The impala muscle from INT and SE regions/production systems had high moisture contents (75.4% and 75.5%, respectively) followed by impala muscle in the EXT (W) region/production system (74.7%) whilst the EXT (N) region/production system had the lowest moisture content (73.7%). The EXT (N) region/production system had the highest protein content (24.2%) that differed significantly ($P \leq 0.05$) from the protein content of other regions/production systems. There was no difference ($P > 0.05$) in protein content between the EXT (W) region/production system and the INT region/production system, and EXT (W) and SE regions/production systems. The fat content was highest in the SE region/production system (2.2%) and did not differ from the EXT (W) region/production system (1.9%). Fat was lowest in the INT region/production system (1.4%) and similar to the EXT (N) region/production system. There was no significant difference in the ash percentage for all regions/production systems ($P > 0.05$).

3.4.2 Macro mineral concentrations in impala liver, muscle and rib from different regions/production systems.

Results from Table 3.3 show the effect of region/production system on macro mineral concentration in impala liver, muscle and rib. The findings showed significant ($P \leq 0.05$) concentrations of Ca and Na in the liver from the EXT (W), INT and SE regions/production systems, whilst the Ca concentration in the liver from SE was similar ($P > 0.05$) to that in the EXT (N) region/production system. A significant concentration of K was detected in the liver from EXT (W), EXT (N) and SE regions/production systems; however, the liver from the SE region/production system was similar ($P > 0.05$) to the liver in the INT region/production system.

Mg was high ($P \leq 0.05$) in the liver from EXT (W), EXT (N) and INT regions/production systems, and the liver from EXT (N) and INT were similar ($P > 0.05$) to that in the SE region/production system. The liver from EXT (W) and EXT (N) regions/production systems had high ($P \leq 0.05$) P concentration, although EXT (N) was similar ($P > 0.05$) to INT and SE regions/production systems. Additionally, the liver from the EXT (W) production system had numerically high macro mineral concentrations than all the other production systems. The region/production system did not affect ($P > 0.05$) any of the macro mineral concentrations in the muscle. K was the most dominant muscle mineral followed by P. The rib in EXT (W), INT and SE regions/production systems all had significantly higher ($P \leq 0.05$) macro mineral concentrations compared to the rib in EXT (N) region/production system.

3.4.3 Micro mineral concentrations in impala liver, muscle and rib from different regions/production systems.

Results from Table 3.4 show the effect of region/production system on the micro mineral concentration in impala liver, muscle and rib. The concentration of B, Al, Fe, and Co in the liver was not influenced by region/production system. However, the liver from the EXT (N) region/production system had high ($P \leq 0.05$) V, Cr, and Cd concentrations. Mn concentrations were high ($P \leq 0.05$) in the liver from EXT (N), INT and SE regions/production systems. Ni concentration was high ($P \leq 0.05$) in the liver from EXT (W) and EXT (N) production systems, however, the liver from EXT (W) was not significantly different from that in the INT production system and liver from the INT production system was similar to that in the SE production system. Cu was high ($P \leq 0.05$) in the liver from the INT region/production system. The concentration of Zn was highest ($P \leq 0.05$) in the liver from INT and EXT (N) regions/production systems followed by EXT (W) and SE production systems. A high ($P \leq 0.05$) concentration of As was found in the liver from the INT, EXT (W) and EXT (N) regions/production systems though

the liver from EXT (W) and EXT (N) regions/production systems had similar levels to that in the SE region/production system. Mo was higher ($P \leq 0.05$) in the liver from EXT (W) and EXT (N) regions/production systems whilst liver from EXT (N) had a similar concentration to the liver in the INT region/production system whilst that in the SE region/production system differed from those in other regions/production systems. Ba was highest ($P \leq 0.05$) in the liver from the SE region/production system. High ($P \leq 0.05$) concentrations of Hg were detected in the liver from the EXT (W) and EXT (N) regions/production systems. A high ($P \leq 0.05$) concentration of Si was found in the liver from the EXT (W), INT and SE regions/production systems; however, liver from the INT and SE regions/production systems did not differ from the liver in the EXT (N) region/production system.

Concentrations of Co, Ni, Sr, Cd, and Si in the muscle did not differ ($P > 0.05$) between production systems. On the other hand, Al, V, Cr, Mn, Mo, Ba, and Pb concentrations were higher ($P \leq 0.05$) in the muscle from the EXT (N) region/production system compared to the other production systems. Fe concentration was high ($P \leq 0.05$) in the muscle from EXT (W) and EXT (N) regions/production systems, and muscle from the EXT (N) did not differ ($P > 0.05$) from the muscle in the INT and SE regions/production systems. High ($P \leq 0.05$) concentrations of Cu were found in muscle from EXT (N) and EXT (W) regions/production systems, whilst the muscle from the EXT (W) region/production system was similar ($P > 0.05$) to that in the SE region/production system which did not differ ($P > 0.05$) from muscle in the INT region/production system. Zn was higher ($P \leq 0.05$) in the muscle from the EXT (N), EXT (W) and SE regions/production systems and was low in muscle from the INT region/production system. The concentration of As differed significantly in muscle from the EXT (N) and INT regions/production systems. The concentration of Se in the muscle varied in all

regions/production systems with muscle from the EXT (W) having the highest ($P \leq 0.05$) concentration.

The results showed no difference ($P > 0.05$) for Co, Ni, Cu, Se, Cd, and Sb concentrations between all the regions/production systems for the rib bone. However, B had a high ($P \leq 0.05$) concentration in the rib from EXT (W) and EXT (N) regions/production systems and was low ($P > 0.05$) in the rib from INT and SE regions/production systems. Al, Mo and Pb had high ($P \leq 0.05$) concentrations in the rib from EXT (N) region/production system. High concentrations for V were detected in the rib from the INT and EXT (N) region/production system, with the exception that EXT (N) did not differ from SE and SE did not differ from EXT (W). Ribs from EXT (W), EXT (N) and INT regions/production systems had high concentrations of Cr; however, EXT (N) and INT were similar to SE. Mn was highest ($P \leq 0.05$) in the rib from EXT (N), INT and EXT (W) regions/production systems, however, EXT (W) and INT did not differ ($P > 0.05$) from SE. Significant ($P \leq 0.05$) differing Fe concentrations were recorded for the ribs from the EXT (W), INT and SE regions/production systems. Zn was high ($P \leq 0.05$) in the ribs from INT and EXT (W) regions/production systems, although, EXT (W) did not differ from SE ($P > 0.05$) and SE was similar to EXT (N) ($P > 0.05$). High concentration of As was recorded in the ribs from the EXT (W), EXT (N) and INT regions/production systems but EXT (W) and INT regions/production systems did not differ ($P > 0.05$) from SE region/production system. Sr and Si had high concentrations in the rib from the EXT (W) region/production system. Ribs from the INT and SE regions/production systems had significant concentration of Ba.

3.5 Discussion

3.5.1 Proximate composition

Game meat has been reported as being healthy as it contains high protein content and has very low fat levels (Hoffman, 2007). Consumer preferences have changed from purchasing and eating only the meat of domesticated animals to eating game meat due to the latter's health advantages (Niewiadomska *et al.*, 2020). According to Hoffman *et al.* (2005b), proximate composition can differ depending on the species, type of muscle, age of the animal, and sex. Additionally, Hocquette *et al.* (2010) noted that proximate composition differs between muscle types because of differences in muscle structure, muscle function and muscle location in the carcass. Nutrition is also considered to effect proximate composition as it can influence the fat and protein contents.

In the current study, impala muscle from different production systems was analysed. INT and SE region/production systems had high ($P \leq 0.05$) moisture content and low protein content, whilst the protein content was significantly high in the EXT (N) production system

Needham *et al.* (2020) reported comparable results for the moisture and protein contents of impala muscle to that of the current study, where the semi-extensive production was high in moisture and low in protein content whilst the opposite was seen for the extensive production system. In this investigation, the fat content was significantly higher for the SE production system followed by the EXT (W) production system, EXT (N) production system and lastly the INT production system. This is possibly influenced by the fact that the impala animals in the SE production system were allowed to graze freely on the vegetation with no limitation and were also provided with supplementary feed when needed. Results in Table 3.1 showed no significant difference in the ash content across all regions/production systems ($P > 0.05$).

3.5.2 Macro mineral concentration in impala liver, muscle and rib from different regions/production systems.

Macro minerals in the liver were found in different concentrations from the impala rams sampled from the different production systems. However, the impala liver from EXT (W) showed higher macro mineral concentrations than any other production system. Results of the high macro minerals in the EXT (W) region/production system might be due to the fact that the EXT (W) is in the Fynbos biome which consists of Renosterveld vegetation. The Renosterveld is a relative clay-based veld type. Clayey soil types are all soils that develop from shale, mudstone, dolomite and igneous rocks. These soil types are rich in nutrients, are very fertile and produce palatable vegetation for animals (Van Rooyen, 2010). Nutrient-rich soils influence mineral absorption by plants, and thus by animals.

There was no significant difference in the concentrations of macro minerals in the muscles in all regions/production systems. However, even though no macro mineral concentrations differences were detected between these regions/production systems, the muscle from the EXT (N) region/production system had numerically higher concentrations of Ca, K and P, whilst the muscle from the EXT (W) region/production system had high concentration of Mg and the SE region/production system had a high concentration of Na. High macro minerals were only observed in the extensive regions/production systems and semi-extensive region/production system whereas the intensive region/production system had low concentrations. This suggests that since the EXT (W), EXT (N) and SE had higher concentrations of macro minerals than the INT production system; supplementary feed containing macro minerals would not be needed as a requirement to animals reared in these production systems, as sufficient minerals seem to be available to the animals during their grazing/browsing. Interesting, although the INT system's

impala received feed, the possibility exists that the feed fed had insufficient minerals and a mineral lick might be required.

Impala ribs from the extensive production system from the Western Cape [EXT (W)], intensive production system from Limpopo (INT) and from the semi-extensive production system from Limpopo (SE) had higher macro mineral concentrations ($P \leq 0.05$) than impala rib in the extensive production system from Namibia [EXT (N)]. Regional factors such as the season in which the animals were hunted, the soil condition, soil pH and the type of vegetation in the different regions could have had an effect on these results. López-Alonso (2012) and Underwood (1999), reported that some minerals become more concentrated in soils with a pH that is higher than 6.5, whereas others become less concentrated. The mineral nutritional value of forage plants for grazing animals is greatly influenced by the condition of the soil which is also dependent on the amount of water it absorbs.

Climate conditions may also have had an effect on these results, considering the fact that the animals in question were hunted during different seasons which was March (end of summer) for EXT (W), INT, and SE and during the dry winter of July for EXT (N). Besides the fact that the Western Cape Province has winter rainfall in the fynbos biome (Rutherford *et al.*, 2006), mineral concentrations were still higher than those in Namibia. Namibia is known to be a very hot and dry country (Lushetile, 2009) with no rainfall at all during winter. These climatic conditions might have contributed to the low concentrations of macro minerals seen in the EXT (N) region/production system since the soils would not be as fertile as soils receiving more water. High mineral concentrations were expected in the INT and SE production system as these regions/production systems are located in the Limpopo Province which has summer rainfall

(Rutherford *et al.*, 2006) as well as the fact that the animals were given supplementary feed containing a mineral pre-mix.

8 Vegetation plays an important role in the mineral concentration of plant-animal pathways since some minerals are obtained through plants. According to Neethling *et al.* (2016), wild species in South Africa are primarily dependant on the natural vegetation for feed. However, these plants have different mineral concentrations as they grow in different soils and receive different amounts of precipitation. The type of vegetation occupying a region depends on the biome in that particular region (Neethling *et al.*, 2016). For instance, in the current study, the INT and SE production systems in the Limpopo region are located in the Savanna biome. This biome is covered by a mixture of a grassy ground layer and an upper layer with woody plants (Huntley, 15 1984). The EXT (W) region/production system is located in the Fynbos biome which consists of evergreen low and tall shrubs, grass-like plants and sedges (Huntley, 1984), whilst EXT (N) comprised mainly of thorn-bush vegetation (mostly acacia trees). According to Lushetile (2009), Namibia has varying soil types; however, most of the soils are poor, making it very difficult for plants to grow. This could in turn influence the animal's nutrition and mineral concentration since animals are primarily dependent on plants.

3.5.3 Micro mineral concentration in impala liver, muscle and rib from different regions/production systems.

The regions/production systems had an influence on the micro mineral concentration of the impala liver. High concentrations of Se, Sr, Mo, Pb, and Si were detected in liver from the EXT (W) region/production system, V, Cr, Ni, Cd, and Hg were highest in liver from the EXT (N) region/production system whilst the liver from the INT region/production system had high 18 concentrations of Mn, Cu, Zn, and As, and lastly, Ba was high in the liver from the SE

region/production system. Biologically, the liver from an intensive production system would be expected to have high mineral concentrations due to the extra supplementary feed animals receive as the latter typically includes a vitamin-mineral premix.

The variation of mineral concentration in these regions/production systems may have been due to nutritional and regional factors. These factors include soil type and soil parent material in a region. According to Singh and Schulze (2015), soil type plays an important role in mineral absorption by plants, and thus, by animals. Plants need ~17 minerals in order to complete their normal life processes (Singh and Schulze, 2015) and these minerals are derived directly from the soil or indirectly, through fertilisers and manure (Parikh and James, 2012). In order to understand the soil type, it is essential to know soil parent material as it directly affects the nutrient mineral content in the soil (Singh and Schulze, 2015). However, this influence is mostly noted in young soil and decreases with increasing soil age and soil weathering. A better understating of the effect of soil parent material on soil mineral composition would require a review of the mineral composition of common rocks that build up the soil parent material. This will then make it easy to know the type of minerals existing in a certain type of soil. For instance, the feldspars which is 59.5% igneous rock contains ²³ macro minerals (K, Ca and Na) and micro minerals (Cu and Mn), whilst micas, a 3.8% igneous rock contains ²³ macro minerals (K, Ca, Na, and Mg) and micro minerals (Fe, Ni, Mn, Co, Zn and Cu) (Klein and Hurlbut, 1993). The concentrations of these minerals in the rocks/soil then ⁴ affect the uptake of these minerals by plants and by animals as they depend primarily on plants for feed. This would most likely influence impala liver from EXT (W), EXT (N) and semi-extensive production systems since these animals graze directly on ⁸ plants grown in the soil. The liver had very high concentrations of Cu (256.273 ⁸ mg/kg) and Zn (46.496 mg/kg) in the INT production system; most likely, due to the supplements the animals

were fed on. The liver is known to be the main storage of Cu and Zn, according to McDonald *et al.* (2002), Cu is known to exist in all body cells, but it is more concentrated in the liver than in any other organs or tissue. ⁵² Similar results were reported by Umaraw *et al.* (2015), where the liver showed a higher concentration of Cu than the muscle and other by-products.

Micro mineral concentrations in the muscle were mostly detected in the EXT (N) production system and had high ⁸ concentrations of Al, V, Cr, Mn, Cu, Zn, As, Mo, Ba, and Pb, whilst EXT (W) had high concentrations of Fe and Se. These high mineral concentrations could have been as a result of the different soil types in these regions/production systems.

Some minerals were below the limit of detection (<LOD) in the muscles and include B which was <LOD in all regions/production systems, V <LOD in EXT, INT and SE, Cr <LOD in EXT (W), As <LOD in EXT (W) and SE, Mo <LOD in EXT (W) and INT, Sb and Hg <LOD in all regions/production systems and Si <LOD in EXT (W).

Fe had high concentrations compared to other minerals in the EXT (W) and EXT (N) regions/production systems. A high Fe concentration is often associated with high myoglobin levels which are mostly found in animals that are active. Deborah and Alan (2013) mentioned that iron is contained in the myoglobin protein and according to Hoffman *et al.* (2005), high myoglobin concentration in muscles is due to systemic exercises. This would, therefore, mean that an increase in myoglobin results in an increase in Fe concentration. The results of the current study concur with Hoffman *et al.* (2005) due to the fact that high Fe concentrations were only observed in the extensive regions/production systems where the animals would have had more exercise.

Besides the liver, bones have high mineral absorption capabilities that are important for bone formation. The level of mineral concentration in ribs differed across the production systems. EXT (W) was found to have high Cr, Sr, and Si concentrations, EXT (N) had the highest concentrations of B, Al, Mn, As, Mo and Pb minerals, V, Fe and Zn were high in the INT production system and SE showed high concentrations of Ba. Of these minerals, Fe (1528.885mg/100g), Zn (647.767mg/100g), Sr (3018.305mg/100g), and Si (654.520mg/100g) had very high concentrations.

Providing animals with these minerals is of paramount importance in ensuring a strong animal skeletal system. Bleich (1990) and Robbins (1993) noted that mineral imbalances in bone results in porous and weak bones and therefore bone breakage. Browning and Cwieson (2013) reported that Sr is 99% concentrated in the bones. Pagano *et al.* (2007) reported that supplementing Sr through feed improves the metatarsal and femur strength, mineral content, and mineral density of bones. Browning and Cwieson, (2013) reported that supplementing adult goats with Sr increases the naturally occurring anabolic hormone insulin-like growth factor which is relevant in animal production.

3.6 Conclusion

The aim of this study was to provide baseline data about the mineral status of impala liver, muscle, and rib harvested from different regions/production systems. The regions/production systems do affect both micro and macro minerals of organs. Normally, animals in an intensive production system would be expected to have significant amounts of minerals due to the supplementary feed they receive. However, this was not the only factor contributing to the deposition of minerals in the organs; regional factors such as seasonal changes and soil types

also need to be considered. The concentration of micro minerals was found to vary in organs according to the production systems.

Chapter 4

Bone, liver and muscle mineral concentration of the bontebok and blesbok harvested from the Western Cape

Abstract

The effect of species on the mineral concentration in organs (liver, *Longissimus thoracis et lumborum* muscle and rib) of the ¹⁷ Bontebok (*Damaliscus pygargus*) and blesbok (*Damaliscus pygargus phillipsi*) was investigated. Both these species were harvested in the Western Cape and were extensively kept. The muscles of both species were first analysed for the ¹ moisture, protein, fat and ash contents. There was significant difference detected in the moisture content between the species, however, the protein ¹ content was high ($P \leq 0.05$) in the bontebok muscle whilst the blesbok was reported to have a high ($P \leq 0.05$) fat and ash contents. The blesbok liver and muscle had the highest ($P \leq 0.05$) concentration of macro minerals, excluding Na which did not differ ($P > 0.05$) between the two species. ²⁸ There was no significant difference in the concentration of macro minerals in the rib of both species, excluding K which was found to be highest in the blesbok. The bontebok liver was found to have a high concentration of Fe, Zn, Se, and Cd whilst the blesbok had high concentration of the Al, Cr, Mn, Co, Ni and Mo. For micro mineral concentrations, the bontebok liver had the highest concentration of Fe, Zn, Se and Cd whilst the ²² blesbok liver had the highest concentration of Al, Cr, Mn, Co, Ni and Mo. On the other hand, the bontebok muscle had a significant concentration of B, Fe, Ni, Zn, Sb and Hg and the blesbok

muscle had a high concentration of Mn, Cu, Se and Ba concentrations. In addition, the bontebok rib was recorded to have a significant concentration of Fe, Zn, Sr and Ba and the blesbok rib had a high concentration of Cu. The research shows that species does have an effect on mineral composition.

4.1 Introduction

Game farming in South Africa has been categorised as the 6th largest agricultural sector (Neethling, 2016) that provides employment to over a hundred thousand individuals (Cloete *et al.*, 2015). Game farming focuses on trophy hunting, live sales, eco-tourism, and game meat production (Neethling, 2016). Blesbok is one of the species that is harvested for meat production and trophy hunting in South Africa whilst the scarce bontebok is typically hunted by trophy hunters. This species is widely distributed and is abundant on privately owned ranches. Blesbok is sometimes mistaken for bontebok because of similar characteristics (Smit, 2004). Both species are known to be complete grazers (Skinner and Chimimba, 2005) and both the bontebok (Novellie 1990; Du Plessis, 1972) and blesbok have a high preference for short grasses and newly burnt veld (Kraaij and Novellie, 2010).

The increasing demand for game meat as a healthier protein source has resulted in an increase in the production of game animals (Dahlan and Norfarizan Hanoon, 2008). Game meat has been classified as having high nutritional value (Hofmeyr, 2014; Costa *et al.*, 2016) when compared to domesticated animals (cattle and sheep), but its mineral nutritional content has been overlooked. Animal mineral status plays a major role in animal production as it provides baseline data on whether mineral supplements are required or not. Failure to meet the mineral requirements of animals can be a problem for the animals as it slows production, leads to poor health, and delays growth. Mineral licks are often provided to animals to supplement the deficient mineral(s) in cases where there are mineral shortages. However, there is insufficient information ⁵ to provide the baseline data ⁴⁰ of what the mineral status of game animals should be. Thus, the aim of this study was to investigate the mineral concentration of the bone, liver, and muscle from blesbok and bontebok species.

4.2 Methods and materials

4.2.1 Study site

The bontebok and blesbok were both harvested in the Western Cape Province (Wellington (33.6405° S, 19.0097° E) and Witsand (34.3964° S, 20.8508° E), respectively). Both these farms are located in the Fynbos biome, although the bontebok was from the Renosterveld vegetation and the blesbok was from the fynbos vegetation. All these Animals depended primarily on natural feed with no supplementary feed. Six rams of ± 15 months old were harvested from each farm.

4.3 Statistical analysis

The experimental data was analysed using XLSTAT version 19.2. Due to physiological differences between the sample types these were not compared, instead the species were compared using one way analysis of variance (ANOVA) for individual sample types of liver, muscle, and rib. Bonferroni post-hoc test was used to identify the statistical significance at the 5% level with all results $P \leq 0.05$ considered to be statistically significant.

The following statistical model was used:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where: Y_{ij} is the response variable (minerals)

μ is the overall mean

α_i is the effect due to treatment (bontebok and blesbok species)

ε_{ijk} is the error

4.4 Results

4.4.1 Proximate composition

Results in Table 4.2 show the proximate composition in the *Longissimus thoracis et lumborum* (LTL) of bontebok and blesbok species. There was no significant difference detected between the moisture of these two species. However, the bontebok had a higher protein content ($P \leq 0.05$) than the blesbok, whereas the fat and ash content were found to be higher in the blesbok ($P \leq 0.05$).

4.4.2 Macro mineral concentration in bontebok and blesbok liver, muscle and rib.

Results from the Table 4.3 show the effect of species on the macro mineral concentration in bontebok and blesbok liver, muscle and rib. All the macro mineral concentrations (except Na) differed significantly between the livers of the two species, with blesbok liver having the highest concentration of all the minerals. There was a significant difference in all macro minerals between the two species with the blesbok showing the highest ($P \leq 0.05$) concentrations for all macro minerals for the muscle. There was no significant difference in all macro mineral concentrations (except K which was significantly high for the rib from the blesbok) in the ribs of the two species.

4.4.3 Micro mineral concentration in bontebok and blesbok liver, muscle and rib.

Results from Table 4.4 show the effect of species on the micro mineral concentration in bontebok and blesbok liver, muscle and rib. There was no significant difference in the concentration of V, Cu, As, Sr, Ba, Hg, Pb and Si. A significant concentration of Al, Cr, Mn, Co, Ni and Mo was detected in the blesbok liver whilst the bontebok liver had a significant concentration of Fe, Zn, Se and Cd. No significant difference was detected in the concentration of Al, V, Cr, Co, As, Sr, Mo, Cd, Pb and Si in the muscle. The bontebok muscle had higher

²² ($P \leq 0.05$) concentrations of B, Fe, Ni, Zn, Sb and Hg whilst the blesbok muscle had higher ¹⁸ ($P \leq 0.05$) concentrations of Mn, Cu, Se and Ba. There were no significant differences in the concentration of ³⁸ B, Al, Cr, Mn, Co, Ni, As, Se, Cd, Sb, Pb and Si between the two species for the rib. The bontebok rib had higher ($P \leq 0.05$) concentration of Fe, Zn, Sr and Ba, whilst the blesbok rib had a higher ($P \leq 0.05$) concentration of Cu.

4.5 Discussion

4.5.1 Proximate composition

Game meat is generally known for having a high nutritional value because of its low-fat content and for being a good source of protein. For this reason, game meat demand is increasing (Costa *et al.*, 2016). Game meat is associated with low risks to diseases such as cholesterol, obesity, heart disease, strokes, and hypertension (Hoffman ⁵³ *et al.*, 2005). Costa *et al.* (2016) reported ¹⁹ that the fat content of game species is considered to be low as it is generally below 5%, whereas the protein content was reported to range between 20% to 25%. In the current study both the bontebok and blesbok had a protein and fat content below 25% and 5% respectively. The proximate component contents for the blesbok in this study were similar to the ranges reported by Costa *et al.* (2016) for the proximate composition of blesbok for different *Longissimus dorsi* muscle cuts, which were 68.20 – 75.33% for moisture, 19.30 – 22.43% protein, 0.21 – 6.8% fat and 1.24 – 4.2% ash. Hoffman and Wiklund (2006) showed similar findings where the blesbok had a moisture content of 75.7%, protein content of 23.5% and fat content of 1.7%.

No significant difference was detected for the moisture content between bontebok and blesbok. However, the protein content (22.42%) of the bontebok differed significantly from that of blesbok (21.41%). Although, according to Hoffman *et al.* (2009), protein content of game meat differs according to species, this difference might be caused by the known negative correlation

between protein and lipid contents. In support of the latter, the fat and ash contents were higher in the blesbok (2.33% and 1.35%, respectively) than the bontebok (1.66% and 1.17%, respectively).

4.5.2 Liver, muscle, and rib macro mineral concentration of the bontebok and blesbok.

In as much the bontebok and blesbok are considered to be similar and were both harvested in the Fynbos biome, these two species had different mineral composition in their organs. According to Richards (1993), the Fynbos biome is categorised into two vegetations, which are the fynbos vegetation and the Renosterveld vegetation. As such in this study, the bontebok species originated from the Renosterveld vegetation while the blesbok species was from the fynbos vegetation. The fynbos vegetation is well-known for consisting of acidic sandy soils that are low in nutrients (Richards, 1993). These soils are largely deficient in essential nutrients for plant growth (Bothma and Du Toit, 2010). Whilst, the Renosterveld vegetation consists of more fertile clay soils thus producing nutritious plants (Bonsma and Du Toit, 2010). This can significantly affect the deposition of minerals by animals since animals depend primarily on plants for nutrients.

⁸ The main aim of this study was to investigate the effect of species on mineral concentration in the bone, liver and muscle. For all three organs (bone, liver and muscle), the blesbok had higher macro mineral concentrations as compared to the bontebok. Based on literature, the blesbok would have shown lower macro mineral concentrations compared to the bontebok, however the climatic conditions during harvesting of the species could also contribute to the macro mineral concentrations in different species as plant growth is affected by climate. The bontebok was harvested during summer, whilst the blesbok was harvested in winter; the Fynbos biome has winter rains, and this could have partly attributed to the high macro mineral concentrations of the blesbok.

4.5.3 Liver, muscle and rib micro mineral concentration of the bontebok and blesbok.

The micro mineral in the liver, muscle and the rib between these species occurred in different concentrations, with some minerals being higher than others. For instance, the bontebok liver had very high concentrations of Fe, Zn, Se and Cd, whereas the blesbok liver had significant concentrations of Al, Cr, Mn, Co, Ni and Mo. These differences might have been due to factors such as the soil type and the vegetation these species were grazing on. Even though the concentration of these minerals differed between species and organs, the concentration of Fe and Zn remained high for all the bontebok organs. It could be that the clay soils in the Renosterveld are a good source of Fe and Zn minerals.

High concentrations of Fe, Zn, Sr and Ba were detected in the bontebok rib; meanwhile the blesbok rib had significant concentration of the Cu. Of these micro minerals, the Sr (438.611 mg/100g) and Ba (262.136 mg/100g) concentrations in the bontebok rib were very high. These two micro minerals are known to be essential for bone formation. According to Browning and Cowieson (2013) an animal's body has 99% of strontium which is found in the bones.

4.6 Conclusion

The results from the study show that species does have an effect in the mineral concentrations from the bone, liver and muscle. However, this may have been influenced by the fact that the Fynbos biome in which these species were harvested has different vegetation types which then suggests different soil types; the latter influencing the mineral absorption by animals, via the plants.

5.1 General discussion

Previous research has shown that game meat is healthy due to its low fat (Hoffman, 2007) and high protein contents. Hoffman (2007) reported that beef had the lowest protein (19.2%) content and high-fat content (14.2%), whereas game animals (springbok, nyala, blesbok, and impala) had high protein contents (ranging between 20 – 23.8%) and low-fat contents (0.8 – 2.45%). According to Neethling *et al.* (2016), the high protein and low-fat contents in game meat have resulted in a growth in its demand amongst discerning consumers. Costa *et al.* (2016) reported the proximate composition of different game animals, where moisture ranged between (68.20 – 76.90%), protein being between (17.4 – 25.71%), fat ranged between (0.21 – 6.8%) and ash was between (1.03 – 4.6%). The proximate composition results for the current study also fell between these ranges.

However, there is limited information about the mineral status of game animals. Researchers have reported on the importance of minerals in domesticated animals but little has been said about what are the expected mineral concentrations in game animals. Nonetheless, the consequences of mineral deficiencies in animals are well known, these include delayed growth, poor health, reduced production rate, etc. Preventative measurements such as the inclusion of the deficient mineral in diets and providing salt licks have been practiced correcting mineral shortages in animals.

Additionally, factors such as age, sex, nutrition, type of species and the physiological status of animals have been reported as factors that affect the mineral concentration of animals. Nutrition

is of greater importance in determining mineral concentrations in animals, this is due to the fact that animals depend mainly on the feed they consume for minerals in their body.

5.2 Conclusion

It can be concluded that game meat is a good source of protein when compared to meat from domesticated animals. Also, minerals are essential to animals as they are important for normal life processes. They are also important because humans depend primarily on meat for protein and minerals as they cannot synthesis their own minerals. The liver and rib have shown to be the better mineral storages whilst the muscle has lower mineral levels. Mineral deficiencies in animals can be corrected by providing mineral supplements in diets and salt licks. However, there is still a need to conduct further research about the mineral profile of other game species so as to have baseline data of what the mineral concentration in these animals is expected to be.

5.3 Recommendations

Since there is no base-line data about the mineral status of game animals, there is a need to conduct further research on this aspect. Factors such as vegetation, soil, water and climate should be taken into consideration when conducting this research about mineral status in animals. Knowing the type of soil and the type of minerals found in these soils would be of great significance in determining the mineral concentrations in animals. Also, it would be of importance to analyse the minerals found in the vegetation that these animals feed on. Given that some game species are selective grazers, others are browsers and others mixed feeders, and there is a variety of vegetation (grasses, trees and shrubs) a general overview of what the mineral concentration is expected to be is essential.



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