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EVANDRA DA SILVA JUSTINO

TRACES ELEMENTS REQUIREMENTS FOR HAIR SHEEP: A MULTI-STUDY APPROACH

FORTALEZA
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Thesis presented to the Integrated Doctorate Program in Animal Science at the Federal University of Ceara, as a partial requirement for obtaining the degree Doctor in Animal Science. Area of concentration: Animal science.

Advisor: Prof. Dr. Elzania Sales Pereira.

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EXAMINATION BOARD

_____
Prof. Dr. Elzania Sales Pereira (Advisor)
Federal University of Ceara (UFC)

_____
Prof. Dr. Edson Mauro Santos
Federal University of Paraíba (UFPB)

_____
Prof. Dr. Luciano Pinheiro da Silva
Federal University of Ceara (UFC)

_____
Prof. Dr. Andréa Pereira Pinto
Federal University of Ceara (UFC)

_____
Prof. Dr. Aderson Martins Viana Neto
Federal University of Ceara (UFC)

To my grandfather, Antônio Felinto da Silva.
(in memoriam)

I dedicate this!

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“The heart of those who surrender to prayer has a thousand stories to tell” (Priest Zezinho).

ABSTRACT

The success of ruminant production in tropical regions largely depends on the productive potential of the animals and their ability to adapt to environmental conditions. Microminerals are involved in many functions of metabolism. The dietary requirements of each mineral correspond to the sum of the net requirements for maintenance and production and the result is divided by the absorption coefficient of the mineral in the animal's digestive tract. However, not every mineral absorbed by the animal has a function in the body. Thus, the use of the absorption coefficient does not seem to be the most appropriate method, but the true retention coefficient, which considers mineral losses via urine. Sex class is involved in animal physiology and metabolism and has effects on growth, body composition, and productive and reproductive functions. As with protein and energy, the sex modulates mineral dynamics and, in consequence, mineral deposition in the animal's body. The objective of this study was to evaluate the micromineral requirements of Cu, Fe, Mn, Zn, Co, and Cr for gain and maintenance for hair sheep. The dataset consists of 248 individual records (139 non-castrated, 75 castrated males, and 34 females) compiled from six studies. The meta-analytical approach was used, employing non-linear mixed-effects models. These selected models account for the observed variability among the sexes, based on a low level of uncertainty ($w \geqslant 0.90$). The net mineral requirement for maintenance and retention coefficient were 0.03485 mg/kg of BW and 23% for Cu; 0.03963 mg/kg of BW and 13% for Fe; 0.00611mg/kg of BW and 0,7% for Mn; 0.194 mg/kg of BW and 28.7% for Zn; 0.00606 mg/kg of BW and 13.8 % for Co; 0.011 mg/kg of BW and 8.6% for Cr, respectively. The net iron requirement for gain decreased with increasing BW from 10 to 30 kg and average daily gain (ADG) of 150 g/day, ranging from 11.54 – 8.93; 10.58 - 6.81; 7.16 - 2.91 mg of Fe for intact males, castrated males, and females, respectively. Intact males followed by females had higher Mn requirements than castrated males. Castrated males had a higher requirement for chromium compared to males and females during growth. The micromineral requirements of this study are relevant for the development of feeding systems for hair sheep, and for optimizing mineral nutrition management of males and females during the growing phase.

Keywords: hair sheep; microminerals; sex class.

RESUMO

O sucesso da produção de ruminantes em regiões tropicais depende em grande parte do potencial produtivo dos animais e de sua capacidade de adaptação às condições ambientais. Os microminerais estão envolvidos em muitas funções do metabolismo. As necessidades dietéticas de cada mineral correspondem à soma das necessidades líquidas de mantença e produção e o resultado é dividido pelo coeficiente de absorção do mineral no trato digestivo do animal. No entanto, nem todo mineral absorvido pelo animal tem uma função no organismo. Assim, a utilização do coeficiente de absorção não parece ser o método mais adequado, mas sim o verdadeiro coeficiente de retenção, que considera as perdas minerais via urina. A classe sexual está envolvida na fisiologia e no metabolismo animal e tem efeitos no crescimento, na composição corporal e nas funções produtivas e reprodutivas. Assim como ocorre com proteína e energia, o sexo modula a dinâmica mineral e, consequentemente, a deposição mineral no organismo do animal. O objetivo deste estudo foi avaliar as exigências de microminerais (Cu, Fe, Mn, Zn, Co e Cr) para ganho e mantença de ovinos deslanados. O conjunto de dados consiste em 248 registros individuais (139 machos não castrados, 75 machos castrados e 34 fêmeas) compilados de seis estudos. A abordagem meta-analítica foi usada, empregando modelos não lineares de efeitos mistos. Esses modelos selecionados levam em conta a variabilidade observada entre os sexos, com base em um baixo nível de incerteza ($w \geqslant 0,90$). A exigência mineral líquida para manutenção e coeficiente de retenção foram 0,03485 mg/kg de PC e 23% para Cu; 0,03963 mg/kg de PC e 13% para Fe; 0,00611mg/kg de PC e 0,7% para Mn; 0,194 mg/kg de PC e 28,7% para Zn; 0,00606 mg/kg de PC e 13,8% para Co; 0,011 mg/kg de PC e 8,6% para Cr, respectivamente. A exigência líquida de ferro para ganho diminuiu com o aumento do PC de 10 para 30 kg e ganho médio diário (GMD) de 150 g/dia, variando de 11,54 a 8,93; 10,58 a 6,81; 7,16 a 2,91 mg de Fe para machos inteiros, machos castrados e fêmeas, respectivamente. Machos inteiros seguidos por fêmeas tiveram maiores necessidades de Mn do que machos castrados. Machos castrados tiveram maior necessidade de cromo em comparação a machos inteiros e fêmeas durante o crescimento. As exigências de microminerais deste estudo são relevantes para o desenvolvimento de sistemas de alimentação para ovelhas deslanadas e para otimizar o manejo da nutrição mineral de machos e fêmeas durante a fase de crescimento.

Palavras-chave: classe sexual; microminerais; ovinos deslanados.

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1 INTRODUCTION

The success of ruminant production in tropical regions largely depends on the productive potential of the animals and their ability to adapt to environmental conditions. These regions are characterized by high temperatures, often associated with seasonality in food supply, causing genotypes originating from these regions to present peculiar physiological characteristics for survival in these conditions (Regadas Filho *et al*., 2011). In this context, locally adapted genotypes such as hair sheep are generally well adapted to the conditions present in tropical regions (Mcmannus *et al*., 2020).

Feed represents the highest cost in animal production; therefore, efforts should focus on better understanding the nutritional aspects of these animals, such as their nutritional requirements and nutrient utilization efficiency. Studies on the nutritional requirements of sheep in Brazil have shown that these requirements differ from those recommended by major International Committees for small ruminants. This discrepancy is attributed to the genetic and environmental differences present in the country (BR – CAPRINOS & OVINOS, 2024).

Feed nutrients can be classified as organic or inorganic (Suttle, 2022). The inorganic nutrients, the minerals, are not catabolized into simpler chemical forms or oxidized to produce energy. Instead, minerals are absorbed in the digestive tract to constitute the body tissues and mediate metabolic processes in the cells (Johnson *et al*., 2012). Minerals are divided into macrominerals and microminerals, according to their concentration in animal tissues, macrominerals are expressed in g/kg of animal tissue, while trace minerals are expressed in mg/kg of animal tissue. Macrominerals are calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl), and sulfur (S), while microminerals are zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), cobalt (Co), chromium (Cr), selenium (Se), fluorine (F), silicon (Si), among others (NRC, 2007; Suttle, 2022).

Minerals perform four broad types of functions in animals. First, they have a structural role, forming essential components of body organs and tissues. Second, they take part in physiological functions, serving as electrolytes in body fluids and tissues. These electrolytes maintain osmotic pressure, acid-base balance, membrane permeability, and nerve impulse transmission, as seen in the blood, cerebrospinal fluid, and gastric juice. Third, minerals serve catalytic purposes, acting as catalysts in enzyme and endocrine systems. They may function as integral components of metalloenzymes and hormones or as activators (coenzymes) in these systems (Suttle, 2022).

The dietary requirements of each mineral correspond to the sum of the net requirements for maintenance and production and the result is divided by the absorption coefficient of the mineral in the animal's digestive tract. However, not every mineral absorbed by the animal has a function in the body, then it is excreted via urine. Thus, the use of the absorption coefficient does not seem to be the most appropriate, but the true retention coefficient, which considers mineral losses via urine (Costa; Silva, 2015; BR-CORTE, 2023).

A better understanding of the nutritional requirements of sheep, especially in terms of minerals, is crucial for improving production. However, few studies have aimed to determine the micromineral requirements of hair sheep, therefore there is a need to increase the amount of research to improve these recommendations. Mineral requirements for sheep are expressed as amounts per day, per unit of product, or as a proportion of the dry matter intake (DMI). Mineral requirements can be influenced by breed or genetic group, age, production level, environmental conditions in addition to sex classes (Suttle, 2022). Sex is involved in animal physiology and metabolism and has effects on growth, body composition, and productive and reproductive functions. As with protein and energy, the sex modulates mineral dynamics and, in consequence, mineral deposition in the animal's body (Sousa *et al*., 2021). We hypothesized that the micromineral requirements of hair sheep differ between males and females. Thus, the objective of this study was to estimate the net requirements for gain (NCu_g, NFe_g, NMn_g, NZn_g, NCo_g, and NCr_g) and maintenance (NCu_m, NFe_m, NMn_m, NZn_m, NCo_m, and NCr_m) in male and female hair sheep.

2 REVIEW

Minerals are essential elements that perform specific functions in the animal's organism (Underwood, 1981), making up approximately 3-5% of body weight (BW) and required for maintenance and production (Mcdowell, 2003). The mineral requirements of animals vary according to their genetic group, age, sex, physiological stage, availability and chemical form of the mineral, and synergistic and antagonistic interactions between minerals. Thus, the study of mineral nutrition presupposes an understanding of the mechanisms of action of the mineral and the interactions between minerals and other nutrients in the diet (Underwood, 1999; Avidar *et al*., 1981; Kaneko, 1989).

Minerals required in larger quantities (g) are referred to as macrominerals including calcium, phosphorus, magnesium, sodium, potassium, chlorine and sulfur. Those elements required in smaller quantities (mg or μg) are referred to as trace elements or microminerals. This group includes copper, iron, manganese, zinc, cobalt, chromium, iodine, molybdenum and selenium. Microminerals are involved in many functions in metabolism, such as blood cell composition, intermediates in metabolic pathways, hormonal regulation, fertility, immunity, antioxidant process, and the metabolism, and composition of rumen microbes (CSIRO, 2007; BR - CAPRINOS & OVINOS, 2024).

2.1 Copper

The discovery of copper as an essential element for growth and hemoglobin formation in rats by Hart and associates in 1928 marked a significant advancement in understanding nutritional requirements in animals. This finding was pivotal as it led to the recognition of copper critical role in preventing various clinical and pathological disorders across different farm animals. Conditions such as enzootic ataxia (swayback) in lambs, bovine falling disease, and other health issues like aortic rupture in rabbits and depigmentation in hair and wool were identified as signs of Cu deficiency. These disorders were found to be preventable through Cu supplementation, highlighting the importance of this trace mineral in animal health (Mcdowell, 2003; Suttle, 1991).

The recognition of copper as essential for ruminants was first established in 1931, following the demonstration of Cu deficiency in grazing cattle in Florida (Becker *et al*., 1965). Cu plays a crucial role in the activity of various enzymes, cofactors, and reactive proteins, which are vital for numerous physiological processes. While its necessity for

reproduction and bone development is generally accepted, only two specific processes have been definitively linked to particular enzymes: pigmentation, which relies on tyrosinase, and connective tissue development, which depends on lysyl oxidases (Suttle, 1987; Prohaska, 2006).

Copper is an essential trace element that plays a crucial role in various physiological processes. It is vital for the regulation of iron metabolism, erythrocyte function, hair pigmentation, and cellular respiration. Additionally, copper is important for the formation and development of bones, connective tissues, the nervous system, and cardiac tissues (Van Saun, 2012; NASEM, 2016).

As a cofactor for several key metalloenzymes, copper facilitates critical biochemical reactions. Notable Cu-dependent enzymes include cytochrome oxidase, lysyl oxidase, Cu-Zn superoxide dismutase, dopamine β-hydroxylase, and tyrosinase (Mcdowell, 2003). Linder (1996) identifies 14 known Cu-containing enzymes, indicating that copper is surpassed only by zinc in the number of enzymes it activates. This underscores the significant role of copper in numerous biological functions and its importance in maintaining overall health (Mcdowell, 2003).

Copper is essential for iron metabolism and the synthesis of hemoglobin. A deficiency in Cu results in decreased levels of hephaestin in enterocytes, which in turn leads to elevated Fe levels, likely due to insufficient ferroxidase activity. This indicates that both hephaestin and ferroxidase II play crucial roles in ferroxidase activity, particularly under conditions of Cu deficiency (Prohaska, 2006). Additionally, ceruloplasmin (Cp), another protein that depends on Cu, is vital for the recycling of Fe and the maintenance of Fe homeostasis in the brain (Collins *et al*., 2010).

Although Cu is not a constituent of hemoglobin, it is necessary as a catalyst for the effective utilization of Fe in hemoglobin formation. Deficiencies in either Fe or Cu can result in anemia, which is characterized by delayed maturation and a reduced lifespan of red blood cells (Baxter; Van Wyk, 1953). Furthermore, Cu is crucial for the absorption and mobilization of Fe, with Cu/Zn superoxide dismutase offering protection against oxidative stress in erythrocytes. Ceruloplasmin and ferroxidase II also contribute to the flow of Fe that supports hematopoiesis (Linder, 1996; Gabrielli *et al*., 2000).

The availability of Cu in soil and its subsequent uptake by plants are influenced by several factors, including soil drainage, pH, and the presence of other metallic antagonists such as molybdenum. The concentration of Cu in pastures does not consistently reflect its bioavailability for livestock, as various elements can impact Cu absorption (Gawthorne,

1987). Sheep are particularly vulnerable to Cu toxicity due to higher retention levels in the liver, while cattle are more likely to experience Cu deficiency because of their faster biliary excretion rates (Howell, 1991; Gooneratne *et al*., 1989).

The estimated daily maintenance requirement for Cu in sheep is approximately 4 µg Cu/kg body weight, a figure that is similarly recommended for cattle (Mcdonald *et al*., 1979; Suttle, 1983). During pregnancy, the fetus requires Cu for essential enzymatic functions, leading to significant accumulation in the liver, kidney, and placenta. At birth, the total Cu content in the conceptus is estimated to be between 20 and 30 mg, with additional Cu needed in colostrum, thereby increasing the demand on ewes in the final days of gestation (Suttle, 1987).

The copper requirements for sheep were initially defined as 5 mg/kg of dry matter (DM) in the NRC (1975), and were later revised to a range of 7 to 11 mg/kg of DM (NRC, 1985). The ARC (1980) suggested that the Cu requirements could vary from 1 to 8.6 mg/kg of DM, depending on the physiological state of the animals. In the NRC (2007), a value of 0.004 mg/kg of body weight (equivalent to 4 µg/kg of BW) was established using the factorial method, considering a copper (Cu) absorption coefficient of 6.0% in lambs under confinement conditions.

In animals fed diets with low copper concentrations there is a reduction in the levels of the proteins ceruloplasmin, cytosolic superoxide dismutase, and cytochrome-C oxidase (Linder, 1996), which can be used as indicators of Cu deficiency. The most common symptoms of Cu deficiency include anemia, severe diarrhea, impaired growth, changes in hair color, neonatal ataxia, temporary infertility, heart failure, and fragility of long bones, which become prone to fractures (Underwood; Suttle, 1999).

2.2 Iron

Iron is the most abundant trace element in the body, and its significance as a dietary component has been recognized for over 2000 years. Early research primarily focused on the heme molecule, which contains four iron atoms, one at the center of each of the four linked porphyrin rings. Heme, in conjunction with globin, forms hemoglobin (Hb), which is packaged within erythrocytes. This complex enables tissues to “breathe” by transporting oxygen (O_2) from the lungs as oxyhemoglobin via arterial blood and returning carbon dioxide (CO_2) as carboxyhemoglobin through the venous circulation (Suttle, 2022).

For many years, nutritional interest in iron (Fe) primarily focused on its role in hemoglobin formation and oxygen transport. Researchers established the presence of iron in hemeprotein enzymes, particularly cytochromes, and emphasized the significance of these enzymes in the oxidative mechanisms of all cells (Underwood; Suttle, 1999).

Initially, the emphasis was on iron's role in hematopoiesis; however, this interest broadened with the discovery of iron in the ubiquitous cytochrome oxidase (CCO) enzyme group (Gubler *et al*., 1957). Today, it is acknowledged that iron plays a crucial role in influencing cell growth, development, and gene expression (Wang; Pantopoulos, 2011).

Iron is an essential component of the heme group in protoporphyrin, primarily found in hemoglobin, which facilitates the transport of oxygen from the lungs to tissues as oxyhemoglobin and returns carbon dioxide to the venous circulation as carboxyhemoglobin. Additionally, iron serves as a cofactor in various enzymes involved in the Krebs cycle and in the metabolic functions of catalases and peroxidases (Suttle, 2022). Specifically, 24 enzymes within the tricarboxylic acid (Krebs) cycle contain iron at their active sites or as essential cofactors. The net iron requirements are determined by the total amount deposited in the blood and tissues during growth, minus the losses incurred through feces, urine, sweat, blood loss, parturition, and lactation (Mcdowell, 2003).

The absorption of iron occurs along the gastrointestinal tract, with the primary sites being the duodenum and jejunum. Although the stomach is not a site of absorption, it contributes hydrochloric acid, which facilitates the removal of iron bound to proteins through protein denaturation, as well as aiding in the solubilization and reduction of ferric iron to ferrous iron. Iron is absorbed in the ferrous form, while in foods it is predominantly present in the ferric form and in combination with organic compounds (Mcdowell, 2003).

The presence of ascorbic acid and cysteine in foods can enhance iron absorption by promoting the reduction of ferric iron to ferrous iron (Cook; Reddy, 2001). The iron content in cereals varies widely among species and regions, with corn generally having a lower concentration of iron compared to oats and wheat. Cereal by-products tend to be richer in iron than the grains, reflecting the uneven distribution of iron in the grains and possible contamination during processing (Mcdowell, 2003).

Iron in foods is found as organic complexes, with some bound to phytate and others stored in phytosiderophores like ferritin. For example, wheat contains iron primarily in the form of monoferric phytate (Morris; Ellis, 1976), while soybeans have significant amounts of iron as ferritin (Lonnerdal, 2009).

Iron requirements are determined by the basal diet, age, and iron status criteria. Three principles should be considered: 1. Requirements should be established based on practical diets and management regimes. 2. Requirements for growth should decrease as animals develop. 3. Requirements should be directed to ensure optimal health and performance (Mcdowell, 2003).

The absorption of non-heme forms of iron is significantly influenced by various dietary chelators. Ascorbic acid promotes iron absorption, while ethylenediaminetetraacetic acid (EDTA) inhibits it. Amino acids such as histidine, lysine, and cysteine increase the uptake of ferrous iron. Conversely, high dietary levels of phosphorus (P) may reduce iron absorption, likely due to the formation of insoluble ferric phytate and phosphate. Dietary phytate decreases the bioavailability of iron and other minerals, thus affecting absorption (Stahl *et al*., 1999; Kamao *et al*., 2000). Additionally, high dietary levels of copper, manganese, lead, and cadmium may increase iron requirements by competing for absorption sites in the intestinal mucosa (Mcdowell, 2003).

Notably, the iron requirements are greater in young animals compared to mature animals (Goff *et al*., 2018). Additionally, iron plays a protective role against oxidative stress in ruminants (Wysocka *et al*., 2020). Iron is also a vital structural component of various non-enzymatic metalloproteins, including ferredoxin, transferrin, hemosiderin, and ferritin. It is integral to several enzymes, such as cytochromes, cytochrome oxidase, peroxidases, catalase, and xanthine oxidase. Generally, forage provide adequate iron levels (50–300 ppm) to meet the dietary requirements of animals, which are approximately 50 ppm on a dry matter basis. However, iron concentrations can significantly decline in older plants and during winter months. Moreover, the availability of iron in forages is highly variable (10–40%), and its intestinal absorption is typically low (González; Silva, 2019). As a key element of heme, iron is predominantly located in hemoglobin and myoglobin, where it serves as an essential cofactor for enzymes in the electron transport chain, specifically for myeloperoxidase, catalase, and cytochrome P-450 (Goff *et al*., 2018). A deficiency in iron can lead to hypochromic microcytic anemia, primarily due to impaired hemoglobin synthesis. Anemia can arise from several factors, including direct blood loss due to blood-sucking parasites, an increased rate of red blood cell degradation, and a suppression of hematopoiesis caused by toxic substances produced by these parasites. In older ruminants, iron deficiency is rare unless there is significant blood loss resulting from parasitic infestations or underlying diseases (Mcdowell, 2003).

Iron requirements are agreed in some reports (ARC, 1980; Towers; Grace, 1983a; Anon, 1984; Grace, 1986) to be between 30 and 40 mg Fe/kg DM, with the higher value applicable to calves weighing less than 150 kg, as well as to pregnant and lactating cows and ewes. The National Research Council (NRC, 1980) sets maximum tolerable limits at 1000 mg Fe/kg DM for cattle and 500 mg Fe/kg DM for sheep; however, the ARC (1980) recommends a lower limit for both species. In grazing animals, higher iron intakes may sometimes be unavoidable due to soil contamination in their feed. While soil-derived iron is likely to be less bioavailable than that found in supplements used to assess iron tolerance and toxicity, it can negatively impact the availability of copper in forage. The estimates obtained for growing male sheep from the NRC (2007) are 8.25 mg/day**.**

2.3 Manganese

Manganese is the fifth most abundant metal on Earth and has been recognized as essential for growth and fertility in animals since the 1930s (Suttle, 2022). It is found in relatively consistent concentrations in the tissues of plants and organs of animals, with particularly high levels in reproductive organs (Scott *et al*., 1982). Manganese plays a critical role in carbohydrate and lipid metabolism, primarily through its activation of the enzyme pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxaloacetate, thereby facilitating the continuation of the tricarboxylic acid (TCA) cycle (Scrutton *et al*., 1972). This enzyme exhibits its highest activity in gluconeogenic tissues, such as the liver and kidneys, as well as in lipogenic tissues, including adipose tissue and the mammary gland. Additionally, manganese is an essential mineral for biological systems, serving as an enzymatic activator (Leach; Harris, 1997) and playing a crucial role in cartilage formation and epiphyseal growth, processes that directly influence the longitudinal growth of bones (Leach; Muenster, 1962). This highlights its importance for normal lipid and carbohydrate metabolism in ruminants.

Manganese absorption occurs throughout the small intestine in two stages: uptake from the gut lumen and subsequent transfer across the mucosal cells (Keen; Zidenberg-Cherr, 1990). However, manganese absorption is generally inefficient across all studied species (Mcdowell, 2003). The absorption process is influenced by the presence of other minerals, including phosphorus, magnesium, calcium, and iron. Notably, deficiencies in either iron (Rodriguez-Matas *et al*., 1998) or magnesium (Sanchez-Morito *et al*., 1999) can enhance manganese absorption (Mcdowell, 2003). Although forages generally contain manganese

levels that exceed the requirements of ruminants, its concentration in certain grains, such as corn, is relatively low.

Manganese also plays a vital role in protecting against reactive oxygen species (ROS) and intracellular oxidative stress through its function in manganese superoxide dismutase (MnSOD). The activity of MnSOD is initially low in newborn lambs (Paynter; Caple, 1985), likely due to the reduced oxidative stress experienced in utero. However, this activity increases significantly in the placentome at parturition and continues to rise in the lungs of neonates as they begin to respire, peaking by four weeks of age.

In species studied, manganese deficiency is associated with significant skeletal abnormalities, including shortened and thickened bones. Manganese is crucial for the development of the organic matrix of bone, which is primarily composed of mucopolysaccharides. Impairments in mucopolysaccharide synthesis due to manganese deficiency have been linked to the activation of glycosyltransferases, enzymes essential for the synthesis of polysaccharides and glycoproteins, with manganese being the most effective metal ion required for this process (Leach; Harris, 1997). Furthermore, manganese is essential for maintaining bone mineralization (Mcdowell, 2003).

Deficiencies in manganese can lead to reproductive problems, including delayed or suppressed estrus, reduced conception rates, and skeletal malformations in offspring (Hignett, 1941; Wilson, 2016). These issues underscore the critical importance of adequate manganese levels for reproductive health and overall physiological function in ruminants.

Manganese concentrations in seeds and seed products can vary from 5 to 50 mg per kg of dry matter (DM), with variations primarily attributed to differences between species. The classification of manganese content in various grains is as follows: corn < sorghum < barley < wheat < oats. Notably, manganese is predominantly found in the outer layers of grains, resulting in milling by-products containing significantly higher levels of manganese compared to the grains themselves. Consequently, these by-products increase the manganese content of the diets in which they are incorporated (Suttle, 2022).

The manganese content in pastures shows considerable variability, with a global average reported at 86 mg per kg of DM. Remarkably, only 3% of the analyzed grass samples contained less than 20 mg of manganese per kg of DM (Minson, 1990). It is important to note that increases in soil pH can significantly hinder the absorption of manganese by various types of plants, including grasses, legumes (Mitchell, 1957), and cereals (Suttle, 2022).

In a factorial model assessing the manganese needs of dairy cows, the National Research Council (NRC, 2001) proposed an Amn coefficient ranging from 0.065 to 0.075 for

dietary manganese. Given that two potential antagonists—phytate and fiber—are degraded in the rumen, it is expected that the Amn will exceed what is achievable in practical diets for non-ruminants.

For optimal weight gain and wool growth, growing sheep require approximately 13 mg of manganese per kg of DM, while slightly higher levels (16 mg per kg of DM) are necessary for testicular growth (Masters *et al*., 1988). However, factorial estimates for manganese needs are often inadequate. The low absorption coefficient and low manganese content in tissues imply that even small inaccuracies in the absorption coefficient can lead to significant errors in need estimates. For example, Grace (1983b) calculated that approximately 0.5 mg of manganese is needed for each kilogram of live weight gain in sheep. If a sheep is growing at a rate of 100 g per day, and the absorption coefficient is 0.01 (SANSOM *et al*., 1978) or 0.14 (Grace, 1975; Ivan *et al*., 1983), the dietary concentrations required would be 5 mg or 0.35 mg per kg of DM, respectively.

Newborn single lambs and other conception products typically contain about 5.0 to 7.0 mg of manganese. The accumulation of manganese in the ovine fetus is more pronounced during the last 50 days of gestation, with a daily accumulation rate of 0.05 to 0.06 mg in the single fetus as it approaches term (Langlands *et al*., 1982; Grace *et al*., 1986). The net daily requirement for fetal growth during this critical period is comparable to the needs of a sheep growing at a rate of 100 g per day (CSIRO, 2007).

2.4 Zinc

The nutritional importance of zinc was first established in 1934 by Bertrand and Bhattacherjee in mice. In 1940, Keilin and Mann isolated and purified the enzyme carbonic anhydrase, demonstrating that it is a metalloenzyme containing 0.33% zinc as part of its structure (Mcdowell, 2003).

Zinc plays a crucial role in various biological processes, including DNA synthesis, gene expression, and cell division. It also contributes to structural integrity and functions as an enzyme activator. In animal organisms, nearly a thousand proteins have been identified as Zn-associated. Notable Zn-dependent enzymes include carbonic anhydrase, carboxypeptidase A, alkaline phosphatase, alcohol dehydrogenase, and cytosolic superoxide dismutase (Mcdowell, 2003; NRC, 2007; Hosnedlová *et al*., 2007). Zinc's interaction with DNA-binding proteins is particularly significant, as it influences cellular replication and transcription (NRC, 2007).

Zinc is absorbed in the small intestine, either as free zinc ions or possibly in complexes with amino acids (Brody, 1999). Arora *et al*. (1969) noted that sheep absorb zinc more effectively in the rumen than in the small intestine. Once absorbed, metal ions are typically incorporated into proteins, which are then released during protein turnover (degradation and resynthesis) and reused within the cell (Brody, 1999).

In plasma, zinc is distributed between two main fractions: approximately two-thirds is loosely bound to albumin, while the remainder is tightly bound to high molecular weight proteins (Chesters, 1997). Zinc can also exist as a metallo-ligand complex with compounds such as amino acids or EDTA, and the metabolism of zinc post-absorption can be influenced by these ligands (Miller, 1975). Although zinc is widely distributed throughout the body, animals have a limited capacity to store it in a form that can be rapidly mobilized to prevent deficiency (Underwood, 1977).

Zinc is integral to immune system function and is involved in nearly every aspect of immunity. A deficiency in Zn is consistently linked to increased morbidity and mortality (Mcdowell, 1992). During immune responses to pathogenic microorganisms, blood Zn levels typically decline. Zinc deficiency impairs macrophage function, reducing both phagocytosis and the ability to kill pathogens. It also leads to a decrease in blood lymphocyte concentrations (Fraker; King, 1998) and causes atrophy of the spleen and thymus. Additionally, Zn deficiency diminishes the responsiveness of lymphocytes to mitogens, further compromising immune competence (Droke *et al*., 1993).

The primary function of zinc in the body is its association with enzymes and proteins, both as a structural component and as an activator. Over a thousand proteins are known to be associated with zinc (Maret, 2002). The most extensively studied zinc metalloenzymes in mammals include carbonic anhydrase, carboxypeptidase A, alkaline phosphatase, alcohol dehydrogenase, and cytosolic superoxide dismutase, the latter of which also contains copper. The first identification of zinc as a component of a metalloenzyme was in carbonic anhydrase in 1939, which contains about 0.3% zinc. Today, many zinc-dependent proteins have been identified, and several biological roles for zinc have been elucidated, particularly in cell replication and differentiation (Hambidge *et al*., 1986).

In cases of severe zinc deficiency, the activities of various enzymes, including plasma alkaline phosphatase, liver and testicular alcohol dehydrogenase, and pancreatic carboxypeptidase A, may be significantly reduced. Zinc plays a crucial role in enzyme systems involved in nucleic acid metabolism, protein synthesis, and carbohydrate metabolism. In rapidly growing tissues, zinc deficiency can severely impair the synthesis of DNA, RNA,

and proteins, thereby hindering cellular division, growth, and repair. Additionally, zinc deficiency can induce DNA damage and compromise the cell's ability to repair this damage (Ho; Ames, 2002).

Zinc is also known to initiate apoptosis (programmed cell death), which may facilitate the destruction of cancerous and pre-cancerous cells. Furthermore, zinc proteins are involved in the transcription and translation of genetic material, highlighting zinc's essential role in all forms of life (Vallee, 1993).

Zinc is arguably the most influential mineral, catalyzing, co-catalyzing, or providing structural integrity to approximately 300 enzymes across various categories, from hydrolases to transferases (Choi *et al*., 2018). Notably, ten times as many transcription factors require zinc for their structural integrity (King *et al*., 2016). The tetrahedral coordination of zinc to cysteine and histidine residues forms 'zinc-finger' domains that stabilize protein structures (Berg, 1990). Most cellular communication and metabolic pathways are zinc-dependent (Beattie; Kwun, 2004; Cousins *et al*., 2006; Choi *et al*., 2018).

Recent discoveries have identified new functions for zinc, including its role in the calcification of bone matrix (Alcantara *et al*., 2011). Additionally, zinc well-established function as a catalyst for angiotensin-converting enzyme 2 (ACE2) extends to hydrolyzing other peptides involved in humoral and innate immunity (Bernstein *et al*., 2018). The growing list of critical functions that zinc fulfills underscores its importance for health and productivity in livestock.

The concentration of zinc in forages ranges from 17 to 60 ppm, in cereal grains from 20 to 30 ppm, in oilseed meals from 50 to 70 ppm, and in animal protein sources from 90 to 100 ppm. The standard for zinc in drinking water is set at 5 ppm, although industrial pollution can significantly elevate zinc levels in both water and plant sources (NRC, 1979). In the ARC (1980), the zinc (Zn) requirements for growing sheep are set at 24 to 51 mg/kg of dry matter (DM), while the NRC (1985) established a value of 20 mg/kg of DM. The NRC (2007) suggests a net maintenance requirement of 0.076 mg of Zn per kg of body weight (BW), with an absorption coefficient of 30% for lambs weighing between 20 and 40 kg.

2.5 Cobalt

Cobalt is an essential element for ruminants, enabling ruminal microorganisms to synthesize vitamin B12, which is critical for the host animal’s metabolism. Vitamin B12 plays a key role in propionate metabolism by facilitating the conversion of methylmalonyl-CoA to

succinyl-CoA, a crucial step in gluconeogenesis (Kozloski, 2002). Without sufficient cobalt, succinate accumulates in the ruminal fluid due to impaired propionate metabolism (Lee *et al*., 2002).

The dietary requirement for Co was discovered in the 1930s when grazing ruminants in many regions could not thrive due to deficient concentrations of this element in forage. The link between Co and vitamin B12 was established in 1948 by Smith in England and Rickes *et al*. (1948) in the United States. They identified cobalt in compounds effective against pernicious anemia in humans, demonstrating that Co deficiency in ruminants is attributable to a lack of vitamin B12 in tissues (Smith *et al*., 1951).

The Agricultural Research Council (ARC, 1980) estimated the Co requirement for cattle and sheep to be 0.11 ppm. However, precise determination of minimum Co requirements for all grazing conditions is challenging due to factors such as seasonal variation in forage Co content, selective grazing habits, and soil contamination. Under grazing conditions, lambs are the most sensitive to Co deficiency, followed by mature sheep, calves, and adult cattle (Andrews, 1956).

Co deficiency can be exacerbated by diets rich in readily fermentable carbohydrates, such as molasses, flaked maize, and maize silage. These diets increase the microbial requirement for Co, as adenosylcobalamin is critical for propionic acid synthesis, a primary energy source for ruminants (Lee *et al*., 2002).

Cobalt deficiency in ruminants disrupts microbial and metabolic functions and leads to secondary effects such as anemia and liver metabolic disorders, as the liver serves as the primary storage site for vitamin B12 (Mcdowell, 1992). Young animals are particularly susceptible to Co deficiency compared to adults (Andrews, 1971).

Geographically, Co deficiency occurs in extensive areas of several countries, particularly in tropical regions. McDowell (1997) reported that, apart from phosphorus and copper, Co deficiency is one of the most significant mineral limitations for grazing ruminants in tropical countries. Low forage Co concentrations have been noted in 24 developing tropical countries across Latin America, Africa, and Asia (Mcdowell, 1985).

Vitamin B12 production in ruminants varies significantly depending on dietary cobalt intake. In sheep, daily B12 production ranges from approximately 0.05 mg on diets containing less than 0.05 mg Co/kg dry matter (DM) to about 1.6 mg on diets with 1 mg Co/kg DM (Smith; Marston, 1970; Hedrich *et al*., 1973). The efficiency of dietary Co conversion to B12 decreases with higher Co intake. Smith and Marston (1970) reported a

conversion efficiency of 0.13 on Co-deficient diets, which dropped to 0.03 when Co intake was adequate.

The proportion of B12 absorbed in sheep varies widely, with estimates ranging from 0.05 to 0.40 (Smith; Marston, 1970; Elliot *et al*., 1971; Hedrich *et al*., 1973; Rickard; Elliot, 1978). The Agricultural Research Council (ARC, 1980) supported Andrews (1965) recommendation that pastures or conserved roughage diets containing 0.11 mg Co/kg DM are sufficient to meet the requirements of sheep and cattle under most conditions.

On high-energy diets, however, cobalt requirements may increase significantly. Schwarz *et al*. (2000) determined that the dietary Co requirement for maximizing feed intake and growth in cattle fed a corn silage-based diet ranged from 0.16 to 0.18 mg Co/kg DM. Similarly, Durand and Kawashima (1988) identified that optimal Co levels for maximum growth of ruminal microbiota were between 0.5 and 1.0 mg Co/kg DM.

Cobalt dietary requirements for ruminants have been established by various authoritative bodies. The NRC (1975) initially recommended dietary levels of 0.1 mg Co/kg DM. Later updates increased these values to 0.1–0.2 mg Co/kg DM in the NRC (1985) and NRC (2007). The CSIRO (2007) suggested a dietary Co concentration of 0.11 mg/kg DM. More recently, the Inra (2018) set recommendations at 0.17–0.28 mg Co/day, reflecting the need for updated precision in dietary formulations.

2.6 Chromium

Chromium is an essential trace element primarily recognized for its role as a cofactor in the activation of insulin (Schwarz, 1975; Mertz, 1981). Although no Cr-dependent enzyme has been identified, Cr potentiates insulin action. Mertz *et al*. (1974) hypothesized that Cr forms a complex, known as chromodulin, which facilitates the interaction between insulin and insulin receptors. Offenbacher *et al*. (1997) reported that Cr binding to chromodulin enhances the tyrosine kinase activity of the insulin receptor, thereby amplifying insulin action. Without Cr, the inactive form, apochromodulin, cannot stimulate tyrosine kinase activity, demonstrating Cr specificity in activating apochromodulin.

The proposed model for chromodulin's role in insulin signaling suggests that it functions as part of a self-amplification system. In this model, apochromodulin is stored in the cytosol and nucleus of insulin-sensitive cells in its inactive form. An increase in circulating insulin triggers two concurrent processes: (i) enhanced mobilization of Cr to target cells, primarily mediated by transferrin, and (ii) movement of transferrin receptors from

intracellular vesicles to the cell membrane. Once transferrin, saturated with Cr, binds to its receptors, the complex is internalized through endocytosis. In the acidic environment of intracellular vesicles, the complex is degraded, releasing Cr into the cytosol. Four Cr^{3+} ions then bind to apochromodulin, converting it into its active form, chromodulin. Chromodulin subsequently binds to the active site of the insulin receptor, completing its activation and amplifying the insulin signal. When insulin levels decline, chromodulin is thought to be released from cells and excreted in the urine, which is the primary route of Cr elimination. Moreover, the consumption of non-fibrous carbohydrates increases blood insulin levels, which in turn elevates urinary Cr excretion (BR - CAPRINOS & OVINOS, 2024).

Chromium (Cr), in addition to its primary role in carbohydrate metabolism, also plays a role in protein metabolism by enhancing the cellular uptake of amino acids, a process directly associated with insulin activity (Kreider, 1999). Evidence also suggests that Cr influences lipid metabolism. This effect is linked to an increase in high-density lipoprotein (HDL) concentrations and a reduction in total cholesterol and low-density lipoproteins (LDL, VLDL). These changes occur through the enhanced activity of the lipoprotein lipase enzyme, particularly in individuals with dyslipidemia (Grant *et al*., 1997).

Zhou *et al*. (2013) reported that chromium (Cr) supplementation reduced the concentrations of insulin, triglycerides, and cholesterol in growing lambs. Similarly, Uyanik (2001) observed a decrease in triglyceride levels in lambs receiving Cr supplementation. Additionally, Domínguez-Vara *et al*. (2009) investigated the effects of Cr supplementation on serum parameters in growing lambs and found that it increased glucose concentrations while reducing insulin levels. The increased serum glucose concentrations observed in the studies suggest that chromium (Cr) supplementation enhances endogenous glucose entry into the bloodstream. In response to increased blood glucose and insulin levels, Cr is known to move into insulin-sensitive cells, where it binds to apochromodulin to form chromodulin. This complex then activates tyrosine kinase activity in insulin receptors, enhancing their function (Stoecker, 1999).

Chromium deficiency in ruminants is characterized by the interaction between chromium and insulin, resulting in low glucose tolerance, high insulin concentrations, reduced production, and a shortened productive lifespan. Furthermore, even diets with adequate chromium levels to meet normal production demands may become deficient in critical situations such as advanced pregnancy, parturition, the onset of lactation, weaning, and transport (Vargas-Rodriguez *et al*., 2014; Mousaie *et al*., 2014; Davis; Vincent, 1997; Yuan *et al*., 2014).

Chromium supplementation can provide supraphysiological benefits for domestic livestock, particularly for growing animals. In general, the positive effects observed in growing ruminants appear to be associated with a shift in energy partitioning from adipose tissue to lean tissue (Vargas-Rodriguez, 2014).

Sheep supplemented with Cr had higher dry matter intake (DMI) and average daily gain (ADG) (Mousaie *et al*., 2014). However, other researchers have reported that Cr supplementation had no beneficial effect on the growth performance of sheep (Kitchalong *et al*., 1995; Dominguez-Vara *et al*., 2009). Sano *et al*. (2000) investigated the effect of Cr supplementation on the performance of sheep reared under cold stress and reported that DMI, final body weight (FBW), average daily gain (ADG), and gain to feed ratio (G/F) were not affected by adding Cr. Haldar *et al*. (2009) investigated the effect of Cr supplementation on goat performance and reported that ADG in goats fed Cr supplementation increased, whereas DMI and FBW were not affected.

The increased dry matter intake (DMI) observed in growing ruminants receiving chromium supplementation is linked to reduced lipolysis, which likely facilitates greater glucose uptake by adipose tissue, thereby explaining the elevated DMI (NRC, 2001). The positive effects of Cr supplementation on average daily gain (ADG) appear to be associated with Cr's critical role in carbohydrate and protein metabolism. Chromium enhances insulin activity by either promoting its binding to target cell receptors or improving post-receptor signaling (Stoecker, 1999).

As previously mentioned, the effects of chromium supplementation on glucose and insulin concentrations may be partly attributed to reduced blood cortisol levels. Stress and cortisol are well-known immune suppressants (Munck *et al*., 1984; Khansari *et al*., 1990). Although the exact mechanism by which Cr enhances the immune system has not yet been established, a consistent finding across studies is that Cr supplementation reduces serum cortisol levels (Soltan, 2010; Kafilzadeh *et al*., 2012; Depew *et al*., 1998; Besong *et al*., 2001).

Elevated cortisol levels are known to suppress the immune system by inhibiting antibody production, impairing their action, and reducing lymphocyte count and activity (Sordillo; Aitken, 2009). Furthermore, insulin and cortisol have antagonistic effects on metabolism, and it is well-established that Cr supplementation can increase insulin levels. This antagonism may contribute to improved immune responses.

Studies show that chromium supplementation can offer many advantages. These benefits include reduced morbidity and mortality, enhanced feed efficiency, improved

performance, yield, and carcass quality in both calves and steers supplemented with chromium, particularly in its organic form (Moonsie-Shageer; Mowat, 1993; Mowat *et al*., 1993; Spears *et al*., 2024). Angus-crossbred steers (n = 400; 369.7 ± 7.6 kg) were used to determine the influence of trace mineral (TM) source and chromium propionate (Cr Prop) supplementation on performance, carcass characteristics, and ruminal and plasma variables in finishing steers. The study found that Cr Prop supplementation improved steer performance and carcass characteristics (Spears *et al*., 2024).

Micromineral supplementation is essential due to the wide variability of its availability in soils, in older plants and during the winter months (González; Silva, 2019). Young animals have a greater demand for iron; however, older animals may be deficient in this mineral in situations of parasite infestation (Mcdowell, 2003). Iron or copper deficiencies can result in anemia (Baxter; Van Wyk, 1953).

Copper plays a fundamental role in iron metabolism and hemoglobin synthesis (Collins *et al*., 2010). Furthermore, in tropical regions, microminerals such as selenium, zinc and copper have been shown to be indispensable due to their critical role in reducing heat-associated oxidative stress and metabolic alterations in the blood. Supplementation of these minerals helps prevent oxidative stress and changes in metabolites caused by heat stress, promoting better health and performance of animals during periods of high temperatures (Rang Son *et al*., 2022).

3 TRACES ELEMENTS REQUIREMENTS FOR HAIR SHEEP: A MULTI-STUDY APPROACH

ABSTRACT

The objective of this study was to evaluate the micromineral requirements of Mn, Co, Fe, Zn, Cu, and Cr for gain (NCu_g, NFe_g; NMn_g; NZn_g, $NMCo_g$, and NCr_g) and maintenance (NCu_m, NFe_m; NMn_m, NZn_m, NCo_m; and NCr_m) for hair sheep. The dataset consists of 248 individual records (139 non-castrated, 75 castrated males, and 34 females) compiled from six studies. The meta-analytical approach was used, employing non-linear mixed-effects models. These selected models account for the observed variability among the sexes, based on a low level of uncertainty ($w \geqslant 0.90$). The net mineral requirement for maintenance and retention coefficient were 0.03485 mg/kg of BW and 23% for Cu; 0.03963 mg/kg of BW and 13% for Fe; 0.00611mg/kg of BW and 0,7% for Mn; 0.194 mg/kg of BW and 28.7% for Zn; 0.00606 mg/kg of BW and 13.8 % for Co; 0.011 mg/kg of BW and 8.6% for Cr, respectively. The NFe_g, decreased with increasing BW from 10 to 30 kg and average daily gain (ADG) of 150 g/day, ranging from 11.54 – 8.93; 10.58 - 6.81; 7.16 - 2.91 mg of Fe for intact males, castrated males, and females, respectively. Intact males followed by females had higher Mn requirements than castrated males. Castrated males had a higher requirement for chromium compared to males and females during growth. The micromineral requirements of this study are relevant for the development of feeding systems for hair sheep, and for optimizing mineral nutrition management of males and females during the growing phase.

Keywords: mixed models; meta-analysis, sex class; sheep; traces elements; tropical areas.

3.1 Introduction

The mineral requirement knowledge has a global importance (Pereira *et al*., 2018a), however few studies address the trace elements requirements of hair sheep representing a large gap that still needs to be studied. Microminerals or trace elements are distributed throughout the animal body in small quantities (Suttle, 2022), corresponding to less than 0.3% of the total minerals deposited in the body (Lee *et al*., 2002), but of great importance for maintaining normal cellular metabolism (Zhang *et al*., 2018). The mineral retained in the animal's body in relation to that consumed is necessary to determine the need for trace elements for the maintenance and growth of the animals. Adequate mineral nutrition is fundamental to optimize animal performance, because microminerals have functions in animals (Suttle, 2022; Wilson *et al*., 2016).

Although Committees contribute significantly to sheep production, they do not consider the effect of sex on trace mineral demands and this can affect mineral requirements because non-castrated males have higher testosterone levels than castrated males and females, and testosterone can modulate mineral dynamics in the body. The sex of an animal affects its energy end protein requirements (Herbster *et al*., 2024; NRC, 2007), and therefore it is expected that mineral requirements will also be affected.

We hypothesized that the micromineral requirements of hair sheep differ between sex classes. Thus, the aim of this study was to estimate the net requirements for gain (NCu_g, NFe_g; NMn_g; NZn_g, $NMCo_g$ and NCr_g) and maintenance (NCu_m, NFe_m; NMn_m, NZn_m, NCo_m; and NCr_m) in males and females hair sheep.

3.2 Materials and methods

3.2.1 Dataset information for growth and maintenance requirements

The microminerals assessed included copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), cobalt (Co), and chromium (Cr). Data utilized in this study for predicting the growth requirements of hair sheep were sourced from the same six studies (Cabral *et al*., 2008; Silva *et al*., 2016; Pereira *et al*., 2016; Pereira *et al*., 2017; Pereira *et al*., 2018a; Pereira *et al*., 2018b). The raw data set was composed of 248 individual records of hair sheep (139 intact males, 75 castrated males, and 34 females) for to estimate growth requirements (Table 1).

Table 1 – Description of the studies that make up the database used to estimate net mineral requirements for maintenance and weight gain of hair sheep

Study	n	Genotype	Sex Class	BW (kg)	EBW (kg)
Cabral *et al*. (2008)	24	Santa Ines	C	17.90 – 24.40	13.13 – 22.65
Pereira *et al*. (2016)	40	Morada Nova	NC	10.12 – 31.62	6.58 – 27.10
Silva *et al*. (2016)	19	Crossbred	NC	10.64 – 33.60	9.77 – 30.10
			C	7.97 – 31.70	7.68 – 29.44
			F	7.98 – 29.40	7.68 – 26.28
Pereira *et al*. (2018a)	47	Brazilian Somali	NC	10.90 – 35.74	7.95 – 31.18
Pereira *et al*. (2018b)[1]	14	Morada Nova	NC	13.66 – 38.28	9.62 – 29.38
	16		C	12.54 – 31.25	8.66 – 23.08
	16		F	12.74 – 26.95	8.89 – 21.71
Pereira *et al*. (2017)[1]	19	Santa Ines	NC	11.20 – 33.20	8.30 – 24.57
	18		C	12.60 – 31.56	8.50 – 29.30

n, Number of observations; C, Castrated males; NC, Non-castrated males; F, Females; BW, Body weight; EBW, Empty body weight, [1]Studies used to estimate net maintenance requirements.
Source: prepared by the author.

The number of animals and studies varied among the different trace minerals. For Zn and iron Fe, data were gathered from 224 animals (139 intact males, 51 castrated males, and 34 female) from four studies (Silva *et al*., 2016, Pereira *et al*., 2016; Pereira *et al*., 2018a; Pereira *et al*., 2018b). The data for Cu were obtained from 224 animals (139 intact males, 51 castrated males, and 34 females), also from four studies. For Mn, data were gathered from 177 animals (112 intact males, 41 castrated males, and 24 F). Finally, the data for Co and chromium Cr were obtained from 83 animals (33 intact males, 34 castrated males, and 16 females) from two studies (Pereira *et al*., 2017; Pereira *et al*., 2018b).

For maintenance requirements, the microminerals were estimated based on EBW (kg) and the mass of microminerals (mg) retained in the EBW. Micromineral data were sourced from two studies (Pereira *et al*., 2017; Pereira *et al*., 2018a). Each micromineral's requirements were determined using data from 52 animals (26 non-castrated and 26 castrated males).

The descriptive analyses of the variables used to generate models for growth and maintenance are provided in Tables 2 and 3, respectively. The variables contained in the maintenance and growth data set were body weight (BW), empty body weight (EBW) and mass of each mineral in the EBW. More detailed information about BW and EBW can be seen in Herbster *et al*. (2023).

Table 2 – Descriptive statistics of the data used to estimate net requirements of microminerals for growing in hair sheep

Variables	N	Mean	SD	Minimum	Maximum
			Non-castrated males		
BW (kg)	139	21.56	6.54	10.12	38.28
EBW (kg)	139	17.23	5.93	6.58	31.18
			Retention (mg)		
Cu	139	58.57	66.29	1.52	309.82
Fe	139	1278.85	921.33	143.60	6829.34
Mn	139	24.84	24.18	0.00	153.25
Zn	139	617.05	921.33	169.30	5612.29
Co	33	23.84	13.65	6.75	60.53
Cr	33	31.83	18.54	18.54	82.18
			Castrated males		
BW (kg)	75	21.50	5.76	7.97	31.70
EBW (kg)	75	16.81	4.87	7.68	29.44
			Retention (mg)		
Cu	51	103.56	90.31	0.90	407.57
Fe	51	1738.81	904.56	215.07	3918.77
Mn	41	22.36	15.88	1.98	93.52
Zn	51	993.18	904.56	215.07	3918.77
Co	34	24.65	15.41	5.88	71.14
Cr	34	30.42	23.51	5.05	106.66
			Females		
BW (kg)	34	18.91	5.36	7.98	29.40
EBW (kg)	34	16.03	4.99	7.68	26.28
			Retention (mg)		
Cu	34	128.39	83.32	30.25	328.77
Fe	34	1530.35	854.65	96.83	3216.52
Mn	34	15.74	84.08	0.00	328.77
Zn	34	1087.53	854.65	96.83	3216.52
Co	34	19.83	13.46	5.67	49.66
Cr	34	18.64	4.86	9.58	26.51

BW, Body weight; EBW, Empty body weight; n, Number of information; SD, Standard deviation.
Source: prepared by the author.

Table 3 – Descriptive statistics of the data used to estimate net requirements of microminerals

Variables	n	Mean	SD	Minimum	Maximum
BW (kg)	52	19.33	2.90	11.20	38.28
EBW (kg)	52	14.24	2.42	8.30	29.38
		Intake (µg/day)			
Cu	52	312.25	68.16	205.61	437.33
Fe	52	3519.4	1298.40	1821.80	6489.90
Mn	52	1755.3	538.20	913.70	2750.90
Zn	52	1303	311.70	797.20	1926.90
Co	52	79.40	24.70	33.10	139.50
Cr	52	28.46	17.03	3.66	59.80
		Retention (µg/day)			
Cu	52	35.76	28.90	-29.34	94.26
Fe	52	418.60	385.3	-262.20	1734.30
Mn	52	6.70	6.90	-2.23	32.70
Zn	52	178.40	105.2	1.41	416.20
Co	52	4.80	6.10	-5.10	19.50
Cr	52	10.28	8.67	-1.23	36.58

BW, body weight; EBW, empty body weight; n, number of information; SD, standard deviation.
Source: prepared by the author.
(Cu, Fe, Mn, Zn, Co, and Cr) for maintenance of hair sheep

3.2.2 Slaughter procedures

In all studies, the same slaughter procedures were adopted. Before slaughter, the animals were fasted from solids and liquids (water) for 18 hours and then weighed to obtain the fasting body weight (FBW). Subsequently, the slaughter was carried out by brain concussion followed by a jugular vein incision until the animals were completely bled out. After that, the animals were skinned and eviscerated. The blood was weighed and sampled.

The gastrointestinal tract (rumen, reticulum, omasum, abomasum, and the small and large intestines) was separated and weighed full. Subsequently, it was emptied, washed, and after draining, weighed empty. The internal organs (liver, kidneys, heart, lungs with trachea, tongue with esophagus, reproductive tract, and spleen), other body parts (carcass,

head, skin, blood, and paws), and fats (omental, perirenal, mesenteric, and heart fats) were also weighed. The empty body weight (EBW) was considered as the FBW minus the contents of the gastrointestinal tract, bladder, and gallbladder.

The carcasses were refrigerated at 4 °C for 24 hours and then divided into right and left half-carcasses. Subsequently, the right half-carcasses, non-carcass components (blood, head, paws, internal organs, and the cleaned gastrointestinal tract), and skins were frozen at -20 °C, then cut with a band saw, ground in an industrial cutter, and sampled separately for subsequent analysis.

3.2.3 Chemical Analyses

To determine body mineral composition, 500 grams of samples were taken from ground samples of right half-carcasses, non-carcass parts, and hides. The samples were initially pre-dried at 55 °C until reaching a constant weight and then the content of dry matter (DM) was determined (AOAC, 1990; method 967.03). The samples were defatted through extraction in a Soxhlet apparatus (AOAC, 1990; method 920.39) for 12 hours according to Pereira *et al*. (2017). Subsequently, the fat-free body component samples were ground using a ball mill and analysed for dry matter (AOAC, 1990; method 967.03). The roughage, concentrate, refusals, and body components samples of each study were analysed for mineral composition through digestion in nitro perchloric acid, as recommended to method INCT-CA M-004/3 according to Detmann *et al*. (2012). Mineral concentrations of Cu, Fe, Mn, Zn, Co, and Cr were determined using inductively coupled plasma-atomic emission spectroscopy with ultrasonic nebulization (Braselton *et al*. 1997).

3.2.4 Models for predicting growth and maintenance requirements

The data on trace minerals used in the growth requirement analyses included information on EBW (kg) and the amount of trace minerals (µg or mg) contained in the EBW. The models for predicting maintenance and retention of trace minerals used, as well as the procedures for fitting and selection of models, are the same as those described in the article by Herbster *et al*. (2023), a complementary paper on macromineral requirements. Therefore, the models used for maintenance were the linear model (Eqn. 1) and for trace mineral gain, the allometric models (Eqn. 2-3).

$R_{mY} = \beta_0 + \beta_1 I$ (1)

The term R_{mY} denotes the retention of the Y-th trace mineral. The parameters β_0 and β_1 in represent, respectively, the intercept and the slope of the linear model used for predicting the maintenance requirement of the Y-th trace mineral. The maintenance requirement of the Y-th trace mineral was estimated when $R_{mY} = 0$. The retention coefficient at maintenance is given by β_1. The intake rate of the Y-th trace mineral (I; mg/day) at the maintenance level was estimated as follows: $I_m = -\beta_0/\beta_1$.

$R_Y = \alpha X^{\beta}$ (2)

$R_{Y\,i} = \alpha_i X_i^{\beta i}$ (3)

The term R_Y represents the expected mean retention of the Y-th trace mineral (Y=1,2,...,6), in milligrams (mg), as a function of empty body weight (EBW, kg), denoted by the letter X. The subscript "i" in Eqn. (3) denotes the i-th category, which corresponds to the prediction of the requirement of the Y-th trace mineral for non-castrated males (1), castrated males (2), and females (3). The parameters α (Eqn. 2) and α_i (Eqn. 3) are proportionality parameters that fit the magnitude of the requirement for the Y-th trace mineral for a given value of EBW. The parameters β and β_i are the allometric exponents that describe how the requirement for the growth of the Y-th trace mineral scales in relation to EBW.

Combined with these models (Eqn. 1-3), different variance functions were tested, including homogeneous variance ($\sigma_Y^2 = \sigma^2$), exponential variance ($\sigma_Y^2 = \sigma^2 \times \exp[\rho X]$), and power of the means variance ($\sigma_Y^2 = \sigma^2 \times |R_Y|^{2\phi}$). Details on how these variance functions were tested can be found in the study by Herbster *et al*. (2023).

The nonlinear mixed models (Eqn. 1-3) were analyzed using the PROC NLMIXED procedure in SAS (University Edition) with the Newton-Raphson algorithm (tech=NEWRAP) to estimate the maximum likelihood function. Assuming, i.e., Y~ Normal (R_Y, σ_Y^2), $e_X \sim \text{Normal}(0, \sigma_Y^2)$.

The selection of the best candidate combination (model and variance function) for predicting each of the trace minerals was carried out using the corrected Akaike information criterion (AICc) (Sugiura, 1978) and some AICc-derived functions for candidate combination (Eqn. (1-3) and variance function combinations) among those analyzed (Burnham; Anderson, 2002).

The retention of trace minerals per unit of daily weight gain was obtained through the first derivative of Eqn. (2) or Eqn. (3) (Vieira *et al*., 2018), according to the model selected for each trace mineral, as presented in Eqn. (4) and (5), respectively.

$$\frac{dR_Y}{dX} = \alpha\beta\widehat{X^{\beta-1}} \quad (4)$$

$$\frac{dR_{Y_i}}{dX} = \alpha_i\widehat{\beta_i X^{\beta_i-1}} \quad (5)$$

The terms $\frac{dR_Y}{dX}$ (Eq. 4) and $\frac{dR_{Y_i}}{dX}$ (Eq. 5) represent the retention in mg of the Y-th (Y = 1, 2,...,and 6) trace mineral per g of EBW. The subscript 'i' denotes the i-th (i = 1, 2, and 3) category (Eq. 5). The predictor variable EBW is represented by the letter X, and the parameters α, β, and α_i, β_i, are the coefficients of the allometric model presented in Eqs. (2) and (3), respectively.

The linear equations suggested by Herbster *et al*. (2020) were used to estimate the empty body weight (EBW) and the gain in empty body weight (EBWG), corresponding to body weight (BW) and average daily gain (ADG), respectively. Accordingly, trace mineral retention predictions were carried out for ADGs of 100, 150, and 200 g for each BW of 10, 20, and 30 kg. The mean predictions were accompanied by ½ amplitude of the 95% confidence intervals (95%CI; Eqn. 6). The predicted mean values with their 95%CI were obtained using EBW and EBWG values equivalent to the respective BW and ADG values previously estimated.

$$95\%CI = MEAN \pm t_{(1-\alpha,\ df)}\ SE \quad (6)$$

95%CI denotes the 95% confidence interval. The term MEAN represents the predicted average value of trace mineral retention (mg) according to Eqns. (4) and (5) for a given ADG (g) and BW. The confidence interval was estimated using the *t*-test, with a significance level (α) of 0.05 and the degrees of freedom (df) multiplied by the standard error (SE). The degrees of freedom were calculated by subtracting the number of parameters (θ) in the allometric model (Eqn. 2 or 3), the variance function, and the random parameters from the total number of observations (n) ($df = n - \theta$).

3.3 Results

The requirements for growth were best fitted by sex class except for Cu and Co, which showed a better fit for the general allometric model (Eqn. 2). The heterogeneous variance function "power of the mean" provided the best fit for all trace minerals. This function allows the residual variance to vary with the mean of the predicted values. The best performance of the "power of the mean" for Zn and Mn was achieved with the standard deviation (σ) parameter fitted for each category, while for the other trace minerals, this variance function was fitted without category separation. The combination fitted for Mn was the only one, among the analyzed trace minerals, that was unanimous among the other candidate combinations (Table 3). The combination of the linear model with exponential variance was the best choice for Mn, Zn, Co, and Cu. However, this combination was unanimous ($w > 0.90$) only for Mn and Cr. For Fe and Cu, the allometric model with homogeneous variance was the best choice among the candidate models (Table 4).

After selecting the combinations between the allometric model and variance functions for the trace minerals, the model parameters and variance function for each trace mineral were used to obtain the growth requirement predictions, as shown in Table 5. Since there was no effect of sex, general equations were generated for NCu_g and NCo_g. Net mineral requirements for gain of hair sheep with respective confidence intervals are shown in Table 6.

Irrespective of sex, NFe_g, decreased with increasing BW from 10 to 30 kg and average daily gain (ADG) of 150 g/day, ranging from 11.54 – 8.93; 10.58- 6.81; 7.16-2.91 mg of Fe for intact male, castrated males, and females, respectively. Non-castrated male during growth presented greater requirements than castrated males for Mn, and female showed higher requirement than castrated males irrespective gain for Mn. Castrated males had a higher requirement for chromium compared to males and females during growth.

Table 4 – Goodness-of-fit measures used in selecting the best fit among all combinations of allometric model, variance function, and random effects fitted to micromineral growth requirement for hair sheep

Micromineral	Models	Variance functions[f]	Random[g]	AICc	w	ER	Θ
Cu	Equation (2)	Power of the mean[a]	a; b	2309.7	0.60	1.00	6
	Equation (3)	Power of the mean[a]	a_i; b_i	2311.6	0.23	2.59	14
	Equation (3)	Power of the mean[b]	a_i; b_i	2313.3	0.10	6.05	16
Fe	Equation (3)	Power of the mean[a]	a_i; b_i	3469.0	0.68	1.00	14
	Equation (3)	Power of the mean[d]	a_i; b_i	3471.4	0.21	3.32	18
	Equation (3)	Power of the mean[c]	a_i; b_i	3473.1	0.09	7.77	16
Mn	Equation (3)	Power of the mean[b]	a_i; b_i	1395.4	0.93	1.00	18
Zn	Equation (3)	Power of the mean[b]	a_i; b_i	3027.4	0.59	1.00	16
	Equation (3)	Power of the mean[c]	a_i; b_i	3028.4	0.36	1.65	16
Co	Equation (2)	Power of the mean[a]	a; b	577.7	0.52	1.00	4
	Equation (2)	Exponential[e]	-	579.6	0.20	2.59	6
	Equation (2)	Exponential[e]	-	580.6	0.12	4.26	4
	Equation (3)	Power of the mean[c]	-	582.9	0.04	13.5	16
	Equation (3)	Power of the mean[c]	-	583.1	0.03	14.9	16
Cr	Equation (3)	Power of the mean[a]	a_i; b_i	615.2	0.69	1.00	14
	Equation (3)	Power of the mean[c]	a_i; b_i	619.0	0.10	6.69	16
	Equation (3)	Power of the mean[b]	a_i; b_i	619.4	0.08	8.17	16
	Equation (3)	Power of the mean[d]	a_i; b_i	619.7	0.07	9.49	18

AICc, Akaike's Information Criterion corrected for small sample; w, model probability; ER, evidence ratio; Θ, number of parameters of the fitted combination.

[a] *power1*: $\sigma^2_Y = \sigma^2 \times |R_Y|^{2\phi}$,

[b] *power2*: $\sigma^2_{Y_i} = \sigma^2_i \times |R_{Y_i}|^{2\phi}$

[c] *power3*: $\sigma^2_{Y_i} = \sigma^2 \times |R_{Y_i}|^{2\phi_i}$

[d] *power4*: $\sigma^2_{Y_i} = \sigma^2_i \times |R_{Y_i}|^{2\phi_i}$

[e] *Exp*: $c = \sigma^2 \times \exp(\rho X)$,

[f] Details of the variance functions are in the text and in Herbster *et al.* (2023)

[g] a, a_i, b, b_i = model parameters.

Source: prepared by the author.

Table 5 – Goodness-of-fit measures for selecting the best combination between the linear model and the variance functions fitted to predict the micromineral maintenance requirement of hair sheep

Microminerals	Variance Function[a]	AICc	W	ER	Θ
Cu	Homogeneous	485.5	0.58	1.00	3
	Exponential	487.6	0.20	2.86	4
	Power of the mean	487.4	0.22	2.59	4
Fe	Homogeneous	761.2	0.49	1.00	3
	Exponential	762.0	0.33	1.49	4
	Power of the mean	763.1	0.19	2.59	4
Mn	Homogeneous	320.4	0.00	1.26×10^{6}	3
	Exponential	292.3	0.98	1.00	4
	Power of the mean	300.5	0.02	60.34	4
Zn	Homogeneous	586.1	0.09	6.05	3
	Exponential	582.5	0.53	1.00	4
	Power of the mean	583.2	0.38	1.42	4
Co	Homogeneous	318.0	0.34	1.92	3
	Exponential	316.7	0.66	1.00	4
	Power of the mean	-	-	-	4
Cr	Homogeneous	373.0	0.00	1.9×10^{6}	3
	Exponential	348.6	1.00	1.00	4
	Power of the mean	-	-	-	4

AICc, Akaike's Information Criterion corrected for small sample; Δ, Akaike difference, w, model probability; ER, evidence ratio; Θ, number of parameters of the fitted combination.

[a] Description of these variance functions are in the text and in Herbster *et al.* (2023).

Source: prepared by the author.

Table 6 – Models for estimating body composition and net requirements for microminerals for gain of hair sheep

Microminerals	Sex class	Model	Variance parameters	Net requirements for gain (g/day)
Cu	General	$10.72\ (7.28)\ EBW^{0.7124\ (0.1508)}$	$\sigma= 0.173;\ \phi= 1.316$	$EBWG^{a} \times [(7.64) \times EBW^{-0.2876}]$
Fe	Non-castrated males	$151.7\ (53.8)\ EBW^{0.8024\ (0.1344)}$	$\sigma= 0.001;\ \phi= 1.769$	$EBWG \times [(121.72) \times EBW^{-0.1976}]$
	Castrated males	$219.5\ (97.4)\ EBW^{0.6597\ (0.1630)}$		$EBWG \times [(144.8) \times EBW^{-0.3403}]$
	Females	$608.3\ (313.2)\ EBW^{0.3067\ (0.2044)}$		$EBWG \times [(186.57) \times EBW^{-0.6933}]$
Mn	Non-castrated males	$3.36\ (1.57)\ EBW^{1.045\ (0.2655)}$	$\sigma= 0.787;\ \phi= 0.8685$	$EBWG \times [(3.51) \times EBW^{0.045}]$
	Castrated males	$3.73\ (1.60)\ EBW^{0.7155\ (0.2679)}$	$\sigma= 0.480;\ \phi= 0.8685$	$EBWG \times [(2.67) \times EBW^{-0.2845}]$
	Females	$3.54\ (1.59)\ EBW^{0.8722\ (0.2743)}$	$\sigma= 0.641;\ \phi= 0.8685$	$EBWG \times [(3.09) \times EBW^{-0.1278}]$
Zn	Non-castrated males	$48.08(10.38)\ EBW^{0.9526\ (0.1191)}$	$\sigma= 0.010;\ \phi= 1.525$	$EBWG \times [(45.8) \times EBW^{-0.0474}]$
	Castrated males	$52.98(11.22)\ EBW^{0.9125\ (0.1237)}$	$\sigma= 0.007;\ \phi= 1.525$	$EBWG \times [(48.34) \times EBW^{-0.0875}]$
	Females	$242.83(134.97)\ EBW^{0.3559(0.2369)}$	$\sigma= 0.013;\ \phi= 1.525$	$EBWG \times [(86.42) \times EBW^{-0.6441}]$
Co	General	$1.37\ (0.51)\ EBW^{1.00\ (0.147)}$	$\sigma= 0.011;\ \phi= 2.127$	$EBWG \times [(1.37) \times EBW^{0.0000}]$
Cr	Non-castrated males	$1.01\ (0.26)\ EBW^{1.305\ (0.1909)}$	$\sigma= 0.179;\ \phi= 1.161$	$EBWG \times [(1.32) \times EBW^{0.3050}]$
	Castrated males	$0.84\ (0.20)\ EBW^{1.390\ (0.1595)}$		$EBWG \times [(1.17) \times EBW^{0.3900}]$
	Females	$9.95\ (16.85)\ EBW^{0.3689\ (0.6394)}$		$EBWG \times [(3.67) \times EBW^{-0.6311}]$

EBW, Empty body weight; EBWG, Empty body weight gain; σ, Standard deviation; ϕ, Scaling parameter of the scaling function.
[a] The EBWG was estimated according to equation recommended by Herbster *et al*. (2020).
Source: prepared by the author.

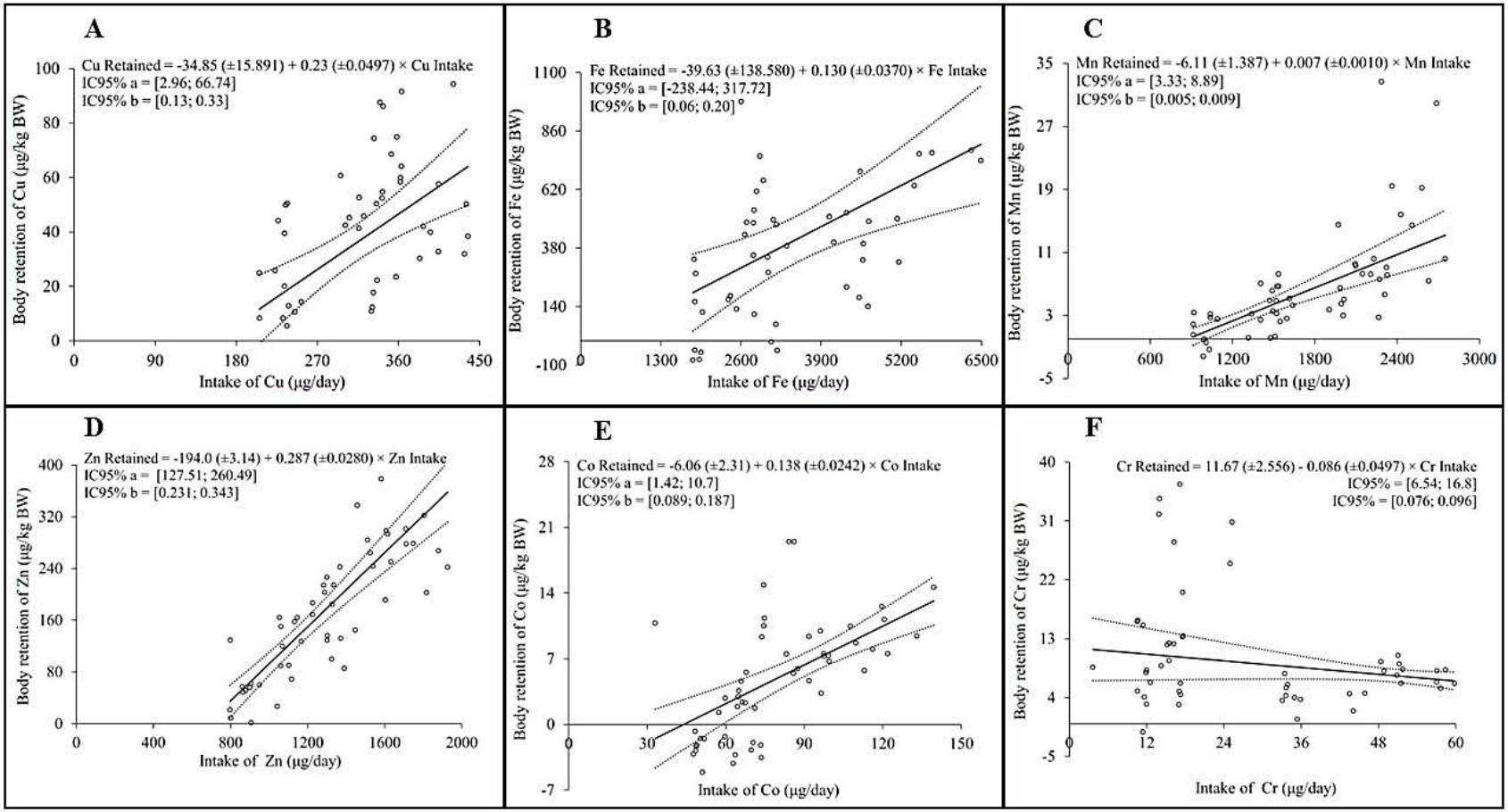
Table 7 – Prediction of net trace mineral requirements for growth of hair sheep with different body weights and average daily gains and respective confidence intervals

BW	ADG (g/day)	Sex class	Cu	Fe	Mn	Zn	Co	Cr
10 kg	100	Non-castrated male		7.72±7.52	0.347±0.396	3.82±3.51		0.210±0.572
	100	Castrated male		7.08±7.92	0.144±0.233	3.75±3.49		0.216±0.643
	100	Female		4.79±15.99	0.222±0.318	2.43±5.15		0.106±0.220
	100	General	0.411±0.54				0.125±0.014	
	150	Non-castrated male		11.54±11.24	0.519±0.591	5.71±5.24		0.313±0.854
	150	Castrated male		10.58±11.84	0.216±0.349	5.60±5.22		0.323±0.961
	150	Female		7.16±23.9	0.332±0.475	3.63±7.70		0.158±0.329
	150	General	0.614±0.807				0.187±0.021	
	200	Non-castrated male		15.36±14.96	0.690±0.787	7.60±6.97		0.417±1.137
	200	Castrated male		14.09±15.76	0.287±0.464	7.46±6.94		0.429±1.278
	200	Female		9.53±31.81	0.442±0.632	4.83±10.25		0.210±0.437
	200	General	0.818±1.074				0.249±0.027	
20 kg	100	Non-castrated male		6.54±9.39	0.360±0.645	3.67±4.34		0.271±0.885
	100	Castrated male		5.32±9.31	0.114±0.262	3.48±4.23		0.300±1.065
	100	Female		2.67±11.52	0.200±0.423	1.41±3.83		0.062±0.148
	100	General	0.323±0.426				0.125±0.042	
	150	Non-castrated male		9.77±14.04	0.539±0.964	5.49±6.49		0.405±1.323
	150	Castrated Male		7.95±13.92	0.170±0.391	5.21±6.33		0.448±1.592
	150	Female		3.99±17.22	0.298±0.632	2.11±5.73		0.093±0.221
	150	General	0.482±0.637				0.187±0.063	
	200	Non-castrated male		13.01±18.68	0.717±1.283	7.31±8.64		0.539±1.760
	200	Castrated male		10.57±18.52	0.226±0.520	6.93±8.42		0.597±2.118
	200	Female		5.31±22.92	0.397±0.842	2.81±7.62		0.123±0.294
	200	General	0.642±0.848				0.249±0.084	
30	100	Non-castrated male		5.98±10.4	0.368±0.794	3.59±4.80		0.311±1.109
	100	Castrated male		4.55±9.75	0.100±0.268	3.35±4.61		0.358±1.384
	100	Female		1.95±9.44	0.188±0.472	1.05±3.20		0.047±0.119
	100	General	0.283±0.390				0.125±0.059	
	150	Non-castrated male		8.93±15.55	0.550±1.186	5.37±7.18		0.465±1.657

150	Castrated male		6.81±14.57	0.149±0.400	5.00±6.89		0.535±2.068
150	Female		2.91±14.11	0.282±0.705	1.57±4.79		0.070±0.178
150	General	0.423±0.582				0.187±0.088	
200	Non-castrated male		11.89±20.69	0.731±1.578	7.15±9.55		0.619±2.206
200	Castrated male	0.563±0.775	9.06±19.39	0.199±0.532	6.66±9.17	0.249±0.117	0.712±2.752
200	Female		3.88±18.78	0.375±0.938	2.09±6.37		0.093±0.237
200	General	0.563±0.775				0.249±0.117	

BW, Body weight; ADG, Average daily gain.
Source: prepared by the author.

Figure 1 – Panels **A**, **B**, **C**, **D**, **E** and **F** show the retention behaviors of the trace minerals copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), cobalt (Co), and chromium (Cr) as a function of their intake. Below the graph are the estimates of the mean values, standard errors, and ½ the amplitude of the 95%CI of the parameters of the linear model and the variance function (only the mean values of the parameters) selected.



I_{m_i} is the prediction of trace mineral intake at the maintenance level ($Rm_i = 0$) its respective ½ the amplitude of the 95%CI. The subscript "i"denotes the i-th trace mineral. The observed retention values of the trace minerals are represented by black circular markers (●), the solid line represents the predicted mean retention values by the linear model (Eq.1), and the dashed lines are the predicted values of the lower and upper bounds of the 95% confidence interval (95%CI).

3.4 Discussion

Mineral elements are either required in low concentrations or are commonly distributed in animal diets, deficiencies are likely to be exceptional under normal practical conditions but of great importance for maintaining normal cellular metabolism (Suttle, 2022; Lee *et al*., 2002). Body composition is not constant during growth and is influenced by factors such as breed, age and sex (Robelin; Geay, 1983).

Hair sheep regardless of sex demand similar Cu and Co requirements as body weight increases. Our study recommends for Cu 0.03485 mg/kg of BW as maintenance requirements and a retention coefficient of 23%, which were significantly higher than those reported by Suttle (2022); Grace and Clark (1991) reported the net Cu requirements for maintenance as 4 µg/kg BW/day) for individuals weighing between 20 to 35 kg BW. This study, considering a 30 kg sheep with an ADG of 150 g (EBWG of 136 g), the demand corresponds to 1.05 and 6,38 mg Cu/day, for the liquid requirements maintenance and dietary, respectively. In NRC (2007), using the factorial method, the value of 0.004 mg Cu/kg of BW (4 µg Cu/kg of BW) is adopted, considering the Cu absorption coefficient of 6.0% in lambs, under feedlot conditions. Cu requirements for sheep were first described as 5 mg/kg DM (NRC, 1975), and were later adjusted to 7-11 mg (NRC, 1985).

In the ARC (1980), it was suggested that requirements vary from 1 to 8.6 mg of Cu/kg of DM, depending on the physiological state of the animals. Cu requirements for ruminants are dependent on interactions with other dietary components, especially Fe, Mo and S. This interdependence makes it necessary to specify the conditions under which the requirements are applied. Generally, sheep in feedlot are more prone to Cu poisoning, as the diets contain higher levels of Cu, making it more available and with lower concentrations of Mo and S (Mcdowell, 2003).

In this study, sex influenced weight gain requirements for Fe, Mn, Zn and Cr. Around 60% of the Fe in the animal's body is associated with hemoglobin. Fe requirement for hair sheep for maintenance is 0.03963 mg of Fe/kg of BW and a retention coefficient of 13%. Sex class influences the requirements for weight gain, for non-castrated, castrated males and females weighing 30 kg with an ADG of 150 g (EBWG of 136), the amount of Fe deposited in the gain and, therefore, the net amount of the mineral required is of 8.93; 6.81 and 2.91 mg Fe/day, respectively. The estimates obtained for males by this study are similar to the general recommendation for growing sheep from NRC (2007), of 8.25 mg/day. Fe is one of the most abundant mineral nutrients in the organism and plays a critical role in the synthesis of nucleic

acids and proteins, electron transport, cellular respiration, proliferation and differentiation (Lieu *et al*., 2001), all of which are intimately related to spermatogenesis and spermatozoa metabolism (Wise *et al*., 2003).

Our study recommends a net Mn requirement for maintenance of 0.00611 mg/kg of BW and a retention coefficient of 0.7%. For a sheep weighing 30 kg BW, the maintenance and dietary Mn requirements are 0.183 and 72.86 mg Mn/day, respectively. In both ARC (1980) and NRC (1985), values of 10 and 20 mg of Mn/kg of DM are suggested, respectively. Masters *et al*. (1988) recommended 13 mg Mn/kg DM. The NRC (2007) suggests a requirement for the weight gain of 0.47 mg of Mn/kg BW. Using the prediction equation of this study to estimate the requirement of females with 30 kg of BW, with an ADG of 150 g (EBWG of 136 g), the demand corresponds to 0.28 mg Mn/day, a requirement lower than intact males, which require 0.55 mg Mn/day. Males exhibit higher manganese (Mn) requirements due to its role in testicular growth. Manganese plays a critical role in cholesterol synthesis (Szentmihályi *et al*., 2006), which is fundamental to various reproductive functions in animals. Cholesterol constitutes a key component of the sperm plasma membrane (Luo, Chen; Zirkin, 2005) and serves as a precursor for testosterone, a sex hormone essential for sperm maintenance (Mcdonald; Pineda, 1989) and production (Mclachlan *et al*., 1994). Testosterone, a steroid hormone produced by Leydig cells, has its secretion regulated by luteinizing hormone (LH) (Santos *et al*., 2000; Moura *et al*., 2002). Elevated LH levels in the bloodstream promote pronounced testicular development, driven by an increase in the number of germ cells and the volume of Sertoli cells (Müller *et al*., 2008).

Additionally, manganese influences the hypothalamus, enhancing LH secretion, which is crucial for testosterone production by Leydig cells. This interaction plays a pivotal role in initiating and sustaining spermatogenesis during pubertal development (Lee *et al*., 2006). Therefore, the Mn demand for spermatogenesis and testicular development is likely significant.

A comparable situation occurs in females, where copper (Cu) and Mn are vital during the reproductive period (Underwood, 1981). Hidiroglou (1975) demonstrated that Mn uptake is higher in the Graafian follicle and corpus luteum of ovines compared to other reproductive tissues, suggesting its essential role in normal ovarian function. Similarly, Corah and Ives (1991) identified reproductive issues associated with copper deficiency, including decreased conception rates, infertility, anestrus, and fetal resorption. These problems may be linked to the function of copper-zinc superoxide dismutase, a major intracellular enzyme. This

copper-dependent enzyme acts as an antioxidant, protecting cellular contents from oxidative stress, and is also crucial for proper immune system function (Miller *et al*., 1979).

Since most trace elements, the key role of Zn in the animal organism is related to its structural function in some proteins or as an enzyme activator. Thus, approximately a thousand proteins associated with Zn are recognized in animal organisms. In our study recommends for net maintenance requirement of Zn 0.194 mg/kg of BW, and a retention coefficient of 28,7% for sheep, regardless of sex class. In ARC (1980), Zn requirements for growing sheep are 24-51 mg/kg DM, while in NRC (1985) the value of 20 mg/kg DM was adopted. NRC (2007) suggests a value of 0.076 mg of Zn/kg of BW as a net maintenance requirement, with an absorption coefficient of 30% for lambs. This study suggests for intact males castrated males, and females weighing 30 kg with an ADG of 150 g (EBWG of 136 g) are 5.37; 5.0 and 1.57 mg Zn/day, respectively. Zinc is a component of many metalloenzymes involved in almost every metabolic pathway of the body (Salgueiro *et al*., 2000). Zinc deficiency alters prostaglandin synthesis, which may affect luteal function (Graham, 1991).

In our study suggests that the net requirement of Co for maintenance is 0.00606 mg of Co/kg of BW, with a retention coefficient of 13.8%, irrespective of sex class. This study suggests values of 0.18 mg of Co/day and 2.63 mg of Co/day for a 30 kg sheep as net requirements for maintenance and dietary, respectively. The dietary requirement is relatively higher than the suggestions INRA (2018), whose recommendations of 0.28 mg of Co/day. The NRC (2007) recommends 0.10 - 0.20 mg Co/kg DM for sheep. CSIRO (2007) suggests a value of 0.11 mg Co/kg DM. According to McDowell (2003), with the exception of for P, Co deficiency is one of the most severe mineral deficiencies affecting ruminants in tropical pastures. Co is essential for the synthesis of vitamin B12 by rumen microorganisms. In the metabolism of ruminants, vitamin B12 is an essential part of multiple enzymes involved in the metabolism of carbohydrates, lipids, some amino acids, and DNA (González-Montaña *et al*., 2020).

Our study makes an interesting contribution to the requirements for chromium, as it is considered an element; suggests the net requirement for maintenance is 0.011 mg Cr/kg BW, and the retention coefficient is 8.6 %. For a 30 kg sheep, the value of 0.35 mg/day is suggested as a net requirement for maintenance. For dietary requirements, 9.47, 10.31 and 4.88 mg of Cr/day are recommended for non-castrated, castrated males and females, respectively. Manganese (Mn) requirements are generally lower in females than in males in animals due to differences in physiological and metabolic processes related to the role of this mineral in the reproductive systems. In males, Mn is crucial for testicular development and

spermatogenesis, as sperm production and testicular growth during puberty require high levels of this mineral, which is involved in hormonal regulation and cellular metabolism necessary for these processes. In addition, males continually require Mn for sperm production and maintenance of high testosterone levels throughout the reproductive life span. Mn also plays an important role in the regulation of luteinizing hormone (LH), which stimulates testosterone production by Leydig cells, necessary for continued spermatogenesis. In females, although Mn is also essential for functions such as the estrous cycle, ovarian function and, in some cases, gestation and lactation, these demands tend to be more stable or limited to specific periods, which may result in a lower overall need for Mn (Mcdowell, 2003; Underwood, 1981; Hostetler *et al*. 2003).

Although chromium (Cr) is an essential trace mineral, the NRC (2007) states that neither deficiency nor toxicity is likely to occur under normal conditions. Consequently, there are no documented reports of mineral deficiency signs in animals or humans. However, considering the relationship of Cr with the immune system and its anti-stress effects, several studies have demonstrated its benefits. These include reduced morbidity and mortality, enhanced feed efficiency, improved performance, yield, and carcass quality in both calves and steers supplemented with Cr, particularly in its organic form (MOONSIE-Shageer; Mowat, 1993; Mowat *et al*., 1993; Spears *et al*., 2024).

Furthermore, Cr has been shown to enhance cattle resistance to diseases by stimulating the immune response, which contributes to improved performance in stressed calves (Wright *et al*., 1994; Almeida; Barajas, 1999). It also promotes increased milk production and improved milk quality (Hayirli *et al*., 2001).

According to the equations suggested in our study for non-castrated, castrated males, and females weighing 30 kg with an ADG of 150 g (EBWG of 136 g) the amount of Cr deposited in the gain and, therefore, the net amount of the mineral required is 0.46; 0.54 and 0.07 mg of Cr/day, respectively. The net Cr requirement for growing sheep is greater for castrated compared to intact males.

In the present study, the requirements of the trace elements evaluated (Cu, Mn, Co, and Cr) increased with higher body weights (BWs), except for Fe and Zinc that showed a stability tendency. Additionally, sex influences the requirements for all evaluated trace elements except for Cu and Co. Accurate estimation of the mineral requirements of growing sheep necessitates precise knowledge of mineral deposition in the body. It is important to emphasize that trace element requirements serve as guidelines for the animal's needs and should be reassessed as new information becomes available. Estimating the various

requirements through multiple approaches is advisable. Achieving reasonable agreement between methods means greater confidence in the estimated trace element requirements.

3.5 Conclusion

The micromineral requirements this study is relevant for the development of feeding systems for hair sheep, and for optimizing mineral nutrition management of males and females during the growing phase.

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