Mineral and digestive responses to dietary challenges in the gastro-intestinal system of ruminants

Biruk Kebede Biru

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Mineral and digestive responses to dietary challenges in the gastro-intestinal system of ruminants

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Dissertation submitted in fulfillment of the requirement for the degree of Doctor of Philosophy (PhD) in Veterinary Sciences

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List of Abbreviations

ADF	acid-detergent fiber
ADL	acid-detergent lignin
AIA	acid-insoluble ash
ANOVA	analysis of variance
BSA	bovine serum albumin
BW	body weight
Cp	ceruloplasmin
CP	crude protein
CA	crude ash
CSA	Central Statistical Agency of Ethiopia
CT	condensed tannin
DM	dry matter
DMI	dry matter intake
DMT1	divalent metal transporter-1 protein
EE	ether extract
FM	fresh matter
ICP-OES	inductively coupled plasma optical emission spectrometry
HT	hydrolysable tannin
JUCAVM	Jimma University, College of Agriculture and Veterinary Medicine
LW	live weight
MT	metallothionein
MTL	maximum tolerable level
NDF	neutral detergent fibre
NRC	National Research Council
OM	organic matter
PRP	proline-rich proteins
PSC	plant secondary compounds
SCFA	short-chain fatty acids
SD	standard deviation
SRB	sulphate-reducing bacteria
SE	standard error
TT	total tannins
VFI	voluntary feed intake
ZIP-4	Zn transporting protein

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CHAPTER 1

General Introduction

Chapter 1 General Introduction

1. Background

In Ethiopia, the national livestock herd is one of the largest in Africa. The Central Statistical Agency of Ethiopia CSA, (2017) estimated there are approximately 30.2 million goats, 30.7 million sheep, 59.5 million cattle. By 2024/25, the cattle, sheep, and goat populations in the sedentary areas of Ethiopia are estimated to reach 75.0, 42.8, and 39.6 million heads, respectively (Leta and Mesele, 2014). Such a large stock of animals has the potential to contribute significantly to national development, including poverty reduction by improving the livelihoods of rural people (Shapiro et al., 2017). The livestock sectors support the livelihoods of about 80% of rural people, generates 16–19% of the foreign exchange earnings of the country and greatly contributes to the overall economy as they account for about 35% of the agricultural GDP (Adem, 2019).

The small ruminant production (sheep and goats) in particular is the backbone of livelihoods for the smallholder farmers and pastoralists and the main foreign exchange earner for Ethiopia (Gizaw et al., 2020). Furthermore, indigenous small ruminant breeds have unique adaptive traits, including disease resistance, water tolerance, and the ability to utilize different browse trees and shrubs more effectively (Jamala et al., 2013) making them more suitable for smallholder production systems than large ruminants (Nwosu, 2019). In addition to their ability to thrive and reproduce in the harsh environment, due to the short generation interval (early maturity) coupled with being small-sized animals, they have a lower production cost

(Gebrekidan, 2018). Due to this socio-economic importance, the demand for small ruminants has been increasing in the tropics (Nasrullah et al., 2013).

Providing appropriate ruminant feeds is a major constraint to achieve sustainable production. In Ethiopia, natural pasture and crop residues continue to be the most important feedstuffs for ruminant production (Tolera et al., 2012). Several researchers reported that global warming (Kara, 2019), the expansion of farmlands, land degradation, erosion and urbanization (Ahmed et al., 2010) has converted semi-humid and semi-arid areas into arid areas, consequently resulting in a shortage of feed resources available for ruminants (Adejoro, 2019). Frequent droughts, prolonged dry periods and uneven distribution of rainfall affecting crop production and re-growth potential of grasses are critical climatic factors causing feed shortage in Ethiopia (Assefa and Nurfeta, 2013). Besides climatic changes, FAO has also estimated in 2009 that 10 to 20% of all grassland was degraded due to overgrazing. By 2028 all agro-ecological zones except lowland areas are thought to become deficient in feeds if the current stock number growth continues (Shapiro et al., 2017). Against this backdrop, nutritional supplements and concentrates are generally expensive for most resource-limited smallholder farmers in rural communities around the world, especially in Africa (Moyo and Nsahlai, 2018). Further, lack of constant supplement supply may reduce their applicability in this setting (Jayanegara and Palupi, 2011), and as a consequence, livestock cannot acquire the required energy and nutrients (Kara, 2019). Thus, smallholder farmers in Ethiopia have explored alternative feed resources such as indigenous multi-purpose browse trees and shrubs to minimize the existing feed gap especially during the dry season of the year (Geta et al., 2014). For instance, browses in the mixed crop-livestock production system have a considerable potential to supplement low quality basal feed. Browse can contribute as much as 30% of cattle and 60% of goats' fodder intake during the dry season (Jamala et al., 2013). Furthermore, sheep often shift to browsing fibrous shrubs and tree leaves, as the dry season progresses, when feed is scarce and less palatable (Shenkute et al., 2012). However, these browse species are also recognized for possessing plant secondary compounds (PSC) such as polyphenols which may comprise 50% of the organic matter, and the latter may have a negative impact on their digestibility (Reed, 1986).

2 Ruminant nutrition

2.1. Preference and intake of ruminants

Dry matter intake (DMI) is fundamentally important in ruminant nutrition as it determines the amount of nutrients available to an animal for health and production. Dry matter intake is commonly expressed based on metabolic body weight, or live weight $(LW)^{0.75}$ (NRC, 2007), and it is largely determined by the animal’s physiological demands (Snyman, 2007). When the voluntary DMI is very low the rate of production is depresses (Van, 2006) and requirements for maintenance become the largest proportion of the metabolisable energy consumption resulting in poor efficiency of feed conversion (Forbes, 1995; Moyo and Nsahlai, 2018). Voluntary feed intake is used to describe the amount of feed eaten by an animal when feed is offered *ad libitum*. Apart from liveweight, the level of voluntary feed intake varies with animal genotype, physiological stage, with diet characteristics and environmental conditions (Ketelaars and Tolkamp, 1992). So, feed intake is an important measure to evaluate in animal production systems, as low nutrient consumption restricts production affecting animal health, while excessive nutrient consumption increases the cost of feeding and may result in excessive excretion of nutrients into the environment (Tseu, 2019).

Grazing ruminants select diet with a variety of plant species and parts with physical and chemical characteristics to reduce the anti-nutritional and toxic effects of unpalatable feeds (Provenza et al., 2003; Van, 2006), and to increase the intake and digestibility of nutrients such as proteins, minerals or energy (Hadjigeorgiou et al., 2003). Preference and voluntary feed intake often related to taste, odor, and feedstuff ability to promote satiety (i.e. the physical feeling of fullness allowing to stop eating) and changes depending on the type of forage, environmental condition, animal genetic and interaction among them.

2.2. Feed intake and digestion difference between sheep, goats and cattle

Under similar conditions, compared to other ruminants like sheep and cattle, goats as intermediate browsers (Hofmann, 1989) are adapted to consume and digest poor-quality forage. Particularly fibrous feeds containing low amounts of nitrogen and high amounts of lignin and tannins (Salem, 2011; Narvaez et al., 2012), are sufficient to allow them to meet their nutrients requirements without any signs of toxicity (Min and Solaiman, 2018). Such an adaptive mechanism to thrive on poor quality might be related to the production of special types of protein, so-called “proline-rich proteins” (PRP), in their saliva (Lamy et al., 2011).

These proteins have the ability to react and readily complex with dietary tannins, overcoming the negative impact on palatability and voluntary feed intake (Alonso-Díaz et al., 2010).

In addition, according to Van Soest (1982) the rumen microbiota of goats has a higher cellulolytic activity, which may contribute to the higher DMI/kg LW$^{0.75}$ in goats than in sheep (Snyman, 2007). The tolerance to tannin-rich browse materials could be explained by the proliferation or growth of microorganisms with the ability to degrade condensed tannins (Muir, 2011). For instance, ruminal bacteria such as *Streptococcus caprinus*, which can degrade tannin-protein complexes have been observed in goats (Brown and Ng'ambi, 2019).

Some researchers argue that sheep and cattle do not produce such PRP in their saliva (Austin et al., 1989; Robbins et al., 1991), hence limited reaction would occur between cattle and sheep saliva PRP and tannin (Makkar, 2003).

Thus, consumption of diets with high level of tannin results in reduction of feed palatability and intake due to the astringent effect caused by tannins. However, the concentration and activity of salivary PRP may differ between ruminant species, physiological state of the animal and geographical regions (Taha, 2015). For example, grazers such as sheep might be subjected to an increase in dietary tannin levels when an area becomes overgrazed and they are forced to include more lignin or tannin-containing browse species in their diets (Marais, 2012). In this situation, several studies revealed evidence that with prolonged tannin exposure, ruminants can develop ways to metabolize tannins or remain active in a high tannin environment and overcome the detrimental effects (Mlambo et al., 2007; Njidda, 2010).

Long exposure to dietary tannins can induce adaptation mechanisms in the animals, such as modifications in salivary gland size as in goats (Jerónimo et al., 2016). Yisehak et al. (2011) also indicated the capability of free ranging zebu cattle to produce higher concentrations of proline in their saliva. This indicates that the animal itself may develop tolerance to tannins by secreting increased amounts proline-rich proteins in the saliva (Patra et al., 2012)**.** This can be explained based on the secretion level and the activity of salivary PRP that have a high affinity to react and readily complex with dietary tannins (Taha, 2015), overcoming the negative impact of tannins on palatability, on voluntary feed intake and digestibility (Alonso-Díaz et al., 2010; Brown and Ng'ambi, 2019). Another mechanism could be through the colonization of rumen with bacteria able to degrade tannins, as it has been reported in sheep adapted to consume tannin-rich diets (Babaei et al., 2015).

The time of exposure to PSC may take up to several months before gut microbes adapted to utilizing the PSC (McArthur et al., 1995), however the induction of microbial adaptations may take only a few days for other plant extracts (Cardozo et al., 2004). For instance when Barbarine lambs fed acacia, rich in condensed tannins (CT) (59.6 g/kg DM), their DMI and growth rate were reduced in the first 6 days, however, the intake and growth rate recovered after continued feeding for 24 days (Salem et al., 2005).

This is because long term consumption of low quality feeds with these PSC can affect the rumen microbial flora and fauna (Mlambo et al., 2016). The degradation of fiber, microbial growth and energy availability, in the form of short-chain fatty acids, are also altered as a result of rumen adaptation with time (Sembiring et al., 2015). This could suggest that ruminal microorganisms adaption to the compounds in time can be one of the key factors in determining how well the defense mechanism works. These mechanisms include the secretion of extracellular polysaccharides to form a protective layer around cells, secretion of a thick extracellular glycoprotein, which has high binding affinity for tannins and dissociation of tannin-substrate complexes (Adejoro, 2019). The tolerance mechanism of bacteria to tannins may also involve degradation of tannins by secretion of tannase enzyme (Patra et al., 2012)**.**

Moreover, efforts have been made to explain the differences between species in diet selection and their ingestive behavior, rumination and swallowing physiology based on anatomical (e.g. body size, salivary gland size, omasum size, height of the reticular crests, intraruminal papillae distribution, etc.), and physiological (passage rate in the reticulo-rumen, etc.) characteristics (Hadjigeorgiou et al., 2003; Clauss and Hummel, 2017). For example, the larger surface area for absorption from the rumen due to broad leaf-like papillae is a general characteristic of intermediate feeders like goats compared to narrow tongue like papillae in grass eaters such as sheep and cattle (Agrawal et al., 2014).

Moreover, the higher intake capacity per metabolic weight (g DM/kg $LW^{0.75}$) by goats than those by sheep (Louca et al., 1982) could be due to the larger rumen volume of goats than that of sheep (Snyman, 2007). Gut fill is referred as rumen fill for ruminants based on the fact that the rumen is the only site in the gastrointestinal tract where distension has an effect of slowing digesta flow to a great extent (Allen, 1996). Rumen fill varies greatly with feeding habits and body weight, and accounts approximately for 9 and 13% of body weight for browsers and grazers, respectively (Moyo and Nsahlai, 2018). However, there is no apparent approximation of rumen fill based on body weight for intermediate feeders such as goats, although it may be assumed to fall within the range of 9–13% when grazers and browsers are regarded as extremes (Moyo and Nsahlai, 2018). Relative to BW goats do have a significantly larger rumen volume, that could result in a longer mean retention time, whereas the lower rumen volume in sheep relative to BW results in higher speed of passage time (Agrawal et al., 2014). This is because ruminants with smaller gut capacity must increase passage rate to ensure they maintain adequate feed intakes to meet metabolic needs (Moyo and Nsahlai, 2018).

In contrast, Clauss et al. (2006) showed that smaller browsing species had much greater fluid and solid passage rates through the rumen than grazers of a similar size, suggesting that goats should have lower rumen fills than sheep. The observed differences can be attributed to different feeding strategies adopted by ruminants influenced by familiarity with the diet, feed accessibility (physical structure), diet chemical composition including concentration of nutrients and antinutrients or toxins (Hadjigeorgiou et al., 2003a; Provenza et al., 2003).

Moreover Silanikove et al., (1993) showed that a 39% higher fluid passage rate in non-desert goats per unit body weight whereas mean retention time of solid particles was 10 h greater for desert goats. Desert goats thus may possess a greater digestive capacity than breeds of non-desert goats as a result of adaptation to feed and climatic conditions in the desert (Silanikove et al., 1993). This finding indicated that differences in passage rates among ruminants of some breeds exist because of differences in habitats in which they live and are adapted, which is dependent on the type of diet available. Passage rate and rumen fill data for sheep and goats adapted to subtropical and tropical climates in Ethiopia are limited, thus necessitating data on how climatic adaptation influences passage rates and rumen fill.

2.3 Source of minerals for ruminants in Ethiopia

In Ethiopia crop residues, range grasses, browse trees and agro-industrial by-products are potential feeds resources and important sources to supply essential minerals to ruminant (Girma, 2016). An earlier mineral study indicated that widespread Cu and Zn deficiencies in the Rift Valley (Faye et al., 1991). Climatic conditions and geo-morphological characteristics of this area associated with high molybdenum or sulphur and normal or sub-optimal levels of copper levels in the soil. Some grass species are deficient in the Cu level (<7 ppm), whereas woody species and grasses in irrigated areas or in swamps have a Cu content above normal (Faye et al., 1991). In addition, contamination of plants, especially grasses by soil also increases their iron content. Moreover, mineral soils such Boole (Aduwaa) soil is abundantly and locally available in the Central Rift Valley and is used by local farmers as a mineral source for cattles (Menta, 2017). However, the optimization of the concentration of Cu, Fe and Mn to improve the use of Aduwaa not yes studied. Therefor to improvement in reproductive performance and overall productivity of animals requires further research.

3 Mineral Bioavailability and Absorption in Ruminants: Focus on Cu, Mo, S, Fe and Zn

Minerals are required in the animal body for numerous functions (Nwosu, 2019). Their excess or deficiency causes detrimental effects on the performance of livestock (Khan et al., 2003). A deficiency or excess of minerals may arise from natural imbalances or may be induced by management practices (intensive animal breeding, intensive land use), inadequate or excess feed supplementation and environmental pollution (Papachristodoulou et al., 2015). Minerals in the soil are being absorbed by plants, which in turn are being ingested by animals. Whether or not this leads to an adequate mineral status for the animals depends on a number of factors playing a role in each step along the soil-plant-animal pathway (Figure 1). Mineral imbalances in soil and forage can equally lead to mineral imbalances in animals. Mineral concentration of forages also depends on soil pH, soil fertility, species and maturity stage of the forage, season and climate factors (Suttle, 2010; Hussein, 2017).

The utilization of minerals by the host animal does not only depend on the mineral content in the feedstuffs but also on the potential bioavailability and absorption of the minerals from the gastrointestinal tract (Ammerman et al., 1995). The potential bioavailability and absorption of most minerals from the gastrointestinal tract of animals is greatly influenced by several factors including breed, species and age of the animal (Spears, 2003). For instance, absorption of copper (Cu) in adult ruminants is low, ranging from 1% to 10% of dietary Cu (Underwood and Suttle, 2001; Spears, 2003), whereas before developing a functional rumen, Cu absorption in lambs can be as high as 70-85% of the dietary supply.

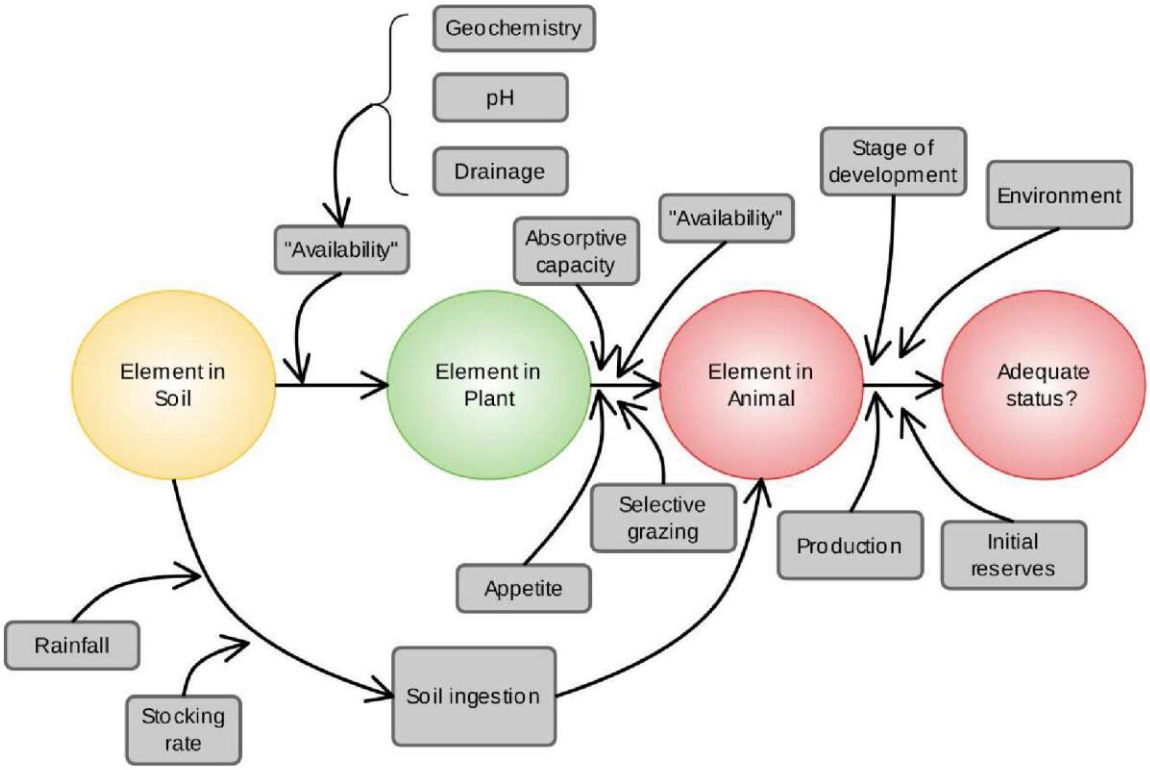

Figure 1. Summary of the many and varied factors, in addition to plants, that can influence the flow of an element from the soil to the grazing animal and whether the supply will meet the animal’s requirement. Source: Dermauw, 2013

3.1. Bioavailability and Absorption of Copper

Dietary Cu is generally present in the cupric form (Cu^{+2}) but only the cuprous form (Cu^{+}) is thought to be transported across the apical membrane in the gut (Goff, 2018). Therefore before uptake, luminal Cu must be reduced to the cuprous (Cu^{+1}) state via metalloreductases, or by the duodenal cytochrome-B reductase-1 enzyme to facilitate absorption (Hussein, 2017; Van Valin, 2019). Once in its reduced state, important brush border protein transporters play a physiological role in Cu absorption, the most important of which is the high-affinity Cu transporter 1 (Ctr1), (Figure, 2; Katulski, 2017). Although CTR is the most important path used to cross the apical membrane, Cu can also be absorbed into the enterocytes through the divalent metal transporter-1 protein (DMT1 also used for Fe, Zn, and Mn) although to lesser extent (Sefdeen, 2017). In ruminants Cu absorption occurs primarily in the proximal section of the small intestines, mainly the duodenum (Turner et al., 1987, 2005; NRC, 2005), via high-affinity copper transporters following the reduction of soluble Cu by metalloreductase enzymes (Faulkner et al., 2017). Jejunum and ileum may also contribute to Cu absorption (Bremner, 1970; Hussein, 2017). It has also been reported that Cu may be absorbed from the large intestine in sheep (Grace, 1975; Sefdeen, 2017).

Homeostasis of copper is influenced by hepatobiliary excretion, sequestration in metallothionein pools (predominantly in the liver) and by intestinal uptake and transfer (Aquilina et al., 2016; López-Alonso and Miranda, 2020). For example, at times of excessive dietary copper exposure, the hepatobiliary excretion increases, hepatic sequestering copper in metallothionein increases and the uptake of copper from the gut is downregulated. When the need for copper increases (e.g. with low intakes) hepatobiliary excretion of copper is reduced, and intestinal uptake and transfer are increased (Aquilina et al., 2016).

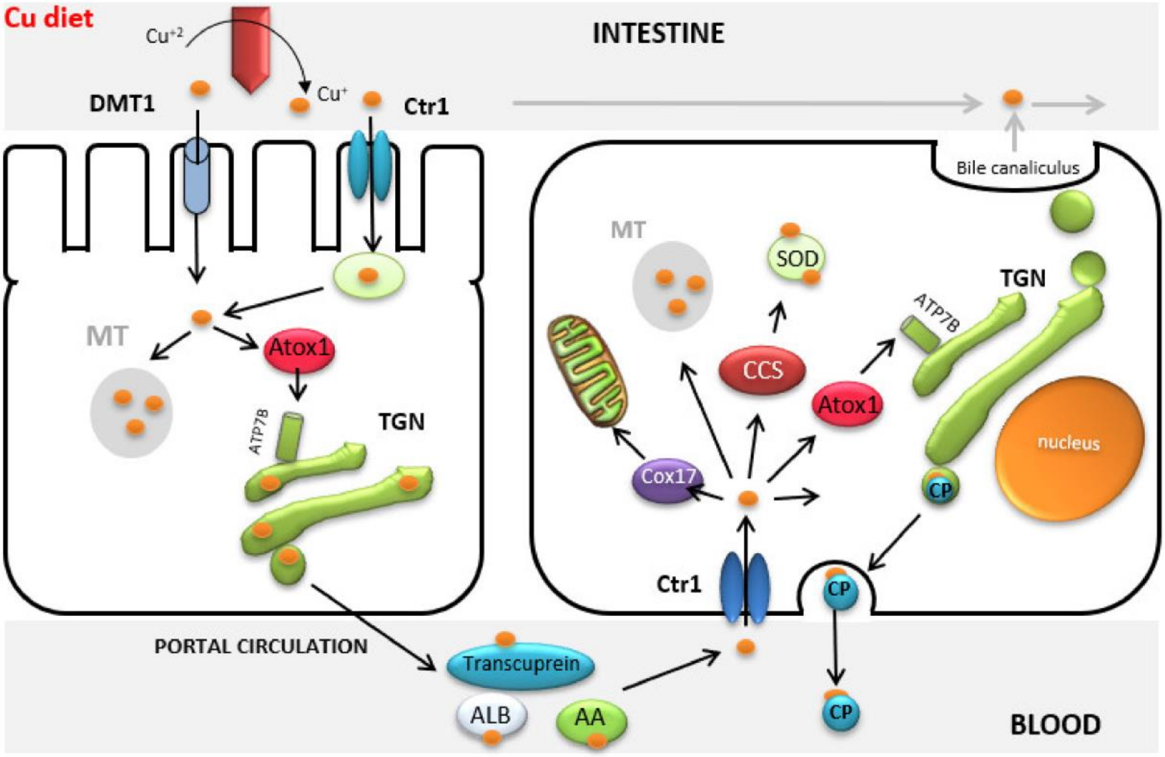

Figure 2 A schematic representation of the main components of the intestinal copper absorption process. Aquilina et al. (2016)

Inside the cell, copper will bind to copper chaperones or metallothionein; the chaperones will carry copper to various copper-dependent proteins such as superoxide dismutase. When high amounts of Cu^+ are entering the cell, the high levels of Cu within the cytosol trigger the ATP7A protein to become more active and hydrolyze more ATP to release Cu^+ at a higher rate into the circulation to get Cu out of the enterocytes (Goff, 2018). As a complex, ATOX1 and ATP7A translocate to the basolateral membrane, and copper is transported across the membrane to the portal circulation where it binds to albumin for transport to the liver.

3.2. Bioavailability and Absorption of Zinc

Zinc homoeostasis in ruminants is achieved primarily through the control of intestinal availability and absorption (Hilal et al., 2016). In ruminants, most of zinc is absorbed through the carrier-mediated transcellular transport or saturable process except at higher intake. Zinc in the intestines enters the enterocytes through the apical membrane and leaves through the basolateral side of enterocytes into circulation (Figure 3). The most important zinc importer is ZIP4 (Rucker et al., 2008; Mir et al., 2020).

Expression of ZIP-4 on the apical membrane is regulated by dietary Zn, up-regulation occurs if dietary Zn deficiency occurs and downregulation takes place when the animal is Zn repleted (Van Valin, 2019), thus intestinal absorption of Zn is more efficient when dietary Zn is limiting. Indeed, an increase in intestinal absorption and retention, together with a decrease in endogenous excretion of Zn in response to deficient dietary Zn intake has been reported (Hamid, 1997). If the requirement is markedly exceeded, additional zinc is not absorbed or endogenously secreted, but passes the gut and ends up in the manure (Suttle et al., 1982). Zinc can also use DMT1 to cross the apical membrane, though it must compete for binding sites on this transporter with Fe, Mn and Cu (Goff, 2018).

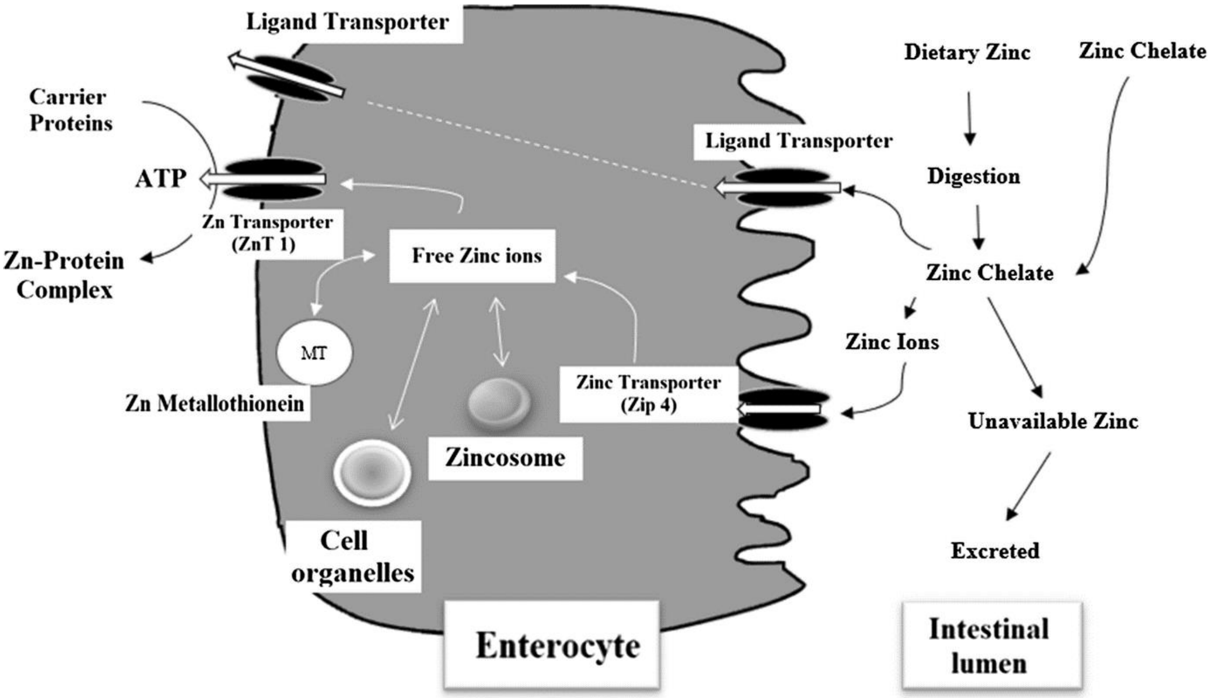

Figure 3. A schematic representation of the main components of the intestinal zinc absorption process. Mir et al., 2020

Inside the cell, each of the extra ion of zinc will be bound to metallothionein. These metallothionein proteins might act as molecular chaperons thereby transferring the zinc ions to the proteins or zinc-dependent enzymes that require these ions as cofactors without releasing the zinc as free ion in the cytoplasm (Mir et al., 2020). Therefore, they increase the cellular capacity of zinc storage. The capacity of cells to handle the extra inflow of zinc is known as its buffering capacity (Mir et al., 2020). Higher buffering potential indicates that cells can increase their zinc load without increasing the free zinc ion concentration within cells.

3.3. Bioavailability and Absorption of Iron

The duodenum and jejunum are the primary sites of Fe absorption, where absorption is rapid (van den Top and Veevoederbureau, 2005b). Most of the Fe is absorbed primarily from the duodenum by a two-stage process involving mucosal uptake and serosal transfer (Suttle, 2010)

Adult animals absorb 5-10% of the Fe in natural feeds, but this proportion may reach 15-20% when the diet is deficient in Fe or during depletion of Fe reserves (Golfman, 1988). If the Fe status of the body is adequate, the amount of DMT1 synthesized and placed onto the apical cell surface of enterocytes is reduced, slowing Fe absorption (Goff, 2018). Given diets deficient in Fe or when animals have a low Fe status, more Fe will be absorbed and retained, as the absorption of Fe is regulated by the animal's physiological needs (Golfman, 1988).

During digestion, before transport, ferric (Fe^{3+}) ions must first be reduced to the ferrous (Fe^{2+}) form by duodenal cytochrome b (Dcytb). Then, Fe can enter the cell from the intestinal lumen through transporter protein DMT1 located on the apical side (lumen) of the cells (Figure 4). After uptake by the mucosal cells, Fe becomes part of a cytosolic pool of weakly bound iron called the cytosolic labile iron pool (cLIP) and from there it can be distributed to all the cellular processes that require Fe, such as cellular respiration in the mitochondria, iron storage in ferritin, or transported outside the cell through the protein FPN located on the basolateral side (bloodstream) (Colins et al., 2017). If the Fe status of the body is adequate, Fe is instead bound to ferritin in the mucosal cell and excreted from the body after the death of the enterocyte (van den Top, 2005b). Once outside the enterocyte, iron is re-oxidized to Fe^{3+} by hephaestin (Hp) and is captured by the protein transferrin in the interstitial fluid and plasma (Colins et al., 2017).

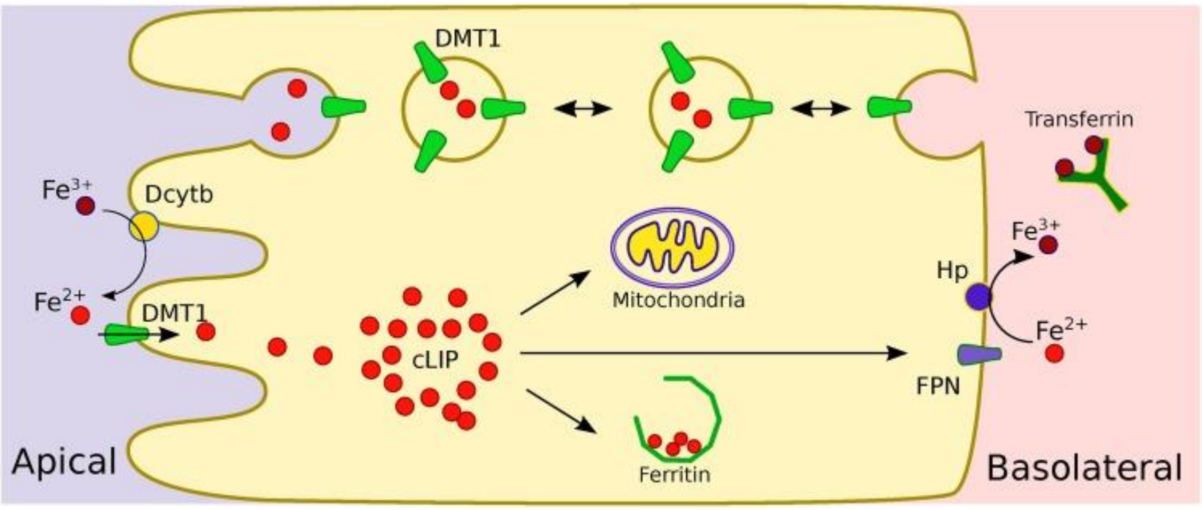

Figure 4 Schematic representation of the main components of the intestinal iron absorption process. Colins et al., 2017

3.4. Bioavailability and Absorption of Molybdenum

Molybdenum is known to be readily absorbed from most feedstuffs or diets, and from inorganic sources and its apparent absorption has ranged from 20 to 36% of dietary intake in cattle and sheep (Golfman, 1988). The amounts of Mo retained by the body and excreted in the urine and faeces increase as Mo intake increases (Grace and Lee, 1992). The absorption of Mo markedly decreases when the dietary intake of S is higher (Grace and Lee, 1992). The primary site of Mo absorption in both sheep and cattle is the small intestine between the duodenum and ileum (Mason et al., 1978). Although the forestomach is not a major site of absorption for Mo, when dietary Mo is high and dietary S is low a higher recycling of Mo into the reticulo-rumen and omasum via saliva or by secretion directly into these compartments occurs (Grace and Suttle, 1979). The process of Mo absorption is both carrier-mediated, involving active transport and by diffusion depending upon the level or concentration of Mo in the gut contents. For example, at low intake levels, absorption of Mo occurs by active transport (Golfman, 1988).

3.5. Bioavailability and Absorption of Sulfur

The supply of S can be achieved from various sources, such as sodium sulfate, ammonium sulfate, calcium sulfate and elemental S, however, the main source of S absorbed in the digestive system is associated with the ingestion of S-containing amino acids (Silva et al., 2014). When S is ingested both inorganic and organic dietary forms are reduced to sulphides (S^{2-}) some of which may be absorbed from the reticulo-rumen as H_2S, or utilised by rumen microorganisms to synthesis S-amino acids which are incorporated into microbial protein (Lee and Grace, 1997). The absorbed sulfides are oxidized to sulfate in the blood and liver, before being recycled to the digestive tract via saliva and other secretions, or excreted in the urine as sulfate and in the faeces as organic S (Lee and Grace, 1997). Sulfates fed to animals are relatively well absorbed and ruminants can absorb 77–87 % of S from sodium sulfate or calcium sulfate (gypsum) (NRC, 2005). Whereas inorganic S in the form of elemental S is relatively insoluble in water and is poorly available for absorption by animals (NRC, 2005).

For lambs, S in the elemental form was required by approximately three times more than the S in the organic form (Silva et al., 2014). In general it is assumed that a 2 Na^+/SO_4^{-2} cotransporter moves S across the apical membrane and SO_4^{-2} diffuses across the cell to the basolateral membrane into the extracellular fluids in exchange for 2 Cl^- or HCO_3^- anions (Goff, 2018).

4 Factors affecting micromineral bioavailability in ruminants

Research has shown that the net Cu requirement in sheep is between 0.5 and 2mg Cu/kg DM (Gould and Kendall, 2011). However, in practice only a small percentage between 3-10% of ingested Cu is absorbed (Dickson, 2016). This might be due to the presence of antagonistic interactions with other minerals such as S, Mo and Fe present in the diet, animal's gastrointestinal tract and body (Suttle, 2010). This is the reason why Cu requirements for sheep are commonly reported to be 7 mg/ kg DM (Table 1).

In addition, minerals may also interact with the high protein content of pastures or with cell wall components such as NDF in the rumen environment (Wang et al., 2020a). The binding of a mineral with other dietary tannins in the rumen (Karamać, 2009; Acharya et al., 2016) further complicates the picture. Tannins and most polyphenols have a catechol group (two consecutive -OH groups on an aromatic ring) that makes them excellent for metal-ion complexation (Zeng et al., 2019)**.** This can affect digestibility of diet and hence influence the mineral bioavailability and absorption at different sites of the gastrointestinal tract (Hartman, 2017). These interactions also depend on the level, type, molecular size and structural conformation of tannins.

Table 1 Micromineral requirements for sheep and goats

Microminerals	Requirements (mg/kg DM)			
	Sheep (NRC, 2007)	MTL (NRC, 2005)	Goats (NRC, 2007)	MTL (NRC, 2005)
Cu	3 - 7.12	15	15 - 25	40
Fe	5.41- 69.11	500	35 - 95	500
Zn	18.57 - 35.04	300	5.41 - 63.15	300
Mn	5.14 - 25.58	2000	7.71 - 23.94	2000
I	0.5 - 0.8	50	0.5 - 0.8	50
Co	0.10 - 0.20	25	0.11	25
Se	0.01-0.03	5	-	-

MTL maximum tolerable levels

There are also other factors that affect interactions in the rumen environment and the bioavailability of minerals e.g. pH, the chemical form in which a mineral is present in the sources, intake of the mineral relative to the amount required or mineral status of the body relative to their requirement (Suttle, 2010). Moreover, mineral status can be influenced by the physiological state of the animal, type (maintenance, growth, reproduction, and lactation) and level of production (Hilal et al., 2016). Together all these factors may lead to a marginal or secondary deficiency (Herdt and Hoff, 2011) that often remains undetected (Schweinzer et al., 2017). The reason could be that the transport pool and storage of microminerals in the body will compensate until the deficiency is well advanced and clinical signs become visible (Kincaid, 2000). It is clear that sub-optimal mineral deficiency could affect growth and production more seriously than the manifested mineral deficiency showing clinical signs (López-Alonso, 2012). Thus, information regarding mineral availability and their status in the body (e.g. liver) are important to improve the accurate composition and formulation of diet as well as, to reduce unnecessary mineral supplementation, and the related environmental pollution and costs (Zanetti et al., 2017).

4.1 Micromineral Antagonists

Cu - S - Mo interaction

It has been known that the interaction of Cu with high levels of antagonistic minerals Mo or S in the rumen can reduce the bioavailability and consequent absorption of Cu thereby causing a secondary deficiency (Hilal et al., 2016). These interactions with Mo and S in ruminants influence requirements and cause fourfold variation in the ability of feeds to provide absorbable copper.

Animals given excess Mo and high levels of S showed severe Cu deficiency due to the formation of an insoluble Cu-thiomolybdate complex in the rumen. These complexes are formed because both organic and inorganic dietary S compounds in feed or water are reduced to sulfides (S^{2-}) by rumen microorganisms (Bradley et al., 2011) and then react sequentially with molybdate from the diet in a stepwise, reversible, and pH-dependent manner to form mono-, di-, tri-, and tetra- thiomolybdates (Gould and Kendall, 2011). These then complex with Cu to give various Cu-thiomolybdates complexes, which are thought to have divergent effects on Cu bioavailability and absorption (Suttle, 1991, 2010). Monothiomolybdates can be broken down by the acidity of the abomasum, releasing complexed Cu for absorption (Price et al., 2017). Tri-thiomolybdate and tetra-thiomolybdates, however, are thought to irreversibly bind Cu, rendering it unavailable for absorption in the small intestine (McCaughern et al., 2020). These interactions of Cu in the gut environment results in a clinical deficiency of Cu which is characterised by low concentrations of Cu and Mo in the tissues (Lee and Grace, 1997). Subsequently, Cu will then be excreted in the feces in the form of these insoluble complexes, without being utilized by the animal (Ivan, 1988; Katulski, 2017).

However, the formation of thiomolybdates is dependent not only on the pH in the rumen but also on the S:Mo ratio in the rumen (Clarke and Laurie, 1980). Thus, at higher S:Mo ratios and over long periods, the extensive formation of tetra-thiomolybdate is favored (Golfman, 1988). The ratio of S:Mo available in the rumen at the time of thiomolybdate formation is indicative of the chemical stability of the thiomolybdate (Kistner et al., 2017). However, the "ideal" S:Mo ratio varies across the literature and is dependent on the relative dietary concentrations of each mineral (Afsah Hejri et al., 2019).

In addition, if the critical Cu:Mo ratio in the diet is or falls below 2:1, conditioned Cu deficiencies can be produced (Ward, 1978). A Cu:Mo ratio of 1:1 indicates a severe decrease in Cu status has or will occur in grazing livestock. A dietary Cu:Mo ratios of 6:1-10:1 are considered optimal for cattle production (Puls, 1994), as the effects of Mo will be minimal (Lee and Grace, 1997). For example, pastures containing 8-10 mg Cu/kg DM and less than 1 mg Mo/kg DM should not cause Cu deficiency in cattle (Lee and Grace, 1997). Thus, the ratio of the antagonistic minerals appears to be as important as the actual amounts of minerals in the diet.

Besides pH and the ratios between Cu, Mo and S, the formation of thiomolybdates also seems to depend on rumen available Cu. For example, when there is no Cu available in the rumen and dietary Mo intake is high, thiomolybdates will either be quickly absorbed through the rumen wall or will be absorbed more slowly via the small intestine and then pass into the bloodstream (Suttle, 1991, 2010). Indeed, the absorbed thiomolybdates have been shown to cause systemic effects on the metabolism of Cu, including an irreversible binding to any available Cu-dependent metalloenzymes such as ceruloplasmin (Cp) (Hilal et al., 2016; Rehman, 2017). These complexes impair the use of Cu in other biological functions or biochemical processes such as lessening cell function and mitochondrial integrity which will manifest as diarrhea (Dickson, 2016) and may result in a clinical deficiency of Cu despite apparently satisfactory concentrations of Cu in the tissues especially blood and liver (Lee and Grace, 1997). This thiomolybdate toxicity will result in clinical problems often wrongly quoted as Cu deficiency (Gould and Kendall, 2011). A Mo induced Cu deficiency therefore not only decreases the Cu concentrations in the plasma and liver but also the Cu available for biochemical and physiological functions (Lee and Grace, 1997).

In addition to its role in the S-Mo-Cu interactions, S if consumed in excess, independent of Mo, can also be detrimental to the bioavailability and absorption of Cu through the formation of CuS or Cu_2S (Goff, 2018). Indeed, when acting independently of Mo, S binds with Cu to form CuS which is unavailable to the animal as it is almost completely insoluble and hence will not be absorbed (Ward, 1978). This interaction between Cu and S is considered more powerful in making Cu unavailable to the animal than the thiomolybdate pathway, where for Mo to reduce Cu availability S must be present (Dickson, 2016). Thus, S has a greater impact on Cu absorption than Mo, on its own or in combination with Mo and regardless of form (inorganic (i.e. sulfate) or organic (i.e. methionine)) (Spears, 2003). For example, when dietary S increases from 0.2-0.4%, and diets contain < 2 mg Mo/kg DM, Cu absorption decreases by 30 to 50% (Suttle, 1991). Thus, the dietary concentration of Cu should be increased under these scenarios.

In general, the amount of S available to interact with Cu is affected by microbial degradation of S compounds in the rumen, the levels of rumen degradable protein and fermentable carbohydrates, the frequency of feeding and ruminal pH (e.g., sulfide production increases when the rumen pH drops suddenly) (López-Alonso and Miranda, 2020).

Iron antagonistic interaction with microminerals

High dietary Fe levels are also known to significantly reduce Cu availability in ruminants. The very soluble form of Fe in water can cause a greater influence on Cu absorption than the less soluble form of Fe from feedstuffs (Breede, 2006). The effect of Fe on Cu absorption can be estimated from the Fe:Cu ratio, with values >100 indicating a high risk of Cu deficiency and values <50 considered safe (López-Alonso and Miranda, 2020).

Increasing dietary Fe concentrations to 500-600 mg/kg DM have been shown to reduce liver and plasma Cu concentrations and utilization in sheep and cattle (Grace and Lee, 1992) but this was not accompanied by a decrease in animal performance (Lee and Grace, 1997). The proposed mechanisms by which Fe antagonism reduces Cu absorption are partly reliant on S concentrations (Rehman, 2017). First, Fe in the rumen is assumed to interact with sulfide and form ferrous sulfide (FeS) complexes (Hussein, 2017) that may become soluble in the acidic environment of the abomasum, where the sulfide may dissociate and react with Cu to form acid-insoluble and unabsorbable CuS complexes (Gould and Kendall, 2011; Sefdeen, 2017). This increases the Cu excretion level (Suttle, 1991). Diets low in S can thus limit the influence of Fe on Cu availability (Dickson, 2016). The formation of FeS in the rumen is a critical step for Fe to antagonize Cu absorption (Suttle, 1975).

Secondly, excess Fe can compete with Cu for its absorption at the intestinal level, by saturating the divalent metal transporter-1 protein (DMT1) Cu transporter which is regulated by Fe status in the body (Rehman, 2017). The significant competition between Cu and Fe for the transport prevents Cu from binding to DMT1 (Katulski, 2017).

Moreover, when high dietary concentrations of Fe are present and bound with Cu, not only do they reduce Cu availability in the rumen, but also, they reduce the Cu available to bind with thiomolybdates (Dickson, 2016). Indeed, when sufficient S and Mo are present in the rumen to form thiomolybdate complexes, lower levels of Cu, due to its affinity to the Fe, will allow these thiomolybdates to be absorbed through the rumen into the body where they can bind to any available Cu (Dickson, 2016). In this manner, Fe can exacerbate thiomolybdate toxicity (Gould and Kendall, 2011).

Excessive dietary Fe concentration may also interfere with Zn absorption (Price et al., 2017). This is because Fe might out-compete Zn for the binding sites on the apical membrane DMT1 (Goff, 2018). For instance, when lambs are fed a diet with adequate Cu and Fe, liver Fe reserves decrease with increasing dietary Zn (Sandoval et al., 1997).

Zinc - copper antagonistic interactions

Besides the discussed interactions between Fe and Zn, both Cu and S can also affect ruminal distribution, bioavailability and absorption of dietary Zn (NRC, 2005; Nwosu, 2019), Cu being the most important antagonist (Baker and Ammerman, 1995; Rehman, 2017).

The mechanism underlying the interaction between Zn and Cu has been proposed to potentially involve metallothionein (Hussein, 2017) which is found in the intestine, liver and kidney, and is involved in the protection of animals from Cu and Zn toxicity (Sefdeen, 2017; VanValin, 2019). The interaction between Cu and Zn is a consequence of the ability of these minerals to induce synthesis of metallothionein (Blanco-Penedo et al., 2006) and their competition for metallothionein binding sites along the brush border membrane of intestinal enterocytes (Katulski, 2017). Metallothionein is one of the important metal-binding proteins and comprises 60 amino acids, that enable a molecule of metallothionein to bind 6 –10 atoms of copper, and may thus function as a sequestrator of excess copper (Aquilina et al., 2016).

In most cases, Zn interferes with Cu absorption by upregulating the synthesis of metallothionein within enterocytes, which can cause Cu deficiency if Cu is marginal (Goff, 2018). When dietary intake of Zn is very high concentration relative to daily requirements, the production of metallothionein is stimulated in the liver. Metallothionein in turn has a higher affinity to bind and complex with Cu than Zn in the liver and in the intestines (López-Alonso et al., 2005). Subsequently, there is a reduced Cu availability and absorption from the small intestine, and a decreased Cu concentration in the liver of ruminants and excretion of Cu through the bile during the detoxification process occurs (Silva Júnior et al., 2015).

Excess Zn reduces the absorption of Cu and has been shown to reduce plasma and liver Cu concentrations due to the mechanism explained above, yet low levels of dietary Zn are associated with Cu accumulation and Cu toxicity in ruminants (Papachristodoulou et al., 2015), pigs (Paulsen, 2005) and laboratory animals (Hall et al., 1979). Nevertheless, the level of dietary Zn required to have a significant negative effect on Cu absorption must be at least 20 times higher than the recommended level (NRC, 2001).

However, dietary Cu appears to have less effect on liver Zn concentrations (Blanco-Penedo et al., 2006). For example, when dietary Cu:Zn ratios are very high (50:1), Cu could interfere with Zn absorption by inducing metallothionein within enterocytes. However, in cattle this is unlikely (Goff, 2018) because they would likely die of Cu intoxication before they became Zn deficient.

4.3. Fiber – mineral interaction

High dietary concentration of both insoluble (cellulose, hemicellulose, and lignin) and soluble (gums and pectin) of dietary fibers can lead to binding of minerals (Cano-Sancho et al., 2014), and thus to the formation of insoluble mineral complexes, adversely affecting their bioavailability and absorption (Baye et al., 2017).

A considerable amount of minerals found associated with the plant cell wall have a high affinity to lignocellulose constituents (Fujihara et al., 2006), escaping digestion, especially if they are intertwined with lignin (Goff, 2018). Rumen soluble microminerals can also bind with fiber in the rumen, where Cu forms an insoluble complex with undigested plant material, which becomes very stable at high pH during passage through the more distal portions, small intestine of the digestive tract in the ruminants (Price et al., 1979; Hernández-Sánchez et al., 2019).

In addition, an increased dietary NDF level leads to lower utilization and increased excretion of minerals (Khan et al., 2003). This might be due to the higher fiber level increasing the passage of undigested dry matter through the intestine, reducing the amount of time microminerals spend in the intestine, with insufficient time to solubilize and be reduced, necessary steps for absorption at intestinal level (Hussein, 2017). Minerals that are associated with plant cell wall in the reticulo-rumen require longer fermentation time for maximal release, this results in lower bioavailability (Emanuele and Staples, 1990) and increased excretion of minerals through the feces. Moreover, Moyo and Nsahlai (2018) concluded that a high level of fibre or NDF in the rumen is a major contributor to increase digesta flow through the rumen. The higher level of fiber in the reticulorumen might increase the intensity and frequency of reticulorumen contractions through tactile stimulation of the rumen wall (Okine and Mathison 1991; Moyo and Nsahlai, 2018). Fractional passage rate of NDF out of the rumen increased by about 34% as a result of increased rumen contractions (Moyo et al., 2017). Due to a similar explanation indicated above the higher fiber intake in sheep might result in faster passage of digesta out of the rumen in sheep compared to goats.

Indeed, the reticulo-rumen is the site where antagonistic interactions between minerals occur and where insoluble complexes are formed (Suttle, 1991). This indicates that the time dietary contents remains in the rumen influences the formation of insoluble complexes that in turn can limit the availability, absorption and utilization of minerals by ruminants (Gould and Kendall, 2011). The negative association may be related to the relatively negative charge of the fiber that can act as a mineral trap and serves to bind the positively charged divalent metal cations making them unavailable for absorption as already reported in non-ruminants (van der Aar et al., 1983), but this has not yet been studied and confirmed in ruminants (Faulkner et al., 2017). On the other hand, diets that are rich in highly digestible and fermentable carbohydrates have been reported to have a higher availability of Cu (Kistner et al., 2017). This higher Cu availability with high concentrate diets may be linked to a lower indigestible fiber content, therefore, reducing the negative impact of fiber on Cu absorption. Another possibility is that these feeds being rich in readily fermentable carbohydrates, such as silage or cereals, will lower the ruminal pH which could increase Cu solubility (Faulkner et al., 2017).

Moreover, when cattle are fed a low forage to concentrate ratio (F:C), they showed significantly higher ruminal Cu, Fe, Mn and Zn concentrations compared to those fed the high F:C diet (Amat, 2012). The release of minerals in the rumen from both grain and forage might be due to differences in the rumen environment (pH, micro-organisms) and the rate of fermentation (Hussein, 2017). This can be related to a more acidic rumen environment that enhances Cu availability by reducing the availability and activity of bisulfide or Mo for thiomolybdate formation (Suttle, 1991). The reduced formation of thiomolybdate results in an increase in both apparent Cu availability and final hepatic Cu concentration (McCaughern et al., 2020).

This is evidence that the solubility and bioavailability of minerals throughout the gut can be influenced by the content or chemical composition of dietary fiber in feedstuffs (Snyman, 2007) and their passage rate in the rumen (Pogge et al., 2014; Zanetti et al., 2017). This may further affect the extent and site of nutrient digestibility (Faulkner and Weiss, 2017).

4.4. Tannin-Minerals Interaction

Tannins are widely distributed in various plants and described as naturally occurring water-soluble polyphenolic plant secondary compounds, with high molecular weights ranging from 500 up to 20,000 Dalton units (Naumann et al., 2013). They are grouped under plant secondary compounds because they are not involved in biosynthesis, biodegradation and other energy conversion metabolism in the plant (Martin, 2016). Based on the reactivity and structural characteristics, tannins are broadly grouped as hydrolyzable tannins (HT) and condensed tannins (CT) or proanthocyanidins.

Condensed tannins (CT)

Condensed tannins are yellowish or brownish bitter-tasting polyphenolic compounds of high molecular weight ranging from 1900 to 28000 Da (Aboagye, 2019). They have strong binding affinity to form complexes with dietary proteins, minerals and other dietary molecules due to the presence of large numbers of phenolic hydroxyl groups (Adejoro, 2019). High CT concentrations above 50 g/kg DM would probably have a negative effect on palatability because constriction, dryness and roughness in the oral cavity develop conditioned aversions (McNabb et al., 1997).

Increasing CT level in the diet may cause an adverse effect on voluntary feed intake, depresses rumen digestibility as well as reduce availability and absorption of nutrients from the small intestines of ruminants (Taha, 2015). The protein-CT complex that cannot be dissociated in the lower gut would thus pass through the GI tract and excreted in the fecal N (Patra and Saxena, 2011).

Hydrolysable tannins (HT)

HT is relatively a low molecular weight tannin (500 to 3000 Da) and is not as widely distributed in plants as the condensed tannins (Adejoro, 2019). HT can be hydrolysed by mild acids or mild bases and by microbial action of the rumen (Adejoro, 2019). For example, some HT can be broken down by rumen bacteria such as *Selenomonas ruminantium* and *Streptococcus Bovis*, *Eubacterium oxidoreducens*, *Coprococcus sp*, *Syntrophococcus* *sucromutans* (McSweeney et al., 2001). It seems that HT are quite susceptible to degradation throughout the GIT of the ruminants. However, this has not been studied in detail and what this would imply for the nutrient utilization and absorption by the animal itself.

Apart from binding to proteins, it is widely believed that tannins in plants also have an excellent binding (chelating) ability to form complexes with minerals (Karamać, 2009) via ion exchange and complexation (Zeng et al., 2019). Ion exchange is indeed the main mechanism explaining the interaction between minerals and tannins, as metal cations displace the adjacent phenolic hydroxyl groups forming a chelate (Zhan and Zhao, 2003). The minerals that are bound with tannins may not be released from feedstuffs and hence become unavailable for absorption by the animal (Acharya et al., 2016) leading to reduced biological availability of minerals (McDonald et al., 1996; Frutos et al., 2004). In addition, high levels of these polyphenolic compounds (tannins) can form insoluble (stable) complexes with many divalent metallic ions through their functional groups of carboxyl and hydroxyl groups more easily than with the interacting groups of proteins (Patra and Saxena, 2011) and hence, the chelating activity of tannin with minerals might have a greater biological effect than other nutrients (Raju et al., 2018).

However, the reports on the influence of CT on ruminant mineral nutrition are quite inconsistent. For instance, Waghorn et al. (1994), reported that CT in fresh-cut *Lotus pedunculatus* (5.5% CT) given to sheep reduced S absorption and increased net absorption of P and Zn, whereas the absorption of other minerals such as Fe, Cu, Ca, and Mg was not affected. Min et al. (2015) observed a significant linear increase in the availability of Mn, Zn and Fe as pine bark was increased in the diets and the effects were more pronounced with the 30% pine bark diet; whereas Cu and S availability linearly decreased in meat goats compared with those receiving the control diet. It is likely that a nutrient-specific effect of CT on minerals in the gastrointestinal tract of ruminants occurs.

The beneficial or negative effect of the mineral-tannin complex is determined by the amount, type, structure, molecular weight, and physicochemical and biological properties of tannins (Karamać, 2009; Naumann et al., 2017). For example, more CT was required as compared to HT to precipitate similar amounts of Cu (McDonald et al., 1996). Therefore, the effects of tannin ingestion at different intake levels and interactions with antagonistic minerals require further research. This is of particular relevance in the tropics where ruminants face a shortage of feed and rely on feedstuffs that contain different forms of tannins, especially during the dry season.

4.5. pH-dependence of tannin - dietary protein interactions

The complexes formed between tannins and proteins, minerals, or other compounds are generally unstable, reversible and interchangeable along the gastrointestinal tract due to pH differences in various parts of the gastrointestinal tract (Mueller-Harvey, 2006; Patra and Saxena, 2011). The pH-dependence of tannin-nutrient (e.g protein) complexes is particularly interesting in ruminant nutrition because the pH variation along the digestive system defines the nutritional behaviour of CT (Dentinho and Bessa, 2016). Beauchemin et al. (2011) indicated that there is considerable variation in ruminal pH among animals fed the same diet, as some animals experience prolonged periods of low pH while for others, pH remains consistently high. Zeitz et al. (2016) also proved that grazer diets (grass hay) caused higher pH values in the rumen than browser diets (e. g. dried leaves of *Castanea sativa, Rubus idaeus*, and *Populus tremula*).

The rumen pH allows any free tannin to bound with most of the dietary proteins and form tannin-protein complexes that are difficult to dissociate, mainly when CT are involved (Taha, 2015; Dentinho and Bessa, 2016). This may protect proteins from microbial degradation and impair at the same time the microbial proteolytic activity. A reduction in proteolysis might be attributed to the direct effect of tannins on reducing proteolytic bacteria numbers and inhibiting proteolytic enzyme activity (e.g., protease activity) due to coating of the protein surface (Patra et al., 2012). This interference with the interaction of enzyme and substrate reduces the rate of proteolysis (Patra et al., 2012). Tannins are also known to cause indirect effects on rumen metabolite concentrations, that can regulate enzymatic activity of rumen bacteria. For instance, the binding of tannins with minerals reduces the availability of some minerals that are important for the growth and function of rumen microbes. Once these stable CT-protein complexes arrive in the acidic abomasal environment (pH 2.5-3.5), some of the bonds uniting the complexes continually dissociate dietary proteins, freeing them for enzymatic hydrolysis and absorption in the small intestine (Frutos et al., 2004; Adejoro, 2019). *In vitro* studies indicated that 95% of protein-CT complex was dissociated and release protein from the complex at more acid pH between 1.0-3.0 but protein and CT complex where stable in the range of pH 4.0 -6.5 (Figure 5). However, the pH is not always low enough in the abomasum to cause dissociation (Martin, 2016), since the pH is affected by the type of diet, feed intake, animal species and water intake.

The protein that reaches the abomasum consists of a mixture of dietary (which escapes microbial degradation) and microbial protein (Tseu, 2019). In addition, following the dissociation of tannin from these proteins, there is also the possibility for tannin molecules to bind with endogenous proteins (Adejoro, 2019).

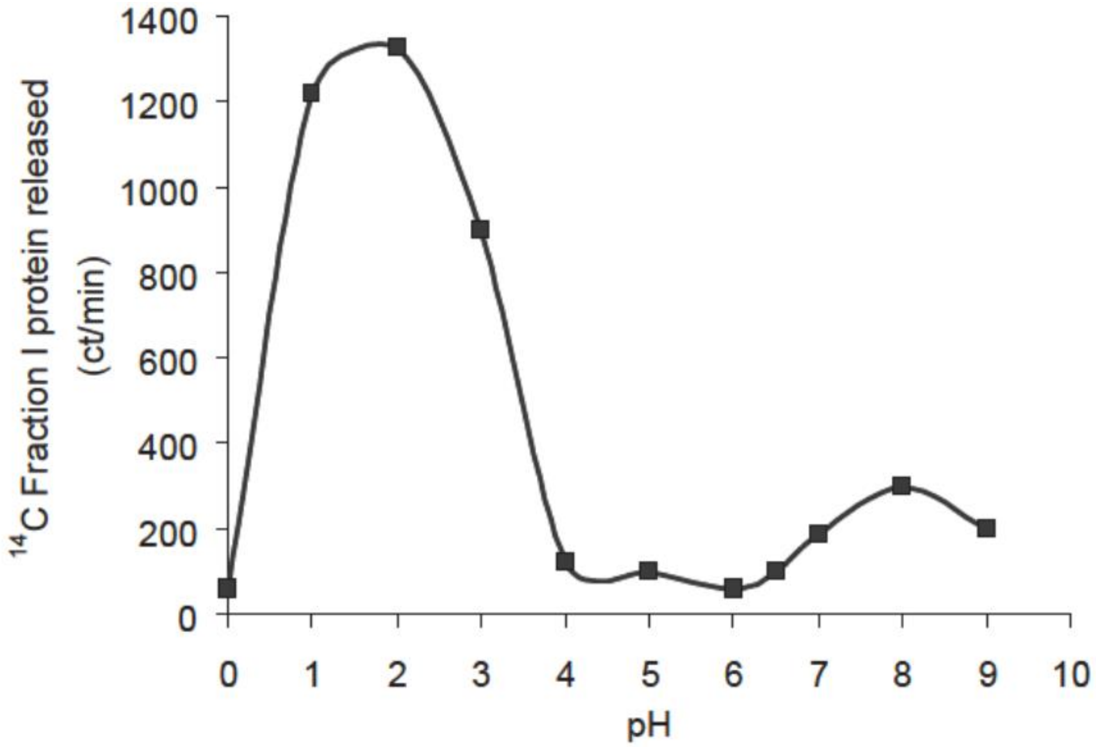

Figure 5: Effect of pH on the tannin - protein complex. Theodoridou, 2010

However, in the alkaline conditions of the small intestine (Frutos et al., 2004; Adejoro, 2019; Tseu, 2019) the hydrogen bonds between the tannin and protein would break down and the complex would dissociate to release the protein (McSweeney et al., 2001; Waghorn, 2008). Early *in vitro* work by Jones and Mangan (1977) also indicated that 30% of the protein-CT complex dissociated at a more alkaline pH between 8.0-8.5. This results in increased digestion and absorption of amino acids in the small intestine (Mueller-Harvey, 2006; Theodoridou, 2010) increasing the overall N-use of CT-fed ruminants (Min and Solaiman, 2018).

McNabb et al. (1997) reported a wider pH range from 3-8 at which white clover proteins and CT (from *Lotus corniculatus* and *L. pedunculatus*) complexes were stable. According to the authors, at a pH 5 about 40% of the protein was complexed with CT and only 10% of the proteins were complexed with CT at pH 5.5. Perez-Maldonado and Norton (1996) in their *in vitro* study found that the most stable protein-CT complexes were formed at pH 3.5-5.5, the highest amount being formed at pH 4.5. The pH level throughout the gut is not the only factor that affects protein-CT complexes but this can also be due to variation in type, concentration, and molecular structures of tannins as well as depend on the composition of diet used in different studies (Mueller-Harvey et al., 2019).

4.6. pH-dependence of minerals solubility and interaction with tannin

There's a close association between the pH of the digesta, the solubility of a mineral, and potential absorption of a mineral. Depending on the nature of their binding, minerals in the ruminal environment can exist in a soluble form (either in the ionic form or as a soluble complex) or insoluble form (Zanetti et al., 2017; Caldera et al., 2019).

In general, the solubility of microminerals is increased by decreasing pH conditions (Keith and Bell, 1987; Serra et al., 1997). For instance, sulfides can interact with Zn to form insoluble ZnS in the rumen, which may be unavailable for absorption by the small intestines (Pogge et al., 2014). However, in an acidic condition of the abomasum, the ZnS complex can interact with the HCl and release soluble and ionized Zn for absorption in the small intestines (Goff, 2018). Likewise, non-heme Fe, which is mainly found in cereals, plants, and the soil is well utilized by ruminants but, much of the dietary Fe exists within feedstuffs in the ferric (Fe^{3+}) form (Golfman, 1988). The reduction of some of the ferric (Fe^{3+}) ion to ferrous (Fe^{2+}) ion occurs in the acid environment of the abomasum (Golfman, 1988). Subsequently, in the gastrointestinal tract soluble ferrous (Fe^{2+}) form is readily absorbed to a much greater extent than as Fe^{3+} ions (van den Top and Veevoederbureau, 2005b; Goff, 2018). Similarly, a reduction of rumen pH could be beneficial to Cu availability (Dickson, 2016).

Moreover, as with proteins, there are different pH values associated with complexation and dissociation of CT with cations (Faithfull, 1984). It is reported that the pH condition influenced not only the precipitation but also the stability of mineral-tannin complexes (McDonald et al., 1996). The pH of the environment plays an important role in the affinity of CT for minerals and hence might influence the availability and absorption of certain metal ions such as Cu in ruminants (Suttle, 1991; Acharya, 2014).

Although most of the metal ion complexes can dissociate in the abomasum (pH 2.5 – 3.0) (Acharya, 2014), Fe will bind with CT and the Fe-CT complex can remain stable in the abomasum and throughout the gastrointestinal tract of ruminants (Faithfull, 1984; Muir, 2011) thus, making their deficiency potentially problematic (Naumann et al., 2013, 2017). Reductions in Fe bioavailability are also found at a higher pH environments, where polyphenol-non-heme Fe complexes prevent Fe^{3+} reduction to Fe^{2+} (Naumann et al., 2017). Zanetti et al. (2017) also indicated that at high pH conditions, minerals typically become insoluble, thus decreasing the chances for their absorption. It is generally accepted that mineral ion-tannin complexes are stable over a wide pH range and throughout the entire gastrointestinal tract (McDonald et al., 1996; Scalbert, 1991). Fe binds to CT at pH levels of 3.2 or less, whereas Zn, and Cu all bind to CT at pH levels of 3.7 or greater (Naumann et al., 2013).

4.7. Association of mineral bioavailability and the rumen fermentation profile

In the rumen, the end-products of ingested feeds are mainly short-chain fatty acids (SCFA), used as energy source by the host, and ammonia (NH_3), used to build microbial protein, whereas methane (CH_4) is a potent greenhouse gas responsible for global warming and energy loss (Arif, 2015). Methane has 23-25 times more global warming potential than carbon dioxide (CO_2) (Gupta et al., 2017). The excess hydrogen generated as a result of rumen fermentation is removed by reducing CO_2 into CH_4, which is required for efficient and continuous microbial growth (Beauchemin et al., 2008). However, 2–12% of the gross energy of feed consumed by the animals can be wasted in the form of CH_4 in ruminants (Gupta et al., 2017). The CH_4 emission can be reduced by substituting the CO_2 with another electron acceptor such as sulfates, nitrates and fumarate (Arif, 2015; Hernández-Sánchez et al., 2019). For example, *in vitro* inclusion or feeding of S has resulted in a reduction in CH_4 production confirming its role as an alternate electron acceptor (Gupta et al., 2017). It has been hypothesized that this might be through a direct effect on the methanogen population or by consuming energetically more efficient terminal electron acceptors as a feed supplement (Arif, 2015).

Although Cu is required for microbial fermentation and digestibility, excessive concentrations can cause modification within the rumen that may adversely affect rumen proteolytic microbial populations and depressed their growth, digestibility of nutrients and products of fermentation (Katulski, 2017). The increase in concentrations from 20, 40 to 60, 80 and 100 mg/kg DM of Cu as $CuSO_4$ mg/kg DM lead to a linear decrease in CH_4 production (mL g^{-1} digestible OM). However, ruminal total bacteria population, N-NH3 and VFA concentrations, OM degradation were unchanged at concentrations from 20, 40 to 60 mg/kg DM of Cu as $CuSO_4$ mg/kg DM, while bacteria populations and VFA concentrations were decreased at the levels of 80 and 100 mg/kg DM of Cu as $CuSO_4$ *in vitro* (Hernández-Sánchez et al., 2019). Moreover, when the level of Cu exceeded 0.5 mg/kg DM in the growth medium, there was bacterial proteolysis, but bacterial growth was greater with a Cu amount below this level (Vázquez-Armijo et al., 2011). Because of the permeability of their membrane, the Gram-negative bacteria are the most Cu-sensitive microorganisms due to concomitant intracellular accumulation of Cu, causing bacterial lysis (Osman and Cavet, 2008). Vázquez-Armijo et al. (2011) also reported increased 96-h *in vitro* gas production, IVDMD, ME, and VFA in ruminal fluid from goats fed 21.7 mg Cu/kg diet DM compared to goats fed 10.3 mg Cu/kg diet DM.

The reduction in rumen CH_4 production might be related to the presence of sulphate-reducing bacteria (SRB), because high level of SRB can compete with methanogens for common substrates, e.g. H_2, formate and acetate (Uniyal et al., 2020). SRB have a greater affinity for hydrogen compared with methanogenic bacteria (Gupta et al., 2017). Richter (2012) also showed that, when dietary S increases, SRB in the rumen were increased two-fold in high S fed steers compared to low S fed steers. In the rumen, some SRB reduce S-containing compounds into hydrogen sulfide by utilizing ruminal hydrogen (Uniyal et al., 2020). In addition, the hydrogen sulfide produced by these bacteria can be utilized by most of the other rumen microorganisms which are unable to use sulphate directly (Gupta et al., 2017). This H_2S can also stimulate cellulose-degrading bacteria and fungi that require S in the form of sulfide and are also involved in the synthesis of S-containing amino acids (Gupta et al., 2017). However, the SRB can perform better than methanogens only in the presence of non-limiting sulphate concentration (Uniyal et al., 2020). Napasirth et al. (2013) indicated that extremely high doses of $CuSO_4$ from 0.2 to 0.4% in a ruminal *in vitro* culture reduced CH_4 emissions. Arif (2015) found a significant reduction of CH_4 emission in goats and sheep fed a low protein forage-based diet supplemented with 0.4% sulfate S (anhydrous $MgSO_4$) compared to unsupplemented animals, indicating that a reduction in CH_4 might not only be caused by the Cu levels but also by the sulfate S. Therefore, an adequate S concentration in diets or in supplementation firstly can improve nutrient utilization through enhancing the rumen microbial growth and secondly can inhibit methane production, which ultimately results in improved animal performance (Uniyal et al., 2020). A sulfur concentration above the requirement usually decreases both feed intake and animal performance (de Oliveira et al., 1996). The consequences of a high sulfur diet depend not only on the dietary level of sulfur but also on the type of sulfur compound, on its mode of administration and on the composition of the ration (de Oliveira et al., 1996).

Moreover, Vázquez-Armijo et al. (2011) reported increases in total gas production from 22 to 36 mg Cu/kg diet DM, without affecting CH_4 production. Gould and Kendall (2011) also emphasized that levels of Cu 20 mg/kg DM stimulate and optimize microbial protein synthesis based on the N-NH3 concentration in the rumen. The higher levels of Cu increasing from 60, 80 and 100 Cu mg/kg DM levels as $CuSO_4$ supplementation decreased N-NH_3 concentration molar proportion of acetate and total VFA concentration, together with a reduction in bacterial population and OM digestibility (Hernández-Sánchez et al., 2019). Excess of dietary $CuSO_4$ concentration may also induce antinutritional effects which could decrease both the feed intake and animal performance, as well as might lead to clinical signs of intoxication and eventually death (de Oliveira et al., 1996; Richter, 2012). In cases of intoxication it is possible to observe the lack of ruminal motility and loss of appetite (Silva et al., 2014).

It is evident from literature that Cu is required for microbial fermentation and digestibility; however, excessive concentrations can cause modification within the rumen that may adversely affect microbial populations, digestibility of nutrients, and products of fermentation. The variation may be due to differences in levels of supplementation or species differences and fermentation duration (Wang et al., 2020). Further research is needed to identify the mechanism of how micro-minerals affect the rumen fermentation profile.

4.8 Effect of animal species and breed on mineral utilization difference

There are major differences in mineral metabolism between non-ruminant and ruminant animals particularly in relation to Cu sensitivity or tolerance levels (NRC, 2005; Hill and Shannon, 2019) which lead to differences in Cu requirements. For instance, compared to ruminant animals, monogastric species (including pigs, poultry and rabbits) have a better ability to accumulate Cu in their liver at different concentrations of dietary Cu (Figure 6).

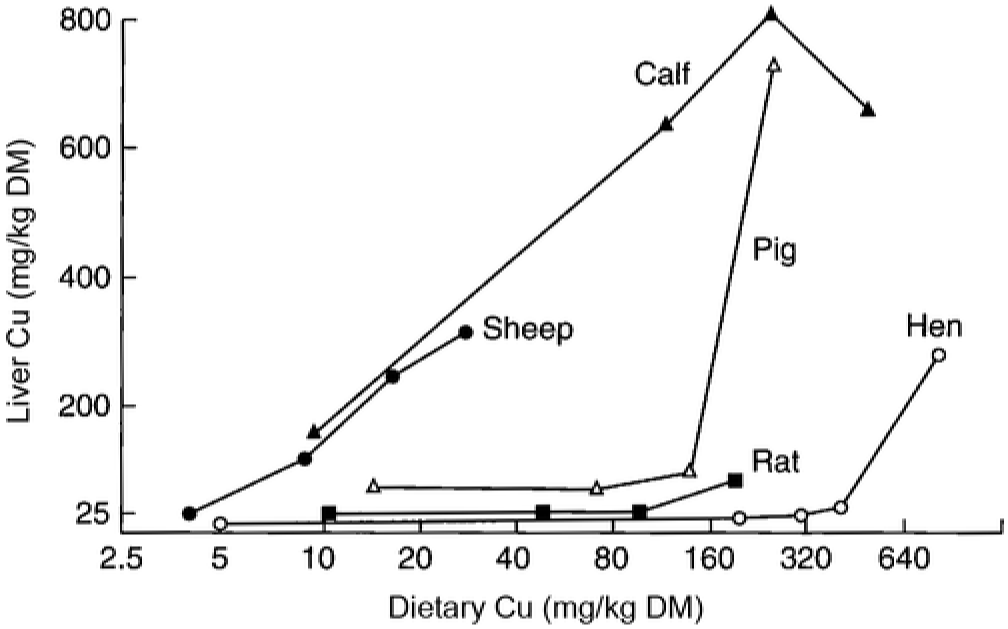

Figure 6. Species differences on responses in liver cu deposition to increases in dietary Cu supply. Source: Suttle, 2010

This difference is mainly related to great differences in the ability to excrete Cu in the bile as well as how Cu is bound within the hepatic cell. Non-ruminants have an adequate biliary excretion of Cu and most of the Cu is bound to metallothionein (MT) in the liver (Hill and Shannon, 2019), and they therefore tolerate high levels of dietary Cu. On the contrary, when exposed to Cu concentrations above physiological requirements, ruminants, particularly sheep have a very limited capacity for copper biliary excretion, and only a very small proportion of copper is bound to MT in their liver (López-Alonso and Miranda, 2020). This results in excessive hepatic Cu accumulation in their liver. Once this storage capacity is overloaded, a sudden and generally fatal haemolytic crisis occurs. When the influx of Cu exceeds the capacity of the metallothionein, the metallothionein transcription in the lysosome of cattle and sheep does not effectively respond to rapid increases in Cu (López-Alonso et al., 2005), explaining why cattle appear to be more Cu tolerant than sheep and why both species appear to be sensitive in comparison to monogastric species such as pigs (Clarkson et al., 2019).

In the past, it has been argued that breed differences in Cu status are mainly due to differences in the proportion of Cu intake that is absorbed, yet over time they appear to be better explained by differences in the hepatic retention of absorbed Cu (Shuttle et al., 2002). For example, better liver Cu storage and increased liver mRNA expression of genes are related to Cu metabolism in Ethiopian zebu (*Bos indicus*) cattle compared to crossbred (*Bos indicus* × *Bos taurus*) cattle when both fed a Cu-deficient diet (Dermauw et al., 2014). The authors also demonstrated higher plasma Zn concentrations in crossbred compared to zebu cattle, suggesting a disparate micromineral utilization between zebu and crossbred cattle.

Breed differences can also affect Cu metabolism in cattle, e.g. Simmental cattle are more susceptible to Cu deficiency and are less tolerant to Cu antagonist, such as Mo than Angus (Fry et al., 2013). This can be due to less apparent Cu absorption in Simmentals than in Angus (Ward et al., 1995), with subsequent greater (two-fold) biliary Cu excretion in Simmental than in Angus (Gooneratne et al., 1994). A breed difference in Cu metabolism for tropical sheep and goats has also been established (Osman et al., 2003). This higher tolerance of goats to Cu than sheep (Hill and Shannon, 2019) could be explained by the fact that goats retain 6 to 9 times less Cu in their liver compared with sheep, when both are supplemented with high Cu levels (Zervas et al., 1990). Genetic differences in Cu metabolism are reported between sheep and deer or between cows and sheep, and between goats and moose, or between individuals within the same breed (Sefdeen, 2017).

A higher incidence of liver Cu deficiency in adults than in growing sheep, are probably an indication that Cu storage reserves decline with age (Mohammed et al., 2016). Sheep are also widely known to have significant age and breed variation to Cu requirements (NRC, 2007; Price et al., 2017). This difference in mineral metabolism between animal species is also true for Zn and Mo. For instance, high Mo levels are least tolerated by cattle, followed by sheep, but are tolerated by goats (Haenlein and Anke, 2011; Schweinzer et al., 2017). Sheep and cattle are also less tolerant for high Zn concentrations in the diet. Levels of 0.9-1.7 g/kg Zn resulted in depressed appetite manifested by wood-chewing and excessive consumption of mineral supplements in ruminants (Hamid, 1997). The lower tolerance of ruminants to high dietary Zn may be related to changes in rumen metabolism brought by a rapid toxic effect of Zn on rumen microflora (Hamid, 1997). Moreover, the variation in liver Zn concentration between species could be a reflection of differences in individual animal Zn metabolism (Lengarite et al., 2012). In general, understanding the differences in micro-mineral bioavailability, absorption, storage, and utilization would enable the development of species or breed-based micro-mineral supplementation strategies, that reduce waste and extra supplementation as some breeds may require less micro-mineral supplementation than others (Price et al., 2017).

4.9 Micromineral function for rumen microorganisms

Carbohydrates including cellulose and hemicellulose are utilized by rumen microbes, and up to 90% of carbohydrate digestion occurs in the rumen (Van Valin, 2019). For optimum cellulose degradation the rumen environment must supply a balance of all nutrients required for optimal microbial growth and activity. Trace minerals such as Zn, Mn, Cu, and Co are required for structural proteins, enzymes, coenzymes, and cellular proteins and participate in many enzymatic processes in the rumen (Pino and Heinrichs, 2016). Rumen microorganisms can use both soluble and insoluble forms of microminerals in the rumen for proper functioning or metabolism (Pino and Heinrichs, 2016). Although minerals are required for microbial fermentation and digestibility, excessive or low concentrations can cause modification within the rumen that may adversely affect rumen microbial populations and depressed their growth, digestibility of nutrients and products of fermentation (Katulski, 2017). *In vitro* studies also suggest that relatively small concentrations of Cu, Mn, and Zn can negatively affect cellulose digestion, whereas *in vivo* experimental results have been less consistent (Genther and Hansen, 2015). For example, cellulose digestion decreases due to micromineral deficiencies in cattle fed high-grain rations (Pino and Heinrichs, 2016). This can occur because fast-growing bacteria increase the microbial micromineral requirement when they are degrading starch and there is not enough microminerals to be used by slow-growing bacteria that are cellulose digesters (Pino and Heinrichs, 2016).

On the other hand, when 25 µg Zn/mL (calculated to be 1,000 mg Zn/kg diet DM) from ZnSO4 was added to *in vitro* cultures, cellulolytic and total bacterial concentrations were not affected, the digestion of cellulose decreased (Eryavuz and Dehority, 2009). The authors conclude that the decrease in the digestion of cellulose may be linked to a direct effect of Zn on the bacterial inactivation of cellulase, rather than a toxic effect of Zn on ruminal bacterial populations (Eryavuz and Dehority, 2009). Factors other than supplemental Zn concentration such as diet type fed to donor animals which could influence rumen fluid content in *in vitro* analysis, and subsequently microbial populations present within the rumen alone may be contributing to the effect of Zn on nutrient digestibility (Van Valin, 2019). Supplementation of Cu (20 mg of Cu/kg of diet DM) had a negative effect on finishing-cattle gain and feed efficiency (Engle and Spears, 2000a); however, the addition of similar concentrations in another study had no effect on rumen fermentation (Engle and Spears, 2000b). Therefore, it is necessary to further determine the specific functions of minerals in the ruminal environment and how they affect microbial metabolic pathways.

References

Aboagye, I.A., 2019. Mitigating enteric methane production and nitrogen excretion from forage-fed ruminants (PhD Thesis). University of Alberta.

Acharya, M., 2014. Improving Lamb Performance with Sericea Lespedeza and Molybdenum (PhD Thesis). University of Arkansas.

Acharya, M., Burke, J.M., Coffey, K.P., Kegley, E.B., Miller, J.E., Smyth, E., Welborn, M.G., Terrill, T.H., Mosjidis, J.A., Rosenkrans, C., 2016. Changes in concentrations of trace minerals in lambs fed sericea lespedeza leaf meal pellets with or without dietary sodium molybdate12. Journal of Animal Science 94, 1592–1599. https://doi.org/10.2527/jas.2015-9910

Adejoro, F.A., 2019. The use of condensed tannins and nitrate to reduce enteric methane emission and enhance utilization of high-forage diets in sheep (PhD Thesis). University of Pretoria.

Adem, M., 2019. Production of hide and skin in Ethiopia; marketing opportunities and constraints: A review paper. Cogent Food & Agriculture 5, 1565078.

Afsah Hejri, S.J., Badiei, K., Pourjafar, M., Oryan, A., Keshavarzi, B., Chalmeh, A., Mesbah, G., 2019. Seasonal Variation and Relationships Between Copper of Serum and Various Tissues in Copper Poisoned Sheep in Kerman Province, Iran. Iranian Journal of Veterinary Medicine 13, 163–173.

Agrawal, A.R., Karim, S.A., Kumar, R., Sahoo, A., John, P., 2014. Sheep and goat production: basic differences, impact on climate and molecular tools for rumen microbiome study. International of Journal Current Microbiol Applied and Science 3, 684–706.

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. Journal of Animal Science 74, 3063–3075.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., 2010. Tannins in tropical tree fodders fed to small ruminants: A friendly foe? Small Ruminant Research 89, 164–173. https://doi.org/10.1016/j.smallrumres.2009.12.040

Amat, S., 2012. Evaluation of sulfur and thiamine metabolism, short chain fatty acid absorption, and mineral status in beef cattle fed high dietary sulfur (PhD Thesis). (University of Saskatchewan).

Ammerman, C.B., Baker, D.P., Lewis, A.J., 1995. Bioavailability of nutrients for animals: Amino acids, minerals, vitamins. Elsevier.

Aquilina, G., Azimonti, G., Bampidis, V., de Lourdes Bastos, M., Bories, G., Chesson, A., Cocconcelli, P.S., Flachowsky, G., Gropp, J., Kolar, B., 2016. Revision of the currently authorized maximum copper content in complete feed.

Arif, M., 2015. Influence of nitrate and sulphate feeding on enteric methane production in sheep and goats (PhD Thesis). University of Agriculture, Faisalabad Pakistan.

Assefa, D., Nurfeta, A., 2013. Assessment of feed resource availability and livestock production constraints in selected Kebeles of Adami Tullu Jiddo Kombolcha District, Ethiopia. African Journal of Agricultural Research 8, 4067–4073.

Austin, P.J., Suchar, L.A., Robbins, C.T., Hagerman, A.E., 1989. Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. Journal of Chemical Ecology 15, 1335–1347.

Babaei, Y., Rouzbehan, Y., Alipour, D., 2015. Effect of rumen bacteria from sheep adapted to a tanninferous diet on *in vitro* fermentation parameters of pistachio hulls using bovine inoculum. Iranian journal of veterinary research 16, 357.

Baker, D.H., Ammerman, C.B., 1995. Zinc bioavailability, in: Bioavailability of Nutrients for Animals. Elsevier, pp. 367–398.

Baye, K., Guyot, J.-P., Mouquet-Rivier, C., 2017. The unresolved role of dietary fibers on mineral absorption. Critical reviews in food science and nutrition 57, 949–957.

Beauchemin, K.A., Janzen, H.H., Little, S.M., McAllister, T.A., McGinn, S.M., 2011. Mitigation of greenhouse gas emissions from beef production in western Canada–Evaluation using farm-based life cycle assessment. Animal Feed Science and Technology 166, 663–677.

Beauchemin, K.A., Kreuzer, M., O'mara, F., McAllister, T.A., 2008. Nutritional management for enteric methane abatement: a review. Australian Journal of Experimental Agriculture 48, 21–27.

Blalock, T.L., Dunn, M.A., Cousins, R.J., 1988. Metallothionein gene expression in rats: tissue-specific regulation by dietary copper and zinc. The Journal of Nutrition 118, 222–228.

Blanco-Penedo, I., Cruz, J.M., López-Alonso, M., Miranda, M., Castillo, C., Hernández, J., Benedito, J.L., 2006. Influence of copper status on the accumulation of toxic and essential metals in cattle. Environment International 32, 901–906.

Bradley, A.S., Leavitt, W.D., Johnston, D.T., 2011. Revisiting the dissimilatory sulfate reduction pathway. Geobiology 9, 446–457.

Breede, D.K., 2006. Evaluation of water quality and nutrition for dairy cattle, in: High Plains Dairy Conference., Albuquerque, NM.

Bremner, I., 1970. Zinc, copper and manganese in the alimentary tract of sheep. British Journal of Nutrition 24, 769–783.

Brown, D., Ng'ambi, J.W., 2019. Effects of dietary vachelia karroo leaf meal inclusion on meat quality and histological parameters in pedi bucks fed a setaria verticillata hay-based diet. Applied Ecology and Environmental Research 17, 2893–2909.

Caldera, E., Weigel, B., Kucharczyk, V.N., Sellins, K.S., Archibeque, S.L., Wagner, J.J., Han, H., Spears, J.W., Engle, T.E., 2019. Trace mineral source influences ruminal distribution of copper and zinc and their binding strength to ruminal digesta. Journal of Animal Science 97, 1852–1864.

Cano-Sancho, G., Rovira, J., Perelló, G., Martorell, I., Tous, N., Nadal, M., Domingo, J.L., 2014. Extensive Literature Search on the bioavailability of selected trace elements in animal nutrition: Incompatibilities and interactions 1136.

Cardozo, P.W., Calsamiglia, S., Ferret, A., Kamel, C., 2004. Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. Journal of animal science 82, 3230–3236.

Clarke, N.J., Laurie, S.H., 1980. The copper-molybdenum antagonism in ruminants. I. The formation of thiomolybdates in animal rumen. Journal of inorganic biochemistry 12, 37–43.

Clarkson, A.H., Paine, S., Martín-Tereso, J., Kendall, N.R., 2019. Copper physiology in ruminants: trafficking of systemic copper, adaptations to variation in nutritional supply and thiomolybdate challenge. Nutrition research reviews 1–7.

Clauss, M., Hummel, J., 2017. Physiological adaptations of ruminants and their potential relevance for production systems. Revista Brasileira de Zootecnia 46, 606–613.

Clauss, M., Hummel, J., Streich, W.J., 2006. The dissociation of the fluid and particle phase in the forestomach as a physiological characteristic of large grazing ruminants: an evaluation of available, comparable ruminant passage data. European Journal of Wildlife Research 52, 88–98.

Colins, A., Gerdtzen, Z.P., Nuñez, M.T., Salgado, J.C., 2017. Mathematical modeling of intestinal iron absorption using genetic programming. PloS one 12, e0169601.

CSA, 2017. Report on livestock and livestock characteristics, (Private peasant holdings) Agricultural sample survey, Statistical Bulletin 585, Addis Ababa, Ethiopia, April, 2017 - Google Search [WWW Document]. URL https://www.google.com/search.

de Oliveira, L.A., Jean-Blain, C., Dal Corso, V., Benard, V., Durix, A., Komisarczuk-Bony, S., 1996. Effect of a high sulfur diet on rumen microbial activity and rumen thiamine status in sheep receiving a semi-synthetic, thiamine-free diet. Reproduction Nutrition Development 36, 31–42.

Dentinho, M.T., Bessa, R.J., 2016. Effect of tannin source and pH on stability of tannin-protein and fibre complexes. Revista de Ciências Agrárias 39, 114–121.

Dermauw, V., 2013. Trace elements in zebu cattle: status and impact: the case of the Gilgel Gibe catchment, Ethiopia (PhD Thesis). Ghent University.

Dermauw, V., De Cuyper, A., Duchateau, L., Waseyehon, A., Dierenfeld, E., Clauss, M., Peters, I.R., Du Laing, G., Janssens, G.P.J., 2014. A disparate trace element metabolism in zebu (Bos indicus) and crossbred (Bos indicus x Bos taurus) cattle in response to a copper-deficient diet. Journal of animal science 92, 3007–3017.

Dickson, H., 2016. Copper deficiency: A review of the economic cost and current constraints to effective management of copper deficiency in southern Australian sheep flocks. Meat and Livestock Australia Limited - Google Search [WWW Document]. URL https://www.google.com/search.

Emanuele, S.M., Staples, C.R., 1990. Ruminal release of minerals from six forage species. Journal of animal science 68, 2052–2060.

Engle, T.E., Spears, J.W., 2000a. Dietary copper effects on lipid metabolism, performance, and ruminal fermentation in finishing steers. Journal of Animal Science 78, 2452–2458.

Engle, T.E., Spears, J.W., 2000b. Dietary copper effects on lipid metabolism, performance, and ruminal fermentation in finishing steers1, 2. Journal of Animal Science 78, 2452–2458.

Eryavuz, A., Dehority, B.A., 2009. Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers *in vitro*. Animal feed science and technology 151, 175–183.

Faithfull, N.T., 1984. The in-vitro digestibility of feedstuffs–a century of ferment. Journal of the Science of Food and Agriculture 35, 819–826. https://doi.org/10.1002/jsfa.2740350802

FAO, 2009. Food and Agriculture Organization of the United Nations (FAOSTAT), 2009, available online: http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID= 567#ancor.

Faulkner, M., St-Pierre, N., Weiss, W., 2017. Effect of source of trace minerals in either forage-or by-product–based diets fed to dairy cows: 2. Apparent absorption and retention of minerals. Journal of dairy science 100, 5368–5377.

Faulkner, M., Weiss, W., 2017. Effect of source of trace minerals in either forage-or by-product–based diets fed to dairy cows: 1. Production and macronutrient digestibility. Journal of dairy science 100, 5358–5367.

Faye, B., Grillet, C., Tessema, A., Kamil, M., 1991. Copper deficiency in ruminants in the Rift Valley of East Africa. Tropical Animal Health and Production 23, 172–180.

Forbes, J.M., 1995. Physical limitation of feed intake in ruminants and its interactions with other factors affecting intake, in: Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction: Proceedings of the 8th International Symposium on Ruminant Physiology. pp. 217–232.

Frutos, P., Hervás, G., Giráldez, F.J., Mantecón, A.R., 2004. Review. Tannins and ruminant nutrition. Spanish Journal of Agricultural Research 2, 191–202. https://doi.org/10.5424/sjar/2004022-73

Fry, R.S., Spears, J.W., Lloyd, K.E., O'Nan, A.T., Ashwell, M.S., 2013. Effect of dietary copper and breed on gene products involved in copper acquisition, distribution, and use in Angus and Simmental cows and fetuses. Journal of animal science 91, 861–871.

Fujihara, T., Serra, A., Serra, S., Orden, E., 2006. Mineral nutrition of grazing goats in Luzon island, Philippines. Bulletin of the Faculty of Life and Environmental Science Shimane University 11, 19–34.

Gebrekidan, T., 2018. Sheep and goat husbandry practices in eastern tigray, and effects of substitution of mulberry (morus alba) leaf meal for concentrate mix on growth and carcass traits of tigray highland lambs in barley straw based feeding (PhD Thesis). Addis Ababa University College of Veterinary Medicine and Agriculture.

Genther, O.N., Hansen, S.L., 2015. The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. Journal of dairy science 98, 566–573.

Geta, T., Nigatu, L., Animut, G., 2014. Evaluation of potential yield and chemical composition of selected indigenous multi-purpose fodder trees in three districts of Wolayta Zone, Southern Ethiopia. World Applied Science Journal 31, 399–405.

Girma, K.B., 2016. Minerals and trace elements in the soil-plant-animal continuum in Ethiopia: a review. African Journal of Food, Agriculture, Nutrition and Development 16, 11219–11235.

Gizaw, S., Abebe, A., Getachew, T., Rischkowsky, B., Haile, A., 2020. Breeding Objectives of Sheep and Goat Keepers in Ethiopia. Amman, Jordan: International Center for Agricultural Research in the Dry Areas (ICARDA).

Goff, J.P., 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of Dairy Science 101, 2763–2813. https://doi.org/10.3168/jds.2017-13112

Golfman, L.S., 1988. The effects of molybdenum and sulfur on the flow and solubility of various minerals along the digestive tract of steers (MSc thesis). University of Manitoba.

Gooneratne, S.R., Christensen, D.A., Bailey, J.V., Symonds, H.W., 1994. Effects of dietary copper, molybdenum and sulfur on biliary copper and zinc excretion in Simmental and Angus cattle. Canadian Journal of Animal Science 74, 315–325.

Gould, L., Kendall, N.R., 2011. Role of the rumen in copper and thiomolybdate absorption. Nutrition Research Reviews 24, 176–182.

Grace, N.D., 1975. Studies on the flow of zinc, cobalt, copper and manganese along the digestive tract of sheep given fresh perennial ryegrass, or white or red clover. British Journal of Nutrition, 34, 73–82. https://doi.org/10.1017/S0007114575000104

Grace, N.D., Lee, J., 1992. Influence of high zinc intakes, season, and staple site on the elemental composition of wool and fleece quality in grazing sheep. New Zealand Journal of Agricultural Research 35, 367–377.

Grace, N.D., Suttle, N.F., 1979. Some effects of sulphur intake on molybdenum metabolism in sheep. British Journal of Nutrition 41, 125–136.

Gupta, V.P., Kamra, D.N., Agarwal, N., Chaudhary, L.C., 2017. Effect of sulphate and blend of plant parts containing secondary metabolites on *in vitro* methanogenesis, digestibility and feed fermentation with buffalo rumen liquor. Indian Journal of Animal Sciences 87, 199–202.

Hadjigeorgiou, I.E., Gordon, I.J., Milne, J.A., 2003. Comparative preference by sheep and goats for Graminaeae forages varying in chemical composition. Small Ruminant Research 49, 147–156.

Haenlein, G.F.W., Anke, M., 2011. Mineral and trace element research in goats: A review. Small Ruminant Research 95, 2–19.

Hall, A., Fielding, A.H., Butler, M., 1979. Mechanisms of copper tolerance in the marine fouling alga Ectocarpus siliculosus—Evidence for an exclusion mechanism. Marine Biology 54, 195–199.

Hamid, T.-E.M., 1997. Zinc and copper levels in the plasma of Nubian goat as affected by the physiological status.

Hartman, S., 2017. Implications of trace mineral supplementation strategies to overcome the effects of high antagonist diets in feedlot cattle (PhD dissertation). Iowa State University. 2https://doi.org/10.31274/etd-180810-4944

Herdt, T.H., Hoff, B., 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Veterinary Clinics: Food Animal Practice 27, 255–283.

Hernández-Sánchez, D., Cervantes-Gómez, D., Ramírez-Bribiesca, J.E., Cobos-Peralta, M., Pinto-Ruiz, R., Astigarraga, L., Gere, J.I., 2019. The influence of copper levels on *in vitro* ruminal fermentation, bacterial growth and methane production. Journal of the Science of Food and Agriculture 99, 1073–1077.

Hilal, E.Y., Elkhairey, M.A., Osman, A.O., 2016. The role of zinc, manganse and copper in rumen metabolism and immune function: a review article. Open Journal of Animal Sciences, 6, 304-324. doi: 10.4236/ojas.2016.64035.

Hill, G.M., Shannon, M.C., 2019. Copper and Zinc Nutritional Issues for Agricultural Animal Production. Biol Trace Elem Res 188, 148–159. https://doi.org/10.1007/s12011-018-1578-5

Hofmann, R.R., 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78, 443–457.

Hussein, A.A., 2017. The effects of forage type and antagonists on copper metabolism in sheep (PhD Thesis). Harper Adams University.

Ivan, M., 1988. Effect of faunation on ruminal solubility and liver content of copper in sheep fed low or high copper diets. Journal of animal science 66, 1496–1501.

Jamala, G.Y., Tarimbuka, I.L., Moris, D., Mahai, S., Adamawa, S., 2013. The scope and potentials of fodder trees and shrubs in agroforestry. IOSR Journal of Agricultural and Veterinary Science 5, 1–17.

Jayanegara, A., Palupi, E., 2011. Condensed tannin effects on nitrogen digestion in ruminants: A meta-analysis from *in vitro* and *in vivo* studies. Media Peternakan 33, 176.

Jerónimo, E., Pinheiro, C., Lamy, E., Dentinho, M.T., Sales-Baptista, E., Lopes, O., Silva, F., 2016. Tannins in ruminant nutrition: Impact on animal performance and quality of edible products. In tannin:

Biochemistry, Food Sources and Nutritional Properties; Combs, C.A., Ed.; Nova Science Publisher Inc.: Hauppauge, NY, USA, pp. 121–168.

Jones, W.T., Mangan, J.L., 1977. Complexes of the condensed tannins of sainfoin (Onobrychis viciifolia Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. Journal of the Science of Food and Agriculture 28, 126–136.

Kara, K., 2019. The *in vitro* digestion of neutral detergent fibre and other ruminal fermentation parameters of some fibrous feedstuffs in Damascus goat (*Capra aegagrus hircus*). Journal of Animal and Feed Sciences 28, 159–168. https://doi.org/10.22358/jafs/108990/2019

Karamać, M., 2009. Chelation of Cu (II), Zn (II), and Fe (II) by tannin constituents of selected edible nuts. International journal of molecular sciences 10, 5485–5497.

Katulski, S.L., 2017. Effects of mineral supplementation on growing cattle and *in vitro* fermentation by ruminal microbes (PhD Thesis). Kansas State University.

Keith, M.O., Bell, J.M., 1987. Effects of pH and canola meal on trace mineral solubility *in vitro*. Canadian Journal of Animal Science 67, 587–590.

Kessler, J., 1991. Mineral nutrition of goats. Goat nutrition 46, 104–119.

Ketelaars, J., Tolkamp, B.J., 1992. Toward a new theory of feed intake regulation in ruminants 1. Causes of differences in voluntary feed intake: critique of current views. Livestock Production Science 30, 269–296.

Khan, Z.I., Ashraf, M., Ashraf, M.Y., Hussain, A., 2003. Mineral status of livestock (goats and sheep) based on soil, dietary components and animal tissue fluids in relation to seasonal changes and sampling periods in specific region of Pakistan. Journal of Animal and Veterinary Advances.

Kincaid, R.L., 2000. Assessment of trace mineral status of ruminants: A review. Journal of Animal Science 77, 1–10.

Kistner, M.J., Wagner, J.J., Evans, J., Chalberg, S., Jalali, S., Sellins, K., Kesel, M.L., Holt, T., Engle, T.E., 2017. The effects of molybdenum water concentration on feedlot performance, tissue mineral concentrations, and carcass quality of feedlot steers. Journal of Animal Science 95, 2758–2766.

Lamy, E., Rawel, H., Schweigert, F.J., Capela e Silva, F., Ferreira, A., Costa, A.R., Antunes, C., Almeida, A.M., Coelho, A.V., Sales-Baptista, E., 2011. The effect of tannins on Mediterranean ruminant ingestive behavior: the role of the oral cavity. Molecules 16, 2766–2784.

Lee, J., Grace, N.D., 1997. New Zealand perspective on copper, molybdenum and sulphur interactions in ruminants, in: Proceedings from the 27th Seminar of the Society of Sheep and Beef Cattle Veterinarians, NZVA: NZVA Conference Week 1997: 29 June-4th July 1997, Wairakei Resort Hotel. Palmerston North, NZ: Veterinary Continuing Education, Massey University, 1997.

Lengarite, M.I., Mbugua, P.N., Gachuiri, C.K., Kabuage, L.W., 2012. Mineral status of sheep and goats grazing in the arid rangelands of Northern Kenya. Pakistan Journal of Nutrition 11, 383.

Leta, S., Mesele, F., 2014. Spatial analysis of cattle and shoat population in Ethiopia: growth trend, distribution and market access. Springer Plus 3, 310.

López-Alonso, M., 2012. Trace minerals and livestock: not too much not too little. International Scholarly Research Notices 2012, 704825-704825.

López-Alonso, M., Miranda, M., 2020. Copper Supplementation, A Challenge in Cattle. Animals 10, 1890.

López-Alonso, M., Prieto, F., Miranda, M., Castillo, C., Hernández, J.R., Benedito, J.L., 2005. Intracellular distribution of copper and zinc in the liver of copper-exposed cattle from northwest Spain. The Veterinary Journal 170, 332–338.

Louca, A., Antoniou, T., Hatjipanayiotou, M., 1982. Comparative digestibility of feedstuffs by various ruminants, specifically goats, in: 3. International Conference on Goat Production and Disease, Tucson, AZ (EUA), 10-15 Ene 1982.

Makkar, H., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small ruminant research 49, 241–256.

Marais, P., 2012. Effects of condensed tannins on browsers and grazers. Grootfontein Agricultural Development Institute, Middelburg EC. 5900

Martin, D.G., 2016. The nutritional and anthelmintic effects of Calliandra calothyrsus condensed tannin in the gastrointestinal tract of merino sheep (Doctoral dissertation). James Cook University.

Mason, J., Lamand, M., Tressol, J.C., 1978. The influence of dietary sulphur, molybdate and copper on the absorption, excretion and plasma fraction levels of 99Mo in sheep. Annales de Recherches veterinaires. Annals of Veterinary Research. 9(3):577-86.

McArthur, C., Sanson, G.D., Beal, A.M., 1995. Salivary proline-rich proteins in mammals: roles in oral homeostasis and counteracting dietary tannin. Journal of Chemical Ecology 21, 663–691.

McCaughern, J.H., Mackenzie, A.M., Sinclair, L.A., 2020. Dietary starch concentration alters reticular pH, hepatic copper concentration, and performance in lactating Holstein-Friesian dairy cows receiving added dietary sulfur and molybdenum. Journal of Dairy Science 103, 9024–9036.

McDonald, M., Mila, I., Scalbert, A., 1996. Precipitation of Metal Ions by Plant Polyphenols: Optimal Conditions and Origin of Precipitation. Journal of Agricultural and food Chemistry, 44(2), 599–606. https://doi.org/10.1021/jf950459q

McNabb, W.C., Aerts, R.J., Brand, A., Peters S. J. S., Foo Y. L., and Waghorn G. C.,1997. Effect of condensed tannin in Lotus corniculatus and Lotus pedunculatus on digestion of rubisco in the rumen. In: Proceedings of the XVIII International Grassland Congress, Section 8, pp. 8-9. Winnipeg, Manitoba, and Saskatoon, Canada.

McSweeney, C.S., Palmer, B., McNeill, D.M., Krause, D.O., 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Animal Feed Science and Technology 91, 83–93.

Menta, A.K., 2017. Determination of Essential Mineral Contents in Salty Soils Used for Supplementary Food to Ruminants in Wolaita Zone. Chemistry and Materials Research, 9(2).

Meschy, F., 2000. Recent progress in the assessment of mineral requirements of goats. Livestock Production Science 64, 9–14.

Min, B.R., Solaiman, S., 2018. Comparative aspects of plant tannins on digestive physiology, nutrition and microbial community changes in sheep and goats: A review. Journal of animal physiology and animal nutrition 102, 1181–1193.

Min, B.R., Solaiman, S., Terrill, T., Ramsay, A., Mueller-Harvey, I., 2015. The effects of tannins-containing ground pine bark diet upon nutrient digestion, nitrogen balance, and mineral retention in meat goats. Journal of Animal Science and Biotechnology 6, 1-8. https://doi.org/10.1186/s40104-015-0020-5

Mir, S.H., Mani, V., Pal, R.P., Malik, T.A., Sharma, H., 2020. Zinc in ruminants: metabolism and homeostasis. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 90, 9–19.

Mlambo, V., Marume, U., Gajana, C., 2016. Utility of the browser's behavioural and physiological strategies in coping with dietary tannins: Are exogenous tannin-inactivating treatments necessary? South African Journal of Animal Science, 45, 441-451. https://doi.org/10.4314/sajas.v45i5.1

Mlambo, V., Sikosana, J.L.N., Mould, F.L., Smith, T., Owen, E., Mueller-Harvey, I., 2007. The effectiveness of adapted rumen fluid versus PEG to ferment tannin-containing substrates *in vitro*. Animal feed science and technology 136, 128–136.

Mohammed, A., Osman, N.E.H., Isam, H., Din Osman, E., G Youssef, F., 2016. Review on Copper's Functional Roles, Copper X Mineral Interactions Affecting Absorption, Tissue Storage, and Cu Deficiency Swayback of Small Ruminants. ARC Journal of Animal and Veterinary Sciences (AJAVS) 2, 1–14. https://doi.org/10.20431/2455-2518.0202001

Moyo, M., Kana, E.G., Nsahlai, I.V., 2017. Modelling of digesta passage rates in grazing and browsing domestic and wild ruminant herbivores. South African Journal of Animal Science 47, 362–377.

Moyo, M., Nsahlai, I.V., 2018. Rate of Passage of Digesta in Ruminants; Are Goats Different? Goat science. 10.5772/intechopen.69745.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. Journal of the Science of Food and Agriculture 86, 2010–2037. https://doi.org/10.1002/jsfa.2577

Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kölliker, R., Lüscher, A., Niderkorn, V., Pellikaan, W.F., Salminen, J.-P., 2019. Benefits of condensed tannins in forage legumes fed to ruminants: importance of structure, concentration, and diet composition. Crop Science 59, 861–885.

Muir, J.P., 2011. The multi-faceted role of condensed tannins in the goat ecosystem. Small Ruminant Research 98, 115–120.

Napasirth, P., Wachirapakorn, C., Saenjan, P., Yuangklang, C., 2013. Effect of Sulfate-Containing Compounds on Methane Production by Using an *In vitro* Gas Production Technique. Pakistan Journal of Nutrition 12, 723–729.

Narvaez, N., Brosh, A., Pittroff, W., 2012. Use of n-alkanes to estimate seasonal diet composition and intake of sheep and goats grazing in California chaparral. Small Ruminant Research 104, 129–138.

Nasrullah, Abdullah, M., Baber, M.E., Jabbar, M.A., Bahtti, J.A., 2013. Feeding Behavior, Voluntary Intake and Digestibility of Various Summer Fodders in Sheep and Goats. Pakistan Journal of Zoology. URL https://eurekamag.com/research/066/270/066270385.php (accessed 12.28.20).

Naumann, H.D., Muir, J.P., Lambert, B.D., Tedeschi, L.O., Kothmann, M.M., 2013. Condensed Tannins In The Ruminant Environment: A Perspective On Biological Activity. Journal of Agricultural Sciences 1, 8-20.

Naumann, H.D., Tedeschi, L.O., Zeller, W.E., Huntley, N.F., 2017. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. Revista Brasileira de Zootecnia. 46, 929–949. https://doi.org/10.1590/s1806-92902017001200009

Njidda, A.A., 2010. Determining dry matter degradability of some semi-arid browse species of north-eastern Nigeria using the *in vitro* technique. Nigerian Journal of Basic and Applied Sciences 18, 160-167.

NRC, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World camelids. Washington, D.C: National Academies Press.

NRC, 2005. Mineral tolerance of animals, 2nd rev. ed. ed. National Academies Press, Washington, D.C.

NRC, N.R., 2001. Nutrient Requirements of Dairy Cattle, Seventh Revised Addition. Academic Press, Washington, D.C.

Nwosu, O., 2019. A Systematic Review of the Impact of Minerals on Pregnant Sheep and Goats and their Offspring in the African Continent (PhD Thesis). University of Pretoria.

Osman, D., Cavet, J.S., 2008. Copper homeostasis in bacteria. Advances in applied microbiology 65, 217–247.

Osman, N., Johnson, E.H., Al-Busaidi, R.M., Suttle, N.F., 2003. The effects of breed, neonatal age and pregnancy on the plasma copper status of goats in Oman. Veterinary research communications 27, 219–229.

Papachristodoulou, C., Stamoulis, K., Tsakos, P., Vougidou, C., Vozikis, V., Papadopoulou, C., Ioannides, K., 2015. Liver concentrations of copper, zinc, iron and molybdenum in sheep and goats from Northern Greece, determined by energy-dispersive X-ray fluorescence spectrometry. Bulletin of environmental contamination and toxicology 94, 460–467.

Patra, A.K., Min, B.-R., Saxena, J., 2012. Dietary tannins on microbial ecology of the gastrointestinal tract in ruminants. Dietary phytochemicals and microbes 237–262.

Patra, A.K., Saxena, J., 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. Journal of the Science of Food and Agriculture 91, 24–37.

Paulsen, H.M., 2005. Sulfur in organic farming, in: Aspects of Sulfur Nutrition of Plants; Evaluation of China's Current, Future and Available Resources to Correct Plant Nutrient Sulfur Deficiencies–Report of the First Sino-German Sulfur Workshop. Braunschweig, Germany.

Perez-Maldonado, R.A., Norton, B.W., 1996. The effects of condensed tannins from Desmodium intortum and Calliandra calothyrsus on protein and carbohydrate digestion in sheep and goats. British Journal of Nutrition 76, 515–533.

Pino, F., Heinrichs, A.J., 2016. Effect of trace minerals and starch on digestibility and rumen fermentation in diets for dairy heifers. Journal of dairy science 99, 2797–2810.

Pogge, D.J., Drewnoski, M.E., Hansen, S.L., 2014. High dietary sulfur decreases the retention of copper, manganese, and zinc in steers. Journal of Animal Science 92, 2182–2191. https://doi.org/10.2527/jas.2013-7481

Price, D.M., O'Neil, M.M., Watson III, W.B., West, R., Rae, D.O., Irsik, D.M., Hersom, M., Yelich, J.V., 2017. Effect of Pre-and Postnatal Trace Mineral (TM) Sources on Growth, Body Composition, Performance, and Liver Mineral Status in Angus and Brangus Bulls. Journal of Animal Science 95, 46.

Price, M.L., Butler, L.G., Rogler, J.C., Featherston, W.R., 1979. Overcoming the nutritionally harmful effects of tannin in sorghum grain by treatment with inexpensive chemicals. Journal of Agricultural and Food Chemistry 27, 441–445.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small ruminant research 49, 257–274.

Puls, R., 1994. Mineral levels in animal health: diagnostic data. Clearbrook. British Columbia, Canada: Sherpa International 241–244.

Raju, J., Sahoo, B., Chandrakar, A., Garg, A.K., Mohanta, R.K., 2018. Effect of Varied Sources of Tannin on Micro-Mineral Bioavailability in Goats Fed Oak Leaves Based Diets. Animal Nutrition and Feed Technology 18, 25–35.

Reed, J.D., 1986. Relationships among soluble phenolics, insoluble proanthocyanidins and fiber in East African browse species. Journal of Range Management 39, 5–7.

Rehman, H., 2017. Fundamentals of Zinc, Manganese and Copper in the Metabolism of the Rumen and Immune Function. International Journal of Livestock Research 7, 55-76 https://doi.org/10.5455/ijlr.20170806111410

Richter, E.L., 2011. The effect of dietary sulfur on performance, mineral status, rumen hydrogen sulfide, and rumen microbial populations in yearling beef steers. (Doctoral dissertation), Iowa State University.

Robbins, C.T., Hagerman, A.E., Austin, P.J., McArthur, C., Hanley, T.A., 1991. Variation in mammalian physiological responses to a condensed tannin and its ecological implications. Journal of Mammalogy 72, 480–486.

Rucker, R., Fascetti, A., Keen, C., 2008. Trace Minerals, in: Clinical Biochemistry of Domestic Animals. 663–693. https://doi.org/10.1016/B978-0-12-370491-7.00022-2

Salem, A.-F.Z., 2011. Plant-Phytochemicals in Animal Nutrition. Nova Science Publishers. New York.

Salem, H.B., Makkar, H.P.S., Nefzaoui, A., Hassayoun, L., Abidi, S., 2005. Benefit from the association of small amounts of tannin-rich shrub foliage (Acacia cyanophylla Lindl.) with soya bean meal given as supplements to Barbarine sheep fed on oaten hay. Animal Feed Science and Technology 122, 173–186.

Sandoval, M., Henry, P.R., Littell, R.C., Cousins, R.J., Ammerman, C.B., 1997. Estimation of the relative bioavailability of zinc from inorganic zinc sources for sheep. Animal feed science and technology 66, 223–235.

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry (oxford). 30, 3875–3884.

Schweinzer, V., Iwersen, M., Drillich, M., Wittek, T., Tichy, A., Mueller, A., Krametter-Froetscher, R., 2017. Macromineral and trace element supply in sheep and goats in Austria. Veterinární medicína 62, 62–73.

Sefdeen, S.M., 2017. Effect of dietary iron on copper metabolism in sheep (PhD Thesis). Harper Adams University.

Sembiring, M., Baba, A.S.H., Dahang, D., 2015. Availability of browse plants to goats fed with Napier grass: voluntary feed intake and effects on body weight. International Journal of Veterinary Sciences and Animal Husbandry. 6, 13-19.

Serra, S.D., Serra, A.B., Ichinohe, T., Fujihara, T., 1997. Ruminal solubility of trace elements from selected Philippine forages. Asian-Australasian Journal of Animal Sciences 10, 378–384.

Shapiro, B.I., Gebru, G., Desta, S., Negassa, A., Nigussie, K., Aboset, G., Mechale, H., 2017. Ethiopia livestock sector analysis. ILRI Project Report. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Shenkute, B., Hassen, A., Assafa, T., Amen, N., Ebro, A., 2012. Identification and nutritive vale of potential fodder trees and shrubs in the mid Rift Valley of Ethiopa. Journal of Animal and Plant Sciences 22,1126-1132.

Shuttle, N.F., Lewis, R.M., Small, J., 2002. Effects of breed and family on rate of copper accretion in the liver of purebred Charollais, Suffolk and Texel lambs. Animal Science 75, 02.

Silanikove, N., Tagari, H., Shkolnik, A., 1993. Comparison of rate of passage, fermentation rate and efficiency of digestion of high fiber diet in desert Bedouin goats compared to Swiss Saanen goats. Small Ruminant Research 12, 45–60.

Silva, C.J. da, Leonel, F. de P., Pereira, J.C., Costa, M.G., Moreira, L.M., Oliveira, T.S. de, Abreu, C.L. de, 2014. Sulfur sources in protein supplements for ruminants. Revista Brasileira de Zootecnia 43, 537–543.

Silva Júnior, S.S., Antonelli, Alexandre.C., Soares, G.W.N., Gomes, I.M.M., Rocha Filho, J.F., 2015. Determinação de cobre e outros minerais em caprinose ovinos criados no sertão do vale do rio São Francisco, Pernambuco. Pesquisa Veterinária Brasileira 35, 767–774.

Snyman, L.D., 2007. Qualitative characteristics of selected Atriplex nummularia (Hatfield Select) (PhD Thesis). University of Pretoria.

Spears, J.W., 2003. Trace mineral bioavailability in ruminants. The Journal of Nutrition 133, 1506S-1509S.

Suttle, N.F., 2010. Mineral nutrition of livestock, 4th ed. ed. CABI, Wallingford, Oxfordshire, UK ; Cambridge, MA.

Suttle, N.F., 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annual review of nutrition 11, 121–140.

Suttle, N.F., 1975. Suttle: Changes in the availability of dietary copper. The Journal of Agricultural Science 84, 255-261.

Suttle, N.F., Davies, H.L., Field, A.C., 1982. A model for zinc metabolism in sheep given a diet of hay. British Journal of Nutrition 47, 105–112.

Taha, V., 2015. Effect of supplemental tannin on silage quality and animal performance (PhD Thesis). Harper Adams University.

Theodoridou, K., 2010. The effects of condensed tannins in sainfoin (Onobrychis viciifolia) on its digestion and nutritive value (Doctoral dissertation). Université Blaise Pascal-Clermont-Ferrand II.

Tolera, A., Yami, A., Alemu, D., 2012. Livestock feed resources in Ethiopia: challenges, opportunities and the need for transformation. Ethiopia Animal Feed Industry Association, Addis Ababa, Ethiopia.

Tseu, R.J., 2019. Associative effect of tannins and monensin as manipulators of rumen fermentation on the mitigation of methane production in cattle (PhD Thesis). Universidade de São Paulo.

Turner, J.C., Shanks, V., Osborn, P.J., Gower, S.M., 1987. Copper absorption in sheep. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology 86, 147–150. https://doi.org/10.1016/0742-8413(87)90157-5

Turner, K.E., Wildeus, S., Collins, J.R., 2005. Intake, performance, and blood parameters in young goats offered high forage diets of lespedeza or alfalfa hay. Small Ruminant Research 59. https://doi.org/10.1016/j.smallrumres.2004.11.007

Underwood, E.J., Suttle, N.F., 2001. The Mineral nutrition off livestock. CABI International Wallingford. Oxford, UK.

Uniyal, S., Chaudhary, L.C., Kala, A., Agarwal, N., Kamra, D.N., 2020. Effect of Sulphur Supplementation on Methane Emission, Energy Partitioning and Nutrient Utilization in Goats. Animal Nutrition and Feed Technology 20, 111–119.

van den Top, A.M., Veevoederbureau, C., 2005b. Reviews on the mineral provision in ruminants (VIII): iron metabolism and requirements in ruminants. Centraal Veevoederbureau (CVB). Lelystad.

van der Aar, P.J., Fahey Jr, G.C., Ricke, S.C., Allen, S.E., Berger, L.L., 1983. Effects of dietary fibers on mineral status of chicks. The Journal of nutrition 113, 653–661.

Van, D.T.T., 2006. Some animal and feed factors affecting feed intake, behaviour and performance of small ruminants (Doctoral thesis). Swedish University of Agricultural Sciences.

Van Soest, P.J., 1982. Nutritional ecology of the ruminant. O and B Books, Corvallis, Oregon. USA.

Van Valin, K., 2019. Influence of stress induction on trace mineral homeostasis and metabolism in ruminants (Doctoral dissertation). Iowa State University.

Vázquez-Armijo, J.F., Martínez-Tinajero, J.J., López, D., Salem, A.-F.Z.M., Rojo, R., 2011. *In vitro* gas production and dry matter degradability of diets consumed by goats with or without copper and zinc supplementation. Biological trace element research 144, 580–587.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. Animal Feed Science and Technology 147, 116–139.

Waghorn, G.C., Shelton, I.D., McNabb, W.C., 1994. Effects of condensed tannins in Lotus pedunculatus on its nutritive value for sheep. 1. Non-nitrogenous aspects. The Journal of Agricultural Science 123, 99–107. https://doi.org/10.1017/S0021859600067824

Wang, C., Han, L., Zhang, G.W., Du, H.S., Wu, Z.Z., Liu, Q., Guo, G., Huo, W.J., Zhang, J., Zhang, Y.L., 2020. Effects of copper sulfate and coated copper sulfate addition on lactation performance, nutrient digestibility, ruminal fermentation and blood metabolites in dairy cows. British Journal of Nutrition 1–27.

Ward, G.M., 1978. Molybdenum toxicity and hypocuprosis in ruminants: a review. Journal of Animal Science 46, 1078–1085.

Ward, J.D., Spears, J.W., Gengelbach, G.P., 1995. Differences in copper status and copper metabolism among Angus, Simmental, and Charolais cattle. Journal of Animal Science 73, 571–577.

Yisehak, K., Becker, A., Belay, D., Bosch, G., Hendriks, W.H., Clauss, M., Janssens, G.P., 2011. Salivary amino acid concentrations in zebus (Bos indicus) and zebu hybrids (Bos indicus x Bos taurus) fed a tannin-rich diet. Belgian Journal of Zoology 141, 93–96.

Zanetti, D., Menezes, A.C.B., Silva, F.A.S., SILVA, L.C.E., Rotta, P.P., Detmann, E., Engle, T.E., Valadares Filho, S.C., 2017. In situ and *in vitro* estimation of mineral release from common feedstuffs fed to cattle. The Journal of Agricultural Science 155, 1160–1173.

Zeitz, J.O., Ineichen, S., Soliva, C.R., Leiber, F., Tschuor, A., Braun, U., Kreuzer, M., Clauss, M., 2016. Variability in microbial population and fermentation traits at various sites within the forestomach and along the digestive tract as assessed in goats fed either grass or browse. Small ruminant research 136, 7–17.

Zeng, X., Du, Z., Sheng, Z., Jiang, W., 2019. Characterization of the interactions between banana condensed tannins and biologically important metal ions (Cu2+, Zn2+ and Fe2+). Food Research International 123, 518–528.

Zervas, G., Nikolaou, E., Mantzios, A., 1990. Comparative study of chronic copper poisoning in lambs and young goats. Animal Science 50, 497–506. https://doi.org/10.1017/S0003356100004980

Zhan, X.-M., Zhao, X., 2003. Mechanism of lead adsorption from aqueous solutions using an adsorbent synthesized from natural condensed tannin. Water Research. 37, 3905–3912. https://doi.org/10.1016/S0043-1354(03)00312-9

CHAPTER 2

Introduction and Scientific Aims

Chapter 2

Introduction to and scientific aims of the study

Nowadays global warming is changing climate patterns throughout the year and turning semi-humid and semi-arid areas into arid areas, hence directly affecting animal health and production (Kara, 2019), and indirectly ruminant production by reducing the availability of feed resources (Aboagye, 2019). Consequently, ruminants in these areas are not able to consume enough feed that gives them the required energy and nutrients for maintenance and production, especially throughout the long dry period when grazing mainly depends on crop residues (McDowell, 2003; Nasrullah et al., 2013). In tropical countries such as Ethiopia, a multitude of factors influence ruminant production, where feed shortage in terms of quantity and quality is one of the major constraints that hinder ruminant production (Shapiro et al., 2017). These ruminants often rely on roughages such as natural grass hays, straws, other crop residues, and agro-industrial by-products to meet their dietary requirements (Ibrahim et al., 1998; Tolera et al., 2012). Subsequently, smallholder farmers have been engaged in using indigenous multi-purpose browse trees and shrubs as alternative feed resources to minimize the existing feed gap especially during the dry season of the year (Tadesse, 2016). Although many shrubs and tree leaves in the tropics contain plant secondary compounds such as tannins, they also contain a high level of protein (Mueller-Harvey, 2006) and minerals (Warly and Fariani, 2010; Gupta, 2018).

Besides macronutrients, it has long been identified that essential microminerals such as Cu, Zn and Fe are normally present in adequate concentrations in commonly used feedstuffs and are vital components in carrying out many biochemical and immunological functions within the body of ruminant livestock (Suttle, 2010; Counotte et al., 2019; Pate, 2019). However, ruminants can develop micromineral deficiencies if no particular source is supplemented, such cases commonly exist in grazing livestock from tropical areas (Dermauw et al., 2013). Indeed, in the smallholder grazing areas, ruminants try to gain their mineral requirements usually through feeds and fodders where most grazing livestock does not receive mineral supplements at all (Khan et al., 2003).

Moreover, because the bioavailability of microminerals can be altered during ruminal fermentation (Katulski, 2017; Gupta, 2018), the ability of forage to supply minerals to ruminants depends not only on the mineral content in the feedstuffs but also on the potential bioavailability and absorption of the minerals from the gastrointestinal tract (Spears, 2003). This, in turn, depends on the species, age, breed and physiological status of the animal and interactions among dietary constituents (Herdt and Hoff, 2011; Pate, 2019). The binding of minerals with dietary tannins in the rumen (Acharya et al., 2016) further complicates the picture, in which the interactions depend on the level, type, molecular size, and structural conformation of both tannins and minerals (Min et al., 2015).

Besides the impact that rumen fermentation and the presence or not of PSC can have on the bioavailability, absorption, and potential utilization of minerals, other factors such as the intake relative to the amount required, and the mineral’s utilization by the animal tissues will also have an impact on the efficient use of dietary minerals by the animals (Nwosu, 2019). Although information regarding the bioavailability of minerals is important to improve the precision of diet formulation, reduce extra mineral supplementation, as well as environmental excretion and costs (Zanetti et al., 2017), the adequacy of the diet in terms of bioavailable micromineral provision is often uncertain. Thus, estimating and optimizing the bioavailable micromineral intakes in ruminant remains a challenge (Kegley et al., 2016).

Despite the numerous publications on the bioavailability of minerals, only a few studies so far evaluated changes in the available concentrations or absorption of microminerals throughout gastrointestinal segments and their storage, as well as how these differ between ruminant types. **Therefore, the current doctoral dissertation was designed to determine the effects of the inhibitory constituent of low-quality diets on the relative bioavailability of microminerals (Cu, Zn and Fe) and fermentation characteristics in ruminants**. More specifically, the objectives were to:

- determine the association between different fermentation traits and micromineral bioavailability in cattle
- evaluate the effect of low-quality diets on the bioavailability of mineral throughout gut segments of tropical intermediate browsers (Kefa goats) and tropical grazers (Bonga sheep).
- evaluate the effect of low-quality diets on the bioavailability of nutrients throughout gut segments of tropical intermediate browsers (Kefa goats) and tropical grazers (Bonga sheep).

References

Aboagye, I.A., 2019. Mitigating enteric methane production and nitrogen excretion from forage-fed ruminants (PhD thesis) University of Alberta.

Acharya, M., Burke, J.M., Coffey, K.P., Kegley, E.B., Miller, J.E., Smyth, E., Welborn, M.G., Terrill, T.H., Mosjidis, J.A., Rosenkrans, C., 2016. Changes in concentrations of trace minerals in lambs fed sericea lespedeza leaf meal pellets with or without dietary sodium molybdate. Journal of Animal Science 94, 1592–1599. https://doi.org/10.2527/jas.2015-9910

Bahtti, A., 2013. Feeding behavior, voluntary intake and digestibility of various summer fodders in sheep and goats. Pakistan Journal of Zoology 45, 53–8.

Counotte, G., Holzhauer, M., Carp-van Dijken, S., Muskens, J., Van der Merwe, D., 2019. Levels of trace elements and potential toxic elements in bovine livers: A trend analysis from 2007 to 2018. PloS ONE 14, e0214584. https://doi.org/10.1371/journal.pone.0214584

Dermauw, V., Yisehak, K., Belay, D., Van Hecke, T., Du Laing, G., Duchateau, L., Janssens, G.P.J., 2013. Mineral deficiency status of ranging zebu (Bos indicus) cattle around the Gilgel Gibe catchment, Ethiopia. Trop Anim Health Prod 45, 1139–1147. https://doi.org/10.1007/s11250-012-0337-4

Gupta, R.K., 2018. A review of copper poisoning in animals: Sheep, goat and cattle. International Journal of Veterinary Sciences and Animal Husbandry 3, 1–4.

Herdt, T.H., Hoff, B., 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Veterinary Clinics: Food Animal Practice 27, 255–283.

Ibrahim, M.N.M., Zemmelink, G., Tamminga, S., 1998. Release of mineral elements from tropical feeds during degradation in the rumen. Asian-Australasian Journal of Animal Sciences 11, 530–537.

Kara, K., 2019. The *in vitro* digestion of neutral detergent fibre and other ruminal fermentation parameters of some fibrous feedstuffs in Damascus goat (*Capra aegagrus hircus*). Journal of Animal and Feed Sciences. 28, 159–168. https://doi.org/10.22358/jafs/108990/2019

Katulski, S.L., 2017. Effects of mineral supplementation on growing cattle and *in vitro* fermentation by ruminal microbes (PhD Thesis). Kansas State University.

Kegley, E.B., Ball, J.J., Beck, P.A., 2016. Bille. Kunkle interdisciplinary beef symposium: Impact of mineral and vitamin status on beef cattle immune function and health. Journal of Animal Science 94, 5401–5413.

Khan, Z.I., Ashraf, M., Ashraf, M.Y., Hussain, A., 2003. Mineral status of livestock (goats and sheep) based on soil, dietary components and animal tissue fluids in relation to seasonal changes and sampling periods in specific region of Pakistan. Journal of Animal and Veterinary Advances 2, 478 - 495.

McDowell, L.R., 2003. Minerals in animal and human nutrition. 2nd ed. Elsevier Science, Amsterdam.

Min, B.R., Solaiman, S., Terrill, T., Ramsay, A., Mueller-Harvey, I., 2015. The effects of tannins-containing ground pine bark diet upon nutrient digestion, nitrogen balance, and mineral retention in meat goats. J Journal of Animal Science and Biotechnology 6, 1-8. https://doi.org/10.1186/s40104-015-0020-5

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. Journal of the Science of Food and Agriculture 86, 2010–2037. https://doi.org/10.1002/jsfa.2577

Nasrullah, Abdullah, M., Baber, M.E., Jabbar, M.A., Bahtti, J.A., 2013. Feeding Behavior, Voluntary Intake and Digestibility of Various Summer Fodders in Sheep and Goats. Pakistan Journal of Zoology 45, 53-58.

Nwosu, O., 2019. A Systematic Review of the Impact of Minerals on Pregnant Sheep and Goats and their Offspring in the African Continent (PhD Thesis). University of Pretoria.

Pate, R.T., 2019. Nutritional and environmental stressors and their effects on performance, metabolism, and the immune system of dairy cattle (PhD Thesis). University of Illinois at Urbana-Champaign.

Shapiro, B.I., Gebru, G., Desta, S., Negassa, A., Nigussie, K., Aboset, G., Mechale, H., 2017. Ethiopia livestock sector analysis. ILRI Project Report. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Spears, J.W., 2003. Trace mineral bioavailability in ruminants. The Journal of Nutrition 133, 1506S-1509S.

Suttle, N.F., 2010. Mineral nutrition of livestock, 4th ed. ed. CABI, Wallingford, Oxfordshire, UK ; Cambridge, MA.

Tadesse, F., 2016. Growth performance and nutritive quality of tree Lucerne (Chamaecytisus palmensis) fodder under different management conditions in the Highlands of Ethiopia (PhD Thesis). Hawassa University.

Tolera, A., Yami, A., Alemu, D., 2012. Livestock feed resources in Ethiopia: challenges, opportunities and the need for transformation. Ethiopia Animal Feed Industry Association, Addis Ababa, Ethiopia.

Warly, L., Fariani, A., 2010. Concentration of Micro Minerals in Fiber Fraction of Forages. International Journal of Agricultural and Biosystems Engineering, 4, 584-590.

Zanetti, D., Menezes, A.C.B., Silva, F.A.S., Silva, L.C.E., Rotta, P.P., Detmann, E., Engle, T.E., Valadares Filho, S.C., 2017. In situ and *in vitro* estimation of mineral release from common feedstuffs fed to cattle. The Journal of Agricultural Science 155, 1160–1173.

CHAPTER 3

The impact of sulfur addition to organic dairy rations on *in vitro* fermentation and micromineral bioavailability

Chapter 3

The impact of sulfur addition to organic dairy rations on *in vitro* fermentation and micromineral bioavailability

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Abstract

Micromineral concentrations in ruminant feedstuffs can vary widely depending on plant species' phenologic stage and soil type. Moreover, micromineral bioavailability and absorption can be altered during ruminal fermentation. Although the macromineral sulfur (S) is needed for adequate ruminal fermentation, it is also a major antagonist of microminerals such as copper (Cu), impacting their post-ruminal bioavailability, absorption, and storage. This study was designed to investigate whether dietary S addition affects fermentation traits and bioavailability of minerals in organic dairy cows. Complete rations of eleven organic dairy farms were collected and subjected to an *in vitro* fermentation with and without the addition of S as $Na_2SO_4.10H_2O$. The 24-hour methane and net short-chain fatty acid (SCFA) production, hydrogen accumulation, and concentrations of the minerals were measured. Sulfur addition reduced Cu bioavailability, while the bioavailability of the other minerals (Fe and Zn) was not significantly affected. Independent of S addition, the ruminal degradation of protein was negatively associated with the bioavailability of Cu and Mo. Also, S addition significantly increased concentrations of fermentation products such as acetate, propionate, butyrate and isobutyrate, resulting in an overall increase in SCFA. This suggests that S provision in the rations was suboptimal for rumen microbial activity. Sulfur may thus be a limiting mineral in organic dairy cow rations but the trade-off of S addition may be a reduced Cu absorption. Therefore, allowing S to promote dairy performance may require sufficient Cu in the diet combined with lower levels of antagonists such as Mo, zinc and iron.

Introduction

Ruminating livestock can develop micromineral shortages if they are not added to the diet, as has been demonstrated in studies across the globe, from free-ranging livestock in tropical areas (Khan et al., 2003; Dermauw et al., 2013) to organic dairy cows in a temperate climate (e.g. Sobry et al., 2012). In organic dairy farming, micromineral deficiencies can arise because the farmers are subject to strict regulations on the use of chemically synthesized mineral sources for plants and animals (Paulsen, 2005).

Micromineral concentrations in feedstuffs can vary largely depending on plant species phenology and soil types (Dermauw et al., 2014). In addition, during ruminal fermentation, the bioavailability of microminerals can be altered, potentially impacting their post-ruminal absorption and storage (Gupta, 2014). The ruminal solubility and bioavailability of microminerals are likely to influence their utilization by rumen microbes (Faulkner and Weiss, 2017). Subsequently this affects rumen microbial populations, rumen fermentation and rumen pH, the extent and site of nutrient digestibility and SCFA production (Genther and Hansen, 2015; Osorio et al., 2016). For instance, when S is deficient due to low concentrated S in forages, no supplementation of the diet, or reduced S availability, feed intake and DM digestibility could be reduced (Mathews et al., 1994; Spears et al., 2011). This might be attributed to the reduced retention time of digesta in the reticulo-rumen (Mathews et al., 1994). Increasing the concentration of dietary S above the requirement may also impact digestibility of the diet in the rumen (Drewnoski et al., 2014). This may indirectly affect the bioavailability of other microminerals.

An element that is often overlooked in formulating ruminant diets is sulfur (Suttle, 2010). This essential element to ruminants plays an important role in the synthesis of the S-amino acids methionine and cystine, needed for maintaining optimum ruminal microbial growth and function (Amat, 2012; Drewnoski et al., 2014). Sulfur deficiency is rarely detected in livestock raised in temperate climates (Ortolani, 2001). However, S may be a forgotten element in the ration formulation of organic dairy cows, because the farmers are subject to strict regulations on the use of chemically synthesized mineral sources for plants and animals (Paulsen, 2005). This might result in a possible deficiency that are often associated with loss of appetite, decreased cellulose and organic matter fermentation (Ortolani, 2001) and reduction of microbial protein synthesis (Suttle, 2010).

On the other hand, the major antagonism of S with microminerals such as Cu, Fe, Zn and Se is widely documented (Suttle, 1991, 2010). For instance, dietary sulfates in the feed are reduced to sulfides (S^{-2}) that possibly react with Cu to form insoluble copper sulfide complexes (Golfman, 1988). In addition, when ruminants are on higher dietary S and excessive Mo is present, sulfides combine with molybdate in a stepwise, reversible, and pH-dependent manner, to form to Cu-thiomolybdate complexes in the rumen (Gould and Kendell, 2011). This will lead to a reduced Cu bioavailability and absorption (Rehman et al., 2017). There is, however, little information on the effect of S supplementation on the bioavailability of microminerals and fermentation profile in organic dairy cows. Estimating and optimizing adequacy of the diet in terms of bioavailable micromineral provision is therefore, often uncertain and remains a challenge (Kegley et al., 2016). Therefore, our aim was to determine the effects of S supplementation on fermentation traits and bioavailability of microminerals in organic dairy cow rations.

Materials and methods

On eleven organic dairy farms in Flanders, Belgium, samples of each ration ingredient were collected in January and ground through milling with a 1-mm grid size mill. Then, these milled samples were proportionally mixed according to the ration used at the respective farm. This way, a homogenous and representative sample was obtained from the rations as fed to the dairy cows in every farm. Rations typically had a basis of grass or grass with clover, with addition of diverse feedstuffs. From each ration sample, two subsamples were created: one without and one with the addition of 1 g inorganic S/kg DM as $Na_2SO_4.10H_2O$. These combinations of 11 rations with or without S addition were then subjected to *in vitro* fermentation.

In vitro rumen incubations were performed in sealed 125 ml flasks, in triplicate according to procedures as described by Van Ranst et al. (2010). Rumen inoculum was collected before morning feeding from three rumen-fistulated sheep on a hay-concentrate-based diet. Rumen fluid was strained (1 mm, kitchen sieve) prior to mixing with buffer. The flasks were incubated at 39°C in a shaking incubator (Edmund Bühler Gmbh, Hechingen, Germany). After 20h incubation, the flasks were removed from the incubator and the gas phase was analyzed for methane production and hydrogen accumulation according to Hassim et al. (2010). Afterwards, the flasks were opened, and the pH was measured. The latter remained between 6.45 and 6.77 for all incubations. This was followed by a 2-hour abomasum simulation by adjusting pH to 4 with HCl and addition of pepsin, and then a 4-hour duodenal phase at pH 9 with pancreatine added. Then, the contents of the flasks were acidified and centrifuged for 10 min at 10,000 × g (Beckman J2-HS, USA). Filtered supernatant (1 ml) was transferred to a glass vial and used for short-chain fatty acid (SCFA) analysis according to Van Ranst et al. (2010). The remainder of the supernatant was then centrifuged at 10 000 × g and analysed for minerals by inductively coupled plasma optical emission spectrometry (ICP-OES) (Iris Intrepid II XSP with dual view (axial and radial), Thermo Fisher Scientific, Aalst, Belgium) (Dermauw et al., 2013). The organic dairy rations underwent proximate analysis (AOAC, 1990) and fiber analysis (Van Soest et al., 1991). Fermentable organic matter, rumen-degradable crude protein, crude protein rumen-degradability and ileal-digestible protein contents of feeds samples were analysed according to Van Duinkerken et al. (2011). Percent bioavailability or solubility was calculated as the amount of soluble mineral in supernatant relative to the total amount of mineral in the test sample Suttle (2010).

$$\text{Bioavailability \%} = \frac{\text{\% mineral in soluble fraction}}{\text{\% mineral in the test food}} \times 100$$

Data analysis

Data were analyzed using SPSS version 24. A paired *t*-test was performed to compare the effect of S addition on microminerals bioavailability and fermentation profile. The significance level was set at $p \leq 0.05$.

Results

The nutrient composition of the organic dairy rations varied widely between farms (Table 1). The mineral profiles of these rations were in line with requirements, with fairly high concentrations of Fe (Table 2). The N:S ratio was distinctly below the ratio of 14, considered as maximum for optimal microbial protein synthesis (Drewnoski et al., 2014; Silva et al., 2014).

Table 1. Nutrient composition of the organic dairy rations (n=11) used for the *in vitro* study

		Mean	SD
Crude protein	g/kg DM	194	59
Rumen-degradable crude protein	g/kg DM	78	23
Crude protein rumen-degradability	% DM	41	5
Crude fibre	g/kg DM	198	28
Sugars	g/kg DM	23	9
Neutral detergent fibre	g/kg DM	469	60
Acid detergent fibre	g/kg DM	216	80
Acid detergent lignin	g/kg DM	36	7
Net energy for lactation	MJ/kg DM	7.1	0.5
Fermentable organic matter	g/kg DM	509	35
Ileal-digestible protein	g/kg DM	75	12
Structure value		1.6	0.3

Table 2. Mineral concentrations in the organic dairy rations (n = 11) used for the *in vitro* study

		Mean	SD	Médian	Minimum	Maximum	Requirements (NRC, 2001)	MTL
Ca	g/kg DM	6,8	2.6	6.3	2.3	12.8	5.7-6.7	1.5%
P	g/kg DM	4,8	1.5	4.9	2.1	7.9	3.2-3.8	0.7%
Mg	g/kg DM	2.6	0.68	2.8	1.4	4.1	1.8-2.1	0.6%
K	g/kg DM	29	13.2	29.2	7.3	52	10.0-10.7	2%
Na	g/kg DM	1.2	0.83	0.92	0.1	3.9	2.0-2.3	-
S	g/kg DM	3.2	1.2	3.1	1.3	5.6	2.0	0.4%
N	g/kg DM	29.6	10.3	31.2	8.5	46.9	-	-
N:S	g/g DM	9.5	1.8	9.3	6.1	14	-	-
	mg/kg DM							
Mn	mg/kg DM	94	60	83	19	319	12-14	2000
Zn	mg/kg DM	52	11	49	31	80	43-55	500
Fe	mg/kg DM	706	447	787	125	1860	13.3-18.0	500
Cu	mg/kg DM	14	4	14	6.7	20	9-11	40
Mo	mg/kg DM	3.6	3.5	2.5	0.6	17	-	5
I	mg/kg DM	0.39	0.2	0.4	0.1	0.8	0.34-0.60	50
Co	µg/kg DM	321.8	171	334.3	71	794	110	25 mg/kg
Se	µg/kg DM	112	62.8	99.5	47	320	300	5 mg/kg

SE : standard error, MTL maximum tolerable level

Sulfur addition reduced the bioavailability of Cu (Table 3; $p < 0.001$). The bioavailability of Mo was only numerically reduced by S addition (Table 3; $p = 0.107$). The other evaluated microminerals (Fe, Zn, Se) remained unaffected, while Mn bioavailability showed a trend to increase ($p = 0.085$) with S addition.

Table 3. Effect of sulfur addition to organic dairy cattle rations on *in vitro* micro-mineral bioavailability

	Without sulfur addition		With sulfur addition		
Bioavailibity (%)	Mean	SE	Mean	SE	*P*
Mn	20	1.8	24	3.1	0.085
Zn	41	1.8	43	2.2	0.158
Fe	20	2.2	21	2.1	0.356
Cu	30	1.9	23	1.3	0.001
Se	19	2.0	19	2.0	0.669
Mo	52	8.0	45	5.0	0.107

SE: standard error

Sulfur addition induced an increase in most fermentation products (Table 4): 14% more acetate, 8% more propionate, 3% more isobutyrate, 4% more butyrate and 4% more valerate. Total SCFA concentrations were 8% higher through S addition. Isovalerate was not affected by S addition. Caproate was the only evaluated volatile fatty acid that significantly decreased (by 19%) due to the S addition. Methane production was independent of S addition whereas hydrogen production level down when adding sulfur (Table 4).

Table 4 Impact of sulfur addition to organic dairy cattle rations on *in vitro* VFA, hydrogen and methane production.

	Without sulfur addition		With sulfur addition		
Volatile fatty acid	Mean	SE	Mean	SE	*P*
Acetate (µmol/g)	625	15	712	14	< 0.001
Propionate (µmol/g)	254	11	274	11	< 0.001
Isobutyrate (µmol/g)	11.6	0.6	12	0.5	0.008
Butyrate (µmol/g)	91	6.4	95	5.5	0.010
Isovalerate (µmol/g)	20.5	1.1	20	0.86	0.986
Valerate (µmol/g)	13.1	0.6	13.6	0.5	0.022
Caproate (µmol/g)	2.6	0.1	2.1	0.1	< 0.001
Total SCFA (µmol/g)	1045	29	1129	27	< 0.001
H_2	0.50	0.02	0.04	0.01	< 0.001
CH_4 (µmol/g)	330	9	326	8	0.146

SE: standard error; VFA: volatile fatty acids (sum):

Discussion

During digestion micromineral in the feed become soluble in the rumen and may bind with ruminal metabolites, or microorganisms, various dietary ingredients including other antagonist minerals, such as S, Mo, Fe, Mn, Se, Zn and thiomolybdates, to form insoluble complexes (Underwood and Suttle, 1999; Caldera et al., 2019). However, the formation of thiomolybdates is dependent on both pH and S:Mo ratio as well as available Cu (Clarke and Laurie, 1980). For example at a neutral pH, when a S:Mo ratio is above 10:1 and at S:Mo ratio of approximately 300:1 results in increased formation of both tri-thiomolybdates and tetra-thiomolybdates that have a higher affinity to bind larger amounts of available Cu (Clarke and Laurie, 1980). According to these authors, the faster conversion to the trithiomolybdate form occurs more rapidly at a lower ruminal pH of 6.5 in comparison to a slower conversion at a pH of 8.0, indicating that the formation of tetra-thiomolybdate form occurs more rapidly at a lower pH.

Subsequently, this results in the formation of Cu - tri - or Cu -tetra-molybdates complexes (Kistner, 2017). However, this is not true in the case of our study where the S:Mo ratio was 3.3 to 3.5. Thus, it is unlikely that Cu reacts with thiomolybdates to produce Cu-thiomolybdates complexes in the rumen of our cows.

Assuming that the conditions for thiomolybdate formation were not met, the negative effect of S on Cu bioavailability is likely caused by the formation of unabsorbable Cu sulfide (Cu_2S and CuS) complexes in the rumen (Goff, 2018) or at sites of the digestive tract beyond the rumen (Rehman et al., 2017). Similarly, a reduction in the percent solubility and flow of soluble Cu were noted for steers on high sulfur diets compared to steers fed low sulfur diets (Golfman, 1988). The relatively higher dietary S intake can modify the recycling of Cu via the saliva or might reduce other endogenous Cu secretions in gut segments (Golfman, 1988).

In addition to a Cu and S interaction, the presence of high dietary Fe levels (> 500 mg/kg, Table 2) can further reduce the bioavailability of Cu in ruminants (Williams, 2004; Pogge et al., 2014). Bremner et al. (1987) emphasized the important role of the rumen microorganisms in reducing S to sulfide (S^{-2}) that will react with Fe first, and then with Cu in the digestive tract, resulting in a reduced Cu availability (Gould and Kendell, 2011). Alternately at high dietary Fe intakes, Fe can also react with sulfide to produce an absorbed Fe–Cu–S complex (Suttle, 1991).

In contrast to our study Pogge et al. (2014) reported a reduced absorption and retention of Zn in steers fed a high-S diet compared to the ones fed a low-S diet. This might be due to rumen sulfide that can interact with Zn to form insoluble zinc sulfide (ZnS) complexes, that are non-absorbable in the small intestine (Rickard and Luther, 2006). Subsequently, a greater percentage of consumed Zn will be excreted in the faeces of the steers on a high-S diet (Pogge et al., 2014). Yet, the impact of S addition on Cu bioavailability is important because we commonly observe Cu deficiencies in organic dairy cows (non-published data) as well as in other situations where little or no concentrates are used, such as in free-ranging cattle in the tropics (Dermauw et al., 2013). It would therefore be a logic advice to accompany S supplementation with a higher provision of dietary Cu or to reformulate other antagonists of Cu such as Fe, Zn and Mo.

The increase of most SCFA due to S addition suggests that the quantity of S in the diet was suboptimal for rumen microbial activity. This is remarkable since guidelines typically indicate that S shortage is only apparent when the N:S ratio increases beyond 14 (Drewnoski et al., 2014; Silva et al., 2014). When S is deficient due to low S intake and bioavailability, the cellulose and organic matter fermentation in the rumen can decrease (Morrison et al., 1990; Drewnoski et al., 2014). Mathew et al. (1994) also indicated that when S is deficient, the digestion rate and retention time of feed particles in the reticulo-rumen might be directly dependent on the rate and extent of S recycling (Mathew et al., 1994). This might also influence the ruminal production of SCFA. Excess levels of $CuSO_4$ supplementation resulted in decreased NH_3-N concentration molar proportion of acetate and total VFA concentration, together with a reduction in bacterial population and OM digestibility (Hernández-Sánchez et al., 2019). Similarly, Arif (2015) found a significant reduction in methane emission in goat and sheep fed 0.4% sulphur, while they found no change in N balance.

In conclusion, S may be limiting for optimal rumen microbial activity, but the trade-off may be a reduced Cu bioavailability or absorption. Therefore, allowing S to promote dairy performance may require sufficient Cu in the diet and lower levels of antagonists such as Mo, Zn and Fe. Under optimal rumen microbial activity, this strategy could result in a greater supply of metabolizable energy and increases metabolizable protein supply in organic dairy cows.

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Animal Welfare Statement

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

References

Amat, S., 2012. Evaluation of sulfur and thiamine metabolism, short chain fatty acid absorption, and mineral status in beef cattle fed high dietary sulfur (PhD Thesis). University of Saskatchewan.

Arif, M., 2015. Influence of nitrate and sulphate feeding on enteric methane production in sheep and goats (PhD Thesis). University of Agriculture, Faisalabad Pakistan.

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists. Arlington, VA.

Clarke, N.J., Laurie, S.H., 1980. The copper-molybdenum antagonism in ruminants. I. The formation of thiomolybdates in animal rumen. Journal of Inorganic Biochemistry 12, 37–43.

Dermauw, V., De Cuyper, A., Duchateau, L., Waseyehon, A., Dierenfeld, E., Clauss, M., Peters, I.R., Du Laing, G., Janssens, G.P.J., 2014. A disparate trace element metabolism in zebu (Bos indicus) and crossbred (Bos indicus x Bos taurus) cattle in response to a copper-deficient diet. Journal of Animal Science 92, 3007–3017.Dermauw, V., Yisehak, K., Belay, D., Van Hecke, T., Du Laing, G., Duchateau, L., Janssens, G.P.J., 2013. Mineral deficiency status of ranging zebu (Bos indicus) cattle around the Gilgel Gibe catchment, Ethiopia. Tropical Animal Health and Production 45, 1139–1147. https://doi.org/10.1007/s11250-012-0337-4

Drewnoski, M.E., Pogge, D.J., Hansen, S.L., 2014. High-sulfur in beef cattle diets: a review. Journal of animal science 92, 3763–3780.

Faulkner, M., Weiss, W., 2017. Effect of source of trace minerals in either forage-or by-product–based diets fed to dairy cows: 1. Production and macronutrient digestibility. Journal of Dairy Science 100, 5358–5367.

Gupta, S., 2014. Studies on the Bioavailability of Copper, Molybdenum and Zinc from Oilseed Cakes in Crossbred Male Calves. (Doctoral thesis). National Dairy Research Institute, Deemed University

Hassim, H.A., Lourenço, M., Goel, G., Vlaeminck, B., Goh, Y.M., Fievez, V., 2010. Effect of different inclusion levels of oil palm fronds on *in vitro* rumen fermentation pattern, fatty acid metabolism and apparent biohydrogenation of linoleic and linolenic acid. Animal Feed Science and Technology 162, 155–158.

Hernández-Sánchez, D., Cervantes-Gómez, D., Ramírez-Bribiesca, J.E., Cobos-Peralta, M., Pinto-Ruiz, R., Astigarraga, L., Gere, J.I., 2019. The influence of copper levels on *in vitro* ruminal fermentation, bacterial growth and methane production. Journal of the Science of Food and Agriculture 99, 1073–1077.

Ivan, M., 1988. Effect of faunation on ruminal solubility and liver content of copper in sheep fed low or high copper diets. Journal of animal science 66, 1496–1501.

Katulski, S.L., 2017. Effects of mineral supplementation on growing cattle and *in vitro* fermentation by ruminal microbes (PhD Thesis). Kansas State University.

Kegley, E.B., Ball, J.J., Beck, P.A., 2016. Bill E. Kunkle Interdisciplinary Beef Symposium: Impact of mineral and vitamin status on beef cattle immune function and health. Journal of Animal Science 94, 5401–5413.

Khan, Z.I., Ashraf, M., Ashraf, M.Y., Hussain, A., 2003. Mineral status of livestock (goats and sheep) based on soil, dietary components and animal tissue fluids in relation to seasonal changes and sampling periods in specific region of Pakistan. Journal of Animal and Veterinary Advances 2, 478 - 495.

Mathew, B.W., Sollenberger, L.E., Staples, C.R., 1994. Sulfur fertilization of bermuda grass and effects on digestion of nitrogen, sulfur and fiber by non-lactating cows. Jornal of Hawaiian Pacific Agriculture 5, 21–30.

McDowell, L.R., 2003. Minerals in animal and human nutrition. Elsevier Science BV.

Morrison, M., Murray, R.M., Boniface, A.N., 1990. Nutrient metabolism and rumen micro-organisms in sheep fed a poor-quality tropical grass hay supplemented with sulphate. The Journal of Agricultural Science 115, 269–275.

Ortolani, E.L., 2001. Sulphur deficiency in dairy calves reared on pasture of Brachiaria decumbens. Ciência Rural 31, 257–261.

Paulsen, H.M., 2005. Sulfur in organic farming, in: Aspects of Sulfur Nutrition of Plants; Evaluation of China's Current, Future and Available Resources to Correct Plant Nutrient Sulfur Deficiencies–Report of the First Sino-German Sulfur Workshop. Braunschweig, Germany.

Pogge, D.J., Drewnoski, M.E., Hansen, S.L., 2014. High dietary sulfur decreases the retention of copper, manganese, and zinc in steers. Journal of Animal Science 92, 2182–2191. https://doi.org/10.2527/jas.2013-7481

Qi, K., Owens, F.N., Lu, C.D., 1994. Effects of sulfur deficiency on performance of fiber-producing sheep and goats: A review. Small Ruminant Research 14, 115–126.

Rehman, H., 2017. Fundamentals of Zinc, Manganese and Copper in the Metabolism of the Rumen and Immune Function. International Journal of Livestock Research 7, 55-76. https://doi.org/10.5455/ijlr.20170806111410

Silva, C.J. da, Leonel, F. de P., Pereira, J.C., Costa, M.G., Moreira, L.M., Oliveira, T.S. de, Abreu, C.L. de, 2014. Sulfur sources in protein supplements for ruminants. Revista Brasileira de Zootecnia 43, 537–543.

Sobry, L., Dermauw, V., Govaerts, W., Du Laing, G., Janssens, G., 2012. Relative contribution of feed, supplements and water to mineral supply on organic dairy farms in Flanders, in: 16th Congress of the European Society of Veterinary and Comparative Nutrition. European Society of Veterinary and Comparative Nutrition (ESVCN), pp. 150–150.

Suttle, N., 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annual review of nutrition 11, 121—140. https://doi.org/10.1146/annurev.nu.11.070191.001005

Suttle, N.F., 2010. Mineral nutrition of livestock, 4th ed. ed. CABI, Wallingford, Oxfordshire, UK ; Cambridge, MA.

Van Duinkerken, G., Blok, M.C., Bannink, A., Cone, J.W., Dijkstra, J., Van Vuuren, A.M., Tamminga, S., 2011. Update of the Dutch protein evaluation system for ruminants: the DVE/OEB2010 system. The Journal of Agricultural Science 149, 351.

Van Ranst, G., Fievez, V., Van Waes, J., De Riek, J., Van Bockstaele, E., 2010. Influence of damaging and wilting red clover on lipid metabolism during ensiling and *in vitro* rumen incubation. Animal 4, 1528–1540.

Van Soest, P. van, Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of dairy science 74, 3583–3597.

Williams, C.L., 2004. The effect of molybdenum, iron and sulphur on copper metabolism and physiology of sheep (PhD Thesis). The Open University.

Chapter 4

Copper, Iron, Zinc and Tannin concentrations throughout the digestive tract of tropical goats and sheep fed a high-fiber tannin rich diet

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Abstract

The dry season in tropical regions urges livestock to feed on nutritionally very poor diets. It has not been explored how tropical sheep – assumed grazers – and tropical goats – intermediate browsers – cope with a high-fiber tannin-rich diet. This study was designed to determine the effects of a high fiber and tannin-rich diet on the flow of important micro-minerals iron (Fe), zinc (Zn), and copper (Cu) throughout the digestive tract of tropical sheep and goats. The feeding trial was set up with twelve adult male animals, six sheep with mean body weight (BW) of 30.3 ± 1.6 kg and six goats with mean BW of 26.4 ± 2.2 kg. The feed consisted of 36% leaves of *Millettia ferruginea*, 61% hay, and 3% concentrate, and was offered at 3% of BW (all on dry matter (DM) basis). The total fecal collection was carried out for 7 consecutive days. At the end of the experimental period, the animals were slaughtered to collect liver and digesta samples from the gastrointestinal tract. Feed, digesta, and fecal samples underwent analysis of Fe, Zn, and Cu and total tannins (TT). Goats had significantly higher reticulum Cu concentrations expressed on DM as compared to sheep. Fecal Cu concentrations were higher for goats compared to sheep. Reticulum and colon digesta Zn levels were higher in goats than sheep. Abomasum and colon Fe levels were higher in sheep than goats when expressed on DM. These results suggest differences in feed intake, micro-mineral absorption, secretion and excretion between sheep and goats, pointing to a divergent mineral metabolism as an adaptation to the challenge of a dry season diet having very low nutritive value.

Introduction

Global climate change, the expansion of farmlands, land degradation and erosion, longer dry season and urbanization (Ahmed et al., 2010) reduces the availability of total feed resources resulting in a shortage of required nutrients available to ruminants (Adejoro, 2019). In tropical regions, alternative feed resources such as indigenous multi-purpose browse trees and shrubs have been applied to minimize the existing feed gap especially during the long dry season (Geta et al., 2014). Most of these browse species are known for a high crude protein and mineral content (Mueller-Harvey, 2006; Gupta, 2014), yet, they also contain high levels of fiber and plant secondary compounds, predominantly tannins (Theodoridou, 2010). The latter considerably bind minerals, proteins, and amino acids in diets (Frutos et al., 2004), making them unavailable for absorption (Muir, 2011; Yisehak et al., 2012).

The ability of the diet to supply minerals indeed not only depends on their concentration but also on the bioavailability of the minerals (Spears, 2003). The potential bioavailability and absorption of minerals from the gastrointestinal tract is greatly influenced by several factors including species, age, and breed of animal, the intake (availability in the feed sources or palatability), the chemical form in which mineral is present, intake of mineral relative to the amount required and the mineral's utilization by the animal tissues (Hilal et al., 2016; Nwosu, 2019). The degree of mineral bioavailability is also influenced by the type and quantity of anti-nutritional components that are ingested (Yun et al., 2004). The solubility of micro-minerals such as Cu, Zn and Fe can greatly affect the total concentration of these minerals that are available to rumen microbes and the ruminants themselves (Genther and Hansen, 2015; Katulski, 2017). The complexation of minerals influences the metabolic capability of microorganisms by altering the physiological uptake of minerals and other nutrients essential for their own metabolism (Scalbert, 1991; McDonald et al., 1996). This may prevent the attachment of rumen microorganisms to plant cell walls necessary for the degradation of these cell walls to occur (Frutos et al., 2004).

Sheep and goats have a better ability to utilize fibrous shrubs and tree leaves than other livestock (Aboagye, 2019). Because goats – as intermediate browsers – produce salivary proline-rich proteins (PRPs), it is assumed that they are better adapted to consume larger amounts of browse. In tropical regions or conditions, although sheep indeed prefer grazing, they shift more to browsing as the dry season progresses, when grass becomes scarce and less palatable (Shenkute et al., 2012). Former work demonstrated adaptations of other tropical livestock ruminants, e.g. zebu cattle, to a tannin-containing browse-diet (Yisehak et al., 2011), as well as diets with low levels of minerals (Dermauw et al., 2014), so it is likely that tropical Kefa goats, as well as Bonga sheep, have adapted to the exposure to such diets. Tropical Kefa goats seem to possess a higher ability than tropical Bonga sheep to neutralize the effect of dietary tannins (Yisehak et al., 2016), but this does not exclude other adaptations in nutrient digestion and absorption to typical dry season diets.

This study evaluated whether tropical sheep and goats have divergently adapted their micro mineral metabolism to a typical dry season, fibrous and tanniferous diet.

Materials and methods

Study area

A feeding trial was set up at the dairy farm of Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM) in Jimma, in the southwestern part of Ethiopia.

Experimental diet

The feed consisted of three components: 61% of a hay mixture, 36% of tannin-rich *Millettia ferruginea* leaves, and 3% of concentrate (all offered on a DM basis). *Millettia ferruginea,* locally called *birbra* is a potential multipurpose nitrogen-fixing legume tree used by local farmers in different parts of Ethiopia for feeding cattle, goats, and sheep especially, during the dry seasons (Alemu et al., 2014). The leaves of *M. ferruginea* were hand-plucked from different trees, pooled, and air-dried for about 5-7 days in shade. After the drying, the twigs were removed and only the leaves were fed to the animals. To prevent mold, the leaves were mixed daily. Similarly, the hay mixture was harvested locally and dried in shade. In the mixed hay, *Cyperus rotundus*, *Phyllanthus amarus*, *Eleusine coracana* and *Satoria verticillate* were identified in increasing order of dominance. The DM digestibility of this diet was previously determined as 48 ± 2 % in goats (mean ± standard deviation (SD)) and 49 ± 2 % in sheep (unpublished data). The analytic nutrient composition and tannin content of the *M. ferruginea* leaves, basal hay mixture and concentrate are shown in Table 1.

Table 1 Nutrient levels in the experimental feedstuffs, *Millettia ferruginea* leaves, hay mixture and concentrate.

	Concentrate	Leaves	Hay mixture
DM (% FM)	90.3	92.4	91.9
CP (% DM)	20.60	19.81	6.42
EE (% DM)	5.54	3.68	0.98
NDF (% DM)	16.8	46.1	43.2
ADF (% DM)	8.9	42.5	38.9
ADL (% DM)	0.2	18.5	25.3
AIA (% DM)	0.4	1.6	4.8
TT (g/kg DM)	0.277	5.41	1.02
Cu (mg/kg DM)	13.6	10.3	5.50
Fe (mg/kg DM)	843	312	188
Zn (mg/kg DM)	75.5	21.2	60.0

The dietary concentrations of Cu, Fe and Zn seem within the range to meet sheep and goats' requirements (NRC, 2007).

Fresh matter (FM), dry matter (DM), copper (Cu), iron (Fe), zinc (Zn), Crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), acid insoluble ash (AIA), total tannin (TT)

Animals and management

Twelve adult male animals, six Bonga sheep with a mean bodyweight of 30.3 ± 1.6 kg and six Keffa goats (26.4 ± 2.2 kg) were purchased at the local livestock market in Seka town near Jimma, Ethiopia. All animals were estimated to be between 12 to 19 months of age, based on their teeth formula inspection (Casburn, 2016). The sheep and goats were transported to JUCAVM and shortly after their arrival, they were individually housed in a ventilated barn with a concrete floor. One day after arrival, the animals were clinically examined and found healthy, and seven days after arrival, all 12 were dewormed with a 1 ml subcutaneous injection of 1% Ivermectin (Shanghai Tongren Pharmaceutical Co. Ltd.) and a 5 ml 20% oxytetracycline (® Oxyvic 20) intramuscular injection. All animals had unlimited access to fresh water. Before the start of the experiment, the animals were kept to adapt to the new diet for 11 days. During this period, the animals were left to graze outside for the first three days to let them adapt to their new environment. Then based on 3% of BW, the hay mixture was given individually, twice a day at 10:00 hours and 16:00 hours for the next eight days. Also, approximately 100-150 g of *M ferruginea* leaves were provided at 8:00 hours before hay supplementation to gradually create a steady state of the metabolism of the animals.

Total fecal collection

The total fecal collection was carried out for 7 consecutive days. During this experimental period, the amount of feed offered was weighed every day based on 3% of BW for the next day feeding (Rashid, 2008; Squires, 2010), but the actual daily intake was recorded through weighing the refusals. The amount of feed offered and refused was collected per animal and weighed daily. Also, all feces produced was collected every morning and weighed. A sub-sample of about 10% of the total amount per animal was collected and stored at -20^{o}C until the end of the trial. At the end of the experimental period, daily fecal subsamples were thoroughly mixed and pooled per animal and oven-dried at 60^{o}C for 48h.

Digesta Sampling and Sample Preparation

The amount of feed offered and refused was collected per animal and weighed daily. Representative samples of both were taken daily every morning before the feeding, stored at room temperature until the end of the trial. Then, samples were pooled per animal, milled in a Wiley Mill (Hanna Instrument, Germany) to pass through a 1mm screen, and the ground material was stored in an airtight container at 25°C until analysis.

At the end of the experimental period, the animals were fasted overnight and weighed. Then, the animals were slaughtered to collect the liver samples and the digesta samples from eight different sites of the gastrointestinal tract: rumen, reticulum, omasum, abomasum, jejunum, caecum and colon. Briefly the whole gastrointestinal tract was removed and each part was tightened up to prevent mixing of the digesta. The representative samples of gut content from respective section were filled into plastic vials.

All samples were immediately stored at -20°C in plastic bags for approximately 12h until processed. The following day, the frozen digesta samples were oven-dried at 45°C for one to three days, until a constant weight was reached, while the livers were dried in the oven at 65°C for three days. The dried feces and liver samples were ground in a mill (Hanna Instrument, Germany) to pass through a 1 mm screen and were stored in an airtight container at 25°C, until analysis.

Mineral Analysis

Feed, digesta, and fecal samples were analyzed for Fe, Zn, and Cu, using inductively coupled plasma mass spectrometry (ICP-MS) as described by Elmer (1996). Acid-insoluble ash (AIA) contents of feeds and faeces samples were analysed according to Sales and Janssens (2003). Apparent micromineral absorption was calculated according to van Riet et al. (2016).

Total Tannin Analysis

The dietary ingredients (leaves of experimental feed, basal diet hay), were analysed for their total tannin (TT) content following the method of International Standards Organization (ISO, 1988). Tannin extraction was done by using dimethylformamide. After centrifugation, ferric ammonium citrate and ammonia were added to a liquid part of the supernatant, followed by spectrometric determination at 525 nm absorbance of the obtained solution.

Apparent absorption were calculated using AIA according to Sales and Janssens (2003).

$$\text{Apparent absorption} = 1 - \frac{\%\ \text{AIA in feed}}{\%\ \text{AIA in faeces}} \times \frac{\%\ \text{nutrient in the faeces}}{\%\ \text{nutrient in feed}}$$

Statistical analysis

Data were analyzed using SPSS version 24. Differences in the parameters studied between sheep and goats and between sample sites (different sections of the gastrointestinal tract) were evaluated using a linear mixed model, with species and sample sites in the intestinal tract, and their interaction as fixed factors, and animal as a random factor. Gut segments were considered as within-subject variable in the repeated measures variance analysis, with species as between subject variable. An unpaired t-test was performed to identify differences between sheep and goats with regards to feed intake. The significance level was set at $p \leq 0.05$.

Results

Table 1 presents the mineral composition (mg/kg DM) and total tannin (g/kg DM) content of the different feed ingredients. The consumption of hay, leaves and concentrate was 67.2, 28.2 and 4.6% of total DM intake in goats while the sheep consumed the feed ingredients in line with what was offered, i.e. 62.2% hay, 34.1% leaves and 3.6% concentrate (Table 2).

Table 2 Total intake of *Millettia ferruginea* leaves, hay mixture and concentrate (g /kg LW $^{0.75}$), with the corresponding intakes of Fe, Cu, Zn (mg /kg LW $^{0.75}$), DM and TT (g /kg LW $^{0.75}$) in tropical sheep and goats.

	Goat		Sheep		
	Mean	SE	Mean	SE	*P*
Feed intake					
Hay	312	1.1	368	1.3	0.008
Leaves	130	1.00	204	1.9	0.006
Concentrate	20.6	0.02	21.2	0.02	0.068
Nutrient intake					
DM	462	1.4	594	2.4	0.001
Cu	3.63	0.01	4.89	0.24	0.001
Fe	127	0.39	164	0.70	< 0.001
Zn	25.1	0.07	30.5	0.09	0.001
TT	1.02	0.005	1.47	0.010	0.003

Dry matter (DM), copper (Cu), iron (Fe), zinc (Zn), total tannin (TT)

Digesta Total Tannin Content

For both species, no significant differences in TT content between sheep and goats were observed across the sampled digestive compartments (Figure 1).

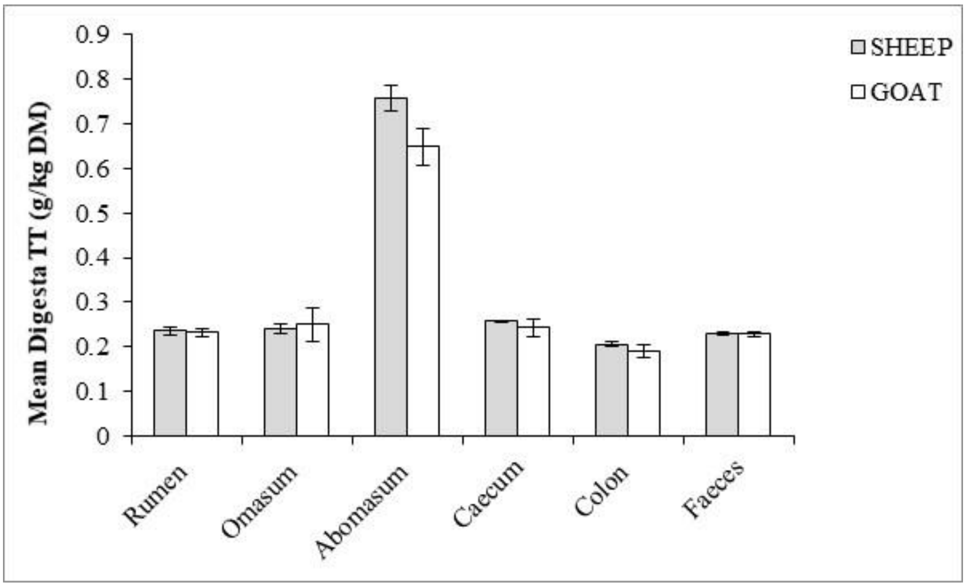

Figure 1. Total tannin (TT) concentrations in digesta on a DM basis for sheep and goats fed a mixture of *Millettia ferruginea* leaves, hay and concentrate. DM: dry matter. TT: total tannin. The error bars display the standard error of means.

Trace-Element Composition of Digesta

Levels of Cu, Fe, and Zn were significantly different among the digesta sampling sites and between sheep and goats on a DM basis. Interactions between sample site and species were seen for all trace-elements, except for Zn on a DM basis.

Goats had higher ($p < 0.05$) Cu levels in the digesta collected from the reticulum and faeces. (Figure 2). Goats also tended to show a higher Cu level than sheep in abomasum ($p = 0.065$). While sheep tended to have higher Cu levels in the rumen and omasum ($p = 0.053$) than goats.

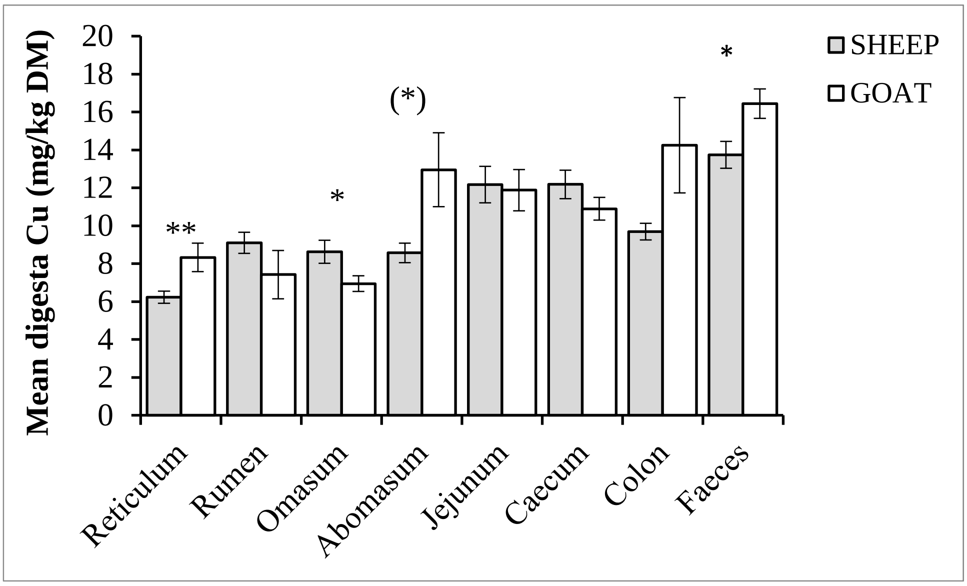

Figure 2. Cu digesta concentration on DM basis for sheep and goats fed a mixture of *Millettia ferruginea* leaves, hay and concentrate. The error bars display the standard error of means. * significance at $p < 0.05$; ** significance at $p < 0.01$; (*) trend ($0.05 < p < 0.10$).

Sheep had higher Fe levels in digesta obtained from omasum (p=0.039) and colon digesta (p = 0.001) than goats (Figure 3).

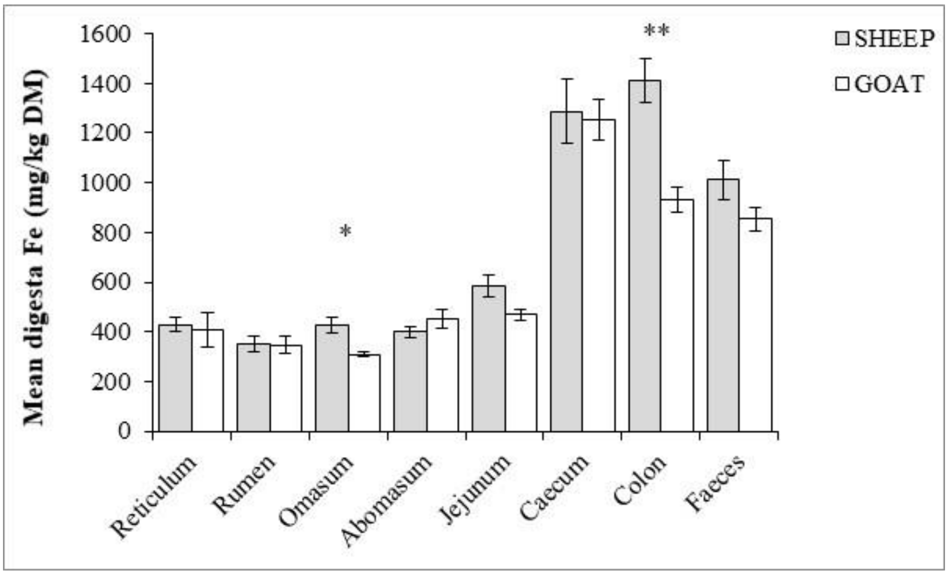

Figure 3. Fe digesta concentration on DM basis, for sheep and goats fed a mixture of *Millettia ferruginea* leaves, hay and concentrate. The error bars display the standard error of means. * significance at $p < 0.05$; ** significance at $p < 0.01$; (*) trend ($0.05 < p < 0.10$).

Both reticulum and colon digesta of goats had higher Zn levels (p = 0.008 for reticulum and p = 0.005 for colon digesta) (Figure 4).

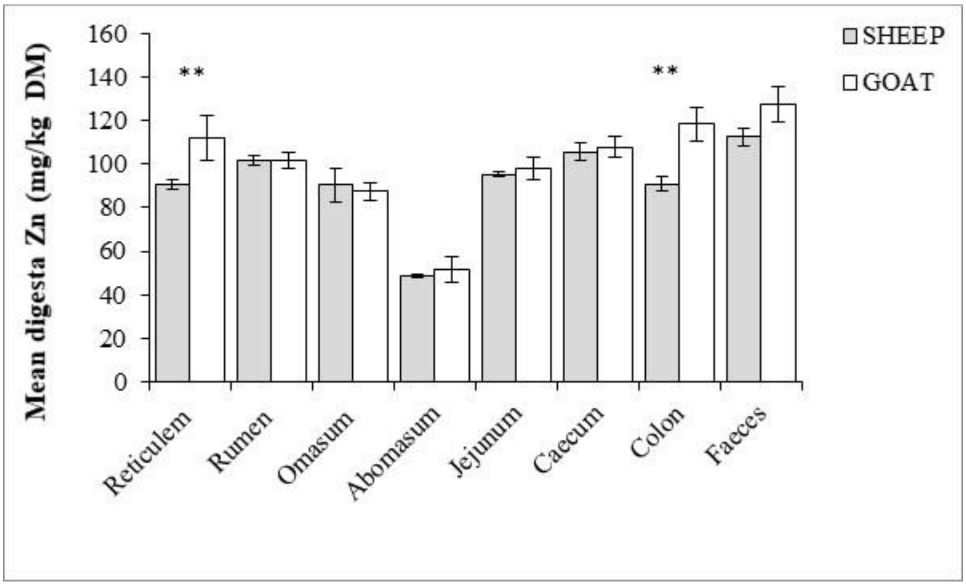

Figure 4. Zn digesta concentration on dm basis for both sheep and goats fed a mixture of *Millettia ferruginea* leaves, hay and concentrate. The error bars display the standard error of means. * significance at $p < 0.05$; ** significance at $p < 0.01$; (*) trend ($0.05 < p < 0.10$).

In other sampled digestive sites and feces, levels of Zn were not significantly different between sheep and goats. Both sheep and goats had negative apparent absorption coefficients for Cu, Fe and Ze (Figure 5), but only Fe absorption showed significant differences between species ($p < 0.05$).

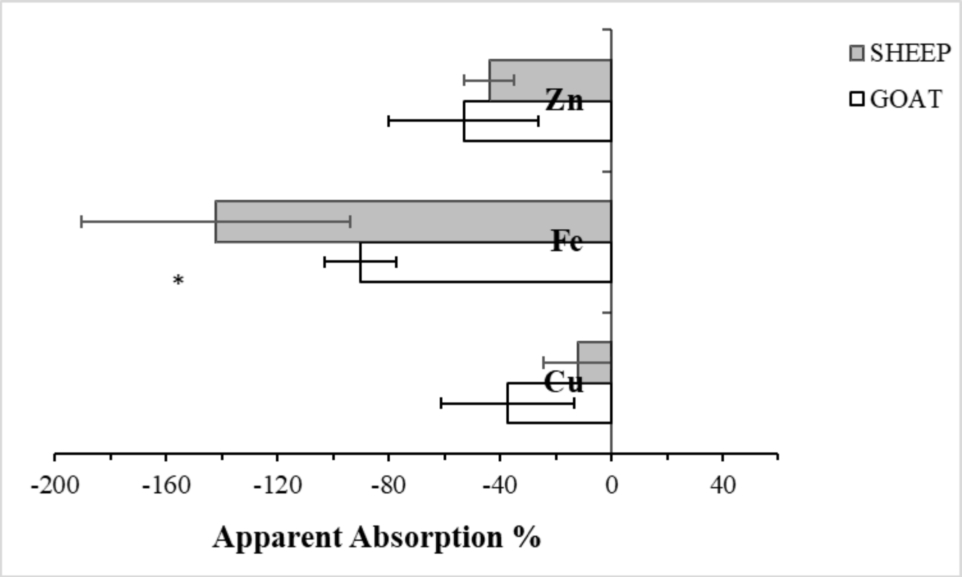

Figure 5. Apparent absorption of Cu, Fe and Zn in tropical goats (n=6) and sheep (n=6) fed a mixture of *Millettia ferruginea* leaves, hay and concentrate.

Mean liver Cu, Fe, and Zn concentrations did not significantly differ between sheep and goats (Table 3).

Table 3 Concentrations of Cu, Fe and Zn (mg/kg DM) in the liver of tropical goats and sheep fed a mixture of *Millettia ferruginea* leaves, hay and concentrate for two weeks.

	Sheep		Goat		*P*
	Mean	SE	Mean	SE	
Cu	310	11	332	10	0.812
Fe	492	71	374	63	0.244
Zn	172	52	175	74	0.855

Discussion

The dry season diet offered in our study induced a similar, prominent loss of the three investigated micro-minerals in sheep and goats. However, micromineral requirements for these local breeds are undocumented and breed type differences in mineral metabolism have been identified for cattle (Ward et al., 1995; Dermauw et al., 2014). The degree of mineral bioavailability is influenced by the types and quantity of inhibitory compounds that are available in the feedstuffs (Zanetti et al., 2017). For example, depending on the concentrations of S and Mo in the diet, antagonistic interactions might cause variation in the ability of feeds to provide absorbable copper and hence influence requirements in ruminants (Underwood and Suttle, 1999). The requirement values are affected by species, age, and breed type as well as physiological factors such as the stage of pregnancy and lactation (Hilal et al., 2016).

Based on the micro-mineral concentrations in the liver (Puls, 1994), with reported deficiency levels for Cu below 20 mg/kg DM (NRC, 2007), it appears that all animals had an adequate micro-mineral status. The liver concentrations of Fe in both group were are higher than the normal levels observed in sheep (Puls, 1994) whereas the Zn concentrations of the liver were much lower than those found for Cu and Fe, though they still range within the normal levels reported by Puls (1994).

It may thus be risky to extrapolate our findings to animals that already have a declined micro-mineral storage, because they may enter a “saving modus” when for instance absorption of these microminerals is an active homeostatic process (Sloup et al., 2017). This is because some animals may have serum or plasma Cu and ceruloplasmin concentrations within the adequate range but at low liver copper concentrations (Herdt and Hoff, 2011). By contrast López-Alonso and Miranda (2020) explained that, animals considered to be deficient, or in the lower margin of the reference interval on the basis of serum Cu concentration have been found to be at or near a toxic state on the basis of necropsy results or examination of hepatic biopsy specimens.

Khalili et al. (1993) demonstrated that Ethiopian cattle seem to have a seasonal deficiency pattern. Likewise, certain tropical dry season diets may actually reduce the sheep and goats' micro-mineral status.

Although the measured micro-minerals apparent absorption was found negative both in goats and sheep, the patterns of the micromineral concentrations throughout the digestive tract differed between species, suggesting a divergent micro-mineral metabolism between tropical goats and sheep. This divergence was also different for every micromineral, since the concentration profiles throughout the digestive tract differed distinctively between Cu, Zn and Fe, and species differences in these profiles were not uniform between these three micro-minerals. For instance, the absorption of Fe is largely dependent on animal body needs: animals of low Fe status or receiving diets deficient in Fe will absorb and retain more Fe in accordance with physiological needs (Golfman, 1988). Similarly, Cu and Zn are absorbed according to the requirements and homeostasis in ruminants, which are regulated by their dietary concentrations and the presence of other antagonists (Rehman, 2017), which primarily interfere with the micromineral's bioavailability and absorption from the gut and subsequent utilization for metabolic processes (Gooneratne et al., 1989; Rehman, 2017). Therefore, the overall process of micromineral absorption is regulated by their dietary concentrations, endogenous secretion and related to the animal's absolute requirements and status of the body (Golfman, 1988; Gupta, 2014).

Compared to the other gut segments both sheep and goats showed lower concentration of Cu in their forestomach, which might be due to possible absorption of Cu. Indeed, it is generally accepted that the absorption of these microminerals occurs in the small intestine (Miller, 1970; Mir et al., 2020). Still, species differences in Zn and Cu concentrations were already observed in the reticulum which may arise from the unequal intake of these elements from the hay versus leaves in the diet. Although this has to be confirmed, differences in salivary gland size, omasum size and reticulo-ruminal structure, degree of rumen content stratification and the relative reticulo-rumen retention times of fluids or particles between grazers and browsers, could be other possible reasons for the detected species differences (Clauss and Hummel, 2017; Sauer et al., 2017).

A major change in concentrations occurs when entering the enzymatic digestion compartments (abomasum and jejunum) by enzymes originate from microbes, where the abomasal concentration suddenly increases for Cu in contrast with a decrease in Zn and little change for Fe. Remarkably, the increase in Cu was distinctly higher for goats than for sheep. The elevation of the concentration of free tannins in the abomasum confirms that the acid pH in that segment disrupts the binding of micro-minerals to tannins (Acharya, 2014; Naumann et al., 2017), allowing these minerals to be absorbed. It is striking that this only occurs for Zn. These net results must be the sum of absorption and re-entering through digestive juices, but unfortunately, we were not able to determine these factors separately. Since Fe, as well as Zn, are antagonists of Cu (Acharya et al., 2016; Hilal et al., 2016), it is plausible that the sudden release of Zn, and maybe Fe, in the abomasum inhibits the absorption of Cu. If goats are better in digestion compared to sheep, a higher secretion of gastric juices may explain why the goats' abomasum showed a much higher increase in Cu.

There is a scarcity of information on the micromineral composition of digestive juices, but previous analyses at our lab found 50.8 mg Cu/kg commercial porcine pepsin versus only 5.37 mg Cu/kg in commercial porcine pancreatine (non-published data). We acknowledge the potential species differences, but it does support our hypothesis that the increased inflow of gastric juice may be responsible for the increased Cu concentrations in the abomasum of goats. That same analysis also found low Zn concentrations in the pepsin (9.38 mg/kg) but very high Zn concentrations in the pancreatin (215 mg/kg).

From the jejunum towards the caecum and colon, Zn and Cu concentrations remained constant on a DM basis. The higher colonic Fe concentrations in sheep versus goats are in contrast with the lower colonic Zn and Cu concentrations in sheep versus goats, indicating that the colonic absorption capacity differs between goats and sheep, with different effects for Fe, Cu and Zn. Because of the higher Cu intake and the consistently higher Cu concentrations in the gut of sheep compared to the situation in goats, the numerically higher Cu concentrations in the liver of goats than sheep was unexpected. Zanetti et al. (2017) indicated that most minerals are associated with organic constituents such as plant cell walls that require a longer fermentation time for maximal release, which may result in lower bioavailability. Poor fermentation of OM content of the ingested feed in the rumen rather than the absolute dietary CP content of feedstuff might be a reason for reduced apparent DM, CP, and ADF digestibility (Adejoro, 2019) or reduced mineral absorption in the case of this study.

It is also important to note that the sheep and goat with the lowest hepatic Cu levels were also the ones with the highest hepatic Fe levels (> 600 mg/kg DM) as well.

In general, the apparent digestibility of Cu was found to be lower compared to Fe and Zn. The significant species difference in apparent digestibility indicates a more efficient Fe recovery in goats during the digestive processing of this very low-quality diet. It may happen that goats compensate the lower Fe intake by a higher Fe hindgut absorption, which might explain the lower colon and fecal Fe content in goats than in sheep.

In conclusion, at least in the present case, a dry season diet with high fiber and tannin concentrations can induce net losses of important micro-minerals such as Fe, Cu, and Zn. Tropical Kefa goats react differently to such dietary challenge compared to tropical Bonga sheep, in a way that tropical goats seem to invest more in abomasal digestion, hence probably losing more micro-minerals through gastric juices, but also showing more efficient colonic absorption. The study demonstrates that dry season diets can deplete micro-mineral reserves, but that differences in digestive strategies between tropical goats and sheep may exert different responses.

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References

Aboagye, I.A., 2019. Mitigating enteric methane production and nitrogen excretion from forage-fed ruminants (PhD Thesis). University of Alberta.

Acharya, M., 2014. Improving Lamb Performance with Sericea Lespedeza and Molybdenum (PhD Thesis) University of Arkansas.

Acharya, M., Burke, J.M., Coffey, K.P., Kegley, E.B., Miller, J.E., Smyth, E., Welborn, M.G., Terrill, T.H., Mosjidis, J.A., Rosenkrans, C., 2016. Changes in concentrations of trace minerals in lambs fed sericea lespedeza leaf meal pellets with or without dietary sodium molybdate12. Journal of Animal Science 94, 1592–1599. https://doi.org/10.2527/jas.2015-9910

Adejoro, F.A., 2019. The use of condensed tannins and nitrate to reduce enteric methane emission and enhance utilization of high-forage diets in sheep (PhD Thesis). University of Pretoria.

Alemu, B., Animut, G., Tolera, A., 2014. Effect of Millettia ferruginea (Birbra) foliage supplementation on feed intake, digestibility, body weight change and carcass characteristics of Washera sheep fed natural pasture grass hay basal diet. SpringerPlus 3, 1 - 11.

Aquilina G, Azimonti G, Bampidis V, et al. Revision of the currently authorised maximum copper content in complete feed EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Efsa J 2016;14:100.

Casburn, G., 2016. How to tell the age of sheep. Department of Primary Industries. Wagga Wagga, New South Wales.

Clauss, M., Hummel, J., 2017. Physiological adaptations of ruminants and their potential relevance for production systems. Revista Brasileira de Zootecnia 46, 606–613.

Dermauw, V., De Cuyper, A., Duchateau, L., Waseyehon, A., Dierenfeld, E., Clauss, M., Peters, I.R., Du Laing, G., Janssens, G.P.J., 2014. A disparate trace element metabolism in zebu (Bos indicus) and crossbred (indicus x Bos taurus) cattle in response to a copper-deficient diet. Journal of animal science 92, 3007–3017.

Elmer, P., 1996. Analytical methods for atomic absorption spectroscopy. USA: The Perkin-Elmer Corporation.

Frutos, P., Hervás, G., Giráldez, F.J., Mantecón, A.R., 2004. Review. Tannins and ruminant nutrition. Spanish Journal of Agricultural Research 2, 191–202. https://doi.org/10.5424/sjar/2004022-73

Genther, O.N., Hansen, S.L., 2015. The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. Journal of dairy science 98, 566–573.

Geta, T., Nigatu, L., Animut, G., 2014. Evaluation of potential yield and chemical composition of selected indigenous multi-purpose fodder trees in three districts of Wolayta Zone, Southern Ethiopia. World Applied Science Journal 31, 399–405.

Golfman, L.S., 1988. The effects of molybdenum and sulfur on the flow and solubility of various minerals along the digestive tract of steers (MSc thesis). University of Manitoba.

Gooneratne, S.R., Buckley, W.T., Christensen, D.A., 1989. Review of copper deficiency and metabolism in ruminants. Canadian Journal of Animal Science 69, 819–845.

Gupta, S., 2014. Studies on the Bioavailability of Copper, Molybdenum and Zinc from Oilseed Cakes in Crossbred Male Calves (Doctoral thesis). National Dairy Research Institute, Deemed University.

Herdt, T.H., Hoff, B., 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Veterinary Clinics: Food Animal Practice 27, 255–283.

Hilal, E.Y., Elkhairey, M.A., Osman, A.O., 2016. The role of zinc, manganse and copper in rumen metabolism and immune function: A review article. Sciences, 6, 304-324.

ISO, 1988. Sorghum Determination of tannin content. ISO 9648:1988. International Organization for Standardization; Geneva, Switzerland. pp 12–15.

López-Alonso, M., Miranda, M., 2020. Copper Supplementation, A Challenge in Cattle. Animals 10, 1890.

Khalili, M., Lindgren, E., Varvikko, T., 1993. A survey of mineral status of soil, feeds and cattle in the Selale Ethiopian highlands. II. Trace elements. Tropical animal health and production 25, 193–201.

McDonald, M., Mila, I., Scalbert, A., 1996. Precipitation of Metal Ions by Plant Polyphenols: Optimal Conditions and Origin of Precipitation. Journal of Agricultural and food Chemistry 44, 599–606. https://doi.org/10.1021/jf950459q

Miller, W.J., 1975. New Concepts and Developments in Metabolism and Hemeostasis of Inorganic Elements in Dairy Cattle. A Review. Journal of dairy science 58, 1549–1560.

Miller, W.J., 1970. Zinc nutrition of cattle: a review. Journal of dairy science 53, 1123–1135.

Mir, S.H., Mani, V., Pal, R.P., Malik, T.A., Sharma, H., 2020. Zinc in ruminants: metabolism and homeostasis. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 90, 9–19.

Muir, J.P., 2011. The multi-faceted role of condensed tannins in the goat ecosystem. Small Ruminant Research 98, 115–120.

Naumann, H.D., Tedeschi, L.O., Zeller, W.E., Huntley, N.F., 2017. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. Revista Brasileira de Zootecnia 46, 929–949. https://doi.org/10.1590/s1806-92902017001200009

NRC, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World camelids. Washington, D.C: National Academies Press.

Nwosu, O., 2019. A Systematic Review of the Impact of Minerals on Pregnant Sheep and Goats and their Offspring in the African Continent (PhD Thesis). University of Pretoria.

Puls, R., 1994. Mineral levels in animal health: diagnostic data. Clearbrook. British Columbia, Canada: Sherpa International 241–244.

Rashid, M., 2008. goats-and-their-nutrition.pdf [WWW Document]. URL https://www.gov.mb.ca/agriculture/livestock/goat/pubs/goats-and-their-nutrition.pdf (accessed 5.1.19).

Rehman, H., 2017. Fundamentals of Zinc, Manganese and Copper in the Metabolism of the Rumen and Immune Function. International Journal of Livestock Research 7, 55-76. https://doi.org/10.5455/ijlr.20170806111410

Sales, J., Janssens, G.P.J., 2003. Acid-insoluble ash as a marker in digestibility studies: a review. Journal of Animal and Feed Sciences 12, 383–401.

Sauer, C., Clauss, M., Bertelsen, M.F., Weisbjerg, M.R., Lund, P., 2017. Rumen content stratification in the giraffe (Giraffa camelopardalis). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 203, 69–76.

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry (oxford). 30, 3875–3884.

Shenkute, B., Hassen, A., Assafa, T., Amen, N., Ebro, A., 2012. Identification and nutritive vale of potential fodder trees and shrubs in the mid Rift Valley of Ethiopia. Journal of Animal and Plant Sciences 22,1126-1132.

Sloup, V., Jankovská, I., Nechybová, S., Peřinková, P., Langrová, I., 2017. Zinc in the animal organism: a review. Scientia Agriculturae Bohemica 48, 13–21.

Spears, J.W., 2003. Trace Mineral Bioavailability in Ruminants. The Journal of Nutrition 133, 1506S-1509S. https://doi.org/10.1093/jn/133.5.1506S

Squires, V.R., 2010. Range and Animal Sciences and Resources Management - Volume II. EOLSS Publications. University of Adelaide, Australia.

Tadesse, W., Kechero, Y., Tolemariam, T., 2018. Comparison of polyethylene glycol and wood ash extract on feeding value and economic efficiency of mixes of high-tannin feed sources in growing Ethiopian Bonga lambs. Tropical animal health and production 50, 161–167.

Theodoridou, K., 2010. The effects of condensed tannins in sainfoin (Onobrychis viciifolia) on its digestion and nutritive value (Doctoral dissertation). Université Blaise Pascal-Clermont-Ferrand II.

Underwood, E., Suttle, N., 1999. The mineral nutrition of livestock, 3rd. Edition, CAB International 614.

van Riet, M.M., Millet, S., Bos, E.-J., Nalon, E., Ampe, B., Sobry, L., Tuyttens, F.A.M., Maes, D., Du Laing, G., Nagels, T., 2016. No indications that zinc and protein source affect Zn bioavailability in sows during late gestation fed adequate dietary Zn concentrations. Animal Feed Science and Technology 213, 118–127.

Ward, J.D., Spears, J.W., Gengelbach, G.P., 1995. Differences in copper status and copper metabolism among Angus, Simmental, and Charolais cattle. Journal of Animal Science 73, 571–577.

Warly, L., Fariani, A., 2010. Concentration of Micro Minerals in Fiber Fraction of Forages. International Journal of Agricultural and Biosystems Engineering, 4, 584-590.

Yisehak, K., Becker, A., Belay, D., Bosch, G., Hendriks, W.H., Clauss, M., Janssens, G.P., 2011. Salivary amino acid concentrations in zebus (Bos indicus) and zebu hybrids (Bos indicus x Bos taurus) fed a tannin-rich diet. Belgian Journal of Zoology 141, 93–96.

Yisehak, K., Becker, A., Rothman, J.M., Dierenfeld, E.S., Marescau, B., Bosch, G., Hendriks, W., Janssens, G.P.J., 2012. Amino acid profile of salivary proteins and plasmatic trace mineral response to dietary condensed tannins in free-ranging zebu cattle (Bos indicus) as a marker of habitat degradation. Livestock Science 144, 275–280.

Yisehak, K., Kibreab, Y., Taye, T., Lourenço, M.R.A., Janssens, G.P.J., 2016. Response to dietary tannin challenges in view of the browser/grazer dichotomy in an Ethiopian setting: Bonga sheep versus Kaffa goats. Tropical animal health and production 48, 125–131.

Yun, S., Habicht, J.-P., Miller, D.D., Glahn, R.P., 2004. An *In Vitro* Digestion/Caco-2 Cell Culture System Accurately Predicts the Effects of Ascorbic Acid and Polyphenolic Compounds on Iron Bioavailability in Humans. The Journal of nutrition 134, 2717–2721. https://doi.org/10.1093/jn/134.10.2717

Zanetti, D., Menezes, A.C.B., Silva, F.A.S., SILVA, L.C.E., Rotta, P.P., Detmann, E., Engle, T.E., Valadares Filho, S.C., 2017. In situ and *in vitro* estimation of mineral release from common feedstuffs fed to cattle. The Journal of Agricultural Science 155, 1160–1173.

CHAPTER 5

Digestive responses of tropical goats and sheep fed a high-fiber tannin rich diet

Chapter 5

Digestive responses of tropical goats and sheep fed a high-fiber tannin rich diet

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Abstract

Within the classical browser-grazer dichotomy theory, it is commonly assumed that browsers are better adapted to digest highly fibrous and tannin-rich plants than grazers. This study aimed to investigate how tropical goats and sheep process protein and fiber from a typical high fiber and tannin-rich diet. Six male adult goats and six male adult sheep of local breeds from Jimma, Ethiopia, were fed an experimental diet for three weeks. The diet consisted of leaves of *Millettia ferruginea*, mixed hay and concentrates (36%, 61%, and 3%), respectively on dry matter (DM) basis), and was offered based on a DM intake of 3% of body weight (BW). After two weeks of adaptation, the total fecal collection was performed for one week, followed by overnight fasting, slaughtering and collection of the digesta as well as pH measurements in different segments of the gut. Feeds, digesta and fecal samples underwent proximate analysis, and voluntary feed intake (VFI) and BW gain were determined. Differences between sheep and goats and between segments were evaluated using a linear mixed model. The VFI in relation to BW of both *M. ferruginea* leaves and hay was higher in sheep than in goats. Digesta crude protein (CP) in the rumen expressed on DM was higher in goats than in sheep, however apparent digestibility of CP was similar between species. Digesta acid-detergent fiber (ADF) concentrations in the reticulum were higher in sheep than in goats. Goats consistently had higher pH values than sheep in all gastrointestinal segments, except for the abomasum. In the present study, tropical sheep seem to outperform tropical goats on a high-fiber, tannin diet, hence questioning the hypothesis that tropical sheep are less adapted to tannin-rich diets than tropical goats.

Introduction

Feed scarcity and poor quality of the available feedstuffs are major constraints for ruminant productivity in the tropics (Franzel et al., 2014). In Ethiopia, 85% of the feed consumption in the smallholder livestock production system is used to meet maintenance requirements while merely 15% is used for animal production (Tolera et al., 2012). This situation is exacerbated during the dry season when natural pastures dry out and are overgrazed and hence, unpalatable forages with low nutrient content are predominantly posing a challenge for ruminants' nutrition, health, and welfare (Distel and Villalba, 2018).

Agrawal et al. (2014) indicated that sheep and goats have the ability to utilize a wide range of unconventional tropical feed resources such as leguminous and non-leguminous fodder trees as well as shrubs (Sujani et al., 2017). However, the potential use of these shrub and tree plants is limited either by their low palatability or the presence of plant secondary compounds such as condensed tannins (Sujani et al., 2017), and other polyphenols that may contribute to 50% of the organic matter (OM) (Reed, 1986).

In the grazer-browser dichotomy theory, sheep are seen as grazers whereas goats are classified as intermediate browsers, with better ability to process substantial amounts of browse leaves that might contain tannins (Salem et al., 2013). Several studies have shown that goats have a higher tolerance for bitter taste than sheep and cattle (Agrawal et al., 2014). Goats also differ from sheep as they select forage species in a complementary way to maximize nutrient intake and minimize the ingestion of secondary compounds to prevent intoxication (Egea et al., 2019).

In tropical regions facing overgrazing and drought, all animals, including assumed grazers, need to turn to drought-resistant feed resources such as trees and shrubs (Achonwa et al., 2017). It is therefore not unlikely that even grazers might have developed coping strategies for low-quality feeds. The present study was therefore designed to compare the digestive responses of tropical Kefa goats and Bonga sheep to a diet of leaves of *Millettia ferruginea* (a low-palatable and tanniferous browse) combined with fibrous hay, to test the hypothesis that not only tropical browsers but also tropical grazers may show ability to ingest and digest a fibrous and tanniferous diet.

Material and Methods

Study Area

This study was carried out at Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM) campus, Jimma, southwestern Ethiopia. Jimma city is situated at 7°40′N and 36°50′E at an altitude of 1780 (m.a.s.l). The area is characterized by a humid tropical climate with bimodal heavy rainfall ranging from 1200 to 2800 mm per year (Jimma Zone, 2017).

Experimental feed

The ration was composed of 36% of leaves of *Millettia ferruginea* (containing 5% of total tannin on DM basis), 61% of mixed hay and 3.0% of concentrate on DM basis. The locally harvested mixed hay consisted of among others and in declining order of importance, *Satoria verticillate*, *Eleusine coracana*, *Phyllanthus amarus* and *Cyperus rotundus*. Additionally, leaves of *M. ferruginea* were hand-plucked from different trees, pooled, and air-dried for about 5-7 days in the shade. After the drying, the twigs were removed and only the leaves were fed to the animals. To prevent mold, the leaves were mixed daily. The chemical composition and tannin content of the *M. ferruginea* leaves, basal hay mixture and concentrate are shown in Table 1.

Table 1. Chemical composition of the *M. ferruginea* leaves, hay mixture and concentrate used in the experimental diet. Dry matter (DM) is expressed in g/100g fresh material, whereas all the other chemical parameters are expressed as % DM.

Feedstuffs	**DM**	**CA**	**CP**	**EE**	**NDF**	**ADF**	**ADL**	**AIA**	**TT**
Hay	91.9	9.79	6.42	0.980	43.2	38.9	18.5	0.400	1.02
Leaves	92.4	8.33	19.8	3.68	46.1	42.5	25.3	1.60	5.41
Concentrate	90.3	8.86	20.6	5.54	16.8	8.9	0.2	4.80	0.277

Crude ash (CA), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), acid insoluble ash (AIA), total tannin (TT).

Animals and Management

Six male adult Keffa goats (mean BW ± standard deviation (SD): 26.4 kg ± 2.15) and six male adult Bonga sheep (30.3 kg ± 1.57), with similar body condition ranging between 12 to 19 months of age, based on dental inspection (Casburn, 2016), were purchased from a local livestock market of Seka town in Jimma, Ethiopia. The animals were transported to the animal facilities of JUCAVM. Shortly after arrival, animals were ear-tagged and numbered. All animals were housed in individual pens with unlimited access to clean water in a well-ventilated barn with a concrete floor throughout the whole trial period. They were left outside to graze for the first three days to let them adapt to their new environment. Next, to assure a steady-state of the metabolism of the animals, they were allowed to adapt to the experimental diet for eleven days (see further below). During this adaptation period, regular health check-ups were performed. Seven days after arrival, all twelve animals were dewormed with a 1.0 ml subcutaneous injection of 1.0% Ivermectin (Shanghai Tongren Pharmaceutical Co. Ltd.) and due to the nasal discharge and slight irregular breathing observed in one of the sheep, 5.0 ml 20% oxytetracycline (® Oxyvic 20) was administered intramuscularly.

The animals received hay mixture *ad libitum* twice a day, around 10:00h and 16:00h. To this hay, approximately 100-150 g of tannin-rich leaves of *M. ferruginea* were added. The concentrate (granulate) was offered to the animals once in the morning around 8:00h before hay supplementation. The overall amount of feed offered to each animal was calculated based on a DM intake set at 3.0% of their BW (Rashid, 2008b; Squires, 2010) and was in line with maintenance protein requirements for sheep and goats (NRC, 2007). The weight of all animals was registered at the beginning and the end of the experimental period.

Total fecal collection

The total fecal collection was carried out for the last seven consecutive experimental days, using feces collection bags harnessed to the animals. During the seven days of the experimental period, all feces produced was collected every morning from the fecal collection bags and weighed, and then a sub-sample of about 10% of the total amount per animal was collected and stored at -20°C until the end of the trial.

The feces samples were dried and milled (Hanna Instrument, Germany) to pass through a 1.0 mm screen and were stored in an airtight container at 25°C, until analysis (AOAC, 2005). The amount of feed offered and refused was collected every morning before feeding per animal and weighed daily. Representative samples were milled in a Wiley Mill (Hanna Instrument, Germany) to pass through a 1.0 mm screen, and the ground material was stored in an airtight container at 25°C until analysis.

Digesta and Sampling

At the end of the experimental period, the animals were fasted overnight and weighed. The animals were slaughtered and digesta samples from eight different segments of the gastrointestinal tract: rumen, reticulum, omasum, abomasum, jejunum, caecum, and colon were collected. Immediately after slaughtering of the animals, the whole gastrointestinal tract was removed, and tightened by string to prevent the missing of digesta then representative samples of each gut content was filled into plastic vials. Before the collection of digesta samples, the pH of each segment was determined using an electronic pH-meter (Hanna Instrument, Singapore). For the other animals, the digesta was first collected in plastic bags and pH measured in these plastic bags in the lab. No systematic differences were however observed between the two ways of measuring. After measuring pH, subsamples from the digesta content were taken and all samples were directly stored at -20°C in plastic bags for approximately 12h until processed. The following day, the frozen samples were oven-dried at 45°C for 1-3 days, until a constant weight was reached. The dried samples were milled (Retsch Haan, Germany) to pass through a 1.0 mm screen, and the ground materials were stored in an airtight container at 25°C, until analysis.

Chemical analysis

Provided feeds (hay, leaves, concentrate), as well as digesta, and fecal samples underwent proximate analysis (AOAC, 1990) and fiber analysis (Van Soest et al., 1991).

Calculations

The intake of dry matter (DM), crude protein (CP), acid detergent fiber (ADF), acid detergent fiber (ADL) and total tannin (TT) was calculated based on the concentrations in the feed offered from which the feed refusals were deducted. Apparent nutrient digestibilities were calculated using AIA according to Sales and Janssens (2003).

$$\text{Apparent absorption } = 1 - \frac{\%\text{ AIA in feed}}{\%\text{ AIA in faeces}}\, x \,\frac{\%\text{ nutrient in the faeces}}{\%\text{ nutrient in feed}}$$

Data analysis

All data were analyzed using SPSS version 24. Differences in intake, BW gain and nutrient concentrations between sheep and goats and between sample sites were evaluated using a linear mixed model, with animal species, sample site of the intestinal tract, and their interaction as fixed factors and animal as a random factor. Gut segments were considered as within-subject variables in the repeated measures variance analysis, with species as between-subject variable. An unpaired t-test was performed to explore significant differences between sheep and goats. The significance level was set at $p \leq 0.05$.

Results

Feed Intake

Table 2 shows the average feed intake (g/day) on a dry matter (DM) basis for both hay, leaves and concentrates. Goats consumed significantly less hay and fewer leaves of *M. ferruginea* compared to sheep.

Table 2. Average intake per day (g) and body weight and body weight change (kg) in tropical goats and sheep before and after the experimental tannin-rich diet, expressed both in kg and proportionally to the initial body weight (n=6).

	Goat		Sheep		
	Mean	SE	Mean	SE	*P*
Intake of hay, g /day LW$^{0.75}$	31.2	1.1	36.8	1.3	0.008
Intake of leaves, g /day LW$^{0.75}$	13.0	0.99	20.4	1.90	0.006
Concentrate, g /day LW$^{0.75}$	2.1	0.020	2.1	0.010	0.068
Body weight at start, kg	26.4	1.00	30.3	0.700	0.009
Body weight at end, kg	24.3	0.750	29.9	0.600	<0.001
Body weight loss, kg	2.08	0.600	0.400	0.300	0.020

Nutrient Intake

Table 3 shows the nutrient intake (g/day) on a dry matter (DM) basis from *Millettia ferruginea* leaves, hay mixture and concentrate. Goats consumed significantly less DM, CP, ADF, ADL and TT compared to sheep.

Table 3. Intakes of DM, CP, ADF, ADL and TT (g /kg LW $^{0.75}$) from *Millettia ferruginea* leaves, hay mixture and concentrate in tropical sheep and goats.

	Goat		Sheep		
Nutrient intake	Mean	SE	Mean	SE	P
DM	462	1.4	594	2.4	< 0.001
CP	5.4	0.02	7.3	0.04	0.002
ADF	19.4	0.06	25.2	0.11	< 0.001
TT	1.0	0.01	1.5	0.01	0.003
ADL	98	0.34	129	0.59	< 0.001

Body Weight

The loss in BW during the experiment was higher for the goats than for the sheep (Table 2). Indeed, goats had an average decrease of 2.08 kg (or 8% of the initial body weight), while the sheep only had an average decrease of 0.4 kg (or 1.3% of the initial body weight).

Apparent digestibility

Figure 1 shows apparent DM, CP, and ADF digestibility was not significantly different between sheep and goats ($p > 0.05$).

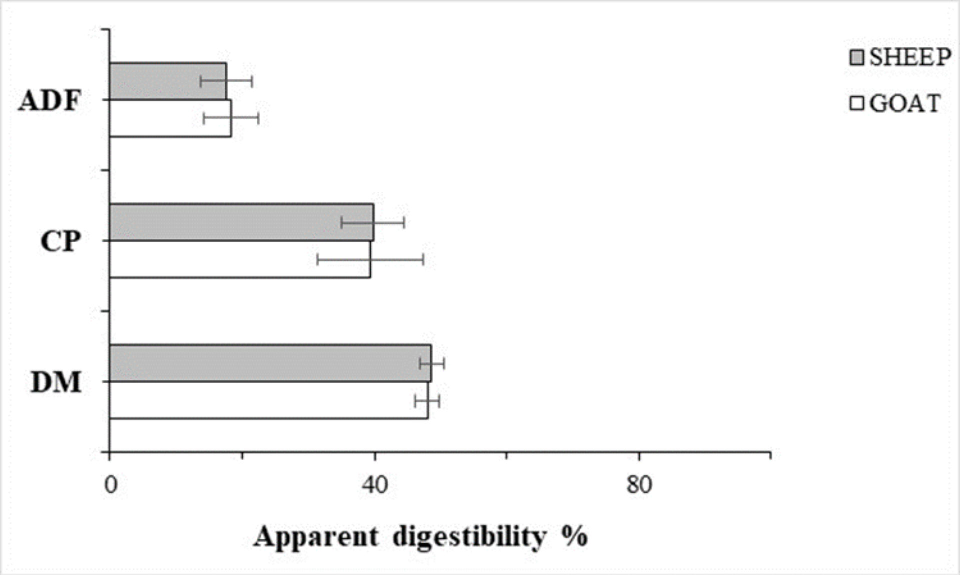

Figure 1. Mean apparent digestibility of DM, CP and ADF of tropical goats (n=6) and sheep (n=6) DM dry matter, CP crude protein, ADF acid detergent fiber

Fiber Digestion

Table 4 shows that ADF concentrations on DM basis remained rather constant throughout the gastrointestinal tract with the lowest concentrations found in the abomasum and jejunum, for both sheep and goats. In the reticulum, sheep had a higher ADF concentration on DM basis than goats (p = 0.022). A significant interaction between gut segment and species was found for ADF, where sheep had consistently higher concentrations of ADF than goats, except for caecum and colon, where ADF concentrations were lower for the sheep than for the goats. However, a significant difference between species was only achieved in the reticulum, where sheep had a higher ADF concentration than goats. Similarly, to what was observed for ADF, ADL concentrations remained rather constant throughout the gastrointestinal tract, with the lowest concentrations found again in the abomasum and jejunum for both sheep and goats. For ADL there was no significant interaction between the gut segment and the species, like for ADF. However, ADL concentrations were significantly higher in sheep in the reticulum and the omasum than for goats.

Protein Digestion

Table 4 shows that crude protein (CP) concentrations varied more than ADF and ADL concentrations across the gastrointestinal sites, where CP concentrations increased from the rumen up to the abomasum and declined again up to the feces. There was a trend for interaction between gut segment and species, with sheep having higher CP concentrations in all gut segments than goats, except for the rumen, where goats had significantly higher CP concentrations than sheep.

Table 4. Crude protein (CP) and acid detergent fiber (ADF) concentrations expressed in g/100 g DM in the different gastrointestinal segments of tropical goats and sheep fed a mixture of fibrous hay, concentrates and tanniferous *Millettia ferruginea* leaves.

Gut segment	Variable	Species		SEM
	(g/100g DM)	Goat	Sheep	
Rumen digesta	CP	15.18^{b}	13.18^{a}	0.79
	ADF	52.07	52.65	1.07
	ADL	21.44	20.48	1.16
Reticulum digesta	CP	14.78	15.58	0.79
	ADF	48.29^{b}	52.07^{a}	1.21
	ADL	20.35^{b}	22.62^{a}	0.93
Omasum digesta	CP	15.83	16.98	0.68
	ADF	49.49	51.71	1.12
	ADL	21.37^{b}	23.35^{a}	0.92
Abomasum digesta	CP	19.41	19.88	0.64
	ADF	42.19	35.26	1.06
	ADL	15.96	13.93	0.57
Jejunum digesta	CP	17.68	18.14	2.71
	ADF	35.26	37.50	3.21
	ADL	13.93	14.71	1.50
Caecum digesta	CP	12.69	11.91	0.59
	ADF	52.23	51.80	0.76
	ADL	23.14	23.06	0.54
Colon digesta	CP	12.12	12.29	0.47
	ADF	53.51	51.01	1.66
	ADL	22.96	21.91	1.03
Feces	CP	10.77	11.21	0.71
	ADF	52.65	53.03	1.04
	ADL	20.41	21.47	0.97

Means with different superscripts in the same column are significantly different at ($P<0.05$). DM dry matter, CP crude protein, ADF acid detergent fiber, ADL acid detergent lignin.

Digesta pH

The mean pH value differed between the different sites of the gastrointestinal tract ($p = 0.001$; Figure 2). There was also a significant effect of species for digesta pH ($p = 0.018$). Goats consistently had higher digesta pH values than sheep, achieving a significant difference in the reticulum ($p= 0.004$) and colon ($p = 0.016$).

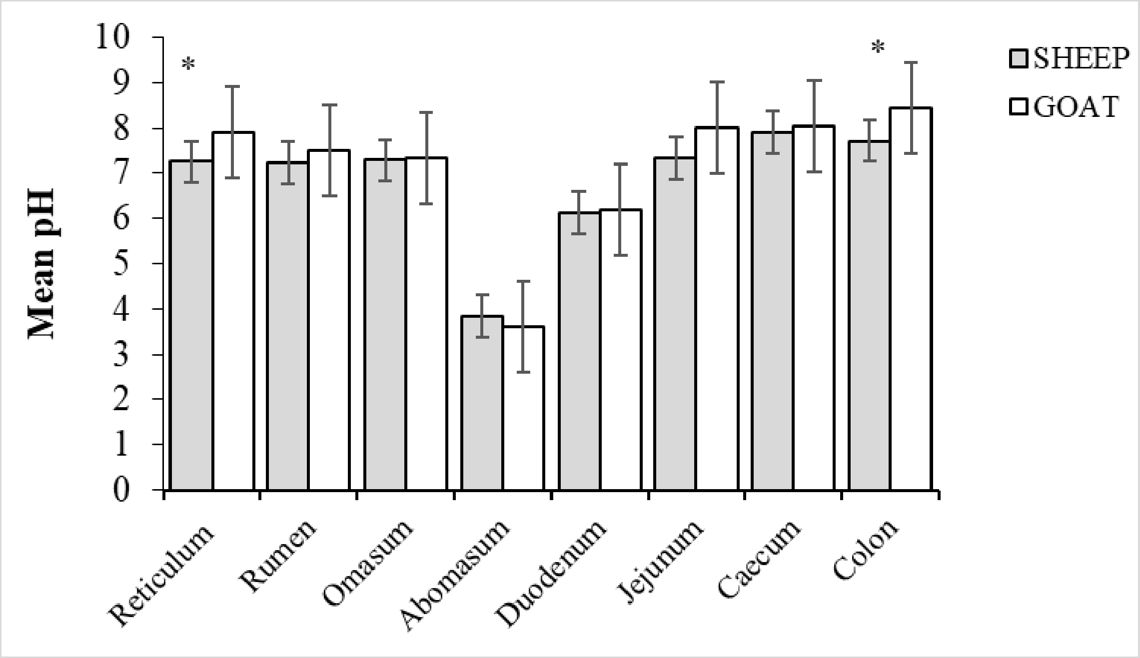

Figure 2. Mean pH levels of the different segments of the gastrointestinal tract of both tropical goats (■) and sheep (■) (n=6) * indicate a significant difference between species ($p<0.05$). Sampletype × species= 0.047

Discussion

The diet in the present study was based on typical ingredients of a fibrous tanniferous diet in Ethiopia. Based on previous comparative studies, the hypothesis was that goats would consume larger amounts of browse leaves than sheep under similar conditions (Narvaez et al., 2012), possibly due to mechanisms such as the secretion of proline-rich proteins in saliva as a response to the consumption of secondary compounds in their diet to alleviate their aversive effects (Salem, 2011). When goats have the choice to choose what to eat, they will select high-quality plants or plant parts in their diet (Rogosic et al., 2006), allowing them to get enough net energy and proteins to meet their requirements, something that sheep fail to achieve as grazers (Jozo et al., 2018). However, when goats have fewer options as to when they are fed in pens and during the dry season under reduced forage availability, they must optimally utilize these available options to meet their nutritional needs. As a result, this would decrease the level of intake, as goats often spend a lot of time trying to select high-quality material if the feed offered or available is of uniformly low quality (Van, 2006). Estell (2010) demonstrated that captive herbivores can modify their intake patterns, by reducing intake rate, or switch their diet composition as an important strategy to minimize the consumption of phenolic compounds (Mkhize et al., 2018). Therefore, it appears that stall-feeding impairs selective feeding behavior and total intake (Alcaide et al., 2000).

Despite the above-mentioned differences in browse utilization by sheep and goats, the amount of feed consumed by sheep in the present study was higher than that of goats for *Millitia ferruginea* leaves hay mixture and concentrates. It was also observed that goats did not compensate by increasing the intake of browse free hay mixture diets to increase the intake of nutrients. Santra et al. (1998) also found that under controlled feeding, DMI was lower in goats than in sheep (57.1 g versus 62.1 g/kg $W^{0.75}$), maybe because the controlled feeding limited the selective ability of goats (Van, 2006). Moreover, the weight gains in goats can be lower than other livestock when goats are fed in confinement situations (McDowell and Woodward, 1982).

Clauss et al. (2010, 2017) have demonstrated that a higher feed intake in sheep might be due to a lack of time for selecting high-quality feed, and less time for mastication, and shorter ingesta retention. As an animal eats more DM, solid material entering the rumen accumulates; this bulky DM builds up more pressure on the rumen contents and as a result forces liquid out of the rumen at a much faster rate compared to low intake levels (Moyo and Nsahlai, 2018).

In contrast, goats consumed higher DM than sheep in the condition where sheep showed faster passage rates of solid digesta than goats (Snyman, 2007). This could be due the fact that goats possesses a unique characteristic of being able to maintain larger rumen fill levels without noticeable rumen distension than sheep when fed medium or low-quality diets (Moyo and Nsahlai, 2018). Demment and Van Soest (1985) also explained that the association between intake and retention time can be explained as a function of gut capacity that increases with body weight, indicating different feeding strategies adopted by sheep and goats.

The disappearance of CP throughout the gastro-intestinal tract was low for both species. The high concentrations of CP (about 15%) in the pre-absorption sites (reticulum, rumen, and omasum) typically reflect the sum of dietary and microbial protein and dropped to an average of about 12% in the post-absorption sites (caecum, colon, and feces). The high CP concentrations found in the abomasum and the jejunum can be explained by enzymes in these particular parts of the digestive tract added to digested feed proteins. Absorption of amino acids from protein digestion is the likely explanation for the decrease after passing the jejunum. Hence, there is an indication of protein degradation and absorption. An increase in CP concentrations in rumen and caecum – the two main fermentation sites – in the goats compared with the sheep was observed. However, the low apparent DM, CP, and ADF digestibility in both goats and sheep might be associated with poor fermentation of OM content of the ingested low-quality feed in the rumen rather than the absolute dietary CP content of feedstuff (Adejoro, 2019). The feeding value of *Millettia ferruginea* leaf seemed to be very low, thus unless the leaves are treated, their contribution to the nutritional status of sheep and goats is extremely low.

In tropical countries such as Ethiopia, it seems that sheep change their feeding strategy upon low nutritional value diets from specialists to generalists, hence becoming exposed to high phenolic compounds or lignin-containing plants (Marais, 2012) due to the necessity of maintaining nutrient intake (Egea et al., 2014; Castro and Fernandez-Nuñez, 2016). Although Jerónimo et al. (2016) indicated that sheep are incapable of salivary proline-rich proteins (PRP) synthesis like goats, long exposure to browses can induce adaptation mechanisms to such modifications of salivary PRP (Taha, 2015).

The time of exposure can be a key factor in how well this works (Marais, 2012): it can take up to several months of exposure before gut microbes have adapted (Arthur et al., 2000), but they can also respond rapidly within a few days to some plant extracts included in the diet (Duncan et al., 1997).

Thus, the apparent differences between sheep and goats might have been due to the different exposure and adaptation of the two species to a similar diet before the experiment. This effect can be attributed to the animals' conditioning to the low-quality tannin-rich forages taste and its post-ingestive feedback (Hadjigeorgiou et al., 2003; Provenza et al., 2003). For instance, unexperienced or naive animals rely more on pre ingestive feedback mechanisms to make decisions on preference towards novel feeds, while previous post ingestive feedback (positive or negative) due to consumption of high fiber or tannin-rich diets determines the preference and intake of similar forages (Mengistu, 2017). It is also important to note that the lower feed intake for goats may be due to the fact that all animals were given hay *ad libitum*: a typical grazers' diet during the experimental period. Haddad and Obeidat (2007) studied the feeding behavior and growth rate of Awassi lambs and Baladi kids that were fed for a period of 60 days, and observed significantly higher intake and average daily weight gain in lambs than kids, suggested that better nutrient digestibility in kids than in lambs might be the reason.

It has been indicated that increased intake of low-quality fibrous diet would cause a fast movement of digesta throughout the entire gastrointestinal tract, which possibly results in a reduced digestibility, but the net intake of digestible material would be maintained constant (Hadjigeorgiou et al., 2003). Thus, the strategy of sheep to cope with such types of diets may, therefore, be to increase feed intake in order to retain enough nutrients and energy. According to Egea et al. (2014), grazing herbivores can cope with variations in nutrient demands through an increase in DM intake in order to maintain nutrient (protein and energy) intake and maintaining their body weight (Van, 2006). The reduction in the amount of DM available for digestion may limit OM digestibility, decrease rumen turnover rate or slow the removal of feed residues from the rumen and increases rumination (McSweeney and Kennedy, 1992; Dludla, 2010; Aboagye, 2019). This might be attributed to the time spent chewing during eating and rumination which was longer in goats (1.92%) in relation to sheep (0.56%). Therefore, goats showed a longer rumination time and a slow removal of particulate dry matter than sheep (McSweeney and Kennedy, 1992), which may explain the reduction of voluntary feed intake in goats.

Anyhow, the strategy of the sheep to increase feed intake seemed more successful in this case than that of the goats as sheep succeeded in maintaining their body weight throughout the trial compared to the goats.

Conclusions

The present tropical dry season livestock diet containing a tannin-rich plant and high-fiber hay led to low digestibility with concomitant loss of body weight in both goats and sheep. Yet, sheep showed a higher intake than goats, leading to a smaller bodyweight loss. The study does not necessarily claim that tropical sheep will always perform better than tropical goats on tannin-rich diets, but at least it shows the opposite is not always the case, leaving scope for research on the exact mechanisms that tropical sheep use to cope with low-quality high fiber diets.

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References

Aboagye, I.A., 2019. Mitigating enteric methane production and nitrogen excretion from forage-fed ruminants (PhD Thesis). University of Alberta.

Achonwa, C.C., Ogbuewu, I.P., Ogundu, E.C., Kubkomawa, I.H., Uchegbu, M.C., Okoli, I.C., 2017. Browse abundance and the methodologies for their selection as candidate feed resources in Nigeria: A review. Nature and Science 15, 82-94.

Adejoro, F.A., 2019. The use of condensed tannins and nitrate to reduce enteric methane emission and enhance utilization of high-forage diets in sheep (PhD Thesis). University of Pretoria.

Agrawal, A.R., Karim, S.A., Kumar, R., Sahoo, A., John, P., 2014. Sheep and goat production: basic differences, impact on climate and molecular tools for rumen microbiome study. International of Journal Current Microbiol Applied and Science 3, 684–706.

Alcaide, E.M., García, A.M., Aguilera, J.F., 2000. A comparative study of nutrient digestibility, kinetics of degradation and passage and rumen fermentation pattern in goats and sheep offered good quality diets. Livestock Production Science 64, 215–223.

AOAC, 2005. Official Methods of Analysis of the Official Analytical Chemists, 18th ed. Association of Official Analytical Chemists. Arlington, VA. USA.

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists. Arlington, VA. USA

Arthur, C.M., Hagerman, A.E., Robbins, C.T., 2000. Physiological strategies of mammalian herbivores against plant defenses. Plant defenses against mammalian herbivory 103–114.

Casburn, G., 2016. How to tell the age of sheep. Department of Primary Industries. Wagga Wagga, New South Wales.

Castro, M., Fernandez-Nuñez, E., 2016. Seasonal grazing of goats and sheep on Mediterranean mountain rangelands of northeast Portugal. Livestock Research for Rural Development 28.

Clauss, M., Fritz, J., Tschuor, A., Braun, U., Hummel, J., Codron, D., 2017. Dry matter and digesta particle size gradients along the goat digestive tract on grass and browse diets. Journal of animal physiology and animal nutrition 101, 61–69.

Clauss, M., Hume, I.D., Hummel, J., 2010. Evolutionary adaptations of ruminants and their potential relevance for modern production systems. Animal 4, 979–992.

Clauss, M., Hummel, J., 2017. Physiological adaptations of ruminants and their potential relevance for production systems. Revista Brasileira de Zootecnia 46, 606–613.

Demment, M.W., Van Soest, P.J., 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. The American Naturalist 125, 641–672.

Distel, R.A., Villalba, J.J., 2018. Use of Unpalatable Forages by Ruminants: The Influence of Experience with the Biophysical and Social Environment. Animals 8, 56.

Dludla, S.P., 2010. The effect of condensed tannins on goats' body weight (PhD Thesis). University of Zululand.

Duncan, A.J., Frutos, P., Young, S.A., 1997. Rates of oxalic acid degradation in the rumen of sheep and goats in response to different levels of oxalic acid administration. Animal Science 65, 451–455.

Egea, A.V., Allegretti, L., Lama, S.P., Grilli, D., Sartor, C., Fucili, M., Guevara, J.C., Passera, C., 2014. Selective behavior of Creole goats in response to the functional heterogeneity of native forage species in the central Monte desert, Argentina. Small Ruminant Research 120, 90–99.

Egea, Á.V., Bakker, M.L., Allegretti, L.I., Paez, S.A., Grilli, D.J., Guevara, J.C., Villalba, J.J., 2019. Seasonal changes in feed intake, diet digestibility and diet composition by lactating and non-lactating goats browsing in a semi-arid rangeland of Argentina. Grass and forage science 74, 115–128.

Estell, R.E., 2010. Coping with shrub secondary metabolites by ruminants. Small Ruminant Research 94, 1–9.

Franzel, S., Carsan, S., Lukuyu, B., Sinja, J., Wambugu, C., 2014. Fodder trees for improving livestock productivity and smallholder livelihoods in Africa. Current Opinion in Environmental Sustainability 6, 98–103.

Haddad, S.G., Obeidat, B.S., 2007. Production efficiency and feeding behavior of Awassi lambs and Baladi kids fed on a high concentrate diet. Small ruminant research 69, 23–27.

Hadjigeorgiou, I.E., Gordon, I.J., Milne, J.A., 2003. Intake, digestion and selection of roughage with different staple lengths by sheep and goats. Small Ruminant Research 47, 117–132.

Jerónimo, E., Pinheiro, C., Lamy, E., Dentinho, M.T., Sales-Baptista, E., Lopes, O., Silva, F., 2016. Tannins in ruminant nutrition: Impact on animal performance and quality of edible products In Tannins: Biochemistry, Food Sources and Nutritional Properties; Combs, C.A., Ed.; Nova Science Publisher Inc.: Hauppauge, NY, USA, 121-168

Jimma Zone, 2017. URL https://en.wikipedia.org/wiki/Jimma Zone

Jozo, R., Ralph, M., Musa, A., Skobic, D., Krvavica, M., Arapovic, M., 2018. Goat preference for phylogenetical diverse compared to similar Mediterranean shrubs. Journal of Mediterranean Ecology 16, 5–13.

Marais, P., 2012. Effects of condensed tannins on browsers and grazers. Grootfontein Agricultural Development Institute, Middelburg.

McDowell, R.E., Woodward, A., 1982. Concepts in animal adaptation: (Comparative suitability of goats, sheep, and cattle to tropical environments), in: Proceedings. International Conference on Goat Production and Disease. Dairy Goat Journal Publishing Co., Scottsdale, AZ (EUA).

McSweeney, C.S., Kennedy, P.M., 1992. Influence of dietary particle size on chewing activity and reticulo-ruminal motility in goats and sheep fed wheaten (Triticum aestivum) hay. Small ruminant research 9, 107–115.

Mengistu, G.F., 2017. Browse species from Ethiopia: role in methane reduction and nematode control in goats. (PhD thesis), Wageningen University.

Mkhize, N.R., Heitkönig, I.M., Scogings, P.F., Hattas, D., Dziba, L.E., Prins, H.H., De Boer, W.F., 2018. Seasonal regulation of condensed tannin consumption by free-ranging goats in a semi-arid savanna. PloS One 13, e0189626. https://doi.org/10.1371/journal.pone.0189626

Moyo, M., Kana, E.G., Nsahlai, I.V., 2017. Modelling of digesta passage rates in grazing and browsing domestic and wild ruminant herbivores. South African Journal of Animal Science 47, 362–377.

Moyo, M., Nsahlai, I.V., 2018. Rate of Passage of Digesta in Ruminants; Are Goats Different? Goat science, Kukovics, S., Ed.; IntechOpen: London, UK, 39-74. DOI: 10.5772/intechopen.69745

Narvaez, N., Brosh, A., Pittroff, W., 2012. Use of n-alkanes to estimate seasonal diet composition and intake of sheep and goats grazing in California chaparral. Small Ruminant Research 104, 129–138.

Okine, E.K., Mathison, G.W., 1991. Reticular contraction attributes and passage of digesta from the ruminoreticulum in cattle fed roughage diets. Journal of animal science 69, 2177–2186.

NRC, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World camelids. Washington, D.C: National Academies Press.

Rashid, M., 2008. Goats and their Nutrition. Manitoba Agriculture, Food and Rural Initiatives.

Reed, J.D., 1986. Relationships among soluble phenolics, insoluble proanthocyanidins and fiber in East African browse species. Journal of Range Management 39, 5–7.

Rogosic, J., Pfister, J.A., Provenza, F.D., Grbesa, D., 2006. Sheep and goat preference for and nutritional value of Mediterranean maquis shrubs. Small Ruminant Research 64, 169–179.

Salem, A.-F.Z., 2011. Plant-Phytochemicals in Animal Nutrition. Nova Science Publishers. New York.

Salem, A.Z.M., López, S., Ranilla, M.J., González, J.S., 2013. Short- to medium-term effects of consumption of quebracho tannins on saliva production and composition in sheep and goats. Journal of Animal Science 91, 1341–1349. https://doi.org/10.2527/jas.2010-3811

Sales, J., Janssens, G.P.J., 2003. Acid-insoluble ash as a marker in digestibility studies: a review. Journal of Animal and Feed Sciences 12, 383–401.

Santra, A., Karim, S.A., Mishra, A.S., Chaturvedi, O.H., Prasad, R., 1998. Rumen ciliate protozoa and fibre utilization in sheep and goats. Small Ruminant Research 30, 13–18.

Snyman, L.D., 2007. Qualitative characteristics of selected Atriplex nummularia (Hatfield Select) (PhD Thesis). University of Pretoria.

Squires, V.R., 2010. Nutrition of small ruminants on rangelands. Range and Animal Sciences and Resources Management-Volume II 25.

Sujani, S., Perera, A., Seresinhe, T., 2017. Tannin binding agents enhanced the *in vitro* rumen fermentation parameters of tanniniferous fodder. International Journal of Agricultural Sciences and Veterinary Medicine 5, 86 -96.

Taha, V., 2015. Effect of supplemental tannin on silage quality and animal performance (PhD Thesis). Harper Adams University.

Tolera, A., Yami, A., Alemu, D., 2012. Livestock feed resources in Ethiopia: challenges, opportunities and the need for transformation. Ethiopia Animal Feed Industry Association, Addis Ababa, Ethiopia.

Van, D.T.T., 2006. Some animal and feed factors affecting feed intake, behaviour and performance of small ruminants (Doctoral thesis). Swedish University of Agricultural Sciences.

Van Soest, P. van, Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of dairy science 74, 3583–3597.

CHAPTER 6

General Discussion

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General discussion

6.1. Background and outline

Ruminant species in tropical areas such as Ethiopia (Dermauw et al., 2013) and organic dairy cows in a temperate climate in Belgium (Sobry et al., 2012) are challenged by shortages of microminerals if not supplemented in their diet. The absorption of minerals from dietary forage in function of animal species, breed and age characteristics (Dermauw et al., 2014; Katulski, 2017), nature of the mineral source (Faulkner and Weiss, 2017; Miller et al., 2020a, 2020b) and season (Mohammed et al., 2016) has already been studied. However, little is known on the kinetics of microminerals throughout the digestive system when facing common challenges such as high amounts of fiber, tannins and S. Therefore, the present thesis work evaluated the impact of these dietary inhibitory constituents on fermentation, digestion and micromineral bioavailability in ruminants.

6.2. Adaptations of tropical ruminants to tropical conditions

The high levels of cell walls and lignin usually found in tropical forages and ruminant diets can include highly lignified fibrous feeds (Van Soest, 1982), which inversely relate to the digestibility of forages (Mathobela, 2018). In the studies presented in this thesis, it has been observed that intake of tanniferous diets is higher in tropical Bonga sheep compared to tropical Kefa goats expressed on the metabolic body weight basis. This seems somehow in contrast with many studies indicating that goats perform better in tropical conditions specifically during the dry season when available feed is scarce in terms of quantity and quality due to seasonal differences in feed availability or overgrazing. The lack of noticeable intake by goats therefore not necessarily contradicts the hypothesis that goats are better in digesting low-quality tropical roughage feeds, because compared to the sheep, goats showed significantly higher CP concentrations in the main fermentation sites of their digestive tract (e.g. rumen and caecum) (Chapter 5). This might be related to differences in terms of the amount or the proportion of rumen fibrolytic microbial species that have the capacity to overcome the detrimental effects of tannin-rich diets through the ability to degrade such type of low-quality feeds in their rumen compared to sheep.

Despite the higher investment of goats in the digestion of proteins from this low-quality diet, the higher weight loss compared to sheep might be due to their low feed intake. Changes in voluntary feed intake of a feedstuff have been associated with the minimum dietary CP level required (> 8% DM) to maintain the rumen ammonia threshold (> 70 mg ammonia N/L) and for optimal rumen microbial fermentation and protein synthesis (Martin, 2016). The fact that goats have the ability to avoid the toxic effects of a high-tannin and high-fiber diet may reduce the total intake. This can be a possible reason for the difference in feed intake between the species, possibly leading to lower dietary CP intake in goats compared to sheep (5.4 vs. 7.3 (g /kg LW$^{0.75}$) as observed in Chapter 5 (Table 3). When the level of CP in forages drops below 8 %, appetite is suppressed and pasture intake by the animal is reduced (Snyman, 2007). The effect becomes more significant when feed availability is scarce in terms of quality and quantity. This can be more pronounced in tropics where ruminants consume diets that contain diverse level and forms of tannin (Kara, 2019).

Moreover, the significantly higher reduction of BW in goats compared to sheep throughout the experimental period (2.1 Vs. 0.4 kg) (Chapter 5, Table 2), can be also related to a difference in nutrient (e.g. CP) intake between species. It is known that when goats are fed in confinement situations or under controlled stable feeding their selective feeding behavior could be impaired (Alcaide et al., 2000), with their weight gain and feed efficiency becoming lower than for other ruminants (Nasrullah et al., 2013). When the requirements for maintenance become a very large proportion of the metabolisable energy consumed, this will result in poor efficiency of feed conversion (Forbes, 1995).

Since feed intake is primarily related to nutrient needs (Van, 2006), the necessity to maintain nutrient intake might force sheep to increase the intake of more lignin or tannin-containing feedstuffs in their diets (Chapter 5). This strategy may be related to the fact that sheep seem to be more successful in increasing their feed intake (to render enough protein, minerals and energy), in order to still maintain their body weight. In ruminants, the decline in NDF digestibility is related to a slow clearance of fiber in which the rate of clearance of undigested feed from the rumen affects the whole tract digestibility (Campbell, 2019). In our trials, the relatively higher intake of indigestible ADF fraction (cellulose, lignin) by sheep than goats (19.4 vs. 25.2) g /kg LW$^{0.75}$ and ADL (98 vs. 129) g /kg LW$^{0.75}$ (Chapter 5, Table 3), will also result in a lower digestibility and a higher passage rate in sheep compared to goats. As the intake of ADF and ADL were higher in sheep than in goats, although this has to be confirmed, the OM digestibility was most probably also lower for sheep than goats, leading to a higher passage rate of feed and linked to a higher feed intake. An increase in the passage of digesta, may also result in an increase of microbial flow or microbial yield to the lower digestive tract from the reticulorumen and enhance ruminant productivity (Clauss et al., 2010; Clauss and Hummel, 2017).

In contrast to our findings, Ndosa (1980) observed a higher DMI by goats than in sheep, together with a slower passage rate of feed particles than in sheep. This higher DMI was associated with a higher rate of digestion in the rumen. Silanikove et al. (1993) also confirmed that goats can maintain a large rumen volume relative to BW, which helps them to quench against the reduction in the quality of the diet. However, from our data it is not clear if a higher passage rate in sheep has allowed higher DMI, or if the higher intake resulted in a higher passage rate.

Besides the effects of low-quality and high-tannin diets on macronutrient availability, the micromineral status of ruminants can also be affected by these diets. It is known that the bioavailability, absorption and utilization of microminerals in the rumen can be affected by the micromineral status of the animal (Baye et al., 2017). As indicated in Chapter 3 (Table 4), the production of SCFA was affected by the level of bioavailable microminerals in the rumen. Most probably this was due to enhanced growth of the rumen microorganisms that effectively utilized the minerals resulting in improved nutrient utilization (Gupta et al., 2017). However, the low apparent DM, CP, and ADF digestibility (Wanyama, 1980; Chapter 5, Figure 1) as well as the negative apparent absorption of Fe, Cu and Zn found in both sheep and goats (Chapter 4, Figure 5), might be associated with a poor OM fermentation of the ingested low-quality feed. This could mean that even high tannin and high roughage diet adapted species are not always capable of coping with severe dietary challenges. This has implications for feeding tropical livestock because it means that even if certain feed resources may still provide energy in times of scarcity such as in the dry season, the negative (long-term) consequences of depleting certain mineral stores remain.

6.3. Species differences in intestinal micromineral kinetics

Dietary intake levels have been recognized to influence the bioavailability, the extent of intestinal absorption, retention and excretion of microminerals including Cu, Fe and Zn (Vellini et al., 2020). This in turn might differ among ruminant species and breeds (Dermauw et al., 2014; Katulski, 2017) due to differences in the animal's absolute requirement and status (Gupta, 2014).

For example, the species differences in Cu, Fe and Zn concentrations in the gastrointestinal segments found in Chapter 4 could be a partial reflection of unequal intake of these elements as a result of the observed difference in DMI between species (Chapter 4, Table 2). However, the hypothesis that the higher intake might lead to increased nutrient utilization was not clearly supported by the findings in this work. For instance, the higher intake and the consistently higher Cu levels in the reticulum and rumen of sheep compared to goats does not seem to be reflected in Cu liver storage, as goats had numerically higher hepatic Cu levels than sheep (Chapter 4, Table 3).

The difference in the efficiency of mineral absorption between species is probably one of the reasons for the observed differences in liver Cu concentration. Alternatively, goats might have higher Cu needs (15–25 mg/kg DM) than sheep (5—8 mg/kg DM) and are less susceptible to Cu intoxication than sheep. In a 96-day experiment, the hepatic Cu concentration of Jianyang Big-ear goats fed diets containing high Cu concentrations (from 40 to 640 mg/kg DM) increased from 502 to 839 mg/kg DM respectively and did not induce any signs of Cu toxicity (Huang et al., 2013).

Goats can also tolerate much higher levels of Mo than cattle and sheep without suffering secondary Cu deficiency due to the antagonist effect. The indicated differences between these species in terms of Cu requirements and tolerance make Cu supplementation easier in goats and could get benefits from copper supplementation above the NRC requirements. Thus, it may be necessary to give Cu supplements to both sheep and goats, but especially when goats are co-raised with sheep.

Since higher dietary intake of Zn results in lower absorption, the higher level of Zn in the reticulorumen of sheep compared to goats (Chapter 4, Figure 3) may be due to the significantly higher Zn intake in sheep compared to goats. This in turn may result in better absorption of Zn from the reticulum of goats compared to sheep. Although there is a significant zinc absorption throughout the small intestine, a potential Zn absorption starts or occurs through the rumen till the jejunum (Mir et al., 2020) which, however, is the function of source, concentration of zinc and the resident time of feed in the reticulorumen.

Evidence on the effect of mineral bioavailability on rumen fermentation kinetics or profile was demonstrated in our study with the addition of dietary S in the diets of organic cows (Chapter 3). However, the dietary inclusion of S beyond certain concentrations is harmful to the animals and potentially affects the bioavailability of other microminerals in ruminants (Suttle, 1991). For example, a reduction in the Cu bioavailability was noted for S supplemented organic cows compared with unsupplemented cows (Chapter 3). Similarly, Golfman (1988) noted a reduction in the solubility and flow of soluble Cu for steers on high S diets compared to steers fed low S diets. Moreover, when an animal is exposed to high levels of dietary S, it may fail to synthesize the required amounts of thiamine pyrophosphate (TPP) for the brain, where high dietary S may increase the metabolic demand for TPP (Amat et al., 2014). This may lead to metabolic insufficiency of TPP and possibly to the development of S-induced malacic lesions or polio-encephalomalacia (PEM), a softening of grey matter (Drewnoski et al., 2014). The importance of thiamine in the pathogenesis of S-induced PEM is further evidenced by the findings that thiamine supplementation reduced the incidence of PEM in lambs fed high dietary S (Amat, 2012). Thiamine plays a key role in the tricarboxylic acid cycle and pentose shunt and thus may increase energy availability to the damaged brain. This suggests a metabolic relationship between excess S intake and thiamine in the development of malacic lesions.

The differences in microminerals bioavailability, absorption and utilization capacity between species are largely caused by different feeding strategies adopted across species and breeds of ruminants (e.g., browsers, grazers) (Hofmann, 1989), that are influenced by the environment where the species live (Hill and Shannon, 2019). All these factors can influence variations in the preference, intake and digestibility of feeds between species and may be important for determining adaptability to the environment. This highlights the importance of identifying the effects of further combinations between species and environmental challenges, for instance, the effect of tannins in cows in temperate regions or the effect of S in tropical goats and sheep.

6.4. The response of microminerals to dietary challenges throughout gut segments

Absorption of microminerals in ruminants primarily occurs in the small intestine, as simple diffusion or through active transport (Turner et al., 1987, 2005). However, Kirk et al. (1994) showed that the pre-intestinal region of sheep was the primary site for apparent absorption of Cu, with approximately 90% of ingested Cu being absorbed in this area. Grace (1975) and Katulski (2017) also showed that mineral absorption can occur in the forestomach and large intestine of ruminants. In the present study both sheep and goats showed lower concentration of Cu in their forestomach compared to the other gut segments (Chapter 4), which might be also due to possible absorption of Cu. In contrast, Turner et al. (1987) and Gooneratne et al. (1989) noted that reticulo-rumen or caecum is not an important site of Cu absorption in ruminants. On the other hand, during digestion, Cu in the diet becomes soluble in the reticulo-rumen, offering ideal conditions for different interactions to happen with various dietary ingredients such as antagonist minerals like S, Mo, Fe, Mn, Se, Zn and thiomolybdates (Suttle, 1991), fibers (Baye et al., 2017; Ghodrat et al., 2017) and tannins

(Naumann et al., 2017), forming insoluble complexes. These ideal conditions can be expressed in terms of either rumen pH or availability of molybdates and sulfides, which promotes the formation of complexes that tend to be irreversibly bound to the solid phase of the digestive environment (Lee and Grace, 1997). This might be also the reason for the reduced Cu concentration in forestomachs.

Moreover, the degree of interaction and level of complex formation is specific to individual mineral as indicated in Chapter 4, Zn concentrations in the forestomach region were relatively higher compared with Cu. This could be due to a higher binding affinity of Cu for fiber than Zn (Faulkner et al., 2017) or due to the lower affinity that Zn has for polyphenols such as tannins, compared to Cu and Fe (McDonald et al., 1996), particularly at neutral pH conditions (Santos-Buelga and Scalbert, 2000). Hattori et al. (2010) found a higher concentration of Zn (more than 40%) in the liquid fraction of the rumen, while there was a greater association of Cu (55% of total intake) than Zn with the rumen solid fraction of digesta in sheep (Bremner, 1970). The higher distribution of Cu, Mo and S in the solid phase of the rumen digesta (undigested plant material, bacteria and protozoa fraction) may result in the formation of different insoluble complexes (Hilal et al., 2016). This may require a longer time for maximal Cu release (Emanuele and Staples, 1990) that is not available in the passage rate seen in sheep. Thus, the minerals may not have adequate time to be solubilized and become available, a necessary step for absorption in the gut, further affecting the extent of mineral bioavailability and site of absorption throughout the gut. So, there is a high chance that this Cu will remain unavailable in the more distal portions of the digestive tract and be excreted through feces without being utilised by the animal.

Moreover, the increase in the concentration of Zn and free tannins in the abomasum (Chapter 4, Figure 4) confirms that the acidic pH in this segment disrupts the binding of Zn to tannins, allowing Zn to be absorbed. Grace and Lee (1992) found increased concentration of Zn in the liquid phase of the abomasum digesta. Similarly, compared to forestomach the concentration of CP was higher in the abomasum of both sheep and goats (chapter 5, table 2). The disassociation of minerals or proteins from the solid phase or digesta is therefore an important aspect that should be considered, especially when the presence of other dietary antagonists like tannins is high.

The microminerals in the small intestine represents not only those from feed ingredients and supplemental sources but also from endogenous sources through intestinal, pancreatic and biliary secretions. The secretion of Zn the pancreatic juice and the secretion of Cu through bile ducts in the small intestine (Hamid, 1997; Suttle, 2010) can explain the observed higher jejunal Zn and Cu concentrations compared to the abomasum and rumen for both sheep and goat (Chapter 4). Indeed, Golfman (1988) noted a net secretion of 6 to 10 mg Cu/day in steers before the proximal duodenum, where the Cu entering the proximal duodenum ranged from 135 to 185% of that ingested. Before Zn is excreted, this micromineral can still be absorbed in the large intestine, as transporters required for Zn absorption have been shown in the colon (Goff, 2018). A considerable amount of Cu and Zn absorption has been found in the large intestine of sheep (Grace, 1975; Rucker et al., 2008) and cattle (Ivan and Grieve, 1975). The observed higher levels Zn in the large intestine of both sheep and goats (Chapter 4) may indicate absorption from these sites.

In ruminants, DMI, cellulose and dry matter digestibility, organic matter fermentation in the rumen (Morrison et al., 1990; Drewnoski et al., 2014), microbial protein synthesis, nitrogen recycling and utilization (Breytenbach, 1999) can be influenced by the addition of S as source of mineral supplement. It is therefore remarkable that cows tend to increase the concentrations of fermentation products when supplemented with S in their diet (Chapter 3); demonstrating that the level of S intake in the present study may not affect optimal rumen microbial activity and fermentation profile, but bioavailability and absorption of the mineral was affected by dietary S intake.

In general, this indicates that the overall availability and absorption process of minerals or the stability of mineral complexes can greatly vary not only depending on species and breed but can also be regulated by dietary concentrations, intake levels, their solubilities, the animal's absolute requirement and mineral status of the body. This is also related to the pH along different regions of the gut segments and endogenous secretion throughout the digestive tract (Golfman, 1988; Chapter 3). However, apparent availability, which is the net absorption of the minerals from the digestive tract expressed as a percentage of the intake, may give only limited information about the extent of the net absorption or net secretion of some microminerals within various gut regions.

6.5. Conclusion and Future perspectives

The findings of the studies presented in this thesis demonstrate a general insight into the different feeding and nutrient utilization or adaptation mechanisms in ruminant species using goats as an example of browsers and sheep and cows as an example of grazers. The findings of this thesis generated questions on how ruminant species have developed evolutionary adaptation mechanisms to cope with low-quality dry season diets and environmental conditions. The impact of the mineral balance or mineral status on the performance of the animal was also demonstrated in this thesis, indicating that mineral status differences between ruminant species may impact the amount of energy provided from feed resources through ruminal fermentation. Moreover, liver Cu status or content between sheep and goats fed the same diet was different and was higher for goats than for sheep. This can be either due to the lower antagonistic effect of dietary nutrients which is caused by relatively lower intake in goats compared to sheep; or might be because goats have a better absorptive efficiency or biliary Cu secretion and excretion capacity than sheep, retaining more Cu in their liver than sheep. However, the adaptation mechanism of this effect was not clear in this thesis.

Therefore, some points to be considered in future research are:

- the effect of tannins in temperate organic cows and the effect of S in tropical goats and sheep.
- the impact of mineral supplementation and dietary tannin contents on ruminal fermentation deserves further attention. At the same time, it will also be necessary to

consider and analyse the concentration of minerals from and blood (plasma or serum).

- Although this is not always easy in practice, the duration of the study, sources of minerals (organic or inorganic), type of tannin (HT or CT) and the type of diet (pasture, silage, and hay) should get attention in future research.
- Future research on the long-term impact of micromineral depletion due to negative micromineral balance with low-quality dry season diets is necessary. At the same time, it will also be necessary to identify whether the differences between species are due to a gut effect or due to a systemic effect of the stored minerals.

The take-home message:

In conclusion, this PhD thesis demonstrated that a ruminant species or breed difference may have a large impact on how an animal deals with dietary feeds. The changes in feed intake, apparent digestibility of diet and nutrient utilization between ruminant species and even the association between mineral status and fermentation profile showed that both dietary factors and animal factors can affect nutrient metabolism in ruminants. In general, it was suggested that further research considering nutrient requirements of ruminant species should include breed and age differences together with species evolutionary adaptations to its local diet and environmental conditions particularly for animals and breeds in Ethiopia.

Limitations of study

- Short duration of experimental period
- Some complications in methods to analyze tannin, so condensed tannin was not

analysed.

- Unable to analyze all important chemical constituents such as S, Mo and Se, mainly due to budget and local availability of equipment.
- Unable to calculate apparent digestibility for each gut segment because of sample shortage. Saliva and blood samples were not analysed.
- Unable to perform *in vitro* trial related to tannin treatment.

References

Alcaide, E.M., García, A.M., Aguilera, J.F., 2000. A comparative study of nutrient digestibility, kinetics of degradation and passage and rumen fermentation pattern in goats and sheep offered good quality diets. Livestock Production Science 64, 215–223.

Baye, K., Guyot, J.-P., Mouquet-Rivier, C., 2017. The unresolved role of dietary fibers on mineral absorption. Critical reviews in food science and nutrition 57, 949–957.

Bremner, I., 1970. Zinc, copper and manganese in the alimentary tract of sheep. British Journal of Nutrition 24, 769–783.

Breytenbach, S., 1999. Sulphur in Ruminant Nutrition. Stephan, Kynoch Feeds, Randburg; AFMAMatrix. South Africa.

Campbell, C., 2019. Improving the utilisation of home grown forage legumes by high yielding dairy cows (PhD Thesis). Harper Adams University.

Dermauw, V., De Cuyper, A., Duchateau, L., Waseyehon, A., Dierenfeld, E., Clauss, M., Peters, I.R., Du Laing, G., Janssens, G.P.J., 2014. A disparate trace element metabolism in zebu (Bos indicus) and crossbred (Bos indicus$\times$ Bos taurus) cattle in response to a copper-deficient diet. Journal of animal science 92, 3007–3017.

Dermauw, V., Yisehak, K., Belay, D., Van Hecke, T., Du Laing, G., Duchateau, L., Janssens, G.P.J., 2013. Mineral deficiency status of ranging zebu (Bos indicus) cattle around the Gilgel Gibe catchment, Ethiopia. Trop Anim Health Prod 45, 1139–1147. https://doi.org/10.1007/s11250-012-0337-4

Drewnoski, M.E., Pogge, D.J., Hansen, S.L., 2014. High-sulfur in beef cattle diets: a review. Journal of animal science 92, 3763–3780.

Emanuele, S.M., Staples, C.R., 1990. Ruminal release of minerals from six forage species. Journal of animal science 68, 2052–2060.

Faulkner, M., St-Pierre, N., Weiss, W., 2017. Effect of source of trace minerals in either forage-or by-product–based diets fed to dairy cows: 2. Apparent absorption and retention of minerals. Journal of dairy science 100, 5368–5377.

Faulkner, M., Weiss, W., 2017. Effect of source of trace minerals in either forage-or by-product–based diets fed to dairy cows: 1. Production and macronutrient digestibility. Journal of dairy science 100, 5358–5367.

Forbes, J.M., 1995. Physical limitation of feed intake in ruminants and its interactions with other factors affecting intake, in: Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction: Proceedings of the Eighth International Symposium on Ruminant Physiology. pp. 217–232.

Ghodrat, A., Yaghobfar, A., Ebrahimnezhad, Y., Aghdam Shahryar, H., Ghorbani, A., 2017. *In vitro* binding capacity of organic (wheat bran and rice bran) and inorganic (perlite) sources for Mn, Zn, Cu, and Fe. Journal of Applied Animal Research 45, 80–84. https://doi.org/10.1080/09712119.2015.1124338

Goff, J.P., 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of Dairy Science 101, 2763–2813. https://doi.org/10.3168/jds.2017-13112

Golfman, L.S., 1988. The effects of molybdenum and sulfur on the flow and solubility of various minerals along the digestive tract of steers. (MSc thesis). University of Manitoba.

Gooneratne, S.R., Buckley, W.T., Christensen, D.A., 1989. Review of copper deficiency and metabolism in ruminants. Canadian Journal of Animal Science 69, 819–845.

Grace, N.D., 1975. Studies on the flow of zinc, cobalt, copper and manganese along the digestive tract of sheep given fresh perennial ryegrass, or white or red clover. British Journal of Nutrition 34, 73–82. https://doi.org/10.1017/S0007114575000104

Gupta, S., 2014. Studies on the Bioavailability of Copper, Molybdenum and Zinc from Oilseed Cakes in Crossbred Male Calves. (Doctoral thesis). National Dairy Research Institute, Deemed University.

Gupta, V.P., Kamra, D.N., Agarwal, N., Chaudhary, L.C., 2017. Effect of sulphate and blend of plant parts containing secondary metabolites on *in vitro* methanogenesis, digestibility and feed fermentation with buffalo rumen liquor. Indian Journal of Animal Sciences 87, 199–202.

Hamid, T.-E.M., 1997. Zinc and copper levels in the plasma of Nubian goat as affected by the physiological status. (Master thesis). University of Khartoum.

Hattori, R., TORII, S., Funaba, M., Matsui, T., 2010. Determination of true absorption and fecal endogenous loss of zinc in goats. Animal science journal 81, 564–568.

Hill, G.M., Shannon, M.C., 2019. Copper and Zinc Nutritional Issues for Agricultural Animal Production. Biological Trace Element Research 188, 148–159. https://doi.org/10.1007/s12011-018-1578-5

Hofmann, R.R., 1989. Evolutionary steps of Eco physiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78, 443–457.

Huang, Y.L., Wang, Y., Spears, J.W., Lin, X., Guo, C.H., 2013. Effect of copper on performance, carcass characteristics, and muscle fatty acid composition of meat goat kids. Journal of animal science 91, 5004–5010.

Ivan, M., Grieve, C.M., 1975. Effects of zinc, copper, and manganese supplementation of high-concentrate ration on digestibility, growth, and tissue content of Holstein calves. Journal of dairy science 58, 410–415.

Kara, K., 2019. The *in vitro* digestion of neutral detergent fibre and other ruminal fermentation parameters of some fibrous feedstuffs in Damascus goat (*Capra aegagrus hircus*). Journal of Animal Feed Science. 28, 159–168. https://doi.org/10.22358/jafs/108990/2019

Katulski, S.L., 2017. Effects of mineral supplementation on growing cattle and *in vitro* fermentation by ruminal microbes (PhD Thesis). Kansas State University.

Kirk, D.J., Fontenot, J.P., Rahnema, S., 1994. Effects of feeding lasalocid and monensin on digestive tract flow and partial absorption of minerals in sheep. Journal of animal science 72, 1029–1037.

Lee, J., Grace, N.D., 1997. New Zealand perspective on copper, molybdenum and sulphur interactions in ruminants, in: Proceedings from the 27th Seminar of the Society of Sheep and Beef Cattle Veterinarians, NZVA: NZVA Conference Week 1997: 29 June-4th July 1997, Wairakei Resort Hotel. Palmerston North, NZ: Veterinary Continuing Education, Massey University, 1997.

Martin, D.G., 2016. The nutritional and anthelmintic effects of Calliandra calothyrsus condensed tannin in the gastrointestinal tract of merino sheep (Doctoral dissertation). James Cook University.

Mathobela, R.M., 2018. Effect of acacia species leaf meal inclusion on methane emission and productivity of yearling male Boer goats fed an avena sativa hay-based diet (PhD Thesis). University of Limpopo.

McDonald, M., Mila, I., Scalbert, A., 1996. Precipitation of Metal Ions by Plant Polyphenols: Optimal Conditions and Origin of Precipitation. Journal of Agricultural and food Chemistry, 44(2), 599–606. https://doi.org/10.1021/jf950459q

Miller, M.D., Lanier, J.S., Kvidera, S.K., Dann, H.M., Ballard, C.S., Grant, R.J., 2020. Evaluation of source of corn silage and trace minerals on lactational performance and total-tract nutrient digestibility in Holstein cows. Journal of Dairy Science 103, 3141-3160

Mkhize, N.R., Heitkönig, I.M., Scogings, P.F., Hattas, D., Dziba, L.E., Prins, H.H., De Boer, W.F., 2018. Seasonal regulation of condensed tannin consumption by free-ranging goats in a semi-arid savanna. PloS one 13.

Mohammed, A., Osman, N.E.H., Isam, H., Din Osman, E., G Youssef, F., 2016. Review on Copper's Functional Roles, Copper X Mineral Interactions Affecting Absorption, Tissue Storage, and Cu Deficiency Swayback of Small Ruminants. ARC Journal of Animal and Veterinary Sciences (AJAVS) 2, 1–14. https://doi.org/10.20431/2455-2518.0202001

Morrison, M., Murray, R.M., Boniface, A.N., 1990. Nutrient metabolism and rumen micro-organisms in sheep fed a poor-quality tropical grass hay supplemented with sulphate. The Journal of Agricultural Science 115, 269–275.

Nasrullah, Abdullah, M., Baber, M.E., Jabbar, M.A., Bahtti, J.A., 2013. Feeding Behavior, Voluntary Intake and Digestibility of Various Summer Fodders in Sheep and Goats. Pakistan Journal of Zoology. URL https://eurekamag.com/research/066/270/066270385.php (accessed 12.28.20).

Naumann, H.D., Tedeschi, L.O., Zeller, W.E., Huntley, N.F., 2017. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. Revista Brasileira de Zootecnia. 46, 929–949. https://doi.org/10.1590/s1806-92902017001200009

Ndosa, J.E.M., 1980. A comparative study of roughage utilization by sheep and goats (PhD Thesis).

Rucker, R., Fascetti, A., Keen, C., 2008. Trace Minerals, in: Clinical Biochemistry of Domestic Animals. 6th ed. Academic, Burlington, pp 663–694. https://doi.org/10.1016/B978-0-12-370491-7.00022-2

Santos-Buelga, C., Scalbert, A., 2000. Proanthocyanidins and tannin-like compounds–nature, occurrence, dietary intake and effects on nutrition and health. Journal of the Science of Food and Agriculture 80, 1094–1117.

Silanikove, N., Tagari, H., Shkolnik, A., 1993. Comparison of rate of passage, fermentation rate and efficiency of digestion of high fiber diet in desert Bedouin goats compared to Swiss Saanen goats. Small Ruminant Research 12, 45–60.

Snyman, L.D., 2007. Qualitative characteristics of selected Atriplex nummularia (Hatfield Select) (PhD Thesis). University of Pretoria.

Sobry, L., Dermauw, V., Govaerts, W., Du Laing, G., Janssens, G., 2012. Relative contribution of feed, supplements and water to mineral supply on organic dairy farms in Flanders, in: 16th Congress of the European Society of Veterinary and Comparative Nutrition. European Society of Veterinary and Comparative Nutrition (ESVCN), pp. 150–150.

Suttle, N.F., 2010. Mineral nutrition of livestock, 4th ed. ed. CABI, Wallingford, Oxfordshire, UK ; Cambridge, MA.

Suttle, N.F., 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annual review of nutrition 11, 121–140.

Turner, J.C., Shanks, V., Osborn, P.J., Gower, S.M., 1987. Copper absorption in sheep. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology 86, 147–150. https://doi.org/10.1016/0742-8413(87)90157-5

Turner, K.E., Wildeus, S., Collins, J.R., 2005. Intake, performance, and blood parameters in young goats offered high forage diets of lespedeza or alfalfa hay. Small Ruminant Research 59. https://doi.org/10.1016/j.smallrumres.2004.11.007
Van, D.T.T., 2006. Some animal and feed factors affecting feed intake, behaviour and performance of small ruminants. (Doctoral thesis). Swedish University of Agricultural Sciences.

Van Soest, P.J., 1982. Nutritional ecology of theruminant. O & B Books. Inc., Corvallis, Oreg. 374pp.

Vellini, B.L., Prados, L.F., Monção, F.P., Fireman, A.K., Resende, F.D., Siqueira, G.R., 2020. Zinc amino acid complex in association with chromium methionine improves the feed efficiency of finished Nellore cattle in the feedlot. Animal Feed Science and Technology 262, 114430.

Wanyama, J.M., 1980. Comparison of high and low tannin sorghum grain with low quality roughage for feeding ruminants. (Master thesis). Oregon state University.

SUMMARY - SAMMENVATTING

Summary

The growth in livestock population for human consumption contributes to the world's increasing demand for protein sources. The increasing demand for protein source for human consumption contributes to an increasing growth in livestock population worldwide. Yet, the expected gradual increase of their population intensifies overgrazing pressure on natural pastures and results in the lack of sustainable feed resources. In addition, global warming or climate change does also affect feed resources; in subtropical and tropical regions, especially in Ethiopia where natural pasture and crop residues continue to be the most important ingredients of ruminant feeds (Chapter 1).

Usually, ruminant species have a preferred nutrient requirement that is considered safe and should allow normal function and development. However, nutritional or concentrate supplements are generally expensive for most resource-limited smallholder farmers in tropical areas like Ethiopia, and on top of that, these ruminants are challenged by micromineral shortages. The general introduction of this thesis further describes the diverse mechanisms of how ruminants have adapted to respond to different environmental and feeding conditions or developed a system for storing minerals in their liver through different adaptation mechanisms. Micromineral bioavailability has been intensively studied depending on the source of the mineral, and age and breed of the animal, but the impact of the nutrient content of some quality diets on the relative absorption and storage of minerals, and their influence on fermentation characteristics in ruminants, often remains uncertain and gets only little attention. Moreover, it is unlikely that mineral deficiency occurs in temperate areas like

Belgium (besides f.i. in organic dairy farming), but any micromineral secondary deficiencies can arise in the presence of antagonists. For this, the current background literature on factors affecting micromineral bioavailability was reviewed to indicate the gap why this research was needed (Chapter 1).

The present PhD thesis work was designed under *in vitro* and *in vivo* conditions, and aimed to investigate and to evaluate the impact of dietary constituents on protein digestion and bioavailability of microminerals, as well as their associated effect on fermentation characteristics in ruminants (Chapter 2). The specific objectives were 1) to evaluate the association between mineral bioavailability and ruminal fermentation profile in organic cows; 2) to investigate the effect of low-quality diets on the bioavailability of nutrients throughout gut segments of tropical sheep and goats.

In Chapter 3, a study was conducted to investigate the association of dietary mineral bioavailability and fermentation profile in the presence and absence of S supplementation using dairy cows under organic farm management in temperate climatic regions. It appeared that S supplementation increased neither Zn nor Fe bioavailability, but Cu bioavailability (% DM) was negatively affected by S addition irrespective of diet composition or fermentation profile. The addition of S also positively increased fermentation products such as acetate, propionate, butyrate and isobutyrate, and short-chain fatty acids (SCFA). The lack of effect on the bioavailability of Zn and Fe with S addition warrants further investigation to unravel the mechanism of S effect on tropical grazing ruminants adapted to grazing copper-deficient pastures, that may contain antagonistic minerals, such as Fe, S or Mo.

Given the association between micromineral bioavailability and fermentation profile as described in Chapter 3, a comparison was made between sheep and goats to evaluate if ruminant type (browser versus grazer) in a tropical setting with fibrous and tanninferous diet would also show such associations between mineral absorption and protein digestion. The feeding trial was set up with twelve adult male animals, six Bonga sheep with a mean body weight (BW) of 30.3 ± 1.6 kg and six Keffa goats with a mean BW of 26.4 ± 2.2 kg. In Chapter 4 an evaluation on the bioavailability of microminerals in tropical sheep and goats throughout their digestive tract when fed a typical dry season diet was made and a significant differences were found in concentrations of Cu, Zn and Fe. The species differences in micromineral concentrations between sheep and goats gastrointestinal digestive segments may arise from the unequal intake of these elements from the hay versus leaves in the diet. Yet the possible higher intake and the consistently higher Cu levels in the gut of sheep compared to the goats was not reflected in Cu liver storage. This demonstrated a different micromineral metabolism between tropical goats and sheep as an adaptation to the challenge of a dry season diet with a very low nutritive value.

Chapter 5 investigated how tropical goats and sheep process protein and fiber from a typical high fiber tannin-rich diet. Results from this study showed that the average voluntary feed intake (g/day) on a dry matter (DM) basis in relation to BW was significantly higher in sheep than in goats. Despite the higher investment of goats in the digestion of proteins from this low-quality diet, they showed higher weight loss compared to sheep partly due to a lower intake of nutrients compared to sheep. A synthesis of the results from the three research chapters is provided in the general discussion (Chapter 6). The present work demonstrated the associations between the bioavailability of some minerals with fermentation profile. Besides, this work also showed the animal species differences in utilization of minerals when fed high-fiber and tannin-rich diets, leaving scope for research on the exact mechanisms that tropical sheep and goats use to cope with low-quality high fiber diets. This can be strategically used to improve mineral utilization, health and performance in ruminants. Therefore, strict comparisons across ruminant species will always remain difficult, so considering breed differences would be important during feed formulation to determine requirements and avoid toxicity or deficiency.

Samenvatting

De groei van de veestapel draagt bij aan de wereldwijde stijgende vraag voor eiwitbronnen voor menselijke consumptie. De verwachte gestage stijging van de bevolking doet de overbegrazingsdruk op natuurlijke graasgebieden toenemen en resulteert in een gebrek aan duurzame voederbronnen. Klimaatopwarming en -verandering beïnvloeden bovendien deze voederbronnen ook in subtropische en tropische gebieden, vooral in Ethiopië waar natuurlijk graasland en teeltresten nog steeds belangrijke onderdelen zijn van rantsoenen voor herkauwers (Hoofdstuk 1).

Doorgaans is er voor elke herkauwersoort een ideale nutriëntenbehoefte die als veilig wordt beschouwd en zou moeten voorzien voor het normaal functioneren en ontwikkelen. Echter, voedingssupplementen en krachtvoeders zijn in het algemeen duur voor de meeste inkomensbeperkte kleine boeren in tropische streken zoals in Ethiopië, waar deze dieren vaak tekorten aan sporenelementen hebben.

De algemene inleiding van dit proefschrift beschrijft de verschillende mechanismen die herkauwers ontwikkelden als antwoord op de verschillende omgevings- en voedingsfactoren of hoe ze een systeem ontwikkelden om mineralen op te slaan in hun lever via diverse adaptatiemechanismen.

De biobeschikbaarheid van micromineralen werd intensief bestudeerd in functie van de mineralenbron, leeftijd en ras, maar de impact van nutriëntenconcentraties in sommige kwalitatieve voeders op de relatieve absorptie en stockage van mineralen en hun invloed op fermentatiekenmerken bij herkauwers kreeg weinig aandacht. Ook stelde zich de vraag of mineralentekorten zich zouden voordoen in gematigde gebieden zoals België (bv. in biologische melkveehouderij), maar eender welk secundaire tekorten aan micromineralen kunnen ontstaan als gevolg van antagonismen. De huidige achtergrondliteratuur over factoren die micromineralenbiobeschikbaarheid aansturen werd geanalyseerd om het hiaat in kennis aan te duiden waarom dit onderzoek nodig was (Hoofdstuk 1).

Dit doctoraatswerk omvat daarom *in vitro* en *in vivo* studies, met als doel de impact van voedingsbestanddelen op eiwitvertering en micromineralenbiobeschikbaarheid te onderzoeken, evenals hun invloed op fermentatiekarakteristieken bij herkauwers (Hoofdstuk 2). De specifieke doelstelling waren 1) om de invloed van mineralenbiobeschikbaarheid op pensfermentatie te evalueren bij biologische melkkoeien, 2) om het effect van nutriënten op de biobeschikbaarheid van micromineralen te onderzoeken doorheen het verteringsstelsel bij tropische schapen en geiten, en 3) om de invloed van diersoort op voeropname en nutriëntenvertering te meten bij tropische schapen en geiten. In Hoofdstuk 3 werd een studie uitgevoerd om het verband tussen micromineralenbiobeschikbaarheid van rantsoenen en fermentatieprofiel te onderzoeken in de aan- of afwezigheid van zwaveltoevoeging bij biologische melkkoeien in gematigd gebied. Hierbij bleek dat zwaveltoevoeging de biobeschikbaarheid van zink noch ijzer verhoogde, maar dat de biobeschikbaarheid van koper negatief werd beïnvloed door zwaveltoevoeging, onafhankelijk van rantsoensamenstelling of fermentatieprofiel.

De zwaveltoevoeging verhoogde fermentatieproducten zoals azijnzuur, propionzuur, boterzuur, isoboterzuur en de korteketenvetzuren in totaal. De afwezigheid van een effect op de biobeschikbaarheid van Zn en Fe door de zwaveltoevoeging noopt tot verder onderzoek over de invloed van zwavel op tropische grazende herkauwers aangepast aan koperdeficiënte graslanden, met antagonistische mineralen zoals ijzer, zwavel of molybdeen.

Gegeven de associatie tussen micromineralenbiobeschikbaarheid en fermentatieprofiel zoals in Hoofdstuk 3, werd een vergelijking gemaakt tussen schapen en geiten om herkauwertype te evalueren (*browser* versus *grazer*) in een tropische setting met een rantsoen rijk aan vezel en tannines. De voederproef werd opgezet met twaalf volwassen mannelijke dieren, zes Bonga schapen en twaalf Keffa geiten van gelijkaardig gewicht. Hoofdstuk 4 evalueerde daarom de biobeschikbaarheid van micromineralen in tropische schapen en geiten doorheen hun verteringsstelsel met een typische droge-seizoendieet. We vonden een significant verschil in de concentraties van Cu, Zn en Fe doorheen het verteringsstelsel. De soortverschillen in micromineralenconcentraties in het verteringsstelsel van schapen en geiten komt mogelijk van de ongelijke inname van deze elementen vanuit hooi versus de bladeren in het rantsoen. De mogelijk hogere inname en hogere koperconcentraties in het darmstelsel van de schapen in vergelijking met de geiten was nochtans niet weerspiegeld in de koperopslag in de lever. Dit wees op een verschillend micromineralenmetabolisme tussen tropische geiten en schapen als aanpassing aan de “challenge” van een droge-seizoenrantsoen met een zeer lage voedingswaarde.

Hoofdstuk 5 onderzocht hoe tropische geiten en schapen het eiwit en de vezel verwerken vanuit een typisch hoog-vezel hoog-tannine rantsoen. De resultaten van deze studie toonden dat de voederopname per eenheid lichaamsgewicht hoger was bij schapen dan bij geiten.

Ondanks de hogere investering van geiten in de vertering van eiwit vanuit dit laagkwalitatieve rantsoen, was er een lager gewichtsverlies bij schapen.

Een synthese van de resultaten van de drie onderzoekshoofdstukken is te vinden in de algemene discussie (Hoofdstuk 6). Het huidige werk toont de verbanden tussen de biobeschikbaarheid van sommige mineralen en fermentatieprofiel. Daarnaast demonstreerde het werk ook de diersoortverschillen in mineralenbenutting met een rantsoen hoog in vezel en tannines, wat perspectief geeft voor onderzoek over de precieze mechanismen waarmee tropische schapen en geiten omgaan met lage-kwaliteit hoog-vezel rantsoenen. Dit kan strategisch worden gebruikt om mineralenbenutting, gezondheid en prestaties bij herkauwers te verbeteren.

Strikte vergelijkingen tussen herkauwersoorten zullen daarom altijd moeilijk blijven, dus zou het in acht nemen van rasverschillen van belang kunnen zijn bij het formuleren van rantsoenen om behoeften te bepalen en zo tekorten en toxiciteit te vermijden.

CURRICULUM VITAE

Curriculum Vitae

Biruk Kebede Biru was born on the 23rd May 1982 in Jimma, Oromia, Ethiopia. In 2002, he completed secondary school in the natural sciences stream and took the Ethiopian school leaving certificate examination at Jiren Senior Secondary School in Jimma. In 2003, he started his education at Mekele University (MU) and graduated with the diploma of Bachelor of Science in Animal Range and Wildlife Science in 2007. After graduation, he was employed by Gera Wereda Agriculture and Rural Development Office, Jimma zone and served as an animal production expert and a team leader for one year. In 2008 he joined Jimma University to pursue his Master of Science (MSc) degree study. He obtained an MSc degree in Animal Production on March 12, 2012. After graduation, he was employed by the Ethiopian Institute of Agricultural Research, Tepi National Spice Research Center and served as a Head of Livestock Research Process and Assistant Researcher II. Since April 2013, he joined Asossa University as a lecturer and researcher in the Animal Science department, until he was admitted to Ghent University as a PhD student. In 2015, he was awarded a PhD fellowship from the BOF Doctoral Grants for Candidates from Developing Countries (DOS) financed by the Belgium federal government. He conducted his PhD thesis research entitled "Mineral and digestive responses to dietary challenges in the gastro-intestinal system of ruminants" under the supervision of the Laboratory Animal Nutrition, Faculty of Veterinary Medicine. Genetics and Ethology department of Ghent University, Faculty of Veterinary Medicine, Belgium.

Bibliography

Articles in international journals

Yisehak, K., **Biruk, K.,** Abegaze, B. and Janssens, G.P.J., 2014. Growth of sheep fed tannin-rich *Albizia gummifera* with or without polyethylene glycol. *Tropical Animal Health and Production*, *46* (7):1113-1118.

Kebede, B., Kechero, Y. and Beyene, A., 2014. Polyethylene glycol improves the utilization of a high tannin forage in sheep. *Livestock Research for Rural Development*, *26:*214.

Kebede, B., Van De Wiel, K., Derix, J., Tolemariam, T., Dermauw, V., Lourenço, M. and Janssens, G.P.J., 2021. Copper, iron, zinc and tannin concentrations throughout the digestive tract of tropical goats and sheep fed a high-fibre tannin-rich diet. *Journal of Animal Physiology and Animal Nutrition, in press*. https://doi.org/10.1111/jpn.13518

Kebede B., Tolemariam T., Dermauw V., Lourenço M., Sobry L., Fievez V. and Janssens, G.P.J.**,** submitted**.** The impact of sulfur addition to organic dairy rations on *in vitro* fermentation and micromineral bioavailability.

Kebede B., Van De Wiel K., Derix J., Kechero Y., Tolemariam T., Dermauw V., Lourenço M., Janssens, G.P.J., resubmitted after minor revisions. Digestive response of tropical goats and sheep fed a high fiber tannin rich diet. *Journal of Animal Physiology and Animal Nutrition*.

Abstracts in conference proceedings

Yisehak, K., **B. Biruk,** Abegaze, G.P.J. Janssens, 2012. Effect of dietary inclusion of polyethylene glycol on *in vivo* and *in vitro* nutritional parameters for a tropical condensed tannin rich diet. Proceeding of the 16th Meeting of European Society of Veterinary and Comparative Nutrition (ESVCN), September 13-15, 2012, Bydgoszcz, Poland.

Congress attended

22nd European Society of Veterinary and Comparative Nutrition (ESVCN 2018), Munich, Germany, September 5-8, 2018