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Copper, iron, zinc and tannin concentrations throughout the digestive tract of tropical goats and sheep fed a high-fibre 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-fibre tannin-rich diet. This study was designed to determine the effects of a high-fibre and tannin-rich diet on the flow of important microminerals 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 faecal 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 faecal 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. Faecal 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, micromineral 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.

KEYWORDS
goats, mineral metabolism, sheep, tannins

1 | INTRODUCTION

Global climate change 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 multipurpose 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 fibre and plant secondary compounds, predominantly tannins (Theodoridou, 2010). The latter considerably bind minerals, proteins and amino acids in the 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 of animals 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 microminerals 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 & Hansen, 2015; Katulski, 2017). The complexation of minerals influences the metabolic capability of micro-organisms by altering the physiological uptake of minerals and other nutrients essential for their own metabolism (McDonald et al., 1996; Scalbert, 1991). This may prevent the attachment of rumen micro-organisms 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, although sheep indeed prefer grazing, they shift to more browsing as the dry season progresses, when grass becomes scarce and less palatable (Shenkute et al., 2012). Former work already demonstrated adaptations of other tropical livestock ruminants, 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 goats, as well as sheep, have adapted to the exposure to such diets. Tropical goats seem to possess a higher ability than tropical 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 micromineral metabolism to a typical dry season diet, very high in fibre and tannins.

2 | MATERIALS AND METHODS

2.1 | Study area

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

2.2 | Experimental diet

The feed consisted of three components: 61% of a hay mixture, 36% of tannin-rich leaves of *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 for feeding cattle, goats and sheep especially during the dry seasons in Ethiopia (Alemu et al., 2014). The hay mixture was harvested locally. 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. The 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	25.3	18.5
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 fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), acid-insoluble ash (AIA), total tannin (TT).

2.3 | 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 in Jimma, Ethiopia. All animals were estimated to be between 12 and 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 under preliminary adaptation 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 around at 10:00 h and 16:00 h for the next eight days. Also, approximately 100–150 g of *Millettia ferruginea* leaves were provided at 8:00 h before hay supplementation to gradually create a steady state of the metabolism of the animals.

2.3.1 | Total faecal collection

The total faecal 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. Also, all faeces produced were collected every morning and weighed. 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. At the end of the experimental period, daily faecal subsamples were thoroughly mixed and pooled per animal and oven-dried at 60°C for 48 h.

2.4 | 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) to pass through a 1 mm 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. All samples were immediately stored at −20°C in plastic bags for approximately 12 h 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 faeces and liver samples were grinded in a mill (Hanna Instrument) to pass through a 1 mm screen and were stored in an airtight container at 25°C, until analysis.

2.5 | Mineral analysis

Feed, digesta and faecal samples were analysed 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).

TABLE 2 Total intake of *Millettia ferruginea* leaves, hay mixture and concentrate (g DM/day), with the corresponding intakes of Fe, Cu, Zn (mg /day), DM and TT (g /day) in tropical sheep and goats

	Goat		Sheep		
	Mean	SE	Mean	SE	*p*
Feed intake					
Hay	361	9.0	475	19	<0.001
Leaves	152	13	262	23	0.002
Concentrate	24	1.0	27	0.7	0.016
Nutrient intake					
DM	538	17	765	28	<0.001
Cu	3.88	0.14	5.68	0.24	<0.001
Fe	136	4.7	194	7.5	<0.001
Zn	26.7	1.0	36.1	1.5	<0.001
TT	1.2	0.07	1.9	0.12	0.001

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

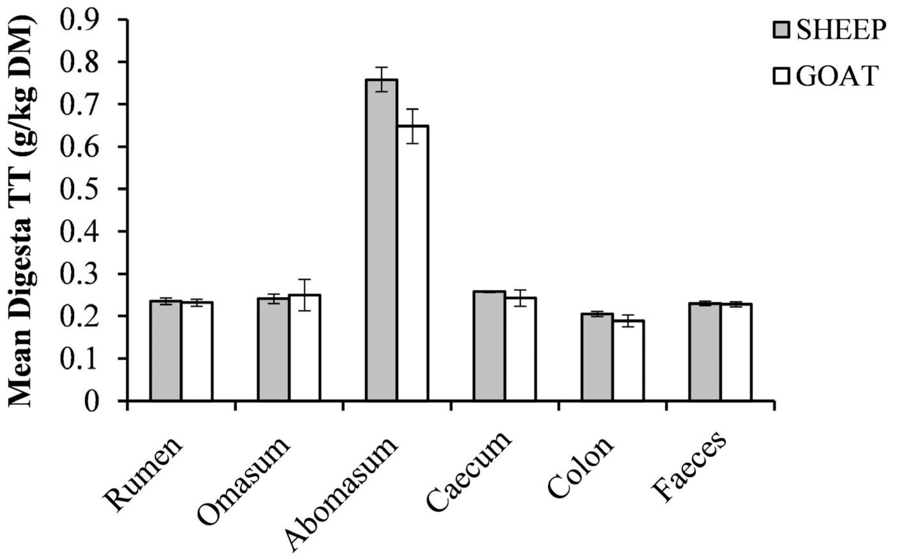

FIGURE 1 Total tannin (TT) concentrations in digesta on a DM basis for sheep and goats fed a mixture of *Millettia ferruginea* leaves, hay and concentrate. DM, dry matter; TT, total tannin. The error bars display the standard error of means

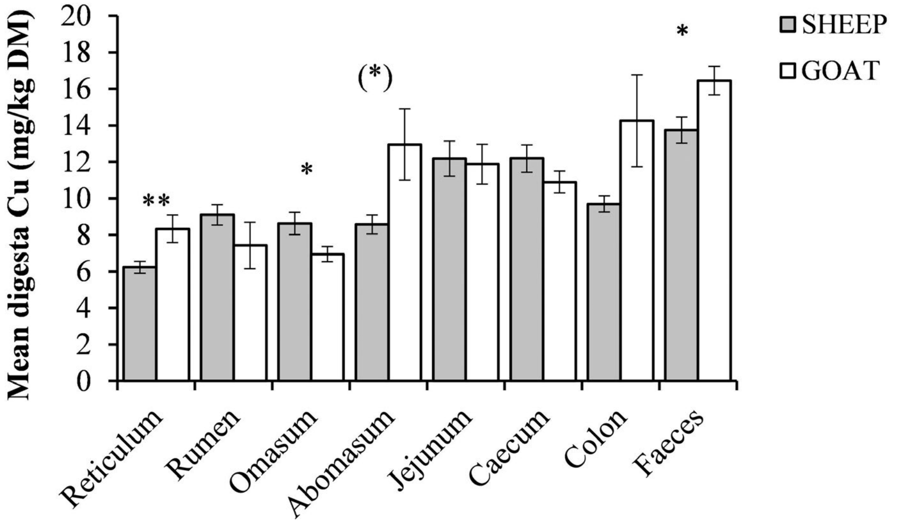

FIGURE 2 Copper (Cu) concentration in digesta on a DM basis for sheep and goats fed a mixture of *Millettia ferruginea* leaves, hay and concentrate. DM, dry matter. The error bars display the standard error of means. *Significance at $p < 0.05$; **Significance at $p < 0.01$; (*) Trend ($0.05 < p < 0.10$)

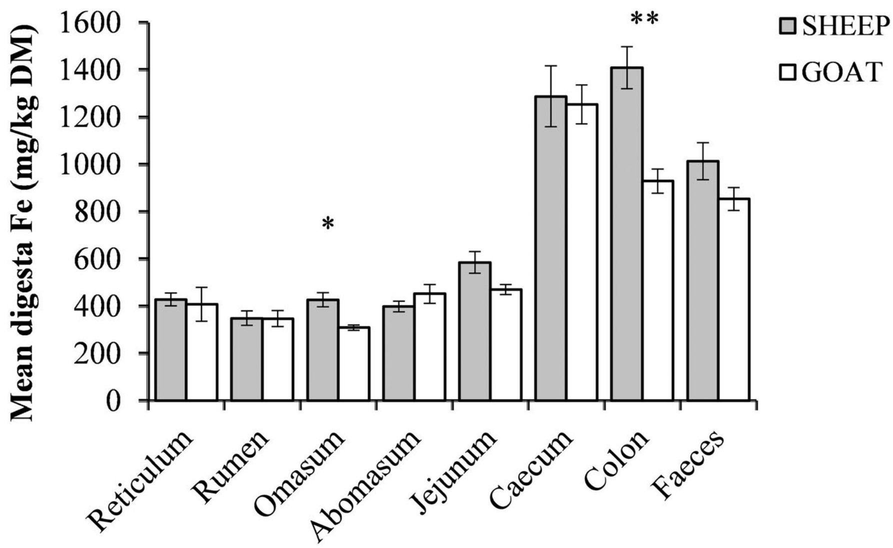

FIGURE 3 Iron (Fe) concentration in digesta on a DM basis, for sheep and goats fed a mixture of *Millettia ferruginea* leaves, hay and concentrate. DM, dry matter. The error bars display the standard error of means. *Significance at $p < 0.05$; **Significance at $p < 0.01$; (*) Trend ($0.05 < p < 0.10$)

2.6 | 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 (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 solution obtained.

2.7 | Statistical analysis

Data were analysed 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, animal as a random factor. An unpaired *t* test was performed to identify differences between sheep and goats with regards to feed intake. The significance level was set at $p \leq 0.05$.

3 | 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, that is 62.2% hay, 34.1% leaves and 3.6% concentrate (Table 2).

3.1 | 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).

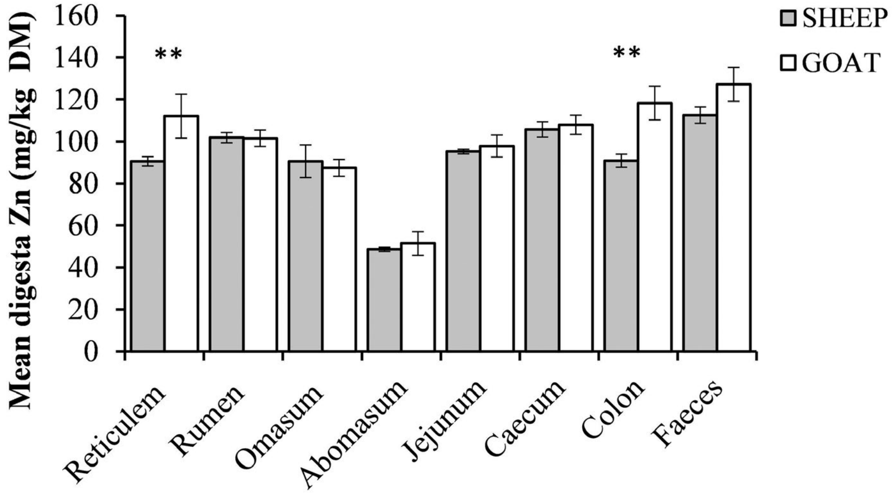

FIGURE 4 Zinc (Zn) concentration in digesta on a DM basis for both sheep and goats fed a mixture of *Millettia ferruginea* leaves, hay and concentrate. DM, dry matter. The error bars display the standard error of means. *Significance at $p < 0.05$; **Significance at $p < 0.01$; (*) Trend ($0.05 < p < 0.10$)

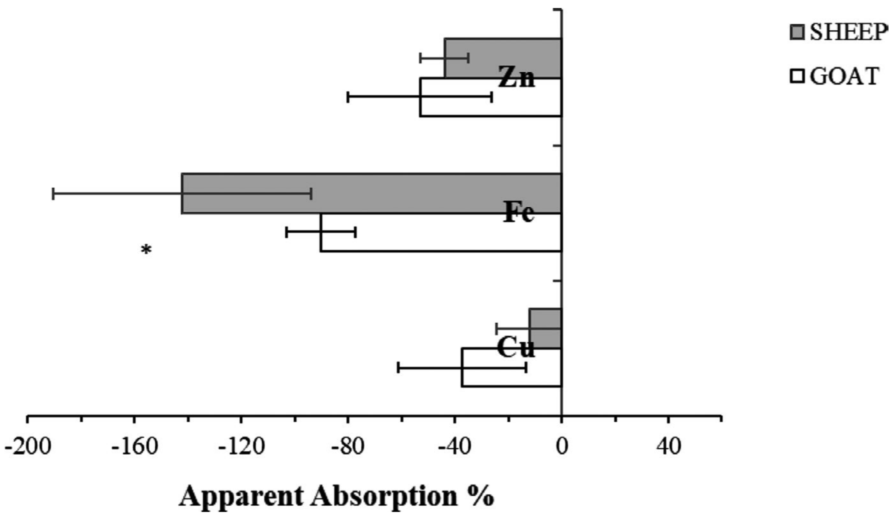

FIGURE 5 Apparent absorption of copper (Cu), iron (Fe) and zinc (Zn) in tropical goats ($n = 6$) and sheep ($n = 6$) fed a mixture of *Millettia ferruginea* leaves, hay and concentrate

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 2 weeks

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

3.2 | 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 Cu levels in the digesta collected from the reticulum ($p = 0.011$) (Figure 2). Sheep tended to have higher Cu levels in the rumen and omasum ($p = 0.053$) than goats. Faecal Cu levels were also significantly higher in goat than sheep ($p = 0.035$).

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

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). In other sampled digestive sites and faeces, 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 ($p < 0.05$). Mean liver Cu, Fe and Zn concentrations did not significantly differ between sheep and goats (Table 3).

4 | DISCUSSION

The dry season diet offered in our study induced a similar, prominent loss of the three investigated microminerals in sheep and goats. Although micromineral requirements for these local breeds are undocumented and breed/type differences in mineral metabolism have been identified for cattle (Dermauw et al., 2014; Ward et al.,), 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 & Suttle, 1999). The requirement values can also be 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 micromineral 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 micromineral status. The liver concentrations of Fe in both species are higher than the normal levels observed in sheep (Puls, 1994) whereas the Zn concentrations of the liver are 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 micromineral storage, because they may enter a 'saving modus' when for instance absorption of these microminerals is an active homeostatic process (Sloup et al., 2017). 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' micromineral status.

Although the measured apparent absorption of the microminerals was found low both in goats and sheep, the patterns of the micromineral concentrations throughout the digestive tract differed between the species, suggesting a divergent micromineral 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 microminerals. 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 availability of other antagonists (Rehman, 2017), which primarily interfere with their bioavailability and absorption from the gut and subsequent utilization for metabolic processes (Gooneratne et al., 1989; Rehman, 2017). Therefore, the overall process of microminerals absorption is regulated by their dietary concentrations, endogenous secretion and related to the animal's absolute requirement and status of the body (Golfman, 1988; Gupta, 2014).

In the first three compartments, the absolute micromineral concentrations are lower than in the diet, likely because of the 'dilution' of the ingested diet with microbial mass. Until that stage, it seems that no absorption is happening. Indeed, it is generally accepted that the absorption of these microminerals occurs in the small intestine (Miller, 1970; Mir et al., 2018). 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 requires confirmation, 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 & Hummel, 2017; Sauer et al., 2017).

A major change in concentrations occurs when entering the enzymatic digestion compartments (abomasum and jejunum), where the abomasal concentration suddenly increased for Cu in contrast with a decrease in Zn and little change for Fe. Remarkably, the Cu increase was distinctly higher for goats than for sheep. The elevation of the free tannins concentration in the abomasum confirms that the acid pH in that segment disrupts the binding of microminerals 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 and Zn are antagonists of Cu (Acharya et al., 2016; Hilal et al., 2016), it is thinkable that the sudden release of Zn and maybe Fe in the abomasum have inhibited the absorption of Cu. If goats tend to invest more in digestion compared with 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 laboratory 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. The numerically higher Cu absorption in sheep was not reflected in Cu liver storage, as goats had numerically higher Cu hepatic levels than sheep. This was unexpected due to the higher Cu intake and the consistently higher Cu levels in the gut of sheep compared to the goats. 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 less low compared to Fe and Zn. The tendency towards a species difference in Fe apparent absorption indicates a more efficient Fe recovery in goats during the digestive processing of this very low-quality diet. It may happen that goats compensated the lower Fe intake by a higher Fe hindgut absorption, the lower colon and faecal Fe content in goats than in sheep might explain this.

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

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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