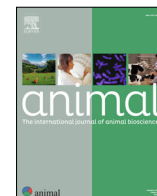




# Animal

## The international journal of animal biosciences



## Review: Homeostatic boundaries to dietary Zn, Cu and Mn supply in cattle



J.B. Daniel <sup>\*</sup>, J. Martín-Tereso

*Trouw Nutrition R&D, Amersfoort, the Netherlands*

### ARTICLE INFO

#### Article history:

Received 16 December 2024

Revised 17 April 2025

Accepted 18 April 2025

Available online 25 April 2025

#### Keywords:

Absorption efficiency

Dietary antagonists

Hepatic Cu

Tolerance

Trace metal

### ABSTRACT

Zinc, Cu, and Mn are critical to sustain animal physiology, and therefore, dietary supplementation to domestic cattle is common practice. However, recent evidence rises concerns about current supplementation levels, as they may exceed dietary tolerance for these nutrients, impairing health and productivity. This generous supplementation has been justified to mitigate the uncertainty in basal mineral supply and its availability. Furthermore, current dietary reference recommendations assume conservative and fixed absorption coefficients. This mathematical simplification greatly limits our ability to precisely define supplemental levels. Apparent absorption efficiency, which comprises absorption and endogenous excretions, is the main mechanism animals use as homeostatic adaptation to a variable dietary supply. The first part of this review summarises quantitative evidence illustrating homeostatic competence of cattle to adapt apparent absorption efficiency, demonstrating a much greater than assumed resilience to low dietary supply. Also, adaptations to high dietary supply are described. These homeostatic adaptations are complex and nutrient-specific, but in all cases, they are tightly orchestrated by the animal's trace metal status, sharply increasing nutritional efficiency when status is low and decreasing it under abundant supply. Identifying the lower and upper boundaries of this homeostatic competence serves much better to prevent dietary deficiencies and excess in trace metal supply by supplementation. The second part of this review is therefore dedicated to estimating the lower boundaries of homeostasis defined as the maximal apparent absorption efficiency that can be achieved under constrained dietary availability. Among the three trace minerals evaluated, Cu and Mn supplies appear the most susceptible to nutritional challenge, despite adaptive upregulation of apparent absorption. Finally, the third and last part of the review intends to identify the upper boundaries of dietary trace metal regulation, that is, the supply levels above which homeostasis is overwhelmed resulting in unregulated trace metal retention. Dry matter intake plays a critical role in this risk, specifically in dairy cows. Indeed, except for Zn, trace metal demands for lactation are negligible. Thus, when DM intake increases to support milk production, dietary trace metal intakes increase disproportionately to their requirements, resulting in a risk for unregulated absorption that may not be compensated by endogenous excretion. Therefore, considering dietary trace metal adequacy in terms of daily intake is better than only considering dietary concentrations. Defining lower and upper boundaries for dietary supply allows for a more adequate prevention of risk for deficiency or excess supply.

© 2025 The Author(s). Published by Elsevier B.V. on behalf of The animal Consortium. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### Implications

Current trace mineral supplementation practices exceed dietary reference levels. These elevated safety margins are primarily intended to mitigate risks associated with variability in trace mineral content of basal diets and interactions with nutrient antagonists. However, they also reflect an incomplete understanding of the role of homeostasis in preventing deficiencies. Excessive sup-

plementation may result in intakes that surpass homeostatic tolerance. In this context, we reviewed homeostatic competence and quantified the lower and upper boundaries for dietary supply of Zn, Cu, and Mn in ruminants. Considering both the opportunity and limitations of trace metal homeostasis in ruminants is crucial to support nutrient adequacy.

### Introduction

Studies in rodents have demonstrated how tightly regulated body balances of Zn (Weigand and Kirchgessner, 1980), Cu

<sup>\*</sup> Corresponding author.

E-mail address: [Jean-Baptiste.Daniel@trouwnutrition.com](mailto:Jean-Baptiste.Daniel@trouwnutrition.com) (J.B. Daniel).

(Owen, 1964) and Mn (Weigand et al., 1986) are over a wide range of dietary supply. A common feature is the modulation of absorption efficiency, regulating the influx from the gut to the body, as the main control system of homeostasis. As dietary supply increases, fractional absorption decreases, but not sufficiently as to maintain absolute absorption constant. Thus, at increasing dietary supply, any excessive absorption of Zn and Cu, resulting from the limitations of gastrointestinal downregulation of absorption, are excreted back to the gut and lost in faeces (Owen, 1964; Weigand and Kirchgessner, 1980), preventing any excessive Zn or Cu retention. Adjustment of endogenous faecal excretion upon excessive dietary supply was also demonstrated for Mn but this was quantitatively small (Weigand et al., 1986). Such comprehensive quantification of trace metal homeostasis in cattle is lacking, and most of the studies that have evaluated the role of apparent absorption regulation in cattle were conducted in the 60–70 s. Since then, scientific progress in the field of mineral nutrition in cattle has mostly focused on evaluating the impact of supplemental form on animal performance and health. The scarcity of recent scientific work in trace metal homeostasis may in part explain the limited awareness of nutritionists of the critical role homeostasis plays in mineral nutrition. In the first part of this review, we summarised evidence and characterisation, of trace metal regulation in cattle. The intention is not to describe homeostasis at cellular level, but to mainly display the quantitative role of the main regulatory fluxes at animal level, such as responses in absorption, endogenous faecal excretion, and urinary excretion, to changes in trace metal dietary availability or physiological requirements.

In part due to the limited availability of studies that quantify absorption efficiency in cattle, current trace metal feeding recommendations applied to cattle assume fixed absorption efficiencies when converting net requirements into gross requirements (INRA, 2018; NASEM, 2021). A risk associated to this approach is to create a self-fulfilling prediction, as high supply results in low absorptive efficiencies, and therefore, low absorptive efficiencies lead to predicting high dietary needs, thus resulting in high recommendations. As an alternative, we propose to calculate expected apparent trace metal absorption efficiencies, based on dietary supply, and factorially calculated net requirements, using published dose–response studies in cattle that evaluated effects of diverse dietary availabilities and/or net requirements. Then, the likelihood that expected apparent trace metal absorption is realised can be judged by the absence or presence of reported adverse effects. This approach is used to determine the lower boundary of upregulation competence, which can be used to propose a minimal level of dietary supply for nutrient adequacy.

Finally, and equally relevant is the quantification of the upper boundaries of downregulation that define the levels tolerable by cattle. The risk of excessive supply has long been recognised and a maximum tolerable level (MTL) has been proposed for trace metals in farm animals (National Research Council, 2005). However, despite compliance with these MTL, there is evidence that current trace metal supplementation practices, which largely exceed reference recommendations (Li et al., 2005; Castillo et al., 2013; Sinclair and Atkins, 2015; Duplessis et al., 2021), may overwhelm homeostasis mechanisms. In particular, the high level of hepatic Cu in dairy cattle represents a growing concern (Kendall et al., 2015; Counotte et al., 2019) and has been associated, in some instances, with reduced milk performance and reproduction (McCaughern, 2019). One critical aspect, often neglected when it comes to assessing the risk of exceeding a tolerable level, is simply the amount of feed cattle consumed. In a study that evaluated Zn, Cu and Mn balance in dairy cows from late lactation to subsequent mid-lactation, increased retention was observed when DM intake was highest (Daniel et al., 2023). Increased trace metal retention above the

expected net requirement may be interpreted as a health risk factor. In the last part of this review, we summarised data that demonstrate the occurrence of unregulated trace metal retention in cattle and proposed a quantitative approach to estimate upper boundaries of downregulation competence, which define this risk related to trace metal tolerance.

## Trace metal homeostasis in ruminants

### Zinc homeostasis

The fraction of Zn absorbed from the diet responds inversely to dietary supply changes. This was demonstrated in primiparous dairy cows (30% at 40 mg/kg DM vs 45% at 17 mg/kg DM, Neathery et al., 1973a), in calves of 2.5 months of age (51% at 38 mg/kg DM vs 71% at 2 mg/kg DM, Miller et al., 1968), in calves of 4.5 months of age (13% at 38 mg/kg DM vs 74% at 2 mg/kg DM, Miller et al., 1968), in lambs (from 11% with 62 mg/kg DM to 23% with 22 mg/kg DM, VanValin et al., 2018) or in growing steers (from 14.5% at 165 mg/kg DM to 19.8% at 36 mg/kg DM, Carmichael et al., 2019). Similarly, true Zn absorption, estimated in ewes, was shown to decrease from 75, 9, 5 to 3% when dietary Zn increased from 17, 149, 320 to 800 mg Zn/kg DM, respectively (Suttle et al., 1982).

In addition to the homeostatic response to change in dietary Zn content, Zn utilisation efficiency is also influenced by Zn status and net Zn utilisation of the animal. As cattle grow from 2.5 to 4.5 month of age, the fraction of an oral dose of  $^{65}\text{Zn}$  retained in the body 7 days after the administration decreased from 51 to 13% (Miller, 1969) when dietary Zn content is maintained at 38 mg/kg DM. This difference in efficiency is not caused by a physiological decline in efficiency, because when calves were fed a Zn-deficient diet (2 mg/kg DM), the fractional retention was no longer different between the two age-groups (71 and 74%, respectively, Miller, 1969). Consistent with this, net  $^{65}\text{Zn}$  absorption was similar between 2-month and 6-month-old calves fed 16.6 mg Zn/kg DM dietary (50 and 47%, respectively, Stake et al., 1974). Thus, the decline in fractional retention as dietary Zn increases is most likely driven by a progressive change in the ratio between the net Zn requirement for growth (i.e., rate of growth decline with age) and dietary Zn supply (i.e., increased by dry-matter intake), parallel to a change in feed efficiency.

Not only growth affects net requirements and consequently dietary Zn utilisation. Vierboom et al. (2003) evaluated the effect of gestation on Zn absorption by feeding two groups of Angus cattle, pregnant ( $250 \pm 7$  days pregnant) and non-pregnant an identical diet containing 17 mg Zn/kg DM. In response to additional Zn demand from the pregnancy, apparent Zn absorption increased from 5 to 10%.

Zinc status can also change the needs to retain Zn from the diet. When goats and calves were fed a diet containing 6 mg Zn/kg DM, those previously fed that same diet for 9–13 weeks as compared to those previously fed a diet with 40 mg Zn/kg DM, had greater apparent Zn absorption (Miller et al., 1967). Interestingly, in rodents, it was established, that the amount of Zn stored in the skeleton was highly correlated with true Zn absorption (Windisch, 2001). Indeed, upon depletion (2 mg Zn/kg DM and added phytate) and repletion (21 mg Zn/kg DM), it was observed that true Zn absorption responded in an orchestrated way with skeletal Zn content, by increasing as skeletal Zn content was being depleted and by decreasing as skeletal Zn was being replenished. Within 2 weeks, adult female rats had lost 21% of the total Zn in the skeleton, providing an important buffer to maintain intake and health. The role of the skeleton to support Zn homeostasis in ruminants has not been quantified but is likely to be important

when dietary Zn supply falls below a critical level transiently or during a longer time frame.

### Copper homeostasis

In contrast to Zn, the role of regulated apparent Cu absorption in Cu homeostasis may seem less evident in ruminants. When non-lactating, non-gestating adult Holstein cows were fed diets with increasing dietary Cu content from 10, 35, to 50 mg/kg DM, apparent Cu absorption increased from 5.5, 10.9, to 17.1%, respectively (Hanauer, 2017). However, when pregnant and non-pregnant Angus cattle were fed an identical diet containing 6.9 mg Cu/kg DM, it was reported that pregnancy increased apparent Cu absorption from 3 to 9%, suggesting an up-regulation of absorption (Vierboom et al., 2003). The explanation to this apparent discrepancy in direction of the response may be in the range of Cu levels fed in relation to the net Cu requirement. In contrast to Vierboom et al. (2003), in the study from Hanauer (2017), Cu supply was evaluated at levels well above their net Cu requirement, so likely exceeding the ability of the system to counteract Cu supply by downregulation of absorption. This response to high Cu supply reported for cattle are directionally opposite to those reported for humans (Turnlund et al., 1989) or rodents (Owen, 1964).

One aspect that appears to limit the ability of cattle to down-regulate apparent Cu absorption is their limited capacity to increase biliary Cu, as reviewed by Clarkson et al. (2019). For example, in Simmental and Angus cattle, biliary Cu secretions were estimated to increase from 0.13 mg/6h to 0.19 mg/6h when dietary Cu was increased from 5 to 40 mg Cu/kg DM (Gooneratne et al., 1994). The extrapolation of those numbers indicates a daily endogenous Cu secretion into the gut of 0.52 and 0.76 mg Cu. This is a moderate amount compared to daily Cu supply (about 1.5 and 0.3% of Cu supply, respectively), indicating limited quantitative competence for Cu homeostatic response by endogenous excretions. By comparison, in the rat, about 9–15% of Cu excreted in faeces originate from bile (Owen, 1964).

Together, the limited competence of ruminants to regulate both Cu absorption in the gut and endogenous excretion through the bile at high dietary Cu intakes support the empirical evidence on the challenge of ruminants to tolerate high dietary Cu levels. This condition seems specific to ruminant species and may be related to the high sulphide concentration inherent to the rumen that poses a constant challenge to the dietary available Cu. This results in a low selection pressure to develop an effective Cu homeostasis against a high dietary Cu supply. We speculate that such conditions may have favoured selection for mechanisms of resilience to low available Cu supply. This is well illustrated by the difficulty to induce Cu deficiency under experimental conditions, for which often supplementation of antagonists (Mo and/or Fe) is required, in addition to the absence of Cu supplementation.

In part as a consequence of their limited capacity to increase biliary Cu secretion, and a trait of resilience to low Cu supply, the liver of ruminants can accumulate a substantial amount of Cu (López-Alonso and Miranda, 2020). However, this seems to be subject to regulation as the rate of hepatic Cu accumulation seems to be related to the initial concentration of Cu in the liver. In growing cattle, the supplementation of 40 mg Cu/kg DM increased hepatic Cu at a rate of 7.5 mg/kg DM in the first 42 days of feeding (growing phase), but only of 0.8 mg/kg DM in the next 84 days of feeding (finishing phase, Engle et al., 2000). When Cu was supplemented at 15 and 30 mg/kg DM in lactating cows fed a diet with basal Cu content of 8 mg/kg DM (Chase et al., 2000), net Cu retention was increased (from 3, to 128, to 229 mg/day) when measurement was made 10 days after the initiation of the supplemental period. However, 49 days and 82 days after, net Cu retention did not differ

from non-supplemented cattle and was close to 0. Similarly, the rate of hepatic Cu depletion in response to a low Cu diet appears to be related to initial hepatic Cu concentration. Ewes whose mean concentration of Cu in the liver was highest at the start of the study had the highest loss of hepatic Cu (Woolliams et al., 1983). Further, when heifers were fed a diet low in Cu (4 mg Cu/kg DM, 0.1 mg Mo/kg DM, 100 mg Fe/kg DM and 2.8 g S/kg DM), hepatic Cu decreased from 129 to 49 mg/kg DM in the first 8 weeks, but only from 49 to 31 mg/kg DM in the following 8 weeks (Phillippo et al., 1987a). In these conditions, the competence to support Cu homeostasis by reduced hepatic Cu mobilisation may be mediated by a hypothetical increase in apparent Cu absorption in response to the depletion of hepatic Cu.

### Manganese homeostasis

Manganese presence in animal tissues is substantially lower than that of Zn (e.g., 0.47 mg Mn vs 24 mg Zn/kg fleece-free empty BW in sheep; Grace, 1983). Also, it is much lower than the typical concentration found in feeds (15–60 mg/kg DM). Uptake of Mn by the intestinal mucosa of weaned calves is not very different in magnitude to that of Zn, but its transport from the small intestine to other tissues was much lower (Miller et al., 1972). The effect of dietary Mn on true Mn absorption and on endogenous Mn excretion is much less studied and understood than that of Zn or Cu. Experimental data in this respect are limited to a few experiments that confirm homeostatic regulation upon dietary supply, also for this trace metal.

In calves fed whole milk (0.5 mg Mn/kg DM), body retention of  $^{54}\text{Mn}$  was highly reduced when 15 mg Mn/kg DM was added to the milk (from 18.2 to 2.2%, Carter et al., 1974). The influence of increasing dietary Mn from 32 to 1 000 mg/kg DM was also evaluated in 80 kg weaned calves, with either intravenous or duodenal administration of  $^{54}\text{Mn}$  (Abrams et al., 1977). Interestingly, when the isotope was administered in the duodenum, the % recovery of  $^{54}\text{Mn}$  in bile over 7-days was not influenced by dietary Mn (0.32 vs 0.30% for 32 and 1 000 mg/kg DM, respectively). However, with intravenous administration, 54% of the  $^{54}\text{Mn}$  dose was recovered through bile over a 7-day period. This data suggest that control of true absorption in the gut is the major factor driving Mn homeostasis, but most importantly, it confirms that endogenous Mn secretion has the competence to excrete substantial amounts of Mn should absorption exceed nutritional needs. Similar evidence is available in rats (Weigand et al., 1986; Davis et al., 1992).

Under normal dietary conditions, ovine data indicate that excretion of Mn through bile and pancreatic juice seem of minor quantitative importance to whole-body Mn homeostasis, since increasing dietary Mn from 24 to 178 mg/d only induced an increase of biliary Mn of about 1 mg/d (Grace and Gooden, 1980).

However, in contrast to expected regulatory mechanisms, a positive correlation between apparent Mn absorption and Mn intake was reported in a meta-analysis including both dry and lactating cattle (Weiss and Socha, 2005). It is unclear if this relationship indicates an accumulation of Mn in the body at high Mn intakes, or if this may simply be an artefact of the experimental model, as an incomplete faecal recovery could translate into a larger absolute error with higher expected Mn content in faeces. Nevertheless, it is interesting to observe that the digestive tract of sheep had 2.9 times more Mn when Mn intake increased from 123 to 473 mg/day (Grace and Lee, 1990). This increase appears mainly driven by an increase in Mn content from ruminal tissue, as ruminal Mn increased linearly from 340 to 1 140 mg/kg DM when growing bulls were fed diets with increasing Mn content, from 47 to 214 mg/kg DM (Daniel and Martín-Tereso, 2024). Linear increase in Mn content of longissimus muscle (+53% with 240 mg

Mn/kg DM compared to no supplementation) was also observed with increasing Mn supplementation in steers (Legleiter et al., 2005).

### The boundaries of homeostatic control of trace metal nutrients in ruminants

The term ‘bioavailability’ is often used in trace metal nutrition as an attempt to describe the nutritional value of trace metal supply, when the apparent or true digestibility of these nutrients is variable and driven by homeostatic absorptive regulation. Availability refers to the potential of the supply of these nutrients to be absorbed, even if actual absorption may not be realised. The prefix “bio” in front of availability is used for trace metals as elements to refer to their potential to be incorporated into a biological system. In nutrition, this should be understood as nutritional uptake to play their biological role as nutrients. This connotation is generally used in trace metal nutrition of farm animals when the boundaries of nutritional upregulation may challenge the trace metal sufficiency of a diet.

In environmental sciences, the term “bioavailability” refers not only to their ability to enter a biological system as nutrients but also to their penetration of organisms or biological systems as undesirable elements, and it is applied to non-nutrient trace metals such as Cd, or Pb, but also to nutrients such as Cu or Zn when their excess presence makes them undesirable to the biological system. To our knowledge, this connotation is less generally used in animal nutrition, although it would be useful to describe trace metal supply beyond the boundaries of downregulation, as it will be described in the later section of this manuscript.

#### *The limits of homeostatic upregulation: dietary deficiency thresholds*

Nutritional bioavailability is a common term in our science, but it refers to a potential and not necessarily to an actual use of the nutrient. Therefore, it cannot be measured directly under nutritionally common conditions. An approximation to determining nutritional bioavailability would be to measure nutrient utilisation under conditions of nutrient undersupply that stimulate the animal to upregulate absorption efficiency. In practice, relative bioavailabilities are determined as the difference in rate of utilisation of the nutrient in two nutritional scenarios, relative to a negative control with lower supply and relative to each other, being all supply levels considered deficient. Utilisation being measured with any metric related to improved supply, from phenotypical observations of mitigation of deficiency, to determination of nutrient contents in tissues or excreta.

In the context of trace metals such as Zn, Cu, and Mn, and restricting the concept to availability for absorption, we can define absorbability as the maximum amount of metal that could be absorbed upon need. This notion of absorbability is obviously different than actual absorption, as the latter is the result of absorbability and homeostasis.

Actual absorption, as we have discussed in the first part of this review, can be down- and up- regulated by homeostatic regulation. In contrast, absorbability is theoretically not influenced by the animal mineral status, but mainly by physicochemical speciation of dietary trace nutrients, most importantly, but not exclusively by solubility and lability to ligand interactions. Hypothetically, absorption is either limited by homeostasis (defined by the animal) or by absorbability (defined by nutrient supply and digestion). The latter is a potential weakness for trace minerals homeostasis, by inducing situations where the animal is unable to absorb sufficient minerals to meet its needs.

It is generally accepted that for effective absorption to occur: the elemental trace metal nutrient should be soluble, either in ionic form, or in a complex that is soluble and labile to the transporting proteins. The pH and composition of the digestive content, as well as the sequence of events during digestions in prior segments of the gut largely determine mineral speciation and solubility (Bremner, 1970). In sheep fed dried grass only (Bremner, 1970), solubility of native Zn and Mn were lowest in the rumen (6 and 8%, respectively), increased until the early section of the duodenum (60 and 82%, respectively), and decreased in the ileum (20 and 12%, respectively). In contrast, Cu solubility remained low throughout the entire gut (19% in the rumen, 17% duodenum, and 23% in the ileum). Consistent with these data, Ivan and Veira (1981) found that in the abomasum of sheep fed corn silage-based diets, the highest to lowest solubility were found for Mn (91–97%), Zn (87–94%) and Cu (21–33%).

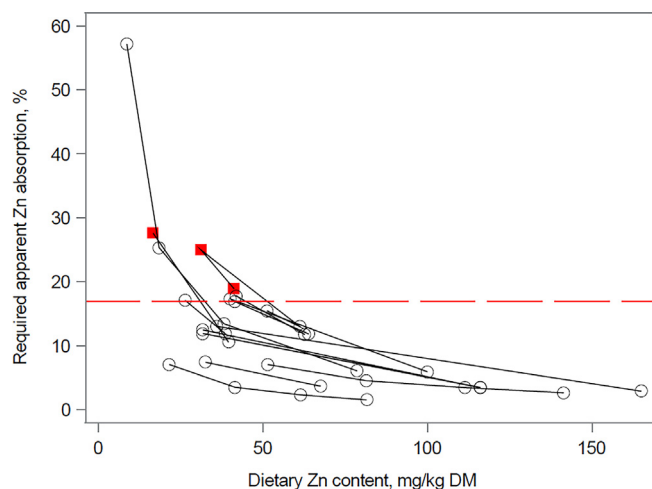
In an additional level of complexity to the process, solubility seems to be a necessary condition but not sufficient for nutritional absorbability. A trace metal nutrient can be in a soluble species, yet unavailable for absorption. This is rather uncommon under natural nutritional conditions, because trace metal transporters have a greater affinity for their metal targets than most naturally occurring nutrient chelators. An exception to this rule would be some endogenously excreted natural chelators or high-affinity synthetic chelators, depending on the chelation strength of the complex binding the metal. The effect of chelating agents on Zn availability was elegantly demonstrated in turkeys fed low dietary Zn (Vohra and Kratzer, 1964).

The consequence of imperfect availability is that under low mineral supply, absorption cannot be upregulated sufficiently to meet nutrient needs. Interestingly, when a diet of 16.6 mg Zn/kg DM was fed to growing bull calves of 2 months, older calves of 6 months (Stake et al., 1975), and primiparous lactating cows (Neathery et al., 1973b), (approximated) true Zn absorption were very similar: 50, 47 and 53%, respectively, potentially providing an indication of maximum true Zn absorption in cattle.

#### *Dietary boundaries of Zn deficiency*

As described in the first part of this review, the adjustment of Zn absorption based on how much Zn is present in the diet is a very effective means for ruminants to ensure that health and performance can be maintained under a wide range of dietary Zn. To illustrate this effect, we selected publications that studied the effect of dietary Zn supply on performance (Miller et al., 1963; Neathery et al., 1973a; Ivan and Grieve, 1975; Kirchgessner et al., 1997; Mandal et al., 2007; Cope et al., 2009; Prados et al., 2017; van Bibber-Krueger et al., 2018; Carmichael et al., 2019; Wang et al., 2021). All these studies used longitudinal designs with a duration that varied from 8 to 44 weeks (Supplementary Table S1). Using BW, average daily gain and or milk yield reported in these studies, net Zn requirements could be factorially calculated (Supplementary Table S2). Fig. 1 illustrates the apparent Zn absorption efficiency that would be expected to meet the calculated net Zn requirement relative to dietary Zn content. As expected, as dietary Zn content decreases, required apparent Zn absorption efficiency increases exponentially. Among all selected publications, two of them (highlighted in Fig. 1) reported adverse effects to lowering dietary Zn content: decrease in milk Zn content (Neathery et al., 1973a) and decreases in both DM intake and milk yield (Wang et al., 2021). The former study reduced dietary Zn from 40 to 17 mg/kg DM for a duration of 6 weeks in primiparous cows producing about 15 kg of milk per day, whereas the latter study reduced dietary Zn from 61 to 31 mg/kg DM for a duration of about 16 weeks in multiparous cows producing about 50 kg of milk per day. The calculated expected apparent Zn absorption efficiencies of 19–28% associated with these lowest Zn supply





**Fig. 1.** Required apparent Zn absorption efficiency to match dietary trace metal supply with net trace metal requirements relative to dietary Zn content in cattle. Lines connect experimental treatments from the same study. Square markers highlighted in red indicate dietary treatments for which impaired productivity was reported in the original manuscripts. A horizontal dotted line was added at 90% of the lowest apparent absorption efficiency associated with impaired productivity.

scenarios may provide quantitative insight into the lower boundary of Zn deficiency, in which bioavailability may limit further increase in absorption efficiency with lower dietary Zn supply. However, a scenario, in which bull calves were fed a diet with 8.6 mg Zn/kg DM for 37 weeks, had calculated apparent Zn absorption efficiency of 55%, and no detrimental effects on growth were reported (Miller et al., 1963). This suggests that this calculated expected efficiency was realised to meet the net Zn requirement. The type of diet fed, considered semi-practical with 90% dried beet pulp, 5% glucose and 1.5% urea, may potentially explain the difference in Zn bioavailability. Considering no adverse effects were reported across all dietary treatments with calculated required apparent Zn absorption efficiency below 19%, the use of such a threshold as a lower boundary may be helpful when defining supplementation strategy. This threshold, illustrated with the horizontal dotted line on Fig. 1, represents the maximum achievable apparent absorption efficiency in practical diets. However, it should be noted that since zootechnical performances were used to define such a threshold, it might overestimate the true limit as animal performance is not very sensitive to change in trace metal supply. Using a more sensitive status parameter would be desirable but those are unfortunately not available at present.

Nutritional factors influencing Zn availability within practical feeding boundaries in ruminants are not clear and have either demonstrated no negative impact (effect of Ca, Pond, 1983) or a modest effect on hepatic Zn (effect of Fe, Standish et al., 1971).

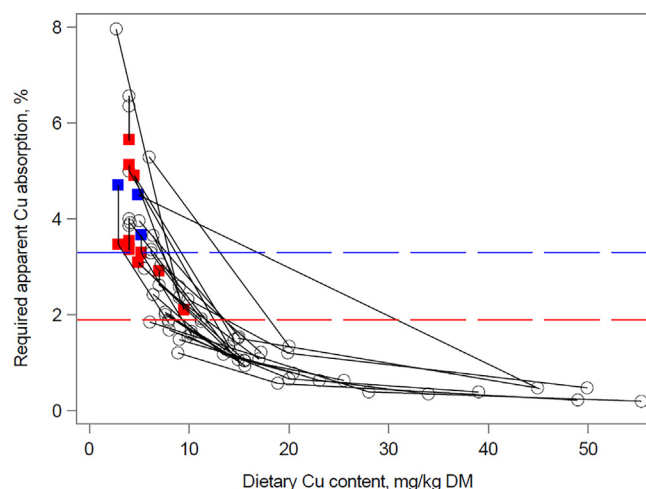
#### Dietary boundaries of Cu deficiency

A total of 20 publications (Ivan and Grieve, 1975; Humphries et al., 1983; Kincaid et al., 1986; Phillippo et al., 1987a; Wittenberg and Boila, 1988; Wittenburg et al., 1990; Ward et al., 1993; Xin et al., 1993; Kegley and Spears, 1994; Ward and Spears, 1997; Kirchgessner et al., 1997; Chase et al., 2000; Engle et al., 2000; Bailey et al., 2001; Engle et al., 2001; Mullis et al., 2003; Spears et al., 2004; Hansen et al., 2009; Prados et al., 2017; VanValin et al., 2019) with dietary treatment designed to evaluate the effect of supply of available Cu on Cu status, health and performance, were used as the basis to estimate maximum achievable apparent Cu absorption (Supplementary Table S3). As for Zn, expected apparent Cu absorption was calculated based on Cu supply reported in the publications and factorially calculated net Cu

requirements based on reported animal characteristics (Supplementary Table S2). A common feature from these selected studies is the use of added Cu antagonists. Out of 75 dietary treatments, 37 fed one or a combination of supplemental antagonists: Mo ( $n = 32$ ), S ( $n = 10$ ), Fe ( $n = 8$ ) or Mn ( $n = 2$ ). Most of the adverse effects reported on reduction of DM intake and growth (10 out of 13) were achieved with supplemental antagonists (Fig. 2). With supplemental antagonists, no adverse effects were reported when required apparent Cu absorption was calculated to be below 2.1%. Without supplemental antagonists, this threshold was higher at 3.7%. From the 33 dietary treatments which had required apparent Cu absorption above 2.1%, 18 fed antagonists, among which 9 reported adverse effects on performance. From the remaining 15 dietary treatments without additional antagonists, only 3 showed adverse effects. These three dietary conditions were reported in growing steers during the growing (5.2 mg Cu/kg DM in Ward and Spears, 1997) and finishing periods (2.9 mg Cu/kg DM in Ward and Spears, 1997; 4.9 mg Cu/kg DM in Engle et al., 2000) with basal diet that had low level of Mo (from 0.58 to 1.16 mg/kg DM) and Fe (from, 55 to 208 mg/kg DM). Interestingly, these reductions in growth were achieved for animals which had plasma Cu concentrations considered adequate (1.22 mg/L in Ward and Spears, 1997 and 0.75 mg/L in Engle et al., 2000), illustrating the difficulty to predict adverse effects.

These data also illustrate the increased likelihood of adverse effects with the presence of a high level of Cu antagonists (e.g., 5 mg Mo/kg DM). Interactions occurring in the rumen between Cu, S, Mo, and Fe have been extensively reviewed previously (e.g., Gooneratne et al., 1989; Gould and Kendall, 2011).

Sulphur, individually, through the formation of ruminal sulphide, can decrease Cu bioavailability (assessed based on plasma Cu from hypocupraemic ewes, Suttle, 1974a). In steers, increasing S from 1.3, 3.1 to 4.6 g/kg DM decreased hepatic Cu content from 230, 140 to 96 mg/kg DM (Spears et al., 2011). The addition of inorganic S or organic S (i.e., methionine or cysteine) had a similar effect on Cu (Suttle, 1974b), and it is thus not surprising to see dietary protein levels negatively affecting Cu solubility numerically in



**Fig. 2.** Required apparent Cu absorption efficiency to match dietary trace metal supply with net trace metal requirements relative to dietary Cu content in cattle. Lines connect experimental treatments from the same study. Square markers highlighted in red (treatments with supplemental Cu antagonists) and blue (treatments without supplemental Cu antagonists) indicate dietary treatments for which impaired productivity was reported in the original manuscripts. Horizontal dotted lines were added at 90% of the lowest apparent absorption efficiency associated with impaired productivity. The red and blue dotted lines represent the maximum achievable apparent absorption efficiency in practical diets with and without supplemental Cu antagonists (i.e., mainly Mo, S, Fe).

the rumen and significantly in the abomasum (Ivan and Veira, 1981).

In contrast, the effect of Mo on Cu bioavailability increases with increasing dietary S, as the formation of thiomolybdates is a prerequisite for the interaction of Mo with Cu in the gut (Suttle and Field, 1983; Al-Kirshi et al., 2011). The negative effect on Cu absorption seems to be maximal when dietary Mo is at about 4–6 mg/kg DM and S about 2–3 g/kg DM (Suttle, 1983). Additionally, thiomolybdates may pose an extra challenge for Cu homeostasis, as when absorbed, the complex can disturb Cu metabolism, decreasing its availability in blood (Gooneratne et al., 1989). Excess availability of thiomolybdates in relation to available Cu is required for systemic Cu metabolism disruption to occur (Smith and Wright, 1975). At abundant thiomolybdate availability, supplementation of Cu has generally proven to be effective to mitigate part of the systemic effect of thiomolybdate (Mason et al., 1978; Suttle and Field, 1983). This practice should however be applied with cautiousness as excess Cu even in the presence of high Mo and S may accumulate in the liver (e.g., White et al., 1989).

Iron, mainly in the presence of abundant dietary S (Standish and Ammerman, 1971), has also been shown to reduce apparent Cu absorption and hepatic Cu levels (Standish et al., 1971; Prabowo et al., 1988; Mullis et al., 2003). As in the case of Mo, the antagonist effect of Fe is dependent on a functional rumen (Bremner et al., 1987). Interestingly, multiple studies comparing the effect of Fe with Mo on Cu status have shown that only Mo was able to negatively affect growth or fertility, despite a similar decrease induced in hepatic Cu and plasma Cu (Humphries et al., 1983; Bremner et al., 1987; Philippo et al., 1987a; Philippo et al., 1987b). It is remarkable that, despite the extremely low hepatic Cu level (3.6 mg/kg DM) and plasma Cu (0.21 mg/L) reached after 32 weeks in heifers fed a 4 mg Cu/kg DM diet supplemented with 800 mg Fe/kg DM, no clinical signs in terms of health nor performance were reported (Humphries et al., 1983). In contrast, in the same study, heifers supplemented with 5 mg Mo/kg DM had, after 20 weeks, reduced growth rate, skeleton lesions and change in hair texture and colour. Such a negative outcome may thus be related with the high Mo and/or thiomolybdate rather than the low Cu status.

In addition to antagonisms described for S, Mo and Fe, supplemental Zn has also proven to affect Cu availability. In bull calves, and addition of 100 mg Zn/kg DM in a high concentrate diet containing 40 mg Zn/kg DM and 6 mg Cu/kg DM decreased hepatic Cu from 239 to 163 mg/kg DM (–32%) after 10 weeks of feeding (Ivan and Grieve, 1975). At higher dietary Cu content (29 mg/kg DM), the data from the study of Bremner et al. (1976) suggested that a much greater amount of Zn was required to affect hepatic Cu in sheep. As much as 220 mg Zn/kg DM only decreased hepatic Cu by 8% and 420 mg Zn/kg DM decreased it by 30%. Similarly, when sheep were supplemented with palm kernel cake, a product characterised with high Cu content (total dietary Cu was 23 mg/kg DM), accumulation of hepatic Cu was reduced by 44% when about 500 mg Zn/kg DM was added in the diet (Al-Kirshi et al., 2011).

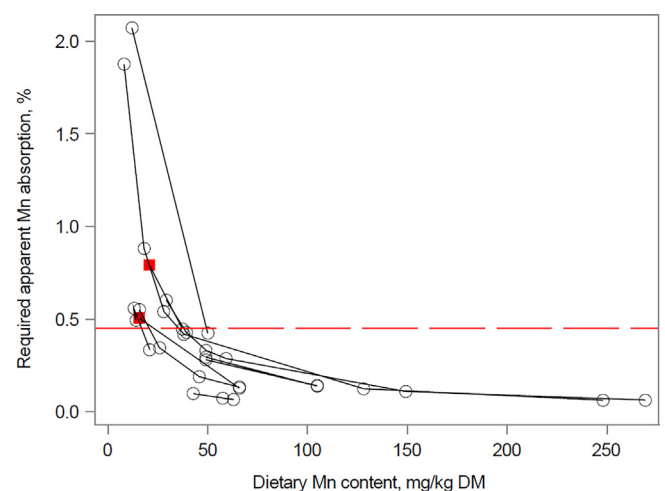
#### Dietary boundaries of Mn deficiency

For Mn, a total of eight publications (Howes and Dyer, 1971; Ivan and Grieve, 1975; Kirchgessner et al., 1997; Legleiter et al., 2005; Weiss and Socha, 2005; Hansen et al., 2006a; Hansen et al., 2006b; Prados et al., 2017) with dietary treatments designed to evaluate the effect of Mn supply on health and performance were used as the basis to estimate maximum achievable apparent Mn absorption (Supplementary Table S4). Interestingly, despite high solubility of Mn in the small intestine (Bremner, 1970), apparent Mn absorption efficiencies as low as 0.50% (Hansen et al., 2006b) or 0.80% (Kirchgessner et al., 1997) appear incompatible with optimal performance. The former study reported anomalies in the development of calf born from dams fed a low Mn diet,

and the latter study identified a potential reduced growth (i.e., statistical tendency) in Simmental bulls during their fastest growth period. Further support for the risk of calf malformation associated with low dietary Mn was published earlier (Bentley and Phillips, 1951; Rojas et al., 1965). The addition of 20 and 10 mg Mn/kg DM prevented these adverse effects in these two studies, respectively. Unfortunately, we were unable to use these two studies in our literature summary (Fig. 3) as DM intake, a critical measure for our calculations was not reported. These issues were described for growing heifers fed a diet with 8.5 mg Mn/kg DM (Bentley and Phillips, 1951) and mature Hereford cows fed diets containing 15.8 and 16.9 mg Mn/kg DM (Rojas et al., 1965).

The reasons why greater Mn absorption may not be possible are unclear and have been speculated to arise by competition between Mn and Fe for common binding sites in enterocytes (Bremner and Davies, 1980). This view may be partly supported by the negative effect of adding clay minerals (high in Fe) on dissolved Mn under ruminal, abomasal and duodenal conditions (Schlattel et al., 2021). Further evidence of Fe impairment of Mn availability was found upon the addition of 750 mg Fe/kg DM to a diet fed to weaned calves, resulting in a significantly reduced Mn concentration in duodenal tissue from 10.7 to 7.4 mg/kg DM (Hansen, 2010). These results are analogous to findings with rats, in which raising dietary Fe content from 19 to 276 mg/kg DM decreased mucosal Mn by 2.8 and 2.4-fold, when dietary Mn content was 48 and 188 mg/kg DM, respectively (Davis et al., 1992).

The typical high dietary Fe content in ruminant diets, in large part explained by soil/dust contamination of forages, may thus be a plausible explanation for the broad difference between gross Mn requirement and net physiological Mn requirement. Furthermore, the availability of dietary Fe is highly uncertain; therefore, so is its influence on Mn availability adding to the unpredictability of the nutritional availability of Mn. *In vitro* evidence shows that soil contamination increases the water-soluble Fe fraction, and thus may contribute to increasing the availability of Fe (Hansen and Spears, 2009; Schlattel et al., 2021) and consequently its effects on Mn. This uncertainty may explain why in some cases, apparent Mn absorption of about 2.0% (Ivan and Grieve, 1975; Legleiter et al., 2005) may appear plausible whereas in other cases (Kirchgessner et al., 1997; Hansen et al., 2006b), adverse effects were reported when required apparent Mn absorption were below 1.0%.



**Fig. 3.** Required apparent Mn absorption efficiency to match dietary trace metal supply with net trace metal requirements relative to dietary Mn content in cattle. Lines connect experimental treatments from the same study. Square markers highlighted in red indicate dietary treatments for which impaired productivity was reported in the original manuscripts. A horizontal dotted line was added at 90% of the lowest apparent absorption efficiency associated with impaired productivity.

Nevertheless, as demonstrated earlier, reports of clinical symptoms driven by Mn deficiency in cattle are extremely scarce in the literature; not only naturally occurring cases are apparently rare but also it seems difficult to provide a practical diet sufficiently low in Mn to study deficiency. Symptoms of Mn deficiency have been characterised using semi-synthetic diets in swine (0.5 mg Mn/kg DM; Plumlee et al., 1956) or chicks (2.4 mg Mn/kg DM; Leach and Muenster, 1962) with a common feature being reduced skeletal growth.

#### *The limits of homeostatic downregulation: Dietary tolerance thresholds*

As elegantly demonstrated *in vitro* for Zn, uptake of trace metals occurs through both a regulated and an unregulated component (Raffaniello and Wapnir, 1989). The former is governed by the body metal status and thus largely under the animal regulatory control. In contrast, unregulated uptake is directly proportional to the labile trace metal concentration (Raffaniello and Wapnir, 1989). Thus, as trace metal supply increased, there is an increasing need to safely handle the excess trace metal uptake. The tolerance to increasing trace metal supply most likely relies on the capacity of the animal to excrete the excess metal back into the gut, but also on the capability of various key tissues (gut, liver, kidney) to chelate the metals to prevent cellular damage (e.g., with metallothionein).

The risk associated with excessive supply of various minerals has long been recognised and MTL have been proposed for farm animals (National Research Council, 2005). These MTL were defined as the highest dietary mineral concentration that will not impair animal health or performance. For cattle, proposed MTL were: 40 mg/kg DM for Cu, 500 mg/kg DM for Zn and Fe, and 2 000 mg/kg DM for Mn. Nevertheless, these proposed MTL have some limitations. The first is that they ignore the key role of DM intake in defining dietary trace metal supply, specially to create unusually high levels of supply. The second is that, largely because of the scarcity of published study with long duration, these levels may not necessarily reflect the upper tolerance of animals chronically exposed to a high supply of trace metals. In this review, we illustrate the potential benefit of using upper boundaries defined as the dietary supply level beyond which apparent absorption empirically has shown to exceed net requirements. Such a threshold reflects dietary conditions at which further down regulation of true absorption efficiency, and, or increase in faecal endogenous trace metal secretion cannot compensate the increase in dietary trace metal supply. An advantage of using such a physiological definition of upper limit is that, when exposed to a high level of trace metals, this physiological imbalance would be expected to occur prior to potential clinical signs of toxicity, and thus, shorter duration studies may be used to define this upper threshold.

#### *Tolerance to high Zn*

As investigated in sheep, a moderate increase in dietary Zn content from 40 to 70 mg/kg DM had no effect on Zn concentration of any of the organs and soft tissues studied (Grace and Lee 1990). However, when dietary Zn was raised from 100 to 500 mg/kg DM in growing heifers and steers, liver Zn content was increased by 208%, pancreas Zn content was increased by 382%, and kidney Zn content was increased by 337% (Ott et al., 1966). These limited data could suggest that within a practical dietary Zn supply; the risk for Zn accumulation in body tissue is limited. In fact, several publications have reported the beneficial effect of Zn supplementation, even when basal dietary Zn appears to be adequate. For example, in steers fed a finishing diet, increasing dietary Zn from 51 to 141 mg/kg DM linearly increased feed efficiency, mainly driven by a linear decrease in feed intake (Van Bibber-Krueger et al.,

2018). During an abrupt weaning, growth was increased by 11% when dietary Zn was raised from 35 to 85 mg/kg DM (Abdollahi et al., 2020). During lactation, increasing dietary Zn from 42 to 63 mg/kg DM decreased milk somatic cell count and milk amyloid A, suggesting an improvement in udder health (Cope et al., 2009). Supplementing 60 mg Zn/kg DM in a basal diet with 37 mg Zn/kg DM also reduced milk somatic cell count by 34% (Ianni et al., 2019). Overall, moderate supplementation of Zn above the reference recommendation seems to induce positive adaptation. Perhaps, the stimulating role of Zn on metallothionein synthesis (Blalock et al., 1988) may partly explain its positive role, as once synthesised, metallothionein may protect against other trace metal toxicity (e.g., Cu; see Cousins, 1985). Additionally, as demonstrated during weaning in pigs, Zn supplementation has been linked to improve intestinal integrity (Hu et al., 2013), which may also partly explain positive performance reported in ruminants.

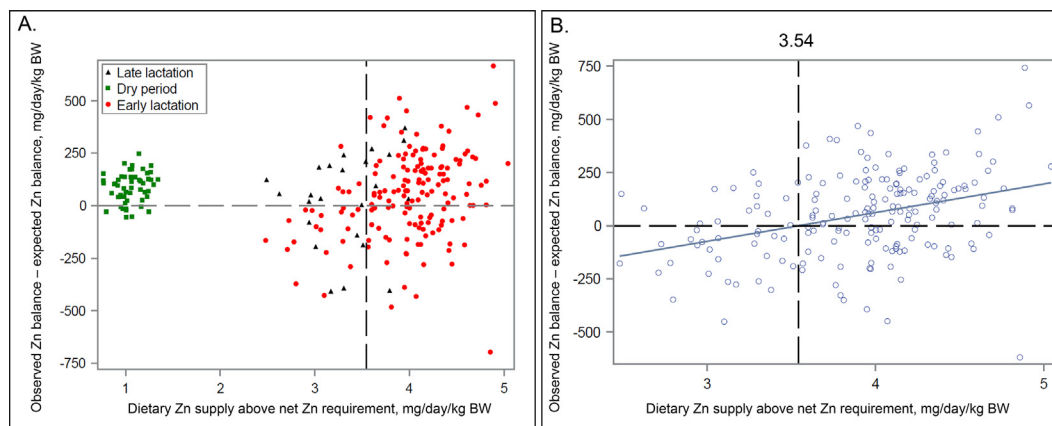
Nevertheless, when dietary Zn is raised to higher levels, negative performance outcomes have also been reported. In beef steers, increased dietary Zn from 90 to 280 mg/kg DM linearly decreased feed intake, although growth was not significantly affected (Malcolm-Callis et al. 2000). A reduction in growth was observed when 150 mg Zn/kg DM was supplemented to growing bulls fed a diet with a basal Zn content of 45 mg/kg DM (Fagari-Nobijari et al., 2012). However, another study supplemented 1 000 mg Zn/kg DM to lactating cows throughout most of their lactation and reported no adverse effects (Miller et al., 1989).

Although most of the literatures indicate practical range of dietary Zn fed to cattle does not negatively affect performance, recent evidence suggested that high DM intake in combination with high supplemental Zn may overwhelm homeostatic capacity in dairy cattle (Daniel et al., 2023). In this study, positive Zn balance, above expected Zn retention based on requirements for growth and gestation (see Supplementary Material S1 for calculation), was reported, mainly when dietary Zn supply was highest because of the increase in DM intake occurring in early lactation. From a physiological standpoint, retention of trace metals, such as Zn, above the net requirement may be interpreted as undesirable. Thus, using cow weekly data collected during this study (Fig. 4a), we performed a regression analysis between, Zn balance above expectation, and dietary Zn supply above net Zn requirement, with data collected during the lactation period. More details about the methodology of this calculation and analysis can be found in Supplementary Material S1. Briefly, the regression was run with PROC MIXED of SAS 9.4 (SAS Institute Inc.) with adjustment for the random effect of cow. The quadratic effect was not significant and was removed from the model. Linear regression had intercept and slopes of  $-476.95 (\pm \text{SE } 123.94)$  and  $134.62 (\pm 31.16)$ , respectively. Using this equation, body Zn balance was greater than expectation when dietary Zn supply above net Zn requirement was above 3.54 mg/day/kg BW (Fig. 4b). In a growing bull study fed increasing level of Zn, Cu and Mn, Zn balance increased linearly when dietary Zn was increased from 38, 61, 78, 123 and 195 mg/kg DM, but only the highest level led to significant increase in Zn balance, as well as significant increase in urinary Zn excretion (Daniel and Martín-Tereso, 2024). The latter may reflect excessive metabolic availability of Zn. Interestingly, the highest level fed during this study supplied 3.96 mg Zn/day/kg BW above net Zn requirement, whereas all other levels had supply between 0.76 and 2.57 mg Zn/day/kg BW, suggesting that using a Zn supply above net requirement of 3.54 mg/day/kg BW as upper limit may have merit to prevent unregulated Zn influx.

#### *Tolerance to high Cu*

As reviewed in the first part of this review, the liver plays a critical role in maintaining Cu homeostasis in ruminants. Nevertheless, excess Cu in the liver has been associated with increased



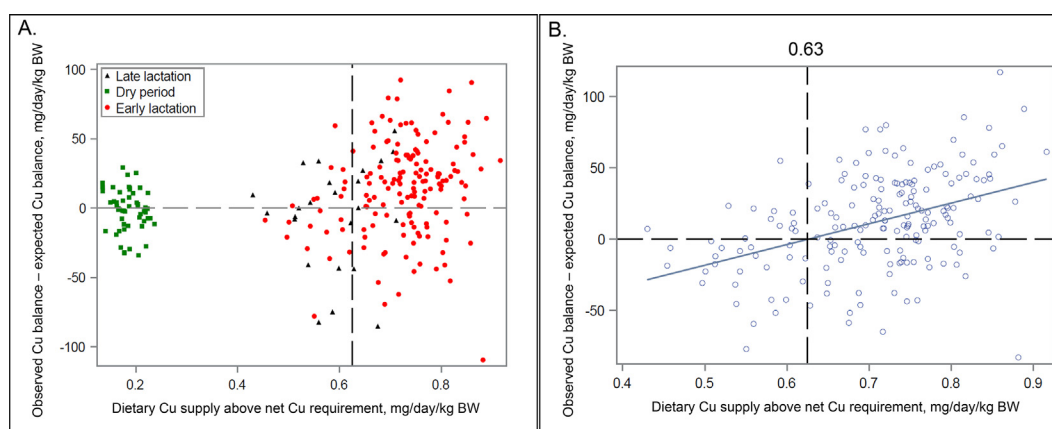


**Fig. 4.** Relationship between deviation from expected Zn balance and dietary Zn supply above net Zn requirements in lactating Holstein cows. In panel A, raw weekly individual cow data are displayed, whereas in panel B, linear regression, and data adjusted for the random effect of cow, applied on the lactation subset, are shown. The expected Zn balance reflects the sum of net Zn requirements for growth and gestation, while the observed Zn balance was calculated based on weekly measurements from individual dairy cows (Daniel et al., 2023) using the total collection method for faeces and urine.

hepatic oxidative stress (Strickland et al., 2019; McCaughern, 2019) and in extreme cases, can cause a hemolytic crisis that may lead to death (Ishmael et al., 1971).

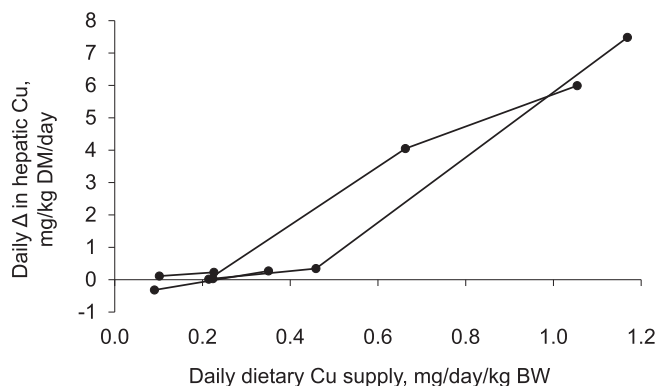
One aspect that has been often overlooked in Cu nutrition is the amount of feed the animal consumes. This is particularly important for the lactating vs non-lactating animals, as feed intake in relation to body size is largely increased. Since the amount of Cu exported per kg of milk is small (0.04 mg/kg, NASEM, 2021), the increased feed intake associated with lactational energy requirement may induce a challenge for Cu homeostasis. As an example, a cow producing 30 kg of milk a day and eating 21 kg of DM from a diet containing 12 mg Cu/kg DM will consume 252 mg of Cu daily, but only export 1.2 mg of Cu in milk. A similar cow fed the same diet and producing 50 kg of milk with a DM intake of 27.5 kg will ingest 330 mg of Cu, and export 2.0 mg of Cu in milk. Thus, the highest producing cow ingests much more Cu (78 mg/d), while milk Cu export is marginally higher (+0.8 mg/d). This simple example illustrates the challenge that high-producing dairy cows may face in maintaining Cu homeostasis when dietary Cu content is moderately high. This risk was further substantiated with a study evaluating Cu balance on a weekly basis throughout the transition period in dairy cattle (Daniel et al., 2023). Whereas Cu balance remained close to 0 from late lactation to early lactation, Cu bal-

ance was significantly positive when DM intake was further increased about 5 weeks after calving (weekly cow data plotted in Fig. 5a). As done for Zn, a regression analysis (see Supplementary Material S1), on the data collected during lactation in Daniel et al. (2023), adjusted for the random effect of cow, resulted in a linear equation with intercept and slope of  $-90.72 (\pm SE 20.57)$  and  $145.07 (\pm 28.32)$ , respectively (Fig. 5b). The equation indicates that Cu supplies above net requirement, in excess of 0.63 mg/day/kg BW results in a Cu balance that exceeded expectations based on calculated net requirements. Studies (Ward and Spears, 1997; Engle et al., 2000; Engle and Spears, 2000; Balemi et al., 2010) evaluating daily changes in hepatic Cu in response to Cu supply in diets that were not supplemented with antagonists give some credit to the use of 0.63 mg/day/kg BW as upper Cu limit. From 0.09 to 0.46 mg Cu/day/kg BW, daily change in hepatic Cu was moderate (within  $-0.3$  to  $0.3$  mg/kg DM/day) whereas with dietary supply above 0.66 mg/day/kg BW, daily increase in hepatic Cu was much greater (4.1–7.5 mg/kg DM/day, Fig. 6). Expressing an upper limit in mg/day/kg BW is expected to better protect lactating dairy cows against excess dietary Cu, as compared to a dietary concentration (as set by National Research Council (2005) to 40 mg/kg DM). Indeed, this upper limit can be met with a dietary Cu content as low as 17.5 mg/kg DM for a 700 kg lactating cow eating 25 kg of



**Fig. 5.** Relationship between deviation from expected Cu balance and dietary Cu supply above net Cu requirements in lactating Holstein cows. In panel A, raw weekly data for individual cows are displayed, whereas in panel B, linear regression results and data adjusted for the random effect of cow, based on the lactation subset, are shown. The expected Cu balance reflects the sum of net Cu requirements for growth and gestation, while the observed Cu balance was calculated based on weekly measurements from individual dairy cows (Daniel et al., 2023) using the total collection method for faeces and urine.





**Fig. 6.** Daily change in hepatic Cu content in response to dietary Cu supply in cattle. Data are from the following studies (number of treatments used in parentheses): Ward and Spears, 1997 ( $n = 2$ , no added Mo); Engle et al., 2000 ( $n = 3$ ); Engle and Spears, 2000 ( $n = 3$ , control and  $\text{CuSO}_4$ ); Balemi et al., 2010 ( $n = 2$ ). Each line connects dietary treatments from the same study.

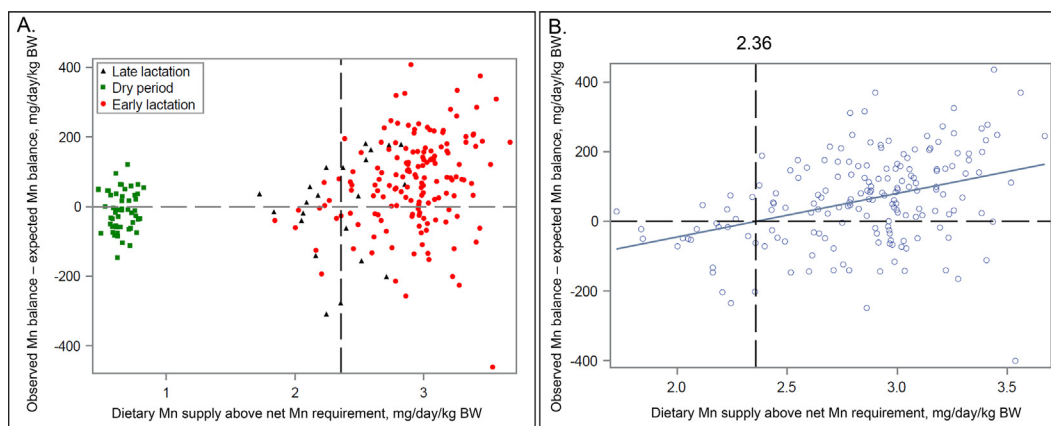
DM daily. In contrast, a growing bull of 450 kg consuming 11 kg of DM daily would need to receive a diet with 25.6 mg Cu/kg DM to reach such an upper limit.

Analysis of Cu content from livers of bovines sent to a slaughterhouse in the United Kingdom confirmed the greater susceptibility of dairy cows compared to beef cattle to Cu overload (Kendall et al., 2015). About 40% of dairy cows (vs 17% for beef breeds) had hepatic Cu levels above the reference range (8 000  $\mu\text{mol/kg}$  DM or 508 mg/kg DM) from the Animal Health and Veterinary Laboratories Agency. The impact of lactation on hepatic Cu accumulation was also evident in a study evaluating the supplementation of 15 mg Cu/kg DM, in the form of a bolus applied every 6 months, from postweaning to 14 weeks after the first calving (McCaughern, 2019). Hepatic Cu concentration decreased for both, the non-supplemented Cu group (15 mg Cu/kg DM basal diet) and the supplemented Cu group, between 7 months (643 and 798 mg/kg DM), 13 months (195 and 350 mg/kg DM), and the precalfing period (128 and 293 mg/kg DM). In contrast, 14 weeks after parturition, hepatic Cu had risen to 424 mg/kg DM for the control group and to 607 mg/kg DM for the Cu supplemented group. In this study, the supplementation of Cu negatively affected milk production within the first weeks after calving. Supplementation of 20 mg Cu/kg DM on a diet containing 4.9 mg Cu/kg DM was also reported to decrease growth rate in finishing steers (Engle and Spears, 2000). Nevertheless, a high supply of Cu does not systematically

result in impaired performance. In contrast, during the last 168 d before slaughter, Angus-cross yearling steers and heifers even had improved feed efficiency when 100 or 200 mg Cu/kg DM was supplemented to a basal diet with 6.4 mg/kg Cu (Felix et al., 2012). Likewise, feeding 80 mg/kg DM of supplemental Cu for 60 days did not induce sign of toxicity in both growing dairy heifers and first lactating cows (Du et al., 1996), nor did it when 40 mg Cu/kg DM was fed to lactating cows for 61 days (Engle et al., 2001). In all those studies, high hepatic Cu was achieved (+847 mg/kg DM in Felix et al., 2012; +424 mg/kg DM in Du et al., 1996; +453 mg/kg DM in Engle et al., 2001). Thus, it could be suspected that longer feeding exposure to high dietary Cu may have impaired health or performance.

#### Tolerance to high Mn

The weekly cow data from Daniel et al. (2023) illustrating deviation from expected Mn balance relative to dietary supply above requirement are shown in Fig. 7a. Details of calculations and analysis can be found in Supplementary Material S1. As for Zn and Cu, the Mn balance was positive when the dietary supply was high. The regression had intercept and slope of  $-295.33 (\pm 81.54)$  and  $125.21 (\pm 28.18)$ , respectively, indicating that above 2.36 mg/day/kg BW, Mn supply above net requirement lead to Mn balance that exceed expectations (Fig. 7b). A positive relationship between Mn supply and apparent Mn absorption was also reported by Weiss and Socha (2005). Interestingly, when Mn intake was raised from 105 to 455 mg/kg DM in sheep, Mn content in the digestive tract was by far the most affected body tissue, with a 188% increase (Grace and Lee, 1990). This increase appears mainly driven by an increase in Mn content from ruminal tissue, as ruminal Mn increased linearly from 340 to 1 140 mg/kg DM when growing bulls were fed diets with increasing Mn content, from 47 to 214 mg/kg DM (Daniel and Martín-Tereso, 2024). Such observation may partly explain the reported positive relationship between Mn intake and Mn balance (Weiss and Socha, 2005; Daniel et al., 2023). The digestive tract may thus hold Mn, limiting unregulated outflow of Mn from the enterocyte to the blood, and thereby minimising the risk for accumulation of Mn in other tissues. This is however insufficient to fully prevent accumulation of Mn in the body, as reported by a range of 20–40% increase in Mn content of most tissue (brain, lungs, heart, spleen, pancreas, kidney) and 58% for the liver when dietary Mn was raised from 105 to 455 mg/kg DM (Grace and Lee, 1990). Similarly, steers fed a diet with 240 mg/kg DM had 23% more Mn in the liver and 53% more Mn in the longissimus muscle as compared to steers fed a basal diet with 29 mg



**Fig. 7.** Relationship between deviation from expected Mn balance and dietary Mn supply above net Mn requirements in lactating Holstein cows. In panel A, raw weekly data for individual cows are displayed, whereas in panel B, linear regression results and data adjusted for the random effect of cow, based on the lactation subset, are shown. The expected Mn balance reflects the sum of net Mn requirements for growth and gestation, while the observed Mn balance was calculated based on weekly measurements from individual dairy cows (Daniel et al., 2023) using the total collection method for faeces and urine.

Mn/kg DM (Legleiter et al., 2005). The supplementation of 250 mg of Mn to sheep grazing a pasture with 140–200 mg Mn/kg DM was sufficient to negatively affect growth rate (48% reduction in the first year and 33% reduction in the second year, Grace, 1973). The addition of 500 mg Mn/kg DM was also reported to decrease DM intake in growing and finishing beef cattle by 24 and 13%, respectively (Hansen et al., 2009).

## Conclusions

Consistent with other species, ruminants tightly regulate the metabolic supply of trace metals to prevent deficiencies and protect against potential toxicity. Increasing apparent absorption efficiency of trace metal is the main response to limited trace metal supply or an increase in physiological demand. The result of this mostly accurate homeostatic control, combined with typical trace metal concentrations in feedstuffs, explains the low incidence of reported clinical deficiency cases.

Based on these observations, the generous safety margins used for supplementation, applied in addition to already conservative trace metal reference feeding recommendations, do not seem justified. There is merit to acknowledge the role of variable absorptive efficiencies when defining a supplemental trace metal strategy. Published epidemiological data were used to estimate the ranges of apparent absorption efficiency compatible with optimal health for all three trace metals evaluated, providing a novel approach to define guidelines of minimum dietary trace metal supply.

Equally important as the consideration of lower boundaries to mitigate deficiencies is the consideration of upper boundaries of trace metal supply. Ruminants accumulate Cu in their liver when the dietary supply is increased. This distinct feature of Cu homeostasis makes ruminants less tolerant to high Cu supply. Second to sheep, high-producing dairy cows, characterised by elevated dry-matter intake relative to their body size, but relatively low net lactation Cu requirement, may be most susceptible to Cu toxicity. An approach based on a novel definition of tolerable supply was presented to estimate upper boundaries for Zn, Cu, and Mn, at which body trace metal retention exceeds expectations. These upper levels were estimated to be 3.54, 0.63, and 2.36 mg/day/kg DM for Zn, Cu, and Mn, respectively.

Aiming to supply levels of trace metal above lower boundaries but also below upper boundaries may help to minimise potential adverse effects associated with inadequate supply.

## Supplementary material

Supplementary Material for this article (<https://doi.org/10.1016/j.animal.2025.101532>) can be found at the foot of the online page, in the Appendix section.

## Ethics approval

Not applicable.

## Data and model availability statement

None of the data or models are deposited in an official repository. The data presented in this article are available upon request from the authors.

## Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

## Author ORCIDs

J.B. Daniel: <https://orcid.org/0000-0001-7627-3708>.

J. Martín-Tereso: <https://orcid.org/0000-0002-6757-4629>.

## CRediT authorship contribution statement

J.B. Daniel: Writing – original draft, Visualisation, Project administration, Conceptualisation. J. Martín-Tereso: Writing – review & editing, Supervision, Conceptualisation.

## Declaration of interest

All authors are employed by Trouw Nutrition (Amersfoort, the Netherlands), a company with broad commercial interests in ruminant nutrition. This review is part of a research line on trace nutrient homeostasis that may result in the development of products and nutritional concepts. Trouw Nutrition R&D adheres to the principles of the European Code of Conduct for Research Integrity (Drenth, 2012).

## Acknowledgements

None.

## Financial support statement

Work of the authors described in this review was funded in part or in full by Trouw Nutrition (Amersfoort, the Netherlands).

## References

- Abdollahi, M., Rezaei, J., Fazaeli, H., 2020. Performance, rumen fermentation, blood minerals, leukocyte and antioxidant capacity of young Holstein calves receiving high-surface ZnO instead of common ZnO. *Archives of Animal Nutrition* 74, 189–205.
- Abrams, E., Lassiter, J.W., Miller, W.J., Neathery, M.W., Gentry, R.P., Blackmon, D.M., 1977. Effect of normal and high manganese diets on the role of bile in manganese metabolism of calves. *Journal of Animal Science* 45, 1108–1113.
- Al-Kirshi, R., Alimon, A., Ivan, M., 2011. Effects of dietary molybdenum, sulfur and zinc on the excretion and tissue accumulation of trace elements in sheep fed palm kernel cake-based diets. *Animal* 5, 1539–1545.
- Bailey, J.D., Ansotegui, R.P., Paterson, J.A., Swenson, C.K., Johnson, A.B., 2001. Effects of supplementing combinations of inorganic and complexed copper on performance and liver mineral status of beef heifers consuming antagonists. *Journal of Animal Science* 79, 2926–2934.
- Balemi, S.C., Grace, N.D., West, D.M., Smith, S.L., Knowles, S.O., 2010. Accumulation and depletion of liver copper stores in dairy cows challenged with a Cu-deficient diet and oral and injectable forms of Cu supplementation. *New Zealand Veterinary Journal* 58, 137–141.
- Bentley, O.G., Phillips, P.H., 1951. The effect of low manganese rations upon dairy cattle. *Journal of Dairy Science* 34, 396–403.
- Blalock, T.L., Dunn, M.A., Cousins, R.J., 1988. Metallothionein gene expression in rats: tissue-specific regulation by dietary copper and zinc. *Journal of Nutrition* 118, 222–228.
- Bremner, I., 1970. Zinc, copper and manganese in the alimentary tract of sheep. *British Journal of Nutrition* 24, 769–783.
- Bremner, I., Davies, N.T., 1980. Dietary composition and the absorption of trace elements by ruminants. In: Ruckebusch, Y., Thivend, P. (Eds.), *Digestive physiology and metabolism in ruminants*. MTP Press Ltd., Lancaster, UK, pp. 409–427.
- Bremner, I., Young, B.W., Mills, C.F., 1976. Protective effects of zinc supplementation against copper toxicosis in sheep. *British Journal of Nutrition* 36, 551–561.
- Bremner, I., Humphries, W.R., Phillippo, M., Walker, M.J., Morrice, P.C., 1987. Iron-induced copper deficiency in calves: dose-response relationships and interactions with molybdenum and sulphur. *Animal Production* 45, 403–414.
- Carmichael, R.N., Genter-Schroeder, O.N., Deters, E.L., Jackson, T.D., Messersmith, E. M., VanValin, K.R., Hansen, S.L., 2019. The influence of supplemental zinc and dietary fiber concentration on mineral retention of beef steers. *Translational Animal Science* 3, 784–795.
- Carter, J.C., Miller, W.J., Neathery, M.W., Gentry, R.P., Stake, P.E., Blackmon, D.M., 1974. Manganese metabolism with oral and intravenous <sup>54</sup>Mn in young calves as influenced by supplemental manganese. *Journal of Animal Science* 38, 1284–1290.

- Castillo, A.R., St-Pierre, N.R., Silva del Rio, N., Weiss, W.P., 2013. Mineral concentrations in diets, water, and milk and their value in estimating on-farm excretion of manure minerals in lactating dairy cows. *Journal of Dairy Science* 96, 3388–3398.
- Chase, C.R., Beede, D.K., Van Horn, H.H., Shearer, J.K., Wilcox, C.J., Donovan, G.A., 2000. Responses of lactating dairy cows to copper source, supplementation rate, and dietary antagonist (iron). *Journal of Dairy Science* 83, 1845–1852.
- 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* 33, 43–49.
- Cope, C.M., Mackenzie, A.M., Wilde, D., Sinclair, L.A., 2009. Effects of level and form of dietary zinc on dairy cow performance and health. *Journal of Dairy Science* 92, 2128–2135.
- 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.
- Cousins, R.J., 1985. Absorption, transport and hepatic metabolism of copper and zinc with special reference to metallothionein and caeruloplasmin. *Physiological Reviews* 65, 238–309.
- Daniel, J.B., Martín-Tereso, J., 2024. Trace metal homeostasis in growing cattle. *Book of abstracts of annual meeting of American Society of Animal Science*, 21–25 July 2024, Calgary, Canada. pp. 79–80.
- Daniel, J.B., Brugger, D., van der Drift, S., van der Merwe, D., Kendall, N., Windisch, W., Doelman, J., Martín-Tereso, J., 2023. Zinc, copper, and manganese homeostasis and potential trace metal accumulation in dairy cows: longitudinal study from late lactation to subsequent mid-lactation. *Journal of Nutrition* 153, 1008–1018.
- Davis, C.D., Wolf, T.L., Greger, J.L., 1992. Varying levels of dietary manganese and iron affect absorption and gut endogenous losses of manganese by rats. *Journal of Nutrition* 122, 1300–1308.
- Drenth, P.J.D., 2012. The European code of conduct for research integrity. In: Mayer, T., Steneck, N. (Eds.), *Promoting Research Integrity in a Global Environment*. World Scientific Publishing Co Pte Ltd, Singapore city, Singapore. pp. 161–168.
- Du, Z., Hemken, R.W., Harmon, R.J., 1996. Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. *Journal of Dairy Science* 79, 1873–1880.
- Duplessis, M., Fadul-Pacheco, L., Santschi, D.E., Pellerin, D., 2021. Toward precision feeding regarding minerals: what is the current practice in commercial dairy herds in Québec, Canada? *Animals* 11, 1320.
- Engle, T.E., Spears, J.W., Xi, L., Edens, F.W., 2000. Dietary copper effects on lipid metabolism and circulating catecholamine concentrations in finishing steers. *Journal of Animal Science* 78, 2737–2744.
- Engle, T.E., Spears, J.W., 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. *Journal of Animal Science* 78, 2446–2451.
- Engle, T.E., Fellner, V., Spears, J.W., 2001. Copper status, serum cholesterol, and milk fatty acid profile in Holstein cows fed varying concentrations of copper. *Journal of Dairy Science* 84, 2308–2313.
- Fagari-Nobijari, H., Amanlou, H., Dehghan-Banadaky, M., 2012. Effects of zinc supplementation on growth performance, blood metabolites and lameness in young Holstein bulls. *Journal of Applied Animal Research* 40, 222–228.
- Felix, T.L., Weiss, W.P., Fluharty, F.L., Loerch, S.C., 2012. Effects of copper supplementation on feedlot performance, carcass characteristics, and rumen sulfur metabolism of growing cattle fed diets containing 60% dried distillers grains. *Journal of Animal Science* 90, 2710–2716.
- Gooneratne, S.R., Laarveld, B., Chaplin, R.K., Christensen, D.A., 1989. Profiles of  $^{67}\text{Cu}$  in blood, bile, urine and faeces from  $^{67}\text{Cu}$ -primed lambs: effect of  $^{99}\text{Mo}$ -labelled tetrathiomolybdate on the metabolism of recently stored tissue  $^{67}\text{Cu}$ . *British Journal of Nutrition* 61, 355–371.
- Gooneratne, S.R., Symonds, H.W., Bailey, J.V., Christensen, D.A., 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., 1973. Effect of high dietary Mn levels on the growth rate and the level of mineral elements in the plasma and soft tissues of sheep. *New Zealand Journal of Agricultural Research* 16, 177–180.
- Grace, N.D., 1983. Amounts and distribution of mineral elements associated with fleece-free empty body weight gains in the grazing sheep. *New Zealand Journal of Agricultural Research* 26, 59–70.
- Grace, N.D., Gooden, J.M., 1980. Effect of increasing intakes of Zn, Cu, and Mn on their secretion via bile and pancreatic juice and their excretion in faeces and urine in sheep fed lucerne pellets. *New Zealand Journal of Agricultural Research* 23, 293–298.
- Grace, N.D., Lee, J., 1990. Effect of Co, Cu, Fe, Mn, Mo, Se and Zn supplementation on the elemental content of soft tissues and bone in sheep grazing ryegrass/white clover pasture. *New Zealand Journal of Agricultural Research* 33, 635–647.
- Hanauer, M.J., 2017. Experimental study on the effect of dose and source of copper supplementation on copper metabolism, rumen microbiota, and rumen fermentation characteristics in cannulated cows. PhD thesis, Technical University of Munich, Munich, Germany.
- Hansen, S.L., 2010. High dietary iron reduces transporters involved in iron and manganese metabolism and increases intestinal permeability in calves. *Journal of Dairy Science* 93, 656–665.
- Hansen, S.L., Spears, J.W., Lloyd, K.E., Whisnant, C.S., 2006a. Feeding a low manganese diet to heifers during gestation impairs fetal growth and development. *Journal of Dairy Science* 89, 4305–4311.
- Hansen, S.L., Spears, J.W., Whisnant, C.S., Lloyd, K.E., 2006b. Growth, reproductive performance, and manganese status of beef heifers fed varying concentrations of manganese. *Journal of Animal Science* 84, 3375–3380.
- Hansen, S.L., Ashwell, M.S., Legleiter, L.R., Fry, R.S., Lloyd, L.E., Spears, J.W., 2009. The addition of manganese to a copper-deficient diet further depresses copper status and growth of cattle. *British Journal of Nutrition* 101, 1068–1078.
- Hansen, S.L., Spears, J.W., 2009. Bioaccessibility of iron from soil is increased by silage fermentation. *Journal of Dairy Science* 92, 2896–2905.
- Howes, A.D., Dyer, I.A., 1971. Diet and supplemental mineral effects on manganese metabolism in newborn calves. *Journal of Animal Science* 32, 141–145.
- Hu, C.H., Song, Z.H., Xiao, K., Song, J., Jiao, L.F., Ke, Y.L., 2013. Zinc oxide influences intestinal integrity, the expressions of genes associated with inflammation and TLR4-mycoid differentiation factor 88 signaling pathways in weanling pigs. *Innate Immunity* 20, 478–486.
- Humphries, W.R., Phillipppo, M., Young, B.W., Bremner, I., 1983. The influence of dietary iron and molybdenum on copper metabolism in calves. *British Journal of Nutrition* 49, 77–86.
- Ianni, A., Innosa, D., Martino, C., Grotta, L., Bennato, F., Martino, G., 2019. Zinc supplementation of Friesian cows: Effect on chemical-nutritional composition and aromatic profile of dairy products. *Journal of Dairy Science* 102, 2918–2927.
- INRA. 2018. *INRA Feeding system for ruminants*. Wageningen Academic Publishers, Wageningen, The Netherlands.
- Ishmael, J., Gopinath, C., Howell, J.M., 1971. Experimental chronic copper toxicity in sheep. Histological and histochemical changes during the development of the lesions in the liver. *Research in Veterinary Science* 12, 358–366.
- 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.
- Ivan, M., Veira, D.M., 1981. Effect of dietary protein on the solubilities of manganese, copper, zinc and iron in the rumen and abomasum of sheep. *Canadian Journal of Animal Science* 61, 955–959.
- Kegley, E.B., Spears, J.W., 1994. Bioavailability of feed-grade copper sources (oxide, sulfate, or lysine) in growing cattle. *Journal of Animal Science* 72, 2728–2734.
- Kendall, N.R., Holmes-Pavord, H.R., Bone, P.A., Ander, E.L., Young, S.D., 2015. Liver copper concentrations in cull cattle in the UK: are cattle being copper loaded? *Veterinary Record* 177, 493.
- Kincaid, R.L., Blauwiel, R.M., Cronrath, J.D., 1986. Supplementation of copper as copper sulfate or copper proteinate for growing calves fed forages containing molybdenum. *Journal of Dairy Science* 69, 160–163.
- Kirchgesner, M., Schwarz, F.J., Stangl, G.L., 1997. Growth performance of beef cattle fed corn silage-based rations without Cu, Zn, Mn, Co and Se supplementation. *Journal of Animal Physiology and Animal Nutrition* 78, 141–153.
- Leach, R.M., Muenster, A.M., 1962. Studies on the role of manganese in bone formation: i. effect upon the mucopolysaccharide content of chick bone. *Journal of Nutrition* 78, 51–56.
- Legleiter, L.R., Spears, J.W., Lloyd, K.E., 2005. Influence of dietary manganese on performance, lipid metabolism, and carcass composition of growing and finishing steers. *Journal of Animal Science* 83, 2434–2439.
- Li, Y., McCrory, D.F., Powell, J.M., Saam, H., Jackson-Smith, D., 2005. A survey of selected heavy metal concentrations in Wisconsin dairy feeds. *Journal of Dairy Science* 88, 2911–2922.
- López-Alonso, M., Miranda, M., 2020. Copper supplementation, a challenge in cattle. *Animals* 10, 1890.
- Malcolm-Callis, K.J., Duff, G.C., Gunter, S.A., Kegley, E.B., Vermeire, D.A., 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing beef steers. *Journal of Animal Science* 78, 2801–2808.
- Mandal, G.P., Dass, R.S., Isore, D.P., Garg, A.K., Ram, G.C., 2007. Effect of zinc supplementation from two sources on growth, nutrient utilization and immune response in male crossbred cattle (*Bos indicus* × *Bos taurus*) bulls. *Animal Feed Science and Technology* 138, 1–12.
- Mason, J., Lamand, M., Tressol, J.C., Lab, C., 1978. The influence of dietary sulphur, molybdate and copper on the absorption, excretion and plasma fraction levels of  $^{99}\text{Mo}$  in sheep. *Annals of Veterinary Research* 9, 577–586.
- McCaughern, J.H., 2019. Optimising the health and performance of dairy cows through improved copper nutrition. PhD thesis, Harper Adams University, Edgmond, UK.
- Miller, W.J., 1969. Absorption, tissue distribution, endogenous excretion, and homeostatic control of zinc in ruminants. *American Journal of Clinical Nutrition* 22, 1323–1331.
- Miller, W.J., Clifton, C.M., Cameron, N.W., 1963. Zinc requirement of Holstein bull calves to nine months of age. *Journal of Dairy Science* 46, 715–719.
- Miller, W.J., Blackmon, D.M., Gentry, R.P., Pitts, W.J., Powell, G.W., 1967. Absorption, excretion, and retention of orally administered zinc-65 in various tissues of zinc-deficient and normal goats and calves. *Journal of Nutrition* 92, 71–78.
- Miller, W.J., Martin, Y.G., Gentry, R.P., Blackmon, D.M., 1968.  $^{65}\text{Zn}$  and stable zinc absorption, excretion and tissue concentrations as affected by type of diet and level of zinc in normal calves. *Journal of Nutrition* 94, 391–401.
- Miller, W.J., Neathery, M.W., Gentry, R.P., Blackmon, D.M., Lassiter, J.W., Pate, F.M., 1972. Distribution and turnover rates of radioactive manganese in various tissues after duodenal dosing in Holstein calves fed a practical-type diet. *Journal of Animal Science* 34, 460–464.



- Miller, W.J., Amos, H.E., Gentry, R.P., Blackmon, D.M., Durrance, R.M., Crowe, C.T., Fielding, A.S., Neathery, M.W., 1989. Long-term feeding of high zinc sulfate diets to lactating and gestating dairy cows. *Journal of Dairy Science* 72, 1499–1508.
- Mullis, L.A., Spears, J.W., McCraw, B.L., 2003. Effects of breed (Angus vs Simmental) and copper and zinc source on mineral status of steers fed high dietary iron. *Journal of Animal Science* 81, 318–322.
- National Academies of Sciences Engineering and Medicine (NASEM), 2021. *Nutrient Requirements of Dairy Cattle*, 8th revised edition. The National Academies Press, Washington, DC, USA.
- National Research Council, 2005. *Mineral Tolerance of Animals*. National Academy Press, Washington, DC, USA.
- Neathery, M.W., Miller, W.P., Blackmon, D.M., Gentry, R.P., 1973a. Zinc-65 Metabolism, secretion into milk, and biological half-life in lactating cows. *Journal of Dairy Science* 56, 1526–1530.
- Neathery, M.W., Miller, W.P., Blackmon, D.M., Gentry, R.P., Jones, J.B., 1973b. Absorption and tissue zinc content in lactating dairy cows as affected by low dietary zinc. *Journal of Animal Science* 37, 848–852.
- Ott, E.A., Smith, W.H., Harrington, R.B., Parker, H.E., Beeson, W.M., 1966. Zinc toxicity in ruminants. IV. Physiological changes in tissues of beef cattle. *Journal of Animal Science* 25, 432–438.
- Owen Jr., C.A., 1964. Absorption and excretion of Cu64-labeled copper by the rat. *American Journal of Physiology* 207, 1203–1206.
- Phillippo, M., Humphries, W.R., Garthwaite, P.H., 1987a. The effect of dietary molybdenum and iron on copper status and growth in cattle. *Journal of Agricultural Science* 109, 315–320.
- Phillippo, M., Humphries, W.R., Atkinson, T., Henderson, G.D., Garthwaite, P.H., 1987b. The effect of dietary molybdenum and iron on copper status, puberty, fertility and oestrous cycles in cattle. *Journal of Agricultural Science* 109, 321–336.
- Plumlee, M.P., Thrasher, D.M., Beeson, W.M., Andrews, F.N., Parker, H.E., 1956. The effects of a manganese deficiency upon the growth, development, and reproduction of swine. *Journal of Animal Science* 15, 352–367.
- Pond, W.G., 1983. Effect of dietary calcium and zinc levels on weight gain and blood and tissue mineral concentrations of growing Columbia- and Suffolk-sired lambs. *Journal of Animal Science* 56, 952–959.
- Prabowo, A., Spears, J.W., Goode, L., 1988. Effects of dietary iron on performance and mineral utilization in lambs fed a forage-based diet. *Journal of Animal Science* 66, 2028–2035.
- Prados, L.F., Sathler, D.F.T., Silva, B.C., Zanetti, D., Valadares Filho, S.C., Alhadas, H.M., Detmann, E., Santos, S.A., Mariz, L.D.S., Chizzotti, M.L., 2017. Reducing mineral usage in feedlot diets for Nellore cattle: II. Impacts of calcium, phosphorus, copper, manganese, and zinc contents on intake, performance, and liver and bone status. *Journal of Animal Science* 95, 1766–1776.
- Raffaniello, R.D., Wapnir, R.A., 1989. Zinc uptake by isolated rat enterocytes: effect of low molecular weight ligands. *Proceedings of the Society for Experimental Biology and Medicine* 192, 219–224.
- Rojas, M.A., Dyer, I.A., Cassatt, W.A., 1965. Manganese deficiency in the bovine. *Journal of Animal Science* 24, 664–667.
- Schlattl, M., Buffler, M., Windisch, W., 2021. Clay minerals affect the solubility of Zn and other bivalent cations in the digestive tract of ruminants in vitro. *Animals* 11, 877.
- Sinclair, L.A., Atkins, N.E., 2015. Intake of selected minerals on commercial dairy herds in central and northern England in comparison with requirements. *Journal of Agricultural Science* 153, 743–752.
- Smith, B.S.W., Wright, H., 1975. Copper: Molybdenum interaction: effect of dietary molybdenum on the binding of copper to plasma proteins in sheep. *Journal of Comparative Pathology* 85, 299–305.
- Spears, J.W., Kegley, E., Mullis, L., 2004. Bioavailability of copper from tribasic copper chloride and copper sulfate in growing cattle. *Animal Feed Science and Technology* 116, 1–13.
- Spears, J.W., Lloyd, K.E., Fry, R.S., 2011. Tolerance of cattle to increased dietary sulfur and effect of dietary cation-anion balance. *Journal of Animal Science* 89, 2502–2509.
- Stake, P.E., Miller, W.J., Blackmon, D.M., Gentry, R.P., Neathery, M.W., 1974. Role of pancreas in endogenous zinc excretion in the bovine. *Journal of Nutrition* 104, 1279–1284.
- Stake, P.E., Miller, W.J., Neathery, M.W., Gentry, R.P., 1975. Zinc-65 absorption and tissue distribution in two- and six-month-old Holstein calves and lactating cows. *Journal of Dairy Science* 58, 78–81.
- Standish, J.F., Ammerman, C.B., Palmer, A.Z., Simpson, C.F., 1971. Influence of dietary iron and phosphorus on performance, tissue mineral composition and mineral absorption in steers. *Journal of Animal Science* 33, 171–178.
- Standish, J.F., Ammerman, C.B., 1971. Effect of excess dietary iron as ferrous sulfate and ferric citrate on tissue mineral composition of sheep. *Journal of Animal Science* 33, 481–484.
- Strickland, J.M., Herdt, T.H., Sledge, D.G., Buchweitz, J.P., 2019. Short communication: Survey of hepatic copper concentrations in Midwest dairy cows. *Journal of Dairy Science* 102, 4209–4214.
- Suttle, N.F., 1974a. Recent studies of the copper-molybdenum antagonism. *Proceedings of the Nutrition Society* 33, 299–305.
- Suttle, N.F., 1974b. Effects of organic and inorganic sulphur on the availability of dietary copper to sheep. *British Journal of Nutrition* 32, 559–568.
- Suttle, N.F., 1983. Effects of molybdenum concentration in fresh herbage, hay and semi-purified diets on the copper metabolism of sheep. *Journal of Agriculture Science* 100, 651–656.
- Suttle, N.F., Field, A.C., 1983. Effects of dietary supplements of thiomolybdates on copper and molybdenum metabolism in sheep. *Journal of Comparative Pathology* 93, 379–389.
- Suttle, N.F., Lloyd Davies, H., Field, A.C., 1982. A model for zinc metabolism in sheep given a diet of hay. *British Journal of Nutrition* 47, 105–112.
- Turnlund, J.R., Keyes, W.R., Anderson, H.L., Acord, L.L., 1989. Copper absorption and retention in young men at three levels of dietary copper by use of the stable isotope 65Cu. *American Journal of Clinical Nutrition* 49, 870–878.
- van Bibber-Krueger, C.L., Vahl, C.I., Narayanan, S.K., Amachawadi, R.G., Taylor, E.A., Scott, H.M., Drouillard, J.S., 2018. Effects of supplemental zinc sulfate on growth performance, carcass characteristics, and antimicrobial resistance in feedlot heifers. *Journal of Animal Science* 97, 424–436.
- VanValin, K.R., Genter-Schroeder, O.N., Carmichael, R.N., Blank, C.P., Deters, E.L., Hartman, S.J., Niedermayer, E.K., Laudert, S.B., Hansen, S.L., 2018. Influence of dietary zinc concentration and supplemental zinc source on nutrient digestibility, zinc absorption, and retention in sheep. *Journal of Animal Science* 96, 5336–5344.
- VanValin, K.R., Genter-Schroeder, O.N., Laudert, S.B., Hansen, S.L., 2019. Relative bioavailability of organic and hydroxy copper sources in growing steers fed a high antagonist diet. *Journal of Animal Science* 97, 1375–1383.
- Vierboom, M.M., Engle, T.E., Kimberling, C.V., 2003. Effects of gestational status on apparent absorption and retention of copper and zinc in mature Angus cows and Suffolk ewes. *Asian-Australasian Journal of Animal Sciences* 16, 515–518.
- Vohra, P., Kratzer, F.H., 1964. Influence of various chelating agents on the availability of zinc. *Journal of Nutrition* 82, 249–256.
- Wang, C., Xu, Y.Z., Han, L., Liu, Q., Guo, G., Huo, W.J., Zhang, J., Chen, L., Zhang, Y.L., Pei, C.X., Zhang, S.L., 2021. Effects of zinc sulfate and coated zinc sulfate on lactation performance, nutrient digestion and rumen fermentation in Holstein dairy cows. *Livestock Science* 251, 104673.
- Ward, J.D., Spears, J.W., 1997. Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance, and carcass characteristics of cattle. *Journal of Animal Science* 75, 3057–3065.
- Ward, J.D., Spears, J.W., Kegley, E., 1993. Effect of copper level and source (copper lysine vs copper sulfate) on copper status, performance, and immune response in growing steers fed diets with or without supplemental molybdenum and sulfur. *Journal of Animal Science* 71, 2748–2755.
- Weigand, E., Kirchgessner, M., 1980. Total true efficiency of zinc utilization: determination and homeostatic dependence upon the zinc supply status in young rats. *Journal of Nutrition* 110, 469–480.
- Weigand, E., Kirchgessner, M., Helbig, U., 1986. True absorption and endogenous fecal excretion of manganese in relation to its dietary supply in growing rats. *Biological Trace Element Research* 10, 265–279.
- Weiss, W.P., Socha, M.T., 2005. Dietary manganese for dry and lactating Holstein cows. *Journal of Dairy Science* 88, 2517–2523.
- White, C.L., Caldwell, T.K., Hoekstra, W.G., Pope, A.L., 1989. Effects of copper and molybdenum supplements on the copper and selenium status of pregnant ewes and lambs. *Journal of Animal Science* 67, 803–809.
- Windisch, W., 2001. Homeostatic reactions of quantitative Zn metabolism on deficiency and subsequent repletion with Zn in 65Zn-labeled adult rats. *Trace Elements and Electrolytes* 18, 122–128.
- Wittenberg, K.M., Boila, R.J., 1988. Supplementary copper for growing cattle consuming diets high in molybdenum or molybdenum plus sulfur. *Canadian Journal of Animal Science* 68, 1143–1154.
- Wittenburg, K.M., Boila, R.J., Shariff, M.A., 1990. Comparison of copper sulfate and copper proteinate as copper sources for copper-depleted steers fed high molybdenum diets. *Canadian Journal of Animal Science* 70, 895–904.
- Wooliams, J.A., Suttle, N.F., Wiener, G., Field, A.C., Wooliams, C., 1983. The long-term accumulation and depletion of copper in the liver of different breeds of sheep fed diets of differing copper content. *Journal of Agricultural Science* 100, 441–449.
- Xin, Z., Waterman, D.F., Hemken, R.W., Harmon, R.J., 1993. Copper status and requirement during the dry period and early lactation in multiparous Holstein cows. *Journal of Dairy Science* 76, 2711–2716.