Nutrition Research Reviews (2020), 33, 43–49
doi:10.1017/S0954422419000180
© The Authors 2019

Copper physiology in ruminants: trafficking of systemic copper, adaptations to variation in nutritional supply and thiomolybdate challenge

A. H. Clarkson[1]* , S. Paine[1], J. Martín-Tereso[2] and N. R. Kendall[1]
[1]*School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK*
[2]*Trouw Nutrition Research & Development, Amersfoort, the Netherlands*

Abstract

Ruminants are recognised to suffer from Cu-responsive disorders. Present understanding of Cu transport and metabolism is limited and inconsistent across vets and veterinary professionals. There has been much progress from the studies of the 1980s and early 1990s in cellular Cu transport and liver metabolism which has not been translated into agricultural practice. Cu metabolism operates in regulated pathways of Cu trafficking rather than in pools of Cu lability. Cu in the cell is chaperoned to enzyme production, retention within metallothionein or excretion via the Golgi into the blood. The hepatocyte differs in that Cu-containing caeruloplasmin can be synthesised to provide systemic Cu supply and excess Cu is excreted via bile. The aim of the present review is to improve understanding and highlight the relevant progress in relation to ruminants through the translation of newer findings from medicine and non-ruminant animal models into ruminants.

Key words: **Ruminants: Copper transport: Liver metabolism: Thiomolybdate**

Introduction

Cu metabolism in ruminants remains poorly understood in practice[1–5]. Developments in the fundamental understanding of Cu physiology have been insufficiently translated into livestock nutrition. While there is some awareness among industry professionals of the effects of 'copper deficiency' and of the potential nutritional effects by antagonists it is inconsistently understood[6]. Vets vary in their response to Cu-related problems; some may discourage supplementation in fear of toxicity problems, while others may continue to supplement[3,5–7]. There is considerable marketing pressure from mineral suppliers for their products and an inclination from producers to seek a 'quick fix' for trace element supplementation[8].

Recent surveys have found UK sheep and cattle are commonly affected by different forms of Cu imbalance, including toxicity and deficiency[9,10]. Kendall *et al.*[10] reported that as many as 40 % of British dairy cattle may be accumulating excessive liver Cu, with up to 52 % of them above the Animal Health Veterinary Laboratories Agency (AHVLA) reference range of 300–8000 μmol/kg DM[10]. Cu imbalance was the most common mineral problem reported between 2004 and 2014; with about 300 fatal occurrences each year reported for cattle and sheep combined for both toxicity and deficiency[11–13]. Indications from academic studies, government reports and industry suggest that Cu imbalance is still highly prevalent[3,5,14,15], highlighting that Cu supplementation remains a problem in ruminant production.

This review focuses on post-absorptive trafficking and systemic regulation of Cu and describes the interference of thiomolybdates on these mechanisms. A review of the role of the rumen in thiomolybdate formation has been previously published[16].

Copper metabolism at the cellular level

Most recent fundamental knowledge generated on Cu biology has been produced with models such as cell culture, *Caenorhabditis elegans*, laboratory animals and human subjects[17]. These selected species concentrate on a medical or nutritional perspective. The lack of emphasis on ruminants, and the limited overlap with human-focused sciences, has prevented dissemination of this new understanding, resulting in a lack of progress from the classic ideas on Cu in ruminants.

The Cu chaperones and enzymes which exist in ruminants are the same as those studied in other mammalian species[17]. At the cellular level, basic Cu metabolism appears to be consistent throughout eukaryotic life and can be traced from laboratory animals to man through their shared evolution[18], demonstrating that Cu in the systemic circulation is trafficked in the same manner in mammalian cells, thus providing opportunities to expand our understanding of Cu metabolism in ruminants[17].

Since 1966 radiolabelled Cu, cell fractionation and isolation of intracellular membrane components have been used to develop mathematical models to describe Cu movement in rat liver[19,20]. This led to the concept that separate pools, of varying availability, existed[21]. Initially, the pools were

Abbreviations: Atx1, antioxidant 1; CCS, Cu chaperone for superoxide dismutase; COMMD1, Cu metabolism MURR1 domain; Cox17, cyclo-oxygenase 17; Ctr1, Cu transporter 1; SOD, superoxide dismutase.

* **Corresponding author:** A. H. Clarkson, email andrea.clarkson@nottingham.ac.uk

https://doi.org/10.1017/S0954422419000180 Published online by Cambridge University Press

designated as 'storage', 'synthetic' and 'excretory'[19]. The relationship between the pools appeared complex, with no evidence of reversible movement between them. It was suggested that the Cu pools were able to become saturated, and the regulation or exchange between the pools was not determined[21,22]. The number and function of the pools were not easily apparent. Most studies agreed that hepatocyte Cu could be divided into at least two pools: one a readily available, extractable Cu pool accounting for the majority of Cu, the second, a less readily available pool containing the remainder of soluble Cu, and potentially a third, non-extractable, insoluble pool which could be considered a potential subset of the second pool[20,22]. By 1987, it was proposed that three separate pools existed within the liver representing bile, caeruloplasmin and 'storage' which was not further defined[21].

Subsequent research has mapped the intracellular movement of Cu and improved our understanding of Cu distribution in cells[23–27]. Fundamentally, this new knowledge does not contradict the description of Cu as cellular pools, but it illustrates Cu physiology in terms of Cu trafficking. Free Cu ions rarely exist within cells, thus Cu is kept complexed to prevent intracellular damage[28]. Distinct intracellular pathways exist where Cu is bound to chaperones and channelled across membranes rather than a series of storage compartments as the older model suggests. However, the persistence of the term 'pool', even in current literature, conjures images of discrete areas. It is perhaps better to update our terminology, and start discussing the 'pathways' of Cu trafficking, rather than its 'pools' of availability to better reflect the process and improve understanding of the process as a continuous regulation instead of discrete compartments of varying lability.

Overview of copper trafficking in enterocytes

The one aspect of Cu metabolism that differentiates ruminants from other species is their unique digestive system. Cu availability in the ruminant gastrointestinal tract presents peculiarities that have been extensively reviewed elsewhere[16,29,30]. However, the process of absorption is well preserved across the animal kingdom[31–33]. In order for Cu to be absorbed, it must be reduced into its most reactive state (Cu^{+}). At the intestinal brush border a Cu specific transporter (Ctr1) is responsible for about 70 % of Cu uptake into the enterocyte; the remainder is taken up by the non-specific transporter divalent metal transporter 1 (DMT1)[34]. Where Cu is trafficked through the DMT1 route direct competition for the transporter with dietary elements such as Fe and Zn may be more biologically relevant[35]. Once inside the cell, Cu chaperone proteins bind Cu and transport it to other specific proteins or incorporate it into enzymes. The pathway via the Golgi is known as the secretory pathway. Cu in excess of cellular requirements enters the secretory pathway to be bound to metallothionein by the Golgi and is stored in the lysosome, which acts as a buffer restricting free cellular Cu. Once the metallothionein reaches its saturation capacity Cu continues through the secretory pathway from the Golgi via its chaperone to the basolateral membrane for efflux from the cell.

The process in detail

Fig. 1 below illustrates the process described.

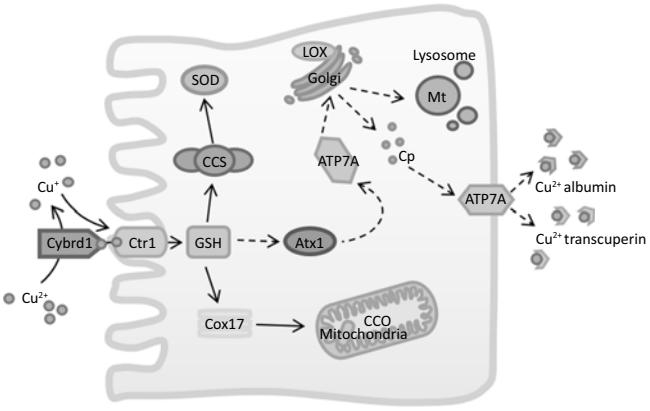

Fig. 1. Copper trafficking pathways using the copper chaperones from the intestinal lumen. Atx1, antioxidant 1; CCO, cytochrome c oxidase; CCS, copper chaperone for superoxide dismutase; Cox17, cyclo-oxygenase 17; Cp, caeruloplasmin; Ctr1, copper transporter 1; Cybrd1, cytochrome B reductase 1; GSH, glutathione; LOX, lysyl oxidase; Mt, metallothionein; SOD, superoxide dismutase.

Upon arrival at the intestinal brush border the membrane reductase Cybrd1 (cytochrome B reductase 1) and ascorbate (vitamin C) reduce any dietary Cu which is present as Cu^{2+} into Cu^{+}[36–38]. Reduced Cu is carried across the membrane by high-affinity Cu transporter 1 (Ctr1)[34,39–41]. Once inside the cell it is immediately incorporated onto its specific chaperones (Cu chaperone for superoxide dismutase (CCS), antioxidant 1 (Atx1) and cyclo-oxygenase 17 (Cox17)) within the cytosol[42,43]. CCS transports Cu within the cytosol where the metalloenzyme superoxide dismutase (SOD) is synthesised[17]. Cox17 transports Cu to proteins in the mitochondria where the metalloenzyme cytochrome c oxidase (CcO) is synthesised[44,45]. Atx1 and ATP7A transport Cu to the Golgi lumen where dopamine β-hydroxylase, peptidylglycine α-amidating mono-oxygenase, lysyl oxidase (LOX), SOD, tyrosinase, caeruloplasmin (Cp) and hephaestin vital for nerve and connective tissue function and for Cu and Fe transport are synthesised[18,46]. Surplus Cu is bound to metallothionein (Mt) and held in the lysosome after processing by the Golgi[18,44,47,48]. Upon reaching the metallothionein-carrying capacity in the lysosome, surplus Cu from the Golgi is transported using the ATP7A secretory pathway and effluxed from the enterocyte into circulation[17,18,45]. At the point of release from the cell membrane the oxygen tension of the interstitial fluid is sufficient to elicit spontaneous oxidation of the Cu^{+} to oxidised Cu^{2+} without the need for an oxidase in the membrane[49].

Copper movement in the blood

Following efflux from the enterocytes Cu is bound to albumin, an abundant plasma protein accounting for 15–20 % of total Cu transport, and transcuprein, a small protein which, in contrast to albumin, is a specific Cu carrier in plasma carrying

https://doi.org/10.1017/S0954422419000180 Published online by Cambridge University Press

10–30 % of total transported Cu[34,50–53]. The concentration of albumin in blood plasma exceeds that of transcuprein, but transcuprein has a higher affinity for Cu. Around a third of the Cu entering the blood from the small intestine is bound to transcuprein[53]. These two proteins transport Cu from the intestines through the systemic circulation to the liver. Metabolic studies have demonstrated that absorbed dietary Cu from the portal circulation is cleared by the liver and appears in newly synthesised caeruloplasmin[54]. Caeruloplasmin is the predominant Cu transporter in the systemic blood and is responsible for distribution of Cu to the tissues after its synthesis in the liver[55,56]. In ruminants about 88 % (range 86–90 %) of total plasma Cu is present bound to caeruloplasmin[57].

Overview of hepatic copper trafficking

The liver has a major role in the regulation of Cu[28]. This homeostatic control acts primarily through regulating the secretion of Cu into bile[36,43,50,58]. Cu reaching the liver is transported in a similar mechanism to the enterocytes. At the membrane the arriving Cu is reduced and trafficked into the cell by the same Cu transporter (Ctr1). Once inside the hepatocyte the chaperones fulfil their respective roles with one notable difference. The secretory pathway for efflux via the Golgi has a unique chaperone (ATP7B) which directs the majority of Cu to be incorporated into caeruloplasmin which is then effluxed into circulation for distribution to other tissues. However, when caeruloplasmin-bound Cu from the peripheral tissues re-enters the circulation and returns to the liver the whole molecule of caeruloplasmin is absorbed for destruction and excretion through the biliary route.

The process in detail

Fig. 2 illustrates the process described below.

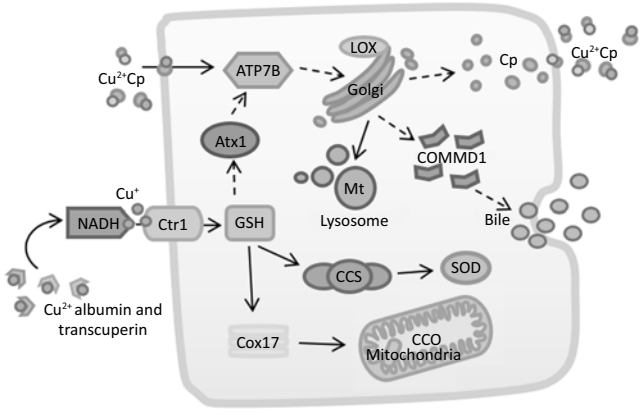

Fig. 2. Copper trafficking pathways using the copper chaperones into hepatocytes and out into the systemic circulation. Atx1, antioxidant 1; CCO, cytochrome c oxidase; CCS, copper chaperone for superoxide dismutase; COMMD1, copper metabolism MURR1 domain; Cox17, cyclo-oxygenase 17; Cp, caeruloplasmin; Ctr1, copper transporter 1; GSH, glutathione; LOX, lysyl oxidase; Mt, metallothionein; SOD, superoxide dismutase.

Cu reaches the liver bound to either transcuprein or albumin which are reduced on arrival by NADH oxidase[52]. Uptake of the reduced Cu into the hepatocyte is mediated by Ctr1[59]. Once inside, CCS and Cox17 traffic their Cu payload to the cytosol and mitochondria respectively and Atx1 delivers Cu to the Golgi body via ATP7B[60]. ATP7A is not expressed in the liver; instead, hepatocytes express a unique version ATP7B[44]. ATP7B directs the majority of Cu to be incorporated into caeruloplasmin to be subsequently returned to the circulation for distribution to other tissues[17,28,40,44,60]. When caeruloplasmin returns from the systemic circulation to the hepatocytes the whole molecule is absorbed. The endothelial hepatocytes must first remove sialic acid residues from the caeruloplasmin to allow the underlying hepatocytes to absorb the caeruloplasmin molecule for proteolysis and destruction through the biliary route[58]. The excess hepatic Cu is exported into the bile using the chaperones COMMD1 (Cu metabolism MURR1 domain) and potentially also XIAP (X-linked inhibitor of apoptosis protein)[36,40,60]. COMMD1 binds to the N-terminal region of ATP7B but not to ATP7A, explaining the difference in ATPase channel expression between hepatocytes and other cells[60,61].

Adaptations to changing dietary copper supply

Under Cu-limiting conditions the movement of Cu into the secretory pathway (Atx1-ATP7A) is diminished in all tissues[25,50]. Cu bound to metallothionein is mobilised using the acidic pH of the lysosome to partially degrade the metallothionein held within the lysosome and release its Cu into the cytosol[18,62,63]. The released Cu is delivered, probably by glutathione (GSH), to the Cu chaperones (cytosolic CCS and mitochondria targeting Cox17) equally, but not into the secretory pathway (Atx1)[25,63,64]. This redirection diminishes Cu supply to the secretory pathway resulting in the production and secretion into the bloodstream of the Cu-empty apo-caeruloplasmin, rather than its Cu-containing holo form[63]. This process inhibits excretion and retains Cu for intracellular use[65].

Under Cu-replete conditions in the tissues each of the Cu transporters and proteins are down-regulated[25,48]. The down-regulation of Cu transporter (Ctr1) in the membrane prevents any further Cu uptake into the cell[66–68]. ATP7A (a chaperone in the secretory pathway) moves out of the trans-Golgi network into vesicles that move towards the membrane. These vesicles accumulate Cu and intermittently fuse with the membrane to efflux the remaining excess Cu from the cell into the blood before returning to the cytoplasm[69]. Increased metallothionein expression (regulated by metal transcription factor MTF1) exerts intracellular homeostatic control through binding excess Cu and acting as a storage buffer protecting the cell[18,65].

When hepatocytes are exposed to increasing Cu concentrations they behave similarly to other cells with one exception; ATP7B (from the hepatocyte secretory pathway) leaves the trans-Golgi network but instead of moving towards the membrane it moves towards the lysosome at the canalicular membrane[50,65]. Here, the ATP7B imports Cu into the lysosomal lumen for temporary storage. Increasing intracellular Cu

https://doi.org/10.1017/S0954422419000180 Published online by Cambridge University Press

concentrations induce exocytosis of the lysosome, releasing the excess Cu into the biliary canal (mediated by the secretory chaperones ATP7B and COMMD1)[25,36,60,70,71].

Ruminant copper sensitivity

When discussing the unique characteristics of ruminant Cu handling it is important to first note that metallothionein knock-out animals, even from single-stomached species, are hypersensitive to Cu[72]. Sheep have a limited ability to synthesise metallothionein in response to rising Cu concentration and they appear to have a restricted capacity to accumulate Cu bound to metallothionein in the liver[56,73]. In comparison with rats, sheep reach a point where metallothionein synthesis is unable to keep up with rising Cu at a much lower dietary inclusion, resulting in less Cu sequestering by the lysosome[73]. Additionally, sheep have a limited ability to increase biliary Cu excretion in response to Cu intake[74]. Cattle also have a lower capacity to store Cu bound to metallothionein in comparison with single-stomached species and a limited capacity to induce metallothionein in response to Cu intake[56,75]. Furthermore, in cattle and sheep the Cu-buffering capacity decreases as hepatic Cu loading increases alongside the Cu:Zn ratio[76]. If the influx of Cu exceeds the capacity of the metallothionein and lysosomal uptake, unbound Cu will occur in the cytosol and begin to enter the nucleus, causing severe cell damage[76,77]. While, pigs and dogs have about 500–600 mg/kg, sheep and cattle have only about 200 mg/kg metallothionein in their livers[77]. Additionally, the metallothionein transcription in the lysosome of cattle and sheep does not effectively respond to rapid increases in Cu[75,78], seemingly reaching a plateau of total Cu concentration about 1607 mg/kg DM (25 347 μmol/kg DM) in cattle and about 571–643 mg/kg DM (9006–10 142 μmol/kg DM) in sheep[74,75,77,78]. Potentially this plateau is linked to the limited production of metallothionein and an inhibited biliary Cu excretion[74], theoretically explaining why cattle appear to be more Cu tolerant than sheep and why both species appear sensitive in comparison with single-stomached species such as pigs.

Further to species differences, breed differences among ruminants have also been documented. Texel sheep are more sensitive to Cu than Landrace breeds[79,80]. In cattle, Holstein and Angus breeds are more Cu tolerant than Jersey, Charolais and Simmental[81–83]. In cattle, the more Cu-tolerant breeds exhibit a greater expression of duodenal Ctr1 and ATP7A, and a higher hepatic expression of Ctr1, Cox17, ATP7B, CCS and SOD where Cu supply is inadequate[84,85]. These suggest that the ability to increase expression of Cu transporters and chaperones allows more effective uptake and utilisation where Cu supply is insufficient, reducing the susceptibility of these breeds to deficiency in comparison with their counterparts[84,85]. This research highlights a potential mechanism for the observed breed differences, but further studies in a wider range of breeds and in sheep, under elevated and Cu-replete conditions, would further clarify the role of transporter expression in Cu sensitivity.

Thiomolybdate disruption

Thiomolybdate is known to interact with Cu. It naturally forms in the reducing environment of the rumen between dietary S and Mo. Thiomolybdate poses a problem for Cu availability and post-absorptive utilisation[29,86–88]. Thiomolybdates interact with available Cu in the digestive tract, forming an insoluble precipitate and greatly reducing Cu availability[29,86–89]. If there is insufficient Cu where thiomolybdates form to 'de-toxify' them they can be absorbed into the systemic circulation, where they exert their affinity for Cu by complexing with Cu contained in biological compounds, rendering them biologically inactive[16,90]. Thiomolybdates are able to cross cell membranes but the mechanism by which this takes place is unknown. However, once inside the cell they have the potential to disrupt Cu transport through binding to Cu located on the Cu chaperones, transporters and enzymes[17].

Thiomolybdates can bind to Cu in cuproenzymes including caeruloplasmin, metallothionein, cytochrome c oxidase (CcO), SOD[90–93] and Atx1[94]. Binding does not remove the Cu component but renders it unable to perform redox reactions (vital to its biological function) through the formation of a stable complex[16,29,95,96]. Superoxide dismutase has been shown to differ and Cu may be partially stripped from this enzyme[97,98]. In the case of the chaperone Atx1, thiomolybdate suppresses the incorporation of Cu into the products of the secretory pathway, disrupting the activity of the Atx1[94]. Thiomolybdates have a high affinity for Cu and they have no effect on other trace metals with similar properties such as Fe, Zn or Cd[99,100].

Practical implications

Cu provision in ruminants requires a careful balance between intake and availability. The inhibited capacity of these species to adapt to Cu influx explains their sensitivity to overloading. Routine calculation of Cu intake at farm level is not routinely undertaken, which can lead to oversupply[11,101]. Calculation of Cu supply in combination with monitoring of biological parameters as part of routine management allows a more accurate assessment of Cu status across the entire flock or herd to be made[102]. At present, liver sampling is underutilised as a measure of herd or flock Cu status, especially where there is a history of oversupply. Annual monitoring of a representative sample, from cull animals or from biopsy, allows more effective long-term decisions to be made for Cu provision. It has been recently demonstrated that a significant linear relationship exists between increasing hepatic Cu concentrations and the abundance of rhodamine-stained granules in hepatic tissue histology[15]. This staining technique detects the Cu-filled lysosomes which occur as the cellular mechanism for Cu storage becomes overwhelmed[15]. In effect, their presence has the potential to be used as an indicator that Cu concentrations are in excess; although this technique is not yet used in practice. Little correlation exists between hepatic Cu concentrations and Cu concentrations in blood parameters[30,103]. It is useful to bear this in mind and employ both techniques in conjunction with each other to establish animal status[30,103].

Nutrition Research Reviews

https://doi.org/10.1017/S0954422419000180 Published online by Cambridge University Press

The potential danger posed through absorption of thiomolybdate causing disruption to systemic Cu chaperones and cuproenzymes should also not be neglected. The use of blood assays is of importance to help monitor changes in shorter-term Cu status. Decreases in caeruloplasmin activity can be a useful indicator of systemic thiomolybdate presence or Cu deficiency over and above the use of caeruloplasmin concentration[91]. Since the apo-protein will continue to be synthesised in the absence of adequate available hepatic Cu its activity can be reduced to nil[104]. This measure is not without flaws, as caeruloplasmin is an acute-phase protein and can be elevated by infection or stress, leading to falsely elevated measures of Cu status[30,105,106]. Unfortunately, a single, reliable measure for Cu status does not yet exist. Therefore, it is important to use both blood and hepatic measures in monitoring ruminant Cu status in addition to monitoring nutritional input[11,101]. Furthermore, it is important in practice to provide an appropriate Cu source, or combination of sources, which will be sufficient to 'de-toxify' thiomolybdate before it is absorbed and retain a sufficient supply of labile Cu for absorption which does not provide an excess or exceed legal restriction[101].

Conclusion

Advances in understanding of the physiology of intracellular Cu transport from fundamental biology have not effectively penetrated the field of ruminant nutrition, leading to widespread misunderstanding and consequently widespread Cu imbalance in practice. The pathways of Cu transport are synonymous with other mammalian species and much information is available to underpin nutritional theory for ruminants. Greater understanding of the trafficking pathways and their response to over- and under-Cu supply allows decisions for Cu supply to be more informed. In ruminants, and in particular sheep, these pathways have a limited ability to respond to changes in dietary Cu supply, which explains this species sensitivity to Cu oversupply. Thiomolybdates formed under ruminal conditions have been shown to be able to interfere with the Cu chaperone pathways, leading to cellular disruption of their function, if they are not effectively 'de-toxified' preventing their entry into the systemic circulation. Considering the cellular pathways for Cu and their potential disruption through thiomolybdate absorption can help to better inform supplemental actions to remedy Cu-related disorders in practice.

Acknowledgements

This research received no specific grant from any funding agency, commercial or not-for-profit sectors. However, funding for the wider project was jointly funded by the University of Nottingham, School of Veterinary Science and Medicine and Trouw Nutrition R&D. In addition to financial support both funders contributed to the review in their role as co-authors.

A. H. C., N. R. K., J. M.-T. and S. P. were all involved in initial planning and selection of areas to review; A. H. C. did the review of research and article writing; N. R. K., J. M.-T. and S. P. were all involved in proofing of concept, article content and wording.

There are no conflicts of interest.

References

1. McDonald J (2000) *The Conditions of Use of Copper Sulphate for Ruminating Cattle and Goats in Areas of Teart Soil*. Addlestone, Surrey: Veterinary Medicines Directorate.
2. Bone PA, Payne JH & Twigge J (2011) Guidance note for supplementing copper to bovines. https://acaf.food.gov.uk/sites/default/files/multimedia/pdfs/committee/guidancesuppcopperbovines.pdf (accessed August 2019).
3. Bidewell CA, Drew JR, Payne JH, *et al.* (2012) Case study of copper poisoning in a British dairy herd. *Vet Rec* **170**, 464–468.
4. Laven RA (2014) Do my goats need more copper? *Goat Vet Soc J* **30**, 69–73.
5. Hunt J (2016) Copper is being overfed and can lead to fatalities. *Dairy Farmer* **63**, 60–63.
6. Black DH & Kendall NR (2010) The attitudes and approach to trace element diagnosis and treatment in the UK. *Cattle Pract* **18**, 67–72.
7. Bowyer L (2016) Copper intake: getting the balance right. *Farmers Guardian*. https://www.fginsight.com/vip/vip/copper-intake-getting-the-balance-right-10343 (accessed August 2019).
8. Whitaker DA (1999) Trace elements – the real role in dairy cow fertility? *Cattle Pract* **7**, 3–7.
9. Clarkson AH, Meades N, Watters B, *et al.* (2017) The liver copper status of finished lambs in the UK. In *Proceedings of the 9th International Sheep Veterinary Congress*, Harrogate, 22–26 May, p. 48.
10. Kendall NR, Holmes-Pavord HR, Bone PA, *et al.* (2015) Liver copper concentrations in cull cattle in the UK: are cattle being copper loaded? *Vet Rec* **177**, 493–496.
11. Sinclair LA & Mackenzie AM (2013) Mineral nutrition of dairy cows: supply vs requirements. In *Recent Advances in Animal Nutrition*, pp. 31–36 [PC Garnsworthy and J Wiseman, editors]. Packington, Leicestershire: Context Publishing.
12. Animal Health Veterinary Laboratories Agency (AHVLA) (2012) *Veterinary Investigation Surveillance Report (VIDA)*. London: AHVLA.
13. Animal Health Veterinary Laboratories Agency (AHVLA) (2014) *Veterinary Investigation Surveillance Report (VIDA)*. London: AHVLA.
14. Agri-Food & Biosciences Institute (AFBI) (2016) AFBI warns of the risk of chronic copper poisioning in sheep. https://www.afbini.gov.uk/news/afbi-warns-about-risk-chronic-copper-poisoning-sheep (accessed August 2019).
15. Strickland JM, Herdt TH, Sledge DG, *et al.* (2019) Short communication: Survey of hepatic copper concentrations in Midwest dairy cows. *J Dairy Sci* **102**, 4209–4214.
16. Gould L & Kendall NR (2011) Role of the rumen in copper and thiomolybdate absorption. *Nutr Res Rev* **24**, 176–182.
17. Suttle NF (2012) Copper imbalances in ruminants and humans: unexpected common ground. *Adv Nutr* **3**, 666–674.
18. Nevitt T, Öhrvik H & Thiele DJ (2012) Charting the travels of copper in eukaryotes from yeast to mammals. *Biochim Biophys Acta* **1823**, 1580–1593.
19. Hazelrig JB, Owen A & Jane B (1966) A mathematical model for copper metabolism and its relation to Wilson's disease. *Am J Physiol* **211**, 1075–1081.
20. Bingham MJ, Sargeson AM & McArdle HJ (1997) Characterization of intracellular copper pools in rat hepatocytes using the chelator diamsar. *Am J Physiol* **272**, G1400–G1407.
21. Bremner I (1987) Involvement of metallothionein in the hepatic metabolism of copper. *J Nutr* **117**, 19–29.
22. McArdle HJ, Gross SM, Creaser I, *et al.* (1989) Effect of chelators on copper metabolism and copper pools in mouse hepatocytes. *Am J Physiol* **256**, G667–G672.

https://doi.org/10.1017/S0954422419000180 Published online by Cambridge University Press

23. Rubino JT & Franz KJ (2012) Coordination chemistry of copper proteins: how nature handles a toxic cargo for essential function. *J Inorg Biochem* **107**, 129–143.
24. Festa RA & Thiele DJ (2011) Copper: an essential metal in biology. *Curr Biol* **21**, R877–R883.
25. Lutsenko S (2010) Human copper homeostasis: a network of interconnected pathways. *Curr Opin Chem Biol* **14**, 211–217.
26. Argüello JM, Raimunda D & Padilla-Benavides T (2013) Mechanisms of copper homeostasis in bacteria. *Front Cell Infect Microbiol* **3**, 73.
27. Puig S & Thiele DJ (2002) Molecular mechanisms of copper uptake and distribution. *Curr Opin Chem Biol* **6**, 171–180.
28. La Fontaine S & Mercer JF (2007) Trafficking of the copper-ATPases, ATP7A and ATP7B: role in copper homeostasis. *Arch Biochem Biophys* **463**, 149–167.
29. Spears JW (2003) Trace mineral bioavailability in ruminants. *J Nutr* **133**, 1506–1509.
30. Suttle NF (2010) *Mineral Nutrition of Livestock*, 4th ed. Cambridge: CABI.
31. Peña MM, Lee J & Thiele DJ (1999) A delicate balance: homeostatic control of copper uptake and distribution. *J Nutr* **129**, 1251–1260.
32. Lutsenko S, Barnes NL, Bartee MY, *et al.* (2007) Function and regulation of human copper-transporting ATPases. *Physiol Rev* **87**, 1011–1046.
33. van den Berghe PV & Klomp LW (2009) New developments in the regulation of intestinal copper absorption. *Nutr Rev* **67**, 658–672.
34. European Food Safety Authority (2016) Revision of the currently authorised maximum copper content in complete feed. *EFSA J* **14**, 4563.
35. Espinoza A, Le Blanc S, Olivares M, *et al.* (2012) Iron, copper, and zinc transport: inhibition of divalent metal transporter 1 (DMT1) and human copper transporter 1 (hCTR1) by shRNA. *Biol Trace Elem Res* **146**, 281–286.
36. Collins JF, Prohaska JR & Knutson MD (2010) Metabolic crossroads of iron and copper. *Nutr Rev* **68**, 133–147.
37. Knöpfel M & Solioz M (2002) Characterization of a cytochrome b_{558} ferric/cupric reductase from rabbit duodenal brush border membranes. *Biochem Biophys Res Commun* **291**, 220–225.
38. Tennant J, Stansfield M, Yamaji S, *et al.* (2002) Effects of copper on the expression of metal transporters in human intestinal Caco-2 cells. *FEBS Lett* **527**, 239–244.
39. Boal AK & Rosenzweig AC (2009) Structural biology of copper trafficking. *Chem Rev* **109**, 4760–4779.
40. Prohaska JR (2008) Role of copper transporters in copper homeostasis. *Am J Clin Nutr* **88**, 826–829.
41. Lutsenko S (2016) Copper trafficking to the secretory pathway. *Metallomics* **8**, 840–852.
42. Maryon EB, Molloy SA & Kaplan JH (2013) Cellular glutathione plays a key role in copper uptake mediated by human copper transporter 1. *Am J Physiol* **304**, C768–C779.
43. Linder MC, Zerounian NR, Moriya M, *et al.* (2003) Iron and copper homeostasis and intestinal absorption using the Caco2 cell model. *BioMetals* **16**, 145–160.
44. Failla ML (1999) Considerations for determining "optimal nutrition" for copper, zinc, manganese and molybdenum. *Proc Nutr Soc* **58**, 497–505.
45. Spears JW (2013) Advancements in ruminant trace mineral nutrition. In *Proceedings of the Cornell Nutrition Conference 2013*, East Syracuse, New York, 22–24 October.
46. Polishchuk R & Lutsenko S (2013) Golgi in copper homeostasis: a view from the membrane trafficking field. *Histochem Cell Biol* **140**, 285–295.
47. Dameron CT & Harrison MD (1998) Mechanisms for protection against copper toxicity. *Am J Clin Nutr* **67**, 1091S–1097S.
48. Thiele DJ (2003) Integrating trace element metabolism from the cell to the whole organism. *J Nutr* **133**, 1579–1580.
49. Gulec S & Collins JF (2014) Molecular mediators governing iron–copper interactions. *Annu Rev Nutr* **34**, 95–116.
50. Stern BR, Solioz M, Krewski D, *et al.* (2007) Copper and human health: biochemistry, genetics, and strategies for modeling dose–response relationships. *J Toxicol Environ Health* **10**, 157–222.
51. Linder MC & Hazegh-Azam M (1996) Copper biochemistry and molecular biology. *Am J Clin Nutr* **63**, 797–811.
52. Crisponi G, Nurchi VM, Fanni D, *et al.* (2010) Copper-related diseases: from chemistry to molecular pathology. *Coord Chem Rev* **254**, 876–889.
53. Weiss KC & Linder MC (1985) Copper transport in rats involving a new plasma protein. *Am J Physiol* **249**, E77–E88.
54. Hellman NE & Gitlin JD (2002) Ceruloplasmin metabolism and function. *Annu Rev Nutr* **22**, 439–458.
55. Nemec LM (2010) The bioavailability of zinc and copper in Holstein steers. MSc Dissertation, University of Delaware.
56. Bremner I & Beattie JH (1995) Copper and zinc metabolism in health and disease: speciation and interactions. *Proc Nutr Soc* **54**, 489–499.
57. Mackenzie AM, Illingworth DV, Jackson DW, *et al.* (1997) The use of ceruloplasmin activities and plasma copper concentrations as an indicator of copper status in ruminants. In *Trace Elements in Man and Animals*, pp. 137–138 [PW Fischer, MR L'Abbé, KA Cockell and RS Gibson, editors]. Ottawa, QC: NRC Research Press.
58. Harris ED (2000) Cellular copper transport and metabolism. *Annu Rev Nutr* **20**, 291–310.
59. Kim H, Son H, Bailey SM, *et al.* (2009) Deletion of hepatic Ctr1 reveals its function in copper acquisition and compensatory mechanisms for copper homeostasis. *Am J Physiol* **296**, G356–G364.
60. De Bie P, Van de Sluis B, Klomp L, *et al.* (2005) The many faces of the copper metabolism protein MURR1/COMMD1. *J Hered* **96**, 803–811.
61. Tao TY & Gitlin JD (2003) Hepatic copper metabolism: insights from genetic disease. *Hepatology* **37**, 1241–1247.
62. Klaassen CD, Choudhuri S, McKim JM, *et al.* (1994) In-vitro and in-vivo studies on the degradation of metallothionein. *Environ Health Perspect* **102**, 141–146.
63. Suzuki KT, Someya A, Komada Y, *et al.* (2002) Roles of metallothionein in copper homeostasis: responses to Cu-deficient diets in mice. *J Inorg Biochem* **88**, 173–182.
64. Vulpe CD & Packman S (1995) Cellular copper transport. *Annu Rev Nutr* **15**, 293–322.
65. Hamza I & Gitlin JD (2003) Hepatic copper transport. In *Molecular Pathogenesis of Cholestasis*, pp. 225–234 [M Trauner and PL Jansen, editors]. Berlin: Springer Science & Business Media.
66. Nose Y, Wood LK, Kim BE, *et al.* (2010) Ctr1 is an apical copper transporter in mammalian intestinal epithelial cells *in vivo* that is controlled at the level of protein stability. *J Biol Chem* **285**, 32385–32392.
67. Petris MJ, Smith K, Lee J, *et al.* (2003) Copper-stimulated endocytosis and degradation of the human copper transporter, hCtr1. *J Biol Chem* **278**, 9639–9646.
68. Leary SC, Winge DR & Cobine PA (2009) "Pulling the plug" on cellular copper: the role of mitochondria in copper export. *Biochim Biophys Acta* **1973**, 146–153.
69. Nyasae L, Bustos R, Braiterman L, *et al.* (2007) Dynamics of endogenous ATP7A (Menkes protein) in intestinal epithelial cells: copper-dependent redistribution between two intracellular sites. *Am J Physiolology* **292**, G1181–G1194.

https://doi.org/10.1017/S0954422419000180 Published online by Cambridge University Press

70. Polishchuk EV, Concilli M, Iacobacci S, *et al.* (2014) Wilson disease protein ATP7B utilizes lysosomal exocytosis to maintain copper homeostasis. *Dev Cell* **29**, 686–700.
71. Petris MJ, Mercer JF, Culvenor JG, *et al.* (1996) Ligand-regulated transport of the Menkes copper P-type ATPase efflux pump from the Golgi apparatus to the plasma membrane: a novel mechanism of regulated trafficking. *EMBO J* **15**, 6084–6095.
72. Tapia L, González-Agüero M, Cisternas MF, *et al.* (2004) Metallothionein is crucial for safe intracellular copper storage and cell survival at normal and supra-physiological exposure levels. *Biochem J* **378**, 617–624.
73. Saylor WW, Morrow FD & Leach RM (1980) Copper- and zinc-binding proteins in sheep liver and intestine: effects of dietary levels of the metals. *J Nutr* **110**, 460–468.
74. López-Alonso M, Prieto F, Miranda M, *et al.* (2005) Intracellular distribution of copper and zinc in the liver of copper-exposed cattle from northwest Spain. *Vet J* **170**, 332–338.
75. Saylor WW & Leach RM (1980) Intracellular distribution of copper and zinc in sheep: effect of age and dietary levels of the metals. *J Nutr* **110**, 448–459.
76. López-Alonso M, Carbajales P, Miranda M, *et al.* (2017) Subcellular distribution of hepatic copper in beef cattle receiving high copper supplementation. *J Trace Elem Med Biol* **42**, 111–116.
77. López-Alonso M, Prieto F, Miranda M, *et al.* (2005) The role of metallothionein and zinc in hepatic copper accumulation in cattle. *Vet J* **169**, 262–267.
78. Corbett WS, Saylor WW, Long TA, *et al.* (1978) Intracellular distribution of hepatic copper in normal and copper-loaded sheep. *J Anim Physiol Anim Nutr (Berl)* **47**, 1174–1179.
79. Menzies PI, Boermans H, Hoff B, *et al.* (2003) Survey of the status of copper, interacting minerals, and vitamin E levels in the livers of sheep in Ontario. *Can Vet J* **44**, 898–906.
80. van der Berg R, Levels FH & van der Schee W (1983) Breed differences in sheep with respect to the accumulation of copper in the liver. *Vet Q* **5**, 26–31.
81. Du Z, Hemken RW & Harmon RJ (1996) Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. *J Dairy Sci* **79**, 1873–1880.
82. National Research Council (2000) *Nutrient Requirements of Beef Cattle*, 7th revised ed. Washington, DC: National Academies Press.
83. Gengelbach GP, Ward JD & Spears JW (1994) Effect of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. *J Anim Sci* **72**, 2722–2727.
84. Fry RS, Spears JW, Lloyd KE, *et al.* (2013) Effect of dietary copper and breed on gene products involved in copper acquisition, distribution, and use in Angus and Simmental cows and fetuses. *J Anim Sci* **91**, 861–871.
85. Dermauw V, De Cuyper A, Duchateau L, *et al.* (2014) A disparate trace element metabolism in zebu (*Bos indicus*) and crossbred (*Bos indicus* × *Bos taurus*) cattle in response to a copper-deficient diet. *J Anim Sci* **92**, 3007–3017.
86. Galbraith H, Chigwada W, Scaife JR, *et al.* (1997) The effect of dietary molybdenum supplementation on tissue copper concentrations, mohair fibre and carcass characteristics of growing Angora goats. *Anim Feed Sci Technol* **67**, 83–90.
87. Gooneratne SR, Buckley WT & Christensen DA (1989) Review of copper deficiency and metabolism in ruminants. *Can J Anim Sci* **69**, 819–845.
88. Price J, Will AM, Paschaleris G, *et al.* (1987) Identification of thiomolybdates in digesta and plasma from sheep after administration of ^{99}Mo-labelled compounds into the rumen. *Br J Nutr* **58**, 127–138.
89. Essilfie-Dughan J (2007) Speciation modelling of Cu(II) in the thiomolybdate contaminated rumen. PhD Thesis, University of Saskatchewan.
90. Ogra Y, Komada Y & Suzuki KT (1999) Comparative mechanism and toxicity of tetra- and dithiomolybdates in the removal of copper. *J Inorg Biochem* **75**, 199–204.
91. Chidambaram MV, Barnes G & Frieden E (1984) Inhibition of ceruloplasmin and other copper oxidases by thiomolybdate. *J Inorg Biochem* **22**, 231–239.
92. Bissig K, Voegelin TC & Solioz M (2001) Tetrathiomolybdate inhibition of the *Enterococcus hirae* CopB copper ATPase. *FEBS Lett* **507**, 367–370.
93. Suzuki KT, Ogra Y & Ohmichi M (1995) Molybdenum and copper kinetics after tetrathiomolybdate injection in LEC rats: specific role of serum albumin. *J Trace Elem Med Biol* **9**, 170–175.
94. Alvarez HM, Xue Y, Robinson CD, *et al.* (2010) Tetrathiomolybdate inhibits copper trafficking proteins through metal cluster formation. *Science* **15**, 331–334.
95. Van Ryssen JB, Van Malsen S & Barrowman PR (1986) Effect of dietary molybdenum and sulphur on the copper status of hypercuprotic sheep after withdrawal of dietary copper. *S Afr J Anim Sci* **16**, 77–82.
96. Hynes M, Woods M, Poole DB, *et al.* (1985) Some studies on the metabolism of labelled molybdenum compounds in cattle. *J Inorg Biochem* **24**, 279–288.
97. Juarez JC, Betancourt O, Pirie-Shepherd SR, *et al.* (2006) Copper binding by tetrathiomolybdate attenuates angiogenesis and tumor cell proliferation through the inhibition of superoxide dismutase 1. *Clin Cancer Res* **12**, 4974–4982.
98. Juarez JC, Manuia M, Burnett ME, *et al.* (2008) Superoxide dismutase 1 (SOD1) is essential for H_2O_2-mediated oxidation and inactivation of phosphatases in growth factor signaling. *Proc Natl Acad Sci U S A* **105**, 7147–7152.
99. Ogra Y, Ohmichi M & Suzuki KT (1996) Mechanisms of selective copper removal by tetrathiomolybdate from metallothionein in LEC rats. *Toxicology* **106**, 75–83.
100. Ogra Y, Ohmichi M & Suzuki KT (1995) Systemic dispositions of molybdenum and copper after tetrathiomolybdate injection in LEC rats. *J Trace Elem Med Biol* **9**, 165–169.
101. Sinclair LA & Atkins NE (2015) Intake of selected minerals on commercial dairy herds in central and northern England in comparison with requirements. *J Agric Sci* **153**, 743–752.
102. Kendall NR & Bone PA (2015) Farm and laboratory assessment of mineral availability in ruminants. In *Recent Advances in Animal Nutrition*, pp. 29–36 [PC Garnsworthy and J Wiseman, editors]. Packington, Leicestershire: Context Publishing.
103. Laven RA, Livesey CT, Harmon RJ, *et al.* (2006) Factors affecting the relationship between caeruloplasmin activity and plasma copper concentration in cattle. *Vet Rec* **159**, 250–251.
104. Gitlin JD, Schroeder JJ, Lee-Ambrose LM, *et al.* (1992) Mechanisms of caeruloplasmin biosynthesis in normal and copper-deficient rats. *Biochem J* **282**, 835–839.
105. Blakley BR & Hamilton DL (1985) Ceruloplasmin as an indicator of copper status in cattle and sheep. *Can J Comp Med* **49**, 405–408.
106. Arthington JD, Martin FG & Blecha F (2003) Effect of molybdenum and sulfur feeding on the acute phase protein response to inflammatory challenge in beef heifers. *Prof Anim Sci* **19**, 221–226.

https://doi.org/10.1017/S0954422419000180 Published online by Cambridge University Press