

1 **Copper Physiology in Ruminants: Trafficking of systemic copper, adaptations to**  
2 **variation in nutritional supply and thiomolybdate challenge**

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7  
8 **ABSTRACT**

9 Ruminants are recognised to suffer from copper responsive disorders. Present understanding  
10 of copper transport and metabolism is limited and inconsistent across vets and veterinary  
11 professionals. There has been much progress from the studies of the 1980s and early 90s in  
12 cellular copper transport and liver metabolism which has not been translated into agricultural  
13 practice. Copper metabolism operates in regulated pathways of copper trafficking rather in than  
14 pools of copper lability. Copper in the cell is chaperoned to enzyme production, retention  
15 within metallothionein or excretion via the Golgi into the blood. The hepatocyte differs in that  
16 copper-containing caeruloplasmin can be synthesized to provide systemic copper supply and  
17 excess copper is excreted via bile. The aim of this review is to improve understanding and  
18 highlight the relevant progress in relation to ruminants through the translation of newer findings  
19 from medicine and non-ruminant animal models into ruminants.

20  
21 **KEYWORDS:** *Ruminant, copper transport, liver metabolism, thiomolybdate*

22  
23 **INTRODUCTION**

24 Copper metabolism in ruminants remains poorly understood in practice <sup>(1-5)</sup>. Developments in  
25 the fundamental understanding of copper physiology have been insufficiently translated into  
26 livestock nutrition. While there is some awareness among industry professionals of the effects  
27 of ‘copper deficiency’ and of the potential nutritional effects by antagonists it is inconsistently  
28 understood <sup>(6)</sup>. Vets vary in their response to copper-related problems some may discourage  
29 supplementation in fear of toxicity problems, while others may continue to supplement <sup>(3,5-7)</sup>.

30 There is considerable marketing pressure from mineral suppliers for their products and an  
31 inclination from producers to seek a ‘quick fix’ for trace element supplementation <sup>(8)</sup>.

32 Recent surveys have found UK sheep and cattle are commonly affected by different forms of  
33 copper imbalance, including toxicity and deficiency <sup>(9,10)</sup>. Kendall et al. <sup>(10)</sup> reported as many  
34 as 40% of British dairy cattle may be accumulating excessive liver copper, with up to 52% of  
35 them above the Animal Health Veterinary Laboratories Agency (AHVLA) reference range of  
36 300-8,000  $\mu\text{mol/kg DM}$  <sup>(10)</sup>. Copper imbalance was the most common mineral problem  
37 reported between 2004 and 2014; with ~300 fatal occurrences each year reported for cattle and  
38 sheep combined for both toxicity and deficiency <sup>(11-13)</sup>. Indications from academic studies,  
39 government reports and industry suggest that copper imbalance is still highly prevalent <sup>(3,5,14,15)</sup>.  
40 Highlighting that copper supplementation remains a problem in ruminant production.

41 This review focusses on post-absorptive trafficking and systemic regulation of copper and  
42 describes the interference of thiomolybdates on these mechanisms. A review of the role of the  
43 rumen in thiomolybdate formation has been previously published <sup>(16)</sup>.

44

#### 45 **COPPER METABOLISM AT CELLULAR LEVEL**

46 Most recent fundamental knowledge generated on copper biology has been produced with  
47 models such as cell culture, *c.elegans*, laboratory animals and humans <sup>(17)</sup>. These selected  
48 species concentrate on a medical or nutritional perspective. The lack of emphasis on ruminants,  
49 and the limited overlap with human focused sciences, has prevented dissemination of this new  
50 understanding; resulting in a lack of progress from the classic ideas on copper in ruminants.

51 The copper chaperones and enzymes which exist in ruminants are the same as those studied in  
52 other mammalian species <sup>(17)</sup>. At cellular level, basic copper metabolism appears to be  
53 consistent throughout eukaryotic life and can be traced from laboratory animals to humans  
54 through their shared evolution <sup>(18)</sup>; demonstrating that copper in the systemic circulation is  
55 trafficked in the same manner in mammalian cells thus providing opportunities to expand our  
56 understanding of copper metabolism in ruminants <sup>(17)</sup>.

57 Since 1966 radiolabelled copper, cell fractionation and isolation of intracellular membrane  
58 components have been used to develop mathematical models to describe copper movement in  
59 rat liver <sup>(19,20)</sup>. This led to the concept that separate pools, of varying availability existed <sup>(21)</sup>.  
60 Initially, the pools were designated as ‘storage’, ‘synthetic’ and ‘excretory’ <sup>(19)</sup>. The

61 relationship between the pools appeared complex, with no evidence of reversible movement  
62 between them. It was suggested the copper pools were able to become saturated, and the  
63 regulation or exchange between the pools was not determined <sup>(21,22)</sup>. The number and function  
64 of the pools was not easily apparent. Most studies agreed hepatocyte copper could be divided  
65 into at least two pools, one a readily available, extractable copper pool accounting for the  
66 majority of copper. The second, a less readily available pool containing the remainder of  
67 soluble copper and potentially a third, non-extractable, insoluble pool which could be  
68 considered a potential subset of the second pool <sup>(20,22)</sup>. By 1987 it was proposed that three  
69 separate pools existed within the liver representing bile, caeruloplasmin and ‘storage’ which  
70 was not further defined <sup>(21)</sup>.

71 Subsequent research has mapped the intracellular movement of copper and improved our  
72 understanding of copper distribution in cells <sup>(23–27)</sup>. Fundamentally, this new knowledge does  
73 not contradict the description of copper as cellular pools, but it illustrates copper physiology in  
74 terms of copper trafficking. Free copper ions rarely exist within cells, thus copper is kept  
75 complexed to prevent intracellular damage <sup>(28)</sup>. Distinct intracellular pathways exist where  
76 copper is bound to chaperones and channelled across membranes rather than a series of storage  
77 compartments as the older model suggests. However, the persistence of the term ‘pool’, even  
78 in current literature, conjures images of discrete areas. It is perhaps better to update our  
79 terminology, and start discussing the ‘pathways’ of copper trafficking, rather than its ‘pools’  
80 of availability to better reflect the process and improve understanding of the process as a  
81 continuous regulation instead of discrete compartments of varying lability.

82

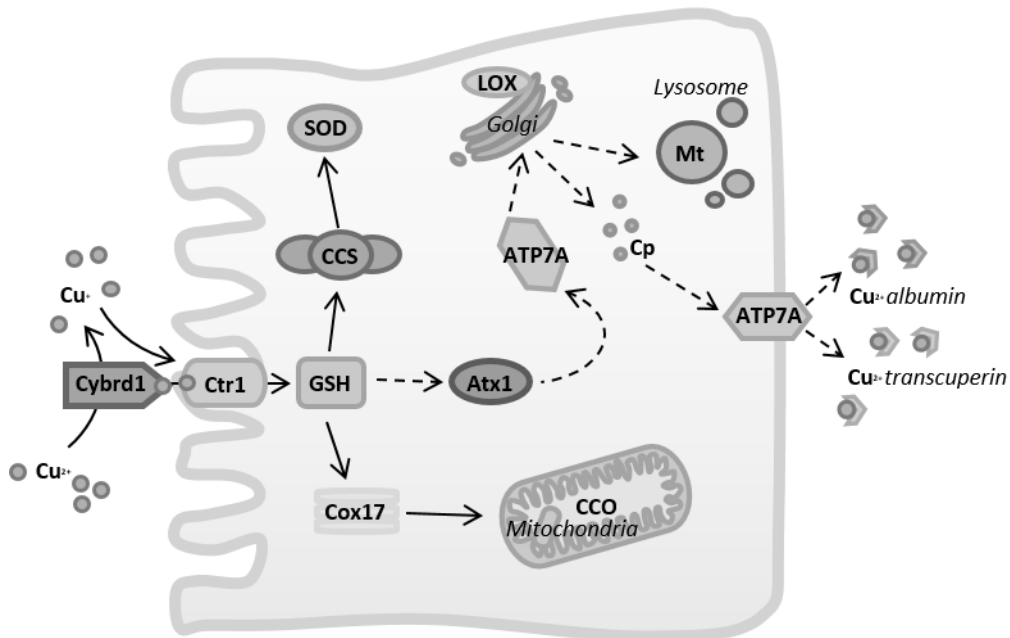
### 83 **OVERVIEW OF COPPER TRAFFICKING IN ENTEROCYTES**

84 The one aspect of copper metabolism that differentiates ruminants from other species is their  
85 unique digestive system. Copper availability in the ruminant gastrointestinal tract presents  
86 peculiarities that are extensively reviewed elsewhere <sup>(16,29,30)</sup>. However, the process of  
87 absorption is well-preserved across the animal kingdom <sup>(31–33)</sup>. In order for copper to be  
88 absorbed, it must be reduced into its most reactive state ( $\text{Cu}^+$ ). At the intestinal brush border a  
89 copper specific transporter (Ctr1) is responsible for ~70% of copper uptake into the enterocyte,  
90 the remainder is taken up by the non-specific transporter Divalent Metal Transporter 1 (DMT1)  
91 <sup>(34)</sup>. Where copper is trafficked through the DMT1 route direct competition for the transporter  
92 with dietary elements such as iron and zinc may be more biologically relevant <sup>(35)</sup>. Once inside

93 the cell, copper chaperone proteins bind copper and transport it to other specific proteins or  
 94 incorporate it into enzymes. The pathway via the Golgi is known as the secretory pathway.  
 95 Copper in excess of cellular requirements enters the secretory pathway to be bound to  
 96 metallothionein by the Golgi and is stored in the lysosome, which acts as a buffer restricting  
 97 free cellular copper. Once the metallothionein reaches its saturation capacity copper continues  
 98 through the secretory pathway from the Golgi via its chaperone to the basolateral membrane  
 99 for efflux from the cell.

100 **The process in detail**

101 *Figure 1 below illustrates the process described.*



102  
 103 *Figure 1: Copper trafficking pathways using the copper chaperones from the intestinal lumen.*  
 104 *Atx1, antioxidant 1; CCO, cytochrome c oxidase; CCS, copper chaperone protein; Cox17,*  
 105 *cyclo-oxygenase 17; Cp, caeruloplasmin; Ctr1, copper transporter 1; Cybrd1, cytochrome B*  
 106 *reductase; GSH, glutathione; LOX, lysyl oxidase; Mt, metallothionein; SOD, superoxide*  
 107 *dismutase.*

108  
 109 Upon arrival at the intestinal brush border the membrane reductase Cybrd1 (Cytochrome B  
 110 Reductase 1) and ascorbate (Vitamin C) reduce any dietary copper which is present as Cu<sup>2+</sup>  
 111 into Cu<sup>+</sup> (36–38). Reduced copper is carried across the membrane by high-affinity Copper  
 112 transporter 1 (Ctr1) (34,39–41). Once inside the cell it is immediately incorporated onto its specific

113 chaperones (CCS, Atx1 and Cox17) within the cytosol <sup>(42,43)</sup>. Copper chaperone protein (CCS)  
114 transports copper within the cytosol where the metalloenzyme Superoxide dismutase (SOD) is  
115 synthesised <sup>(17)</sup>. Cyclo-oxygenase 17 (Cox17) transports copper to proteins in the mitochondria  
116 where the metalloenzyme Cytochrome c oxidase (CCO) is synthesised <sup>(44,45)</sup>. Anti-oxidant 1  
117 (Atx1) and ATP7A transport copper to the Golgi lumen where dopamine  $\beta$ -hydroxylase,  
118 peptidylglycine  $\alpha$ -amidating monooxygenase, lysyl oxidase (LOX), SOD, tyrosinase,  
119 caeruloplasmin (Cp) and hephaestin vital for nerve and connective tissue function and for  
120 copper and iron transport are synthesised <sup>(18,46)</sup>. Surplus copper is bound to Metallothionein  
121 (Mt) and held in the lysosome after processing by the Golgi <sup>(18,44,47,48)</sup>. Upon reaching the  
122 metallothionein carrying capacity in the lysosome, surplus copper from the Golgi is transported  
123 using the ATP7A secretory pathway and effluxed from the enterocyte into circulation <sup>(17,18,45)</sup>.  
124 At the point of release from the cell membrane the oxygen tension of the interstitial fluid is  
125 sufficient to elicit spontaneous oxidation of the  $\text{Cu}^+$  to oxidised  $\text{Cu}^{2+}$  without the need for an  
126 oxidase in the membrane <sup>(49)</sup>.

127

## 128 **COPPER MOVEMENT IN THE BLOOD**

129 Following efflux from the enterocytes copper is bound to albumin; an abundant plasma protein  
130 accounting for 15-20% of total copper transport, and transcuprein; a small protein which in  
131 contrast to albumin, is a specific copper carrier in plasma carrying 10-30% of total transported  
132 copper <sup>(34,50-53)</sup>. The concentration of albumin in blood plasma exceeds that of transcuprein, but  
133 transcuprein has a higher affinity for copper. Around a third of the copper entering the blood  
134 from the small intestine is bound to transcuprein <sup>(53)</sup>. These two proteins transport copper from  
135 the intestines through the systemic circulation to the liver. Metabolic studies have demonstrated  
136 that absorbed dietary copper from the portal circulation is cleared by the liver and appears in  
137 newly synthesised caeruloplasmin <sup>(54)</sup>. Caeruloplasmin is the predominant copper transporter  
138 in the systemic blood and is responsible for distribution of copper to the tissues after its  
139 synthesis in the liver <sup>(55,56)</sup>. In ruminants around 88% (range 86-90%) of total plasma copper is  
140 present bound to caeruloplasmin <sup>(57)</sup>.

141

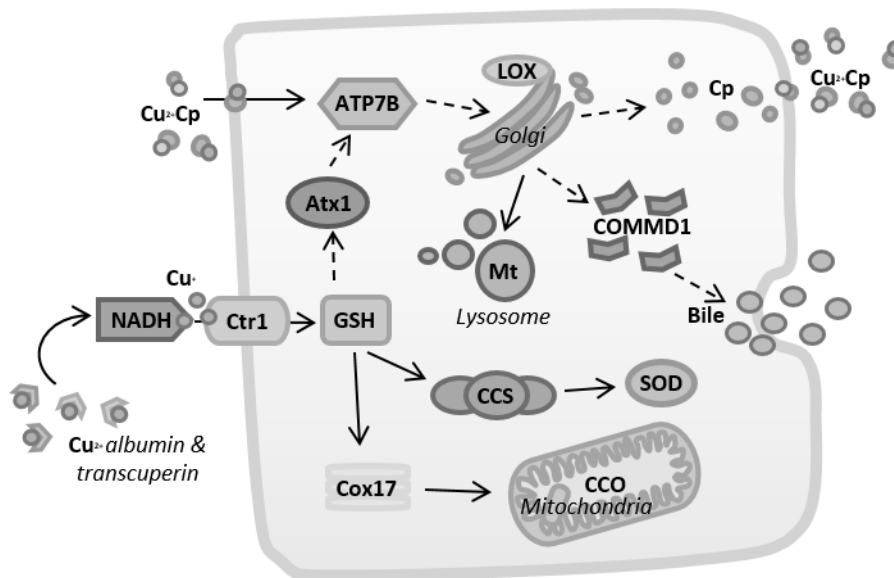
## 142 **OVERVIEW OF HEPATIC COPPER TRAFFICKING**

143 The liver has a major role in the regulation of copper <sup>(28)</sup>. This homeostatic control acts  
144 primarily through regulating the secretion of copper into bile <sup>(36,43,50,58)</sup>. Copper reaching the

145 liver is transported in a similar mechanism to the enterocytes. At the membrane the arriving  
 146 copper is reduced and trafficked into the cell by the same copper transporter (Ctr1). Once inside  
 147 the hepatocyte the chaperones fulfil their respective roles with one notable difference. The  
 148 secretory pathway for efflux via the Golgi has a unique chaperone (ATP7B) which directs the  
 149 majority of copper to be incorporated into caeruloplasmin which is then effluxed into  
 150 circulation for distribution to other tissues. However, when caeruloplasmin bound copper from  
 151 the peripheral tissues re-enters the circulation and returns to the liver the whole molecule of  
 152 caeruloplasmin is absorbed for destruction and excretion through the biliary route.

153 **The process in detail**

154 *Figure 2 illustrates the process described below.*



155  
 156 *Figure 2: Copper trafficking pathways using the copper chaperones into hepatocytes and out*  
 157 *into systemic circulation. Atx1, antioxidant 1; CCO, cytochrome c oxidase; CCS, copper*  
 158 *chaperone protein; COMMD1, copper metabolism MURR1 domain; Cox17, cyclo-oxygenase*  
 159 *17; Cp, caeruloplasmin; Ctr1, copper transporter 1; Cybrd1, cytochrome B reductase; GSH,*  
 160 *glutathione; LOX, lysyl oxidase; Mt, metallothionein; SOD, superoxide dismutase.*

161

162 Copper reaches the liver bound to either transcuprein or albumin which are reduced on arrival  
 163 by NADH oxidase <sup>(52)</sup>. Uptake of the reduced copper into the hepatocyte is mediated by Ctr1  
 164 <sup>(59)</sup>. Once inside, CCS and Cox 17 traffic their copper payload to the cytosol and mitochondria

165 respectively and Atx1 delivers copper to the Golgi body via ATP7B <sup>(60)</sup>. ATP7A is not  
166 expressed in the liver, instead hepatocytes express a unique version ATP7B <sup>(44)</sup>. ATP7B directs  
167 the majority of copper to be incorporated into caeruloplasmin to be subsequently returned to  
168 the circulation for distribution to other tissues <sup>(17,28,40,44,60)</sup>. When caeruloplasmin returns from  
169 systemic circulation to the hepatocytes the whole molecule is absorbed. The endothelial  
170 hepatocytes must first remove sialic acid residues from the caeruloplasmin to allow the  
171 underlying hepatocytes to absorb the caeruloplasmin molecule for proteolysis and destruction  
172 through the biliary route <sup>(58)</sup>. The excess hepatic copper is exported into the bile using the  
173 chaperones COMMD1 (copper metabolism MURR1 domain) and potentially also XIAP (X-  
174 linked inhibitor of apoptosis protein) <sup>(36,40,60)</sup>. COMMD1 binds to the N-terminal region of  
175 ATP7B but not to ATP7A, explaining the difference in ATPase channel expression between  
176 hepatocytes and other cells <sup>(60,61)</sup>.

177

#### 178 **ADAPTATIONS TO CHANGING DIETARY COPPER SUPPLY**

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

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

197 When hepatocytes are exposed to increasing copper concentrations they behave similarly to  
198 other cells with one exception; ATP7B (from the hepatocyte secretory pathway) leaves the  
199 trans-Golgi network but instead of moving towards the membrane it moves towards the  
200 lysosome at the canalicular membrane <sup>(50,65)</sup>. Here, the ATP7B imports copper into the  
201 lysosomal lumen for temporary storage. Increasing intracellular copper concentrations induce  
202 exocytosis of the lysosome releasing the excess copper into the biliary canal (mediated by the  
203 secretory chaperones ATP7B and COMMD1) <sup>(25,36,60,70,71)</sup>.

204

## 205 **RUMINANT COPPER SENSITIVITY**

206 When discussing the unique characteristics of ruminant copper handling it is important to first  
207 note that metallothionein knock-out animals, even from monogastric species, are  
208 hypersensitive to copper <sup>(72)</sup>. Sheep have a limited ability to synthesise metallothionein in  
209 response to rising copper concentration and they appear to have a restricted capacity to  
210 accumulate copper bound to metallothionein in the liver <sup>(56,73)</sup>. In comparison to rats, sheep  
211 reach a point where metallothionein synthesis is unable to keep up with rising copper at a much  
212 lower dietary inclusion resulting in less copper sequestering by the lysosome <sup>(73)</sup>. Additionally,  
213 sheep have a limited ability to increase biliary copper excretion in response to copper intake  
214 <sup>(74)</sup>. Cattle also have a lower capacity to store copper bound to metallothionein in comparison  
215 to monogastric species and a limited capacity to induce metallothionein in response to copper  
216 intake <sup>(56,75)</sup>. Furthermore, in cattle and sheep the copper-buffering capacity decreases as  
217 hepatic copper loading increases alongside the Cu:Zn ratio <sup>(76)</sup>. If the influx of copper exceeds  
218 the capacity of the metallothionein and lysosomal uptake, unbound copper will occur in the  
219 cytosol and begin to enter the nucleus, causing severe cell damage <sup>(76,77)</sup>. While, pigs and dogs  
220 have around 500-600 mg/kg, sheep and cattle have only ~200 mg/kg metallothionein in their  
221 livers <sup>(77)</sup>. Additionally, the metallothionein transcription in the lysosome of cattle and sheep  
222 does not effectively respond to rapid increases in copper <sup>(75,78)</sup>, seemingly reaching a plateau of  
223 total copper concentration ~1,607 mg/kg DM (25,347  $\mu\text{mol/kg DM}$ ) in cattle and ~571-643  
224 mg/kg DM (9,006- 10,142  $\mu\text{mol/kg DM}$ ) in sheep <sup>(74,75,77,78)</sup>. Potentially this plateau is linked  
225 to the limited production of metallothionein and an inhibited biliary copper excretion <sup>(74)</sup>,  
226 theoretically explaining why cattle appear to be more copper tolerant than sheep and why both  
227 species appear sensitive in comparison to monogastric species such as pigs.



228 Further to species differences, breed differences among ruminants have also been documented.  
229 Texel sheep are more sensitive to copper than Landrace breeds <sup>(79,80)</sup>. In cattle, Holstein and  
230 Angus breeds are more copper tolerant than Jersey, Charolais and Simmental <sup>(81–83)</sup>. In cattle,  
231 the more copper tolerant breeds exhibit a greater expression of duodenal Ctr1 and ATP7A, and  
232 a higher hepatic expression of; Ctr1, Cox17, ATP7B, CCS and SOD where copper supply is  
233 inadequate <sup>(84,85)</sup>. These suggest the ability to increase expression of copper transporters and  
234 chaperones allows more effective uptake and utilisation where copper supply is insufficient;  
235 reducing the susceptibility of these breeds to deficiency in comparison to their counterparts  
236 <sup>(84,85)</sup>. This research highlights a potential mechanism for the observed breed differences, but  
237 further studies in a wider range of breeds and in sheep, under elevated and copper replete  
238 conditions would further clarify the role of transporter expression in copper sensitivity.

### 239 **THIOMOLYBDATE DISRUPTION**

240 Thiomolybdate is known to interact with copper. It naturally forms in the reducing environment  
241 of the rumen between dietary sulphur and molybdenum. Thiomolybdate poses a problem for  
242 copper availability and post-absorptive utilisation <sup>(29,86–88)</sup>. Thiomolybdates interact with  
243 available copper in the digestive tract forming an insoluble precipitate greatly reducing copper  
244 availability <sup>(29,86–89)</sup>. If there is insufficient copper where thiomolybdates form to ‘de-toxify’  
245 them they can be absorbed into systemic circulation, where they exert their affinity for copper  
246 by complexing with copper contained in biological compounds rendering them biologically  
247 inactive <sup>(16,90)</sup>. Thiomolybdates are able to cross cell membranes but the mechanism by which  
248 this takes place is unknown. However, once inside the cell they have the potential to disrupt  
249 copper transport through binding to copper located on the copper chaperones, transporters and  
250 enzymes <sup>(17)</sup>.

251 Thiomolybdates can bind to copper in cuproenzymes including; caeruloplasmin,  
252 metallothionein, CCO, SOD <sup>(90–93)</sup>, and Atx1 <sup>(94)</sup>. Binding does not remove the copper  
253 component but renders it unable to perform redox reactions (vital to its biological function)  
254 through the formation of a stable complex <sup>(16,29,95,96)</sup>. Superoxide dismutase has been shown to  
255 differ and copper may be partially stripped from this enzyme <sup>(97,98)</sup>. In the case of the chaperone  
256 Atx1, thiomolybdate suppresses the incorporation of copper into the products of the secretory  
257 pathway disrupting the activity of the Atx1 <sup>(94)</sup>. Thiomolybdates have a high affinity for copper  
258 and they have no effect on other trace metals with similar properties such as iron, zinc or  
259 cadmium <sup>(99,100)</sup>.

260

261 **PRACTICAL IMPLICATIONS**

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

280 The potential danger posed through absorption of thiomolybdate causing disruption to systemic  
281 copper chaperones and cuproenzymes should also not be neglected. The use of blood assay is  
282 of importance to help monitor changes in shorter-term copper status. Decreases in  
283 caeruloplasmin activity can be a useful indicator of systemic thiomolybdate presence or copper  
284 deficiency over and above the use of caeruloplasmin concentration <sup>(91)</sup>. Since the apo-protein  
285 will continue to be synthesised in the absence of adequate, available hepatic copper while its  
286 activity can be reduced to nil <sup>(104)</sup>. This measure is not without flaws, as caeruloplasmin is an  
287 acute phase protein and can be elevated by infection or stress leading to falsely elevated  
288 measures of copper status <sup>(30,105,106)</sup>. Unfortunately, a single, reliable measure for copper status  
289 does not yet exist. Therefore, it is important to use both blood and hepatic measures in  
290 monitoring ruminant copper status in addition to monitoring nutritional input <sup>(11,101)</sup>.  
291 Furthermore, it is important in practice to provide an appropriate copper source, or combination

292 of sources, which will be sufficient to ‘de-toxify’ thiomolybdate before it is absorbed and retain  
293 a sufficient supply of labile copper for absorption which does not provide an excess or exceed  
294 legal restriction <sup>(101)</sup>.

295

## 296 **CONCLUSION**

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

## 311 **FINANCIAL SUPPORT**

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

## 316 **CONFLICT OF INTEREST**

317 None

## 318 **AUTHORSHIP**

319 Initial planning and selection of areas to review- AH Clarkson, NR Kendall, J Martin-Tereso,  
320 S Paine

321 Review of research and article writing- AH Clarkson

322 Proofing of concept and article content and wording- NR Kendall, J Martin-Tereso, S Paine

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