The Vets role in vitamin and mineral supplementation on farm – ensuring food chain safety

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ABSTRACT

Supplementation of animals with minerals and trace elements may be required to ensure health, welfare and productivity. Maximum permitted levels of trace elements under EU regulations are determined by assessments of risk to animals, food consumers, the environment and human material handling. Levels are corrected to 88% dry matter including all feeds, supplements and water intake. However, concentrations may vary in forage, moist feeds, straights, co-products and blends before ration supplementation. Some compounds are available as drenches, long acting boluses or in other therapeutic agents such as wormers under different legislative frameworks. Labels may declare the element, such as copper, or the compound, such as copper sulphate pentahydrate – where the majority of weight is water of crystallisation.

Deficiencies may be suspected when health or production appear compromised. It is tempting to trial additional supplementation without accurate diagnosis and a full audit of current sources within the total ration and any therapeutics previously given.

There is evidence of a risk to the food supply chain if over supplementation occurs.

Vets are ideally placed as part of the farm management team including the farmer, nutritionist, agronomist, and agricultural advisors, to investigate all possible routes of supply and audit total supplementation to ensure both animal health and food safety.

INTRODUCTION

Supplementation of animals with trace minerals may be required to ensure health, welfare and productivity. In animal feed, vitamins and trace elements are classified as feed additives and are subject to rigorous safety assessments before they are authorised for use. Details of authorised products can be found in the European Union Register of Feed Additives and their conditions of use are detailed in individual implementing regulations, available on-line from hyperlinks in the Register (European Commission 2017a).

Whilst mineral supplementation is generally considered a good thing by the farming industry evidence of harm to animal health from over-supplementation have started to appear in UK and other countries. Chronic copper toxicity is an emerging problem in cattle (Livesey and others 2002, Bidewell and others 2012), with 20 cases diagnosed by APHA in 2015 compared to 30 cases of copper deficiency (VIDA 2015). There were 60 diagnosed cases of copper toxicity in sheep compared to 25 cases of deficiency reported in the same period. Both species show a trend for yearly diagnoses of deficiency to be reducing and toxicity to be stable or increasing over the last decade. This suggests the focus of the Veterinary profession and agricultural industry may need to shift to prevention of toxicity by supplementation only after a clear diagnosis of deficiency. Problems are also being reported in New Zealand (Grace and others 2010), and the USA (Engle and others 2001). Diagnosis of specific deficiencies can also be difficult (Suttle 2010) and there is controversy regarding the most appropriate methods for some minerals (Suttle 1993; Telfer and other 2006).

Routes, legislation and methods to assess total dietary intake will be discussed to give participants the knowledge and confidence to be able to audit supply and determine if there is a real need for further supplementation or not.

Maximum permitted levels of feed additives are set, where appropriate, in the specific authorising regulations, all coming under the umbrella of EU regulation 1831/2003 on additives for use in animal nutrition. Veterinary medicinal products are specifically excluded from the scope of the regulation. The maximum permitted levels are determined by assessments of risk to the target animals, food consumers, the environment and human handlers of the materials. The importance of each of these areas varies between additives and the European Food Safety Authority (EFSA) advisory panel on Additives and Products or Substances used in Animal Feed (FEEDAP) provides scientific advice on the safety and/or efficacy of additives and products or substances used in animal feed to the EU commission. A list of authorised feed additives is available (https://ec.europa.eu/food/safety/animal-feed/feed-additives/eu-register_en).

A new review by EFSA in 2016 suggested a maximum permitted level of 30 mg/kg for copper in feeds for dairy cows rather than the current 35 mg/kg (EFSA FEEDAP 2016). Levels are set corrected to 88% dry matter including contributions from all feeds, supplements and water intake. However in UK, feed manufacturers have been advised by the Food Standards Agency Advisory Committee on Animal Feedstuffs (ACAF) to limit the copper in the total rations to 20 mg/kg if significant antagonism has not been diagnosed (ACAF 2011). This paper stems from further consideration of current practice and risks by ACAF and the need to publicise the issue within the agriculture sector.

However, many trace element compounds may be present in varying quantities in forage, moist feeds, straights, co-products and blends before any supplements are added to the ration. Some compounds are also incorporated into drenches and boluses supplied as "dietetic supplements" that come under EU Directive 2008/38/EC (European Commission 2008). Their use should require assessment of the whole diet to allow determination that the maximum permitted level has not been breached. However, this requirement is not always clearly displayed on websites or at point of sale to farmers looking for an improvement in animal performance. Some minerals are also incorporated in other therapeutic preparations such as anthelmintics and short or long acting boluses authorised under the UK Veterinary Medicines Regulations. These have specific doses and frequencies of administration specified which should be followed to meet the requirements of the medicines regulations but may not lead to compliance with the maximum permitted level or ACAF guidance. The Summary of Product Characteristics (SPCs) for the anthelmintics tend to give details of the added trace element compounds but do not provide any context of the required control on their use. For example, one product states "Selenium and cobalt are included in this product as nutritional supplements and are not intended to be used therapeutically" whilst another states "The product should only be used in areas where deficiencies of cobalt and selenium are likely to occur", the latter product actually has a lower concentration of trace elements per Kg dose of anthelmintic. None include overdose information for the trace element component, only the anthelmintic (see VMD Product information database). The tables below indicate the percentage of dietary maximum permitted levels for each trace element that a market authorisation indicated dose of a typical long acting bolus (Table 1), oral drench (Table 2) and anthelmintic with trace elements (Table 3). If these products were added to the diet it would be legally required to calculate the total concentration and remain within the MPL for the whole intake of the animal. Bolus release doses do not exceed MPL but total diet may exceed MPL if forage or ration has not taken the bolus into consideration. Both the example nutritional and anthelmintic drenches provide more than the legal MPL for daily intake.

Adverse reactions, including death in response to administration of selenium/vitamin E and copper in sheep and cattle have been reported via the Veterinary Pharmacovigilance Adverse Reaction reports system (Woodward 2009) although the incidence per dose of product sold in the UK are very low (VMD quoted by ACAF 2016 and VMD personal communication 2017).

Additive compounds may arrive on farm and be incorporated into the diet as the additive itself (subject to feed hygiene controls) as part of a micronutrient supplement, within a feed blend or a pelleted feed. All feeds containing added trace elements have to be labelled with the added amounts. For the last few years, the levels declared have related to the compounds, not the actual mineral elements. This has created confusion as the level of the element within a trace element compound may be variable, according to the water of crystallisation and the purity of the product used. For example one feed company may declare the mg/Kg of feed of copper element, whilst another declares the amount of copper sulphate pentahydrate crystals added per Kg so including the sulphur and water of crystallisation. Newly adopted EU legislation (European Commission 2017b) will soon revert to requiring the added level of the actual element to be declared. This will take some time to implement. Even then, background levels intrinsically present in the feed materials are not accounted for on feed labels, but must be considered when evaluating total levels.

FACTORS AFFECTING MINERAL INTAKE AND ABSORPTION

Soil pH and stage of growth / degree of grazing and thus the part of the plant ingested can also influence mineral intake at grass (Suttle 2010). Concentration of copper in pasture in New Zealand tends to be higher (mean 10.4 vs 8.2 mg/kg DM) in the autumn than spring, while concentration of molybdenum is lower in the autumn (mean 0.35 vs 1.07 mg/kg DM) suggesting that availability of copper may vary through the grazing season (Grace and others 2010) and possibly in forages harvested at different times.

In a recent study no difference in availability of copper was observed between inorganic and organic sources in dairy cattle, but differences have been observed in monogastric species (Sinclair and others 2013). Organic copper sources (e.g. copper glycinates) may be more available in the face of antagonists molybdenum and sulphur than inorganic sources (e.g. copper sulphate or oxide) (Du and other 1996). There is also a variation between studies. Feeding of organic copper and molybdenum and sulphur was associated with an increase in mRNA for ATP7B protein that exports copper out of the liver into bile (Sinclair and others 2013). In this study liver copper was depleted in animals fed molybdenum and sulphur and

either inorganic and organic copper suggesting lack of absorption with inorganic copper and secretion via ATP7B for organic copper sources.

Palm kernel cake (PKC) (used as a protein supplement) is rich in copper but also that copper is very available (Alimon and others 2011). Use of high feed rates of PKC in New Zealand dairy herds was associated with high liver copper concentrations in cull cows (Grace and others 2010). UK farmers considering swapping to feeding straights and tempted to use palm kernel as a cheap protein source need to be aware of these issues if they choose to formulate rations themselves (Suttle and others 2013).

Genetic differences between individuals and breeds of cattle have been reported. Jersey cows have higher plasma and liver copper than Friesians (Du and others 1996, Gipson and others 1987, Morales and others 2000) and duodenal concentration of copper transporter1 (CTR1) protein and transcripts tend to be reduced in Simmentals vs. Angus. This was associated with lower plasma and liver copper in Simmental than Angus animals on the same low copper diet (Fry and others 2013). These data suggest that supplementation requirements may be breed specific and the risk of over supplementation may be greater in some breeds of cattle than other as (analogous to that seen in sheep breeds, but to a lesser degree).

DIETARY AND SUPPLEMENTAL COPPER AND TOXICITY / DEFICIENCY RISK

Parenteral injections circumvent any problems with antagonism of intestinal absorption with many minerals also sharing use of the divalent cation transporters in the small intestine and some also having specific membrane protein transporters. Injection can result in prolonged supplementation of both blood and liver copper concentrations relative to the same dose given orally. For example, a single dose of 100mg can increased plasma copper for over 60 days (Garcia-Diaz and others 2012). A reduction in milk production and fertility has also been reported after parenteral injection of 200mg of copper in dairy cattle (Hawkins 2014) suggesting supplementing “just in case” with no diagnosis of deficiency may be detrimental to herd performance. Authorised copper boluses given in the USA to younger, pre-ruminant calves have also caused toxicity (Hamar and others 1997). Forage in the area was considered to usually be deficient so copper supplementation had been considered as a farm routine. Changing the type of supplementation from a parenteral injection to a bolus and administration to calves younger physiologically and half the minimum body weight advised on the marketing authorisation resulted in toxicity. Due to increased efficiency of absorption, EU MPL in pre-ruminant animals is 15mg/Kg DM of ration (EFSA FEEDAP 2016) so the physiological age of the animal needs to be considered rather than just body weight.

In a UK survey of on-farm feeding practices, in early lactation 6 out of 50 herds were feeding above 40 mg/kg DM of copper in total rations and 40 were feeding above 20 mg/kg DM. In late lactation 2 out of 50 herds were feeding above 40 mg/kg DM and 27 above 20 mg/kg DM suggest over-supplementation is widespread in the UK (Sinclair and Atkins 2015) (Figures 1 and 2).

Clinical disease due to over supplementation has been reported in the UK (Hunter and others 2012) where the animals have not shown the classical signs (Livesey and others 2002). In this case copper sulphate and a bioplex copper were both being added into a TMR ration for both milking and dry cows (45mg Cu/kg DM dry cow ration and 60mg Cu/kg DM lactating cow ration and in calf creep feed (75mg Cu/kg DM), exceeding the current maximum permitted level (MPL) of 35 mg/kg at 88% DM (40mg in 100% DM) (European Commission 2002).

Monitoring of liver copper concentrations from 510 cull cattle at one abattoir over three days in the UK, with animals from all regions of the UK represented, showed 40 per cent of dairy breed and 17 per cent of beef breed livers to have copper content above the upper limit of the APHA reference range (8000 µmol Cu/kg DM). Age of the animal and topsoil copper concentration in the geographical region of origin of the animal was not related to liver copper content suggesting a source other than grazed / home grown forage for the excess copper (Table 4) (Kendall and others 2015).

Grace and others (2010) reported high and variable liver copper concentrations in commercial dairy cows in New Zealand when samples were collected at slaughter or biopsy, with values ranging from approximately 150 to 600 mg/kg of DM.

Any copper excess to requirements will accumulate in the liver at a slow rate. In a recent UK study, liver copper concentrations at the end of the study averaged 397 and 283 mg/kg of DM in cows receiving no or additional molybdenum and sulphur, respectively representing a net increase of 0.33 mg Cu/kg of DM per day in the non-molybdenum and sulphur group. In a New Zealand study dairy cows were supplemented at 10 mg/kg of DMI as copper sulphate, liver concentrations increased by 1.9 mg/kg of DM per day (Engle and others 2001). There was a relatively small difference in percentage total dietary copper for this difference in percentage accumulation (16.9 vs. 18.9 mg/kg of DM, respectively) but the amount accumulated suggest the requirements were similar and the molar amount in excess was accumulated. The inclusion of additional molybdenum and sulphur in the UK study resulted in a depletion of liver copper concentrations of 0.89 mg/kg of DM per day. At this rate of depletion, cows would have been expected to have exhibited clinical signs of deficiency after a further 300 d on treatment and so would only then require a higher copper

inclusion rate, although these levels of antagonists are rarely encountered under commercial feeding conditions (Sinclair and others 2013)

In the absence of marked exposure to copper-antagonists, such as molybdenum and sulphur it has been suggested that the copper requirement of TMR can be met by 5.2 mg/kg DM (Suttle 2010). If supplementation over MPL or supplementation without calculation of the total diet MPL is being considered we recommend accurate diagnosis of deficiency, calculation of total diet copper and periodic monitoring of cull animal liver copper concentration. If there is a history of over supplementation there is a risk that liver copper concentrations will only very slowly decline and prolonged periods with no supplementation may be required to achieve normal liver copper concentrations (Grace and others 2012). Although plasma copper concentrations will increase in deficient animals if supplemented they are not a good indication of risk of over-supplementation when oral or parenteral copper has been given to animals with varying liver copper concentrations (Balemi and others 2011). Antagonists molybdenum and sulphur reduce liver copper reserves more in cows fed a grass silage compared to a maize silage diet. Maize would also be expected to have lower mineral concentrations than grass grown on the same farm (Sinclair and others 2017). In the face of these antagonists in the diet less supplementation may be required in maize based diets so if a farm changes to growing and feeding more maize the traditional level of supplementation needs to be reviewed.

When animals are purchased, particularly from other geographical locations, it is not possible to predict liver copper stores without direct liver biopsy or identify animals that have been administered a long acting bolus. Caution is needed regarding supplementation over the ACAF recommendations and the MPL in these circumstances.

IS THE FOOD CHAIN RISK REAL?

The myriad of possible routes by which animals may be supplemented with minerals, the diverse legislative framework and multiple actors mean that there is a risk of over supplementation that could, unwittingly, leading to a risk to animal and public health. Exposure in the human diet can be viewed “on average” but it is important to consider individual variation in diet choice and the possibility for the food supply chain to focus risk. For example, a consumer who frequently purchases liver from a specific farm shop supplied by one herd would be exposed to risk from management practices on that particular supply chain that an average member of the public would not be. There have been APHA Chemical Food Safety investigations due to diagnosis of copper toxicity in flocks owned by butchers and sold directly to the public at the farm gate (APHA). Such focus of risk was evident in

clustering of new variate CJD cases in the human population due to variation in food supply and processing practices (Ashraf, 2001).

The APHA normal reference range for liver copper concentrations in cattle and sheep is 300 to 8000 µmol/kg DM, equivalent to approximately 5 to 125 mg/kg wet weight (APHA). In the UK, a liver with a copper concentration greater than 500 mg/kg wet weight trigger a Food Standards Agency / APHA investigation. For example, at post mortem examination of a cow which died following a short malaise with jaundice liver copper concentration was 876 mg/kg WM. Two other cows from the herd of 250 had died the previous month but the cause of these deaths had not been investigated. The affected cattle were all from the same high yielding group. This group is housed continually and receives a total mixed ration (TMR). Cows from the low yielding group which are turned out to graze were unaffected. APHA gave advice that the cattle diets were reviewed and that the total copper fed was accurately calculated and reduced. Additional minerals added to the TMR were removed and no further cases occurred. None of the affected group of cows in this case were intended for slaughter but cull dairy cows entering the food chain can be required to have liver condemned or Cu concentration monitored. However, fatal copper toxicity can be diagnosed at lower liver copper concentrations. As an example, in a fatal case in 2016 liver copper concentration in a twelve-month old beef fattening bullock was 20993 µmol/kg DM), equivalent to 397 mg/kg wet weight. In February 2017 a case was reported in sheep associated with animal feed (APHA). Table 5 summarises recent APHA food safety incident reports.

POTENTIAL FOR COPPER TOXICITY IN HUMANS

The level of copper in milk as a food source is not considered to pose a risk to food safety. Milk copper concentrations do not increase as liver copper increases during over supplementation. As a storage organ for copper, liver consumption is the clearest route for over supplementation of animal diets to affect human health. Dietary intake of liver on average has fallen from 36g per person per week in 1974 to 3 g in 2015, chiefly lamb's liver (Defra, 2015). However, specific age groups may still eat traditional amounts and with declining popularity also comes declining price, possibly resulting in some individuals making price related consumption choices. Liver may also be a component in other meat products, notably pâtés. A UK retail sample of veal liver contained 442 mg/Kg copper in an FSA survey (2006). A single weekly portion of 100 g would represent 18% of the Provisional Tolerable Weekly Intake (PTWI) recommended by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA). This assumes individual adherence to typical portion size and intake frequency.

Within the EU adequate copper intakes of 1.3 mg / day for women and 1.6 mg/day for men are recommended (EFSA NDA Panel, 2015) based on studies showing copper balance in women to be zero if eating 1.25 +/- 0.20 mg Cu/day (Johnson and others 1988). A case report of chronic ingestion of a high-dose copper supplement in man (30 mg per day for 2 years followed by 60 mg per day for 1 year) resulting in liver disease (O'Donohue and others 1993). A range of genetic mutations of the ATP7B protein that exports copper out of the liver into bile in humans are collectively known as Wilson's disease. These lead to accumulation of copper in the liver in humans with normal dietary copper intake. Wilson's disease is thankfully very rare (1 in 30,000 to 40,000 of the world population) but those affected represent a small proportion at risk from normal dietary copper intake. Copper has been implicated in the pathogenesis of Alzheimer's Disease (Brewer 2017). Water and meat have been implicated as sources of the copper but these hypotheses remain controversial.

OTHER TRACE ELEMENTS

This review has focussed on copper as over-supplementation of this element is the most recorded and has the clearest risk to food safety. Over-supplementation of other trace elements (and vitamins) have also been recorded and may have an impact on animal or human health. Some, such as zinc, may also have a potential environmental impact. As mentioned above there have been pharmacovigilance reports of fatalities due to overdose of medicines containing other trace elements and APHA have investigated animal deaths so we need to be vigilant of risks from over-supplementation of other minerals too.

CONCLUSIONS

EU regulation 1831/2003 does describe the principle of maximum residue limits for additives, analogous to those for veterinary medicines, however for compounds that may be given daily in feed to meet nutritional requirements and where excess accumulates in specific tissues a withdrawal period is a difficult concept. For a veterinary audience it may be a useful analogy though. We would be careful to calculate the correct dose and make sure that if a product was given by several routes of administration the total dose by all routes was considered when determining the dose. If a new vet examined the case they would want the history of all medicines given by other vets, the farmer or supplied by SQPs that may be still active in the body and consider this before deciding if further treatment was needed and what withhold period was needed for animal and human safety. When vets are prescribing authorised veterinary products for mineral supplementation or advising on diet

supplementation they need to follow the same process. Practicing cattle veterinary surgeons are the route for correct diagnosis of deficiency rather than assumption and trial treatment. They are also likely to be the first to suspect toxicity and clinical examination and full post mortem have been a key part in identification of this issue and the toxicity events. If no deficiency is diagnosed there is no indication to administer minerals over one of the maximum permitted level in feed or the dose on the authorised veterinary product summary of product characteristics (SPC). Prevention is better than diagnosis or cure and, vets are ideally placed as part of the farm management team including the farmer, nutritionist, agronomist, and agricultural advisors, to investigate all possible routes of supply and audit total supplementation to ensure both animal health and food safety.

REFERENCES

ACAF (2011) https://acaf.food.gov.uk/committee/acaf/papers/supplementingcoppertobovines. Accessed September 7 2017

ACAF (2016) https://acaf.food.gov.uk/sites/default/files/finalminutesofoctober2016meeting_0.pdf. Accessed September 7 2017

Alimon, A.R., Ivan, M., Jalaludin S. (2011) Effects of different levels of dietary sulphur and molybdenum on concentrations of copper and other elements in plasma and liver of lambs. British Journal of Nutrition **106**: 1224–1230

APHA chemical food safety reports (livestock) https://www.gov.uk/government/publications/chemical-food-safety-reports Accessed September 7 2017

Ashraf, H. (2001) UK investigators put forward theory for CJD cluster. Lancet **357**:937

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

Bidewell, C.A., Drew, J.R., Payne, J.H., Sayers, A.R., Higgins, R.J. Livesey, C.T. (2012) Case study of copper poisoning in a British dairy herd. Veterinary Record **170**: 464

Brewer, J. (2017) Copper-2 Hypothesis for Causation of the Current Alzheimer's Disease Epidemic Together with Dietary Changes That Enhance the Epidemic. Chemical Research in Toxicology **30:** 763.

Defra (2015) (https://www.gov.uk/government/statistics/family-food-2015) accessed 1.8.17.

Du, Z., Hemken, R.W., HARMON, R.J. (1996) Copper metabolism in Holstein and Jersey cows and heifers fed diets high in cupric sulphate or copper proteinate. Journal of Dairy Science **79**: 1873–1880

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

EFSA FEEDAP (2016) http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4563/epdf Accessed September 7 2017

EFSA NDA Panel (2015) https://www.efsa.europa.eu/sites/default/files/consultation/150629a%2C0.pdf. Accessed September 7 2017

European Commission (2002) List of the authorized additives in feeding stuffs, in Official Journal 2002/C329/1 https://ec.europa.eu/food/safety/animal-feed/feed-additives/eu-register_en) Accessed September 7 2017

European Commission (2008) Directive 2008/38/EC. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008L0038-20141112&from=EN. Accessed September 7 2017

European Commission (2017a) European Union Register of Feed Additives https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03_annex2.pdf Accessed September 7 2017

European Commission (2017b) Draft Commission Regulation amending Annexes II, IV,VI,VII and VIII to Regulation (EC) No 767/2009 of the European Parliament and of the Council on the placing on the market and use of feed https://ec.europa.eu/info/law/better-regulation/initiatives/ares-2017-2679857_en. Accessed September 7 2017

FSA (2006) http://webarchive.nationalarchives.gov.uk/20120403220636/http://www.food.gov.uk/multimedia/pdfs/fsis1406.pdf. Accessed September 9, 2017

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

Garcia-Diaz, J.R., Munyori-Nderitu, H., Cuesta-Mazorra, M., Quinones-Ramos, R., Figueredo-Ross, J.M., Artiles, E.N., Mollineda-Trujillo, A. (2012) Therapeutic efficacy and pharmacological safety of parenteral supplementation of different concentrations of copper in cows. Archiv Fur Tierzucht-Archives of Animal Breeding **55**: 25

Gipson, P., Field, A. C., Wiener, G. (1987) Concentrations of blood constituents in genetically high and low milk production lines of British Friesian and Jersey cattle around calving and in early lactation. Animal Production **44**: 183–199

Grace, N.D., Knowles, S.O., Hittmann, A.R.. (2010) High and variable copper status identified among dairy herds in the Waikato region by concentrations of Cu in liver sourced from biopsies and cull cows. New Zealand veterinary journal **58**: 130

Grace, N.D., Knowles, S.O., West, D.M., Smith, S.L. (2012) The role of liver Cu kinetics in the depletion of reserves of Cu in dairy cows fed a Cu-deficient diet. New Zealand Veterinary Journal **60**: 142

Hamar, D.W., Bedwell, C.L., Johnson, J.L., Schultheiss, P.C., Raisbeck, M., Grotelueschen, D.M., Williams, E.S., O'Toole, D., Paumer, R.J., Vickers, M.G., Graham, T.J. (1997) Iatrogenic copper toxicosis induced by administering copper oxide boluses to neonatal calves. Journal of Veterinary Diagnostic Investigation **9**: 441

Hawkins, D. (2014). Effects of subcutaneously injected Ca Cu EDTA on concentrations of Cu in liver, milk production and reproductive performance in New Zealand dairy cows. New Zealand Veterinary Journal **62**: 244

Hunter, A.G., Suttle, N.F., Martineau, H.M., Spence, M.A., Thomson, J.R., Macrae, A.I., Brown, S. (2012) Mortality, hepatopathy and liver copper concentrations in artificially reared Jersey calves before and after reductions in copper supplementation. Veterinary record **172**: 46

JEFCA http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=2824. Accessed September 9 2017

Johnson, P.E., Stuart, M.A., Hunt, J.R., Mullen, L., Starks, T.L. (1988) ^{65}Copper absorption by women fed intrinsically and extrinsically labelled goose meat, goose liver, peanut butter and sunflower butter. Journal of Nutrition **118**: 1522-1528

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. Erratum in: Veterinary Record (2015) **177**: 596

Livesey, C.T., Bidewell, C.A., Crawshaw, T.R. David, G.P. (2002) Investigation of copper poisoning in adult cows by the Veterinary Laboratories Agency. Cattle Practice **10**: 289-294

Morales, M.S., Palmquist, D.L. Weiss, W.P. (2000) Milk fat composition of Holstein and Jersey cows with control or depleted copper status and fed whole soya beans or tallow. Journal of Dairy Science **83:** 2112–2119

O'Donohue, J., Reid, M., Varghese, A., Portmann, B., Williams, R. (1999) A case of adult chronic copper self-intoxication resulting in cirrhosis. European Journal of Medicine Research **28**: 252

Sinclair, L.A., Atkins, N. (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

Sinclair, L.A., Hart, K.J., Johnson, D., Mackenzie, A.M. (2013) Effect of inorganic or organic copper fed without or with added sulfur and molybdenum on the performance, indicators of copper status, and hepatic mRNA in dairy cows. Journal of Dairy Science **96**: 4355.

Sinclair, L.A., Johnson, D., Wilson, S., Mackenzie, A.M. (2017) Added dietary sulfur and molybdenum has a greater influence on hepatic copper concentration, intake, and performance in Holstein-Friesian dairy cows offered a grass silage- rather than corn silage-based diet. Journal of Dairy Science **100**: 4365.

Suttle, N.F. (1993) Overestimation of copper deficiency. Veterinary Record **133**:123-124

Suttle, N.F. (2010) Copper. In Mineral Nutrition of Livestock. 4th edn. Eds S. Hulbert, K. Hill. CABI, pp255-305

Suttle, N.F., Martineau, H.M., Hunter, A.G., Thomas, J.R., Macrae, A.I. (2013) Atypical copper poisoning in Jersey dairy herds. Veterinary Record **172**: 82

Telfer, S.B., Kendall, N.R., Illingworth, D.V., Mackenzie, A.M. (2006) Caeruloplasmin: plasma copper ratios in cows. Veterinary Record. **159**: 607

VIDA 2015 https://www.gov.uk/government/publications/veterinary-investigation-diagnosis-analysis-vida-report-2015. Accessed September 9, 2017

VMD Product information database
https://www.vmd.defra.gov.uk/ProductInformationDatabase. Accessed September 7, 2017

Woodward, K.N. (Ed) (2009) Veterinary Pharmacovigilance Adverse Reactions to Veterinary Medicinal Products. John Wiley & Sons, Ltd.