Paper

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Liver copper concentrations in cull cattle in the UK: are cattle being copper loaded?

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With the release of the Department for the Environment, Food and Rural Affairs/Advisory Committee on Animal Feed Guidance Note for Supplementing Copper to Bovines it was noted that the current copper status of the national herd was not known. Liver samples were recovered from 510 cull cattle at a single abattoir across a period of three days. The samples were wet-ashed and liver copper concentrations determined by inductively coupled plasma mass spectrometry analysis. Breed, age and previous location information were obtained from the British Cattle Movement Service. Dairy breeds had higher liver copper concentrations than beef breeds. Holstein-Friesian and 'other' dairy breeds had 38.3 per cent and 40 per cent of cattle above the Animal Health and Veterinary Laboratories Agency (AHVLA) reference range (8000 μmol/kg dry matter), respectively, whereas only 16.9 per cent of animals in the combined beef breeds exceeded this value. It was found that underlying topsoil copper concentration was not related to liver copper content and that age of the animal also had little effect on liver concentration. In conclusion, over 50 per cent of the liver samples tested had greater-than-normal concentrations of copper with almost 40 per cent of the female dairy cattle having liver copper concentrations above the AHVLA reference range, indicating that a significant proportion of the UK herd is at risk of chronic copper toxicity.

Background

The release of the Department for the Environment, Food and Rural Affairs/Advisory Committee on Animal Feed (ACAF) Guidance Note for Supplementing Copper to Bovines in 2011 (ACAF 2011) outlined some of the issues with overfeeding copper. The guidance noted that the incidence of clinical copper toxicosis had increased over recent years but during discussions it was acknowledged that the extent of copper loading was currently unknown. However, this could be assessed from liver copper concentrations, measured across the national herd. At this stage it is useful to define what is meant by copper loading and copper toxicity. Copper loading is the accumulation of copper within the liver, often the precursor to chronic copper toxicity. Copper toxicity is the clinical syndrome, summarised by Bidewell and others (2012) as being characterised as hepatic necrosis resulting in a haemolytic crisis which on postmortem typically presents as a swollen, orange liver, with haemolytic anaemia, jaundice, haemoglobinuria, methaemoglobinaemic discolouration of blood and tissues with dark swollen kidneys. Copper toxicity can either be due to a large acute dose of copper or the release of elevated copper from the liver, possibly due to a stress trigger or liver insult (Grace and others 2010, Johnson and others 2014).

The cases of chronic copper poisoning had been reported as increasing according to Bidewell and others (2012) with a maximum of 41 clinical cases reported to Animal Health and Veterinary Laboratories Agency (AHVLA) in 2007 from the period 2000 to 2010.

The maximum dietary level of elemental copper permitted without veterinary prescription under domestic and EU legislation is 35 mg/kg (88 per cent dry matter (DM)) which equates to 40 mg/kg of total diet DM. Sinclair and Atkins (2014) found that 6 of the 50 farms that they studied within a region of the UK exceeded this limit. In addition they found that 32 of the 50 herds studied were also supplemented above the guidance note recommendation for total copper of 20 mg/kg DM (including supplements) (ACAF 2011).

The aim of the current study was to survey liver copper concentrations within the UK dairy and beef herd using abattoir cull cow samples.

Methods

Liver samples (caudal edge of left lobe) were collected from 510 cattle from a single abattoir on three consecutive days in October 2012. The samples were frozen at −20°C at the end of each day before subsequent analysis. Cattle ear tags were recorded and data was obtained from the British Cattle Movement Service (BCMS) to give breed, sex, date of birth and county and parish of the holding before slaughter.

Frozen liver samples had the outside of the liver removed with a scalpel and a sample (0.5–1.0 g) was freeze dried. Approximately 0.1 g of freeze dried liver was wet-digested in

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3 ml 70 per cent HNO_3 (Fisher Scientific, UK), 2 ml 30 per cent H_2O_2 (Fisher Scientific, UK) and 3 ml Milli-Q water (18.2 MΩ cm) for 45 minutes in a Multi-wave 3000 microwave (Anton Paar, UK). Postdigestion samples were washed from digestion vessels into universal tubes (Sarstedt, Leicester, UK) with sequential aliquots (3 ml and 4 ml) of Milli-Q water. Blanks and reference material (standard reference material 1577c, bovine Liver, National Institute of Standards and Technology, US) were run with each digestion batch.

Elemental copper concentrations in liver digests were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo-Fisher XSeries[II]). Samples and calibration standards were diluted identically (0.5 ml into 20 ml) in a diluent containing 0.1 per cent of a non-ionic surfactant ('Triton X-100'and 'antifoam-B', Sigma-Aldrich Company, Dorset, UK), 2 per cent methanol and 1 per cent HNO_3 (Trace Analysis Grade, Fisher, UK) including the internal standards Ir (5 µg/l), Rh (10 µg/l), Ge (50 µg/l) and Sc (50 µg/l). The ICP-MS was run in 'collision cell with kinetic energy discrimination' mode using 7 per cent H_2 in He as the hexapole collision cell gas to reduce polyatomic interferences. Aspiration was through a single sample line via a glass concentric nebuliser (Thermo-Fisher Scientific; 1 ml/min). Following standard dilutions, calibrations were effectively in the range 0–50 µg/l (Claritas-PPT grade CLMS-2 from Certiprep/Fisher, UK).

The data was categorised according to the liver copper concentration categories employed by the NUVetNA laboratory (School of Veterinary Medicine and Science, University of Nottingham) to aid result interpretation, these values are based on values used by the University of Wisconsin Veterinary Diagnostic Laboratory (WVDL 2015) expressed on a DM basis using a typical liver DM of 280 g/kg (unpublished data). Data groups were collated using pivot tables in Microsoft Excel (2007). Categorical distribution analysis was carried out using χ^2 analysis.

Results

Of the 510 livers, 14 were from bulls (eight beef and six dairy breeds), 77 were from females of beef breeds or beef breed crosses and 419 were from females of dairy breed and dairy breed crosses. Table 1 shows the categorical distribution of the liver copper concentrations. Across all classifications 52.2 per cent of liver copper concentrations were above 'normal' (>5618 µmol/kg DM). Within the cohort groups, Holstein-Friesians had the highest relative number of cattle at 58.6 per cent above 'normal' whilst beef cows had the lowest at 23.4 per cent. When compared with the upper limit of AHVLA reference range (8000 µmol/kg DM) 38.3 per cent of Holstein-Friesian dairy cows and 40 per cent of other dairy cows were over this value; 21.4 per cent of bulls and 16.9 per cent of beef cows also exceeded this concentration.

The effect of age is shown in Fig 1. Age had little effect except that the liver copper concentrations of the youngest cows appeared to differ from the rest of the cattle: higher for beef breeds and lower for dairy breeds. There is a suggestion that liver copper concentrations might decrease with age in beef cow culls, but dairy culls over two years old were remarkably constant.

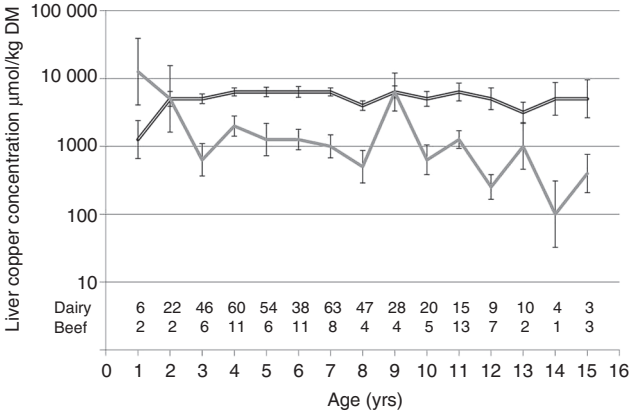

FIG 1: Mean (±se) liver copper concentration of all female cattle (µmol/kg dry matter (DM)) plotted on a log scale against cow age (rounded down to whole years) for dairy (double black line) and beef breeds (solid grey line) with numbers per category

Parish data obtained via BCMS confirmed the widespread geographical distribution of the cattle surveyed. This geographical distribution was compared with the British Geological Survey soil geochemical database (Johnson and others 2005) to determine whether there was a relationship between the concentration of copper in bovine liver and in the underlying topsoil. No significant relationship was found (data not shown).

The regional distribution of the liver copper concentrations found, shown in Table 2, was not significant for female dairy cows when comparing those over the 'normal' NUVetNA range or those above the AHVLA reference range (χ^2, P>0.05).

Discussion

This survey found that liver copper concentrations were above the NUVetNA 'normal' range (5618 µmol/kg DM) in over 50 per cent of the animals surveyed and this was highest in the dairy breeds. Age was not found to be a significant factor, suggesting that the elevated copper concentrations are not a long-term chronic issue of marginal overfeeding but much more likely to be a shorter-term accumulation of copper due to current feeding practices.

Case studies on herds with chronic copper toxicity have found variability in the liver copper concentrations of cattle succumbing to chronic copper toxicity and those not exhibiting clinical signs. Bidewell and others (2012) recorded eight cows with chronic copper toxicity that had liver copper concentrations ranging from 8148 µmol/kg to 23493 µmol/kg DM (mean

TABLE 1: The numerical distribution (%) of liver copper concentration by NUVetNA (School of Veterinary Medicine and Science, University of Nottingham) category for bulls, beef cows, Holstein-Friesian (HF) dairy and other dairy cows

NUVetNA category	Liver copper µmol/kg DM	HF dairy cow	Other dairy cow	Beef cow	Bull	Total
Deficient	<281	13 (3.3)	0 (0.0)	22 (28.6)	2 (14.3)	37 (7.3)
	281–561	5 (1.3)	3 (10.0)	12 (15.6)	0 (0.0)	20 (3.9)
	562–1404	12 (3.1)	3 (10.0)	8 (10.4)	3 (21.4)	26 (5.1)
Normal	1405–5618	131 (33.7)	10 (33.3)	17 (22.1)	3 (21.4)	161 (31.6)
	5619–7999	79 (20.3)	2 (6.7)	5 (6.5)	3 (21.4)	89 (17.5)
	8000–11237	84 (21.6)	6 (20.0)	8 (10.4)	1 (7.1)	99 (19.4)
High	11238–14046	34 (8.7)	2 (6.7)	3 (3.9)	1 (7.1)	40 (7.8)
Toxic	14047–44952	31 (8.0)	4 (13.3)	2 (2.6)	1 (7.1)	38 (7.5)
Total		389	30	77	14	510
Above AHVLA reference range	>8000	149 (38.3)	12 (40.0)	13 (16.9)	3 (21.4)	177 (34.7)

DM, dry matter

TABLE 2: The geographical distribution of high liver copper concentrations, number and (%) for those above the 'normal' NUVetNA (School of Veterinary Medicine and Science, University of Nottingham) range (>5618 μmol/kg DM) and those above the AHVLA reference range (8000 μmol/kg DM)

Area	Counties	Total number	above 'normal'	above 8000
South-West	Devon, Cornwall	119	58 (48.7%)	40 (33.6%)
North-West	Cheshire, Cumbria, Lancashire, Greater Manchester	77	50 (64.9%)	29 (37.7%)
Midlands	Staffordshire, Shropshire, Leicestershire, Derbyshire, Warwickshire	76	45 (59.2%)	36 (47.4%)
Scotland	Ayr, Dumfries, Lanark, Kirkcudbright, Renfrew, Wigtown, Aberdeen	73	46 (63.0%)	32 (43.8%)
Wales	Dyfed, Clwyd, Gwynedd, Powys	32	18 (56.3%)	9 (28.1%)
North-East	Durham, Northumberland, N Yorkshire, W Yorkshire, Humberside, Lincolnshire	21	13 (61.9%)	6 (28.5%)
West	Gloucestershire, Wiltshire, Hereford and Worcestershire, Somerset	12	9 (75.0%)	7 (58.3%)
South-East	E Sussex, Berkshire, Hampshire, Oxfordshire	9	3 (33.3%)	2 (22.0%)

DM, dry matter

14223.5 μmol/kg DM), whilst four herd mates with no clinical signs of chronic copper toxicity had liver copper concentrations of 5260, 9628, 10084 and 21850 μmol/kg DM. Similarly in New Zealand Johnson and others (2014) in an investigation into six deaths from chronic copper toxicity found the liver copper concentrations of 10 random herd mates to range from 1900 μmol/kg to 3500 μmol/kg wet weight with a mean of 2620 (±188) μmol/kg (6785 μmol/kg DM to 12500 μmol/kg DM, with a mean of 9357 (±671) μmol/kg assuming a DM of 280 g/kg). In only three of the dead cows were liver copper concentrations analysed and these were 2300 μmol/kg, 2400 μmol/kg, 2700 μmol/kg wet weight (8214 μmol/kg DM, 8571 μmol/kg DM, 9643 μmol/kg DM). Both of these studies illustrate that a high liver copper concentration per se does not necessarily cause chronic copper toxicity but does increase the risk. Many apparently healthy cull cattle in this study had liver copper concentrations above those of cattle which had succumbed to liver toxicity in these studies. Both of the case studies were on herds predominantly composed of Jersey cattle which are thought to be more susceptible to copper toxicity than the more common Holstein-Friesian types.

In New Zealand, Grace and others (2010) found that average liver copper concentrations of herds studied ranged from 520 μmol/kg to 2610 μmol/kg (~1860 μmol/kg to 9321 μmol/kg DM). Although some values were above 'normal', the loading was not as severe as has been found in the UK or in the case studies discussed. The Grace study also compared the gold standard liver biopsies to abattoir-collected cull cow samples, as used in the current study, and found large variations between animals on farms, especially when copper was 'replete' and not deficient with biopsy samples tending to be higher than cull-collected liver samples. However, this was only significant on two farms both of which were lower in copper status (normal range and below). The biopsy and cull-collected samples were much more comparable when collected from herds with above 'normal' liver copper concentrations.

Currently many herds are fed to the maximum permitted level (MPL) or above. Sinclair and Atkins (2014) conducted a survey of mineral feeding on 50 dairy farms in central/west Britain and found that the mean total copper intake was 27.9 mg/kg DM (sd 9.85) with a range of 12.9 mg/kg to 57.9 mg/kg DM. This included two of the 50 farms which were feeding above the MPL; the MPL for copper is 35 mg/kg at 88 per cent DM which equates to approximately 40 mg Cu/kg DM. The question raised is: *'why are farms feeding this high level, especially when the most recently estimated requirement for dairy cattle (NRC 2001) is only 11 mg Cu/kg DM¿'* Sinclair, L.A. (personal communication) recently found 16 mg Cu/kg DM to be sufficient to satisfy the requirements of cattle in a trial on overwintered heifers. Sinclair and Mackenzie (2013) concur with the ACAF guidelines that 20 mg Cu/kg DM should be adequate in the majority of situations and that if feeding above this then caution should be exercised.

Why is copper still being overfed¿ There are probably several reasons:

1. There is still a culture of 'if I add a little and I get a response then I will add a lot and get a better response'. Unfortunately nature's law of diminishing returns applies and there is the additional issue that too much is toxic. Therefore, there is a requirement to optimise supplementation of copper – which might include reduction rather than continued increases, or changing the form of copper used.
2. The interactions of copper are still poorly understood or even misunderstood. Many people believe that the only effect of molybdenum and sulfur on ruminants is to lock up copper and prevent its absorption. In fact molybdenum and sulfur will combine in the rumen to form thiomolybdate which has a high affinity for copper. The binding of copper to thiomolybdates in the rumen will detoxify the thiomolybdate preventing its absorption into the animal. Once in the animal thiomolybdate will bind to copper engaged in the metabolic functions (enzymes) and thus reduce the activity of copper involved in physiological processes. The ruminal interactions of copper, molybdenum, sulfur and iron have been extensively reviewed by Gould and Kendall (2011). The thought that copper is locked up, rather than acting as a detoxicant for thiomolybdate in the rumen has led to the feeding of high concentrations of copper and copper sources being used which allegedly bypass the rumen making the copper more available to the animal (and potentially less available to detoxify thiomolybdates in the rumen). This can lead to the feeding of excessive amounts of copper and still allow thiomolybdate toxicity to occur within the animal.
3. Often multiple copper sources are fed. Frequently forage mineral reports indicate low copper, or high antagonists, and diets are formulated in response to this rather than with consideration of the complete diet (including concentrates, straights and so on). Often other direct supplement types, such as boluses or drenches, are also given and these are rarely included in the overall calculation of copper intake.
4. Liver copper stores are not assessed before copper supplementation, which is possible using biopsy or abattoir-recovered tissue. This should be done for a representative proportion of the herd and copper supplementation reduced, or stopped completely, if concentrations are above reference/normal levels, or are shown to be increasing over time within normal ranges.

Once the animal has a high liver copper concentration it takes a significant amount of time for this to be reduced. For example, Grace and others (2012) calculated that a liver concentration of 1000 μmol/kg (~3600 μmol/kg DM) would take over 300 days to reach deficiency levels (<95 μmol/kg (~340 μmol/kg DM)). It must be noted that these changes in liver copper concentration are from 'normal to deficient' and not 'toxic to normal'. However, the authors' practical experience in the field

suggests that it often takes between one year and two years to reduce copper loading in livers from toxic to normal levels in commercial situations (unpublished data).

Although the authors have mainly focused on overfeeding of copper it should also be noted that 16.3 per cent of the cattle surveyed had liver copper concentrations below the 'normal' range (<1405 μmol/kg DM). The majority of these were either beef breeds or bulls suggesting that they may have been kept on extensive grazing systems with little or no concentrate fed. The lack of a relationship between the topsoil copper concentrations and the animals was expected as the majority of dairy cattle are fed purchased feeds and supplements. It is much more likely that the lower status extensive grazing animals with no additional inputs would be at more risk in relation to soil concentrations. However, this does not take into account the effect of other interacting elements (Fe, Mo, S) and the nutritional history of the animals within this study is not known. The seasonality of copper supply has not been studied in great depth, but could be related to direct soil ingestion which tends to be increased in hot dry weather and in cold wet weather (close grazing). However, although the study only collected liver samples during a three-day period it is unlikely that the distribution of copper loading would vary significantly across the year given the long times required to reduce liver copper concentrations from elevated values as discussed above.

This work needs to be continued with samples evaluated from different times of the year. There is also a need for a more longitudinal approach where nutritional histories are known so that copper loading can be related to the nutritional history.

The best way to assess risk of copper toxicity is to measure liver copper loading. Practically, this can be done in a number of ways: recovery of cull cattle liver samples from abattoirs, liver biopsy and/or recovery of livers from on-farm trauma culls (casualty slaughters). Livers can be collected and frozen before analysis enabling routine monitoring to be a relatively simple process.

In conclusion, over 50 per cent of the liver samples tested had greater-than-normal concentrations of copper with almost 40 per cent of the female dairy cattle having liver copper concentrations above the AHVLA reference range of 8000 μmol/kg DM. This means that a significant proportion of the UK herd is at risk of chronic copper toxicity.

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