Overfeeding copper during rearing affects the liver function and fertility of replacement dairy heifers

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ORIGINAL RESEARCH

Overfeeding copper during rearing affects the liver function and fertility of replacement dairy heifers

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Abstract

Background: Oversupply of dietary copper (Cu) is common among UK dairy herds, but studies on the long-term outcomes of this oversupply are scarce.
Methods: A longitudinal study was undertaken to determine the long-term implications when 80 Holstein–Friesian heifers with a mean (±standard error) age of 4.1 ± 0.1 months and a mean liveweight of 137 ± 2.4 kg were fed a recommended (R; 16 mg/kg dry matter [DM]) or high (H; 32 mg/kg DM) dietary Cu concentration until 6 weeks prior to calving.
Results: Hepatic Cu concentrations in both treatment groups were elevated into the ranges used to diagnose chronic Cu toxicity in cattle at 6.9 months of age (798 ± 46.4 mg/kg DM for H vs. 643 ± 35.4 mg/kg DM for R), with associated evidence of liver damage. Hepatic Cu concentrations then returned to normality but remained higher ($p < 0.001$) for heifers fed H than for those fed R and were associated with a reduced ($p = 0.044$) conception rate to first and second services (73.7 ± 8.05% for H vs. 91.2 ± 7.68% for R).
Limitation: This retrospective analysis identified pre-study liver damage, which may have affected results.
Conclusions: Supplying Cu in excess of requirements resulted in liver damage and reduced conception rates.

INTRODUCTION

Copper (Cu) is an essential trace element in ruminant diets that is involved in numerous enzyme systems with implications for the health and performance of dairy cattle.[1] The biological consequences of an inadequate dietary supply of Cu have been widely studied and include anaemia and impaired growth,[2,3] as well as reduced fertility.[4] A deficiency in Cu may result from an inadequate dietary supply or an interaction between Cu and antagonists, such as sulfur, molybdenum and iron, that reduce Cu absorption or function.[1] There is, however, evidence of Cu oversupplementation in housed dairy cows in the UK, where a survey of 50 herds reported a mean dietary Cu concentration of 27.9 mg/kg dry matter (DM),[5] which is well in excess of the nutritional recommendation of 11 mg/kg DM proposed by the National Research Council (NRC).[6] Indeed, six of the herds surveyed were feeding above the maximum permitted level of 40 mg/kg DM set by EU regulation 1831/2003, while 10 farms were feeding above the current revised legal limit of 34 mg/kg DM (EU regulation 2018/1039).[5] This issue of Cu oversupply in dairy diets is not limited to the UK, as Castillo et al.[7] reported a median dietary Cu concentration in Californian dairy herds that was 1.9 times the NRC[6] recommendations. It has also been reported that 38% of the livers from Holstein–Friesian cull cows within the UK contained Cu concentrations in excess of 508 mg/kg DM, which is generally considered the liver threshold for chronic Cu toxicity diagnosis in cattle,[8,9] although others have proposed a marginal toxicity range of 350–1500 mg/kg DM.[1] Despite this evidence of oversupplementation, anecdotal evidence suggests that a number of UK dairy herds continue to supply Cu at excessive levels.[10]

The reasons for the excessive feeding of Cu on farms are unclear but may result from a perception within the industry that, in the absence of clinical toxicity, feeding Cu above requirements may benefit cow

health and performance.[8] However, evidence is growing to suggest harmful subclinical consequences of oversupplementation, such as decreased average daily gain[11] and impaired rumen function,[12] and anecdotal links between disease incidence and Cu status have been reported by practising veterinarians. Studies examining the effects of dietary Cu concentrations have tended to be short term in duration,[11] focused primarily on beef cattle and have often neglected aspects related to fertility and health.[13] This paper describes an investigation into the long-term implications for liver function, fertility and performance of rearing replacement Holstein–Friesian heifers at either the recommended (R) or a higher (H) dietary Cu concentration.

MATERIALS AND METHODS

Heifer nutrition and management

This study was conducted at Harper Adams University, Newport, Shropshire, UK, between September 2016 and September 2018. All the procedures that involved animals received local ethical approval (103-201512) and were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 (amended 2012; PPL 70/7636). Eighty Holstein–Friesian heifer calves that were (mean ± standard error) 4.1 ± 0.1 months of age and weighed 137 ± 2.4 kg at the beginning of the study were paired, based on their liveweight, body condition score (BCS; five-point scale with 0.25 increments)[14] and predicted transmitting ability (based on parent averages) for milk, fat and protein yield, and randomly allocated to one of two dietary treatments. Prior to the commencement of the study, the heifers were reared on milk replacer, weaned at 8 weeks of age and fed straw and a commercial pelleted concentrate that contained Cu at a concentration of 40 mg/kg DM and provided a maximum of 700 g/kg DM intake.

Dietary treatments consisted of an R or an H dietary Cu concentration, with animals remaining on their respective treatments from the beginning of the study until 6 weeks prior to their expected calving date. Treatment R consisted of a basal diet that was formulated to contain Cu at a concentration of approximately 16 mg/kg DM and met the animal's Cu requirements according to the nutritional recommendations of the NRC.[6] This basal diet comprised a combination of feedstuffs typically fed throughout the rearing period on UK dairy farms. A total mixed ration (TMR) was fed throughout the winter period (see Table S1), with heifers subsequently grazed during the summer months. The basal Cu concentration of R was maintained during summer grazing by the provision of concentrate to supplement the low Cu content of the grass swards (mean = 7.8 mg/kg DM). During the first grazing season, this Cu supplementation took the form of a commercial pelleted concentrate, and a small quantity of wheat was mixed with youngstock vitamin/mineral premix (mineralised wheat) to provide sufficient Cu during the second grazing season (see Table S2). For heifers receiving H, the basal diet was further supplemented with additional Cu at 16 mg/kg DM intake by the provision of two slow-release intraruminal copper oxide (CuO) boluses (Agrimin), giving a total dietary Cu concentration of approximately 32 mg/kg DM intake. Boluses were administered every 6 months to reflect the predicted rate of erosion, and bolus mass was adjusted to reflect changes in predicted animal intake[15,16] throughout the rearing period, with subsequent administration of boluses containing 4.95, 7.64 and 16.55 g of CuO at 4.1, 10.1 and 16.1 months of age, respectively, with a predicted Cu supply of 55, 73 and 160 mg/day. Treatment H was selected to be similar to the mean dietary Cu concentration of 27.9 mg/kg DM reported on UK dairy farms by Sinclair and Atkins.[5]

All heifers were reared by the Harper Adams University Farm under commercial management conditions. Both animals within each pair received the same management and environmental conditions throughout the duration of the study, were kept in the same pen/paddock for the same period of time and received the same basal diet.

Data collection

Fresh feed samples were collected fortnightly, with a subsample being analysed for dietary minerals[17] and the remainder stored at –20°C. All feed samples were then bulked on a 6-monthly basis, and their proximate compositions were determined according to McCaughern et al.[17] (see Table S3). Mean Cu concentrations of 14.6, 35.9, and 151.9 mg/kg DM were observed for the TMR, pelleted concentrate and mineralised wheat, respectively, throughout the study period, producing a predicted mean overall basal dietary Cu concentration of 16.2 mg/kg DM for heifers on R and H. Based on the observed growth rates and predicted intakes, heifers on H received a further 15.6 mg of Cu per kg DM throughout the study period, resulting in a mean overall Cu concentration of 31.8 mg/kg DM.

Liver biopsy samples were collected at 6.9 and 12.4 months of age and 6 weeks prior to predicted calving via the 11th intercostal space, as described by Davies and Jebbett.[18] Biopsy samples were immediately snap frozen in liquid nitrogen and stored at −80°C prior to subsequent analyses at the end of the study. Blood samples were collected via jugular venepuncture at 11am in the week prior to commencing the study, and every 8 weeks thereafter, into vacutainers (Becton Dickson Vacutainer Systems) containing silica gel (to determine glutamate dehydrogenase [GLDH]) and dipotassium EDTA (to determine plasma minerals, and gamma-glutamyl transferase [GGT]). The samples were separated by centrifugation and stored at –20°C until the end of the study, where plasma samples were analysed for GGT (Randox Laboratories; kit

catalogue no. GT553) and serum samples were analysed for GLDH (Randox Laboratories; kit catalogue no. GL441). The analysis of all enzymes in plasma or serum was conducted using a Cobas Miras Plus autoanalyser (ABX Diagnostics). The Cu concentrations of liver and plasma samples were determined by inductively coupled plasma mass spectrometry, performed according to McCaughern et al.[17] A certified EU bovine liver sample (BCR-185) and a certified ClinCheck plasma control sample 2 (product no. 8885) were used to check the accuracy of liver and plasma Cu analyses.

All heifers were weighed and scored for BCS[14] in the week prior to commencing the study, fortnightly throughout the duration of the study and at the start of the breeding period. Oestrus detection was undertaken from 5 months of age by visual observation according to Van Erdenburg et al.,[19] and the breeding period commenced at 13 months of age, where heifers were artificially inseminated 12 hours after the visual observation of oestrus. Pregnancy was diagnosed by transrectal ultrasound, which was conducted between days 32 and 39 postinsemination by the farm's veterinarians. The conception rate was defined as the proportion of heifers inseminated that were diagnosed as pregnant. Heifers that were observed to return to oestrus before the ultrasound scan or were diagnosed as negative were assumed not to have conceived. The breeding period lasted for 260 days, with heifers that failed to conceive during this time frame being removed from the study. All staff undertaking weighing, scoring of body condition or oestrus detection were blinded to the treatment allocation of the heifers.

Statistical analysis

Liveweight, body condition score, hepatic Cu concentration, fertility index and number of inseminations were analysed in a randomised block (temporal) design using a one-way ANOVA. Data relating to plasma Cu concentration and liver function enzymes were analysed using one-way repeated-measures ANOVA in a randomised block (temporal) design. In both instances, pre-experimental variables collected in the week prior to commencing the study were included as covariates when analysing corresponding dependent variables if shown to be significant ($p < 0.05$) in the model. The number of days to first observed oestrus and from the planned start of mating (PSM) to conception were analysed using Kaplan–Meier survival curves. Initially, for days from PSM to conception, liveweight and body condition at PSM were considered in a multivariate parametric model, but these factors were subsequently excluded after Cox proportional hazards and Kaplan–Meier analyses, respectively, were performed to determine associations. Conception rates were analysed using generalised linear mixed model (GLMM) regression analysis with the logit link function. Initially, for conception rates, liveweight and body condition at PSM were considered for inclusion in the final GLMM analysis, but univariate association probabilities of greater than 0.2 following logistic regression and chi-squared analyses, respectively, excluded them from the final model. All the statistical analyses were conducted using Genstat version 22 (VSN International), with a p-value of less than 0.05 considered the threshold for significance.

RESULTS

There was no difference in fertility index between the groups ($p = 0.528$). One animal on R died with suspected pneumonia, another was removed from the study because it was accidentally bolused, and a third was diagnosed with ovarian scarring and removed. One animal was removed from H due to a spinal skeletal defect, and a second was removed as it tested positive for bovine TB. One heifer on treatment R had not conceived by the end of the breeding period and was removed from the study. All of these animals were censored during the fertility analysis according to event occurrence in relation to the start of the breeding period. Of the heifers that were initially included in the study, 36 on treatment R and 38 on treatment H had conceived by the end of the breeding period, respectively. There was no clinical Cu toxicity in heifers in either treatment group.

Copper status and liver function

The highest hepatic Cu concentration was observed at 6.9 months of age for heifers on either treatment, with those on H having a mean concentration 155 mg/kg DM higher ($p = 0.002$) than those on R (798 ± 46.4 mg/kg DM for H vs. 643 ± 35.4 mg/kg DM for R; Table 1). There was subsequently a decrease in hepatic Cu for heifers receiving either treatment at 12.4 months of age, to concentrations of 350 ± 19.7 mg/kg DM for H and 195 ± 13.5 mg/kg DM for R ($p < 0.001$). This was followed by a further decrease in hepatic Cu concentrations before calving, to 293 ± 26.5 mg/kg DM for H and 128 ± 16.3 mg/kg DM for R ($p < 0.001$).

Plasma Cu concentration was unaffected ($p = 0.812$) by dietary treatment, with a mean of 15.5 ± 0.07 µmol/L observed for animals in either group throughout the study. There was an effect ($p < 0.001$) of time on plasma Cu concentration, which fluctuated throughout the duration of the study, but there was no interaction ($p = 0.966$) between treatment and time (Figure 1). In contrast, heifers receiving treatment H had a mean serum GLDH activity that was 30.5 U/L higher ($p = 0.008$) than that of those on treatment R. However, the mean plasma GGT activities were unaffected ($p = 0.066$) by dietary treatment. Time had an effect ($p < 0.001$) on blood GGT and GLDH activity, with GGT increasing at 5.9 months of age in animals receiving R or H before decreasing until the end of the study, while GLDH increased in animals receiv-

TABLE 1 Hepatic and plasma copper concentration, liver function and performance of replacement dairy heifers fed either a recommended (R) or a high (H) dietary copper concentration from 4.1 to 20.7 months of age. Values are presented as mean +/- standard error unless otherwise specified

Explanatory parameter	Dietary copper concentration		*p*-Value
	R	H	
Hepatic Cu concentration			
6.9 months (mg/kg DM)	643 ± 35.4	798 ± 46.4	0.002
12.4 months (mg/kg DM)	195 ± 13.5	350 ± 19.7	<0.001
Pre-calving (mg/kg DM)	128 ± 16.3	293 ± 26.5	<0.001
Plasma Cu concentration[a]			
Plasma Cu (μmol/L)	15.6 ± 0.09	15.3 ± 0.10	0.812
Liver function[a]			
GGT (U/L)	22.8 ± 0.92	27.0 ± 2.20	0.066
GLDH (U/L)	39.0 ± 5.46	69.5 ± 10.54	0.008
Growth performance			
Initial LW (kg)	137 ± 3.7	138 ± 3.3	0.593
Final LW (kg)	527 ± 9.0	537 ± 6.2	0.154
LW change (kg/day)	0.76 ± 0.019	0.79 ± 0.015	0.033
Initial BCS	2.93 ± 0.036	2.89 ± 0.031	0.405
Final BCS	3.02 ± 0.046	3.16 ± 0.048	0.022
BCS score change	0.09 ± 0.051	0.26 ± 0.059	0.025
Fertility			
Study start to first observed oestrus[b] (days)	209 (174–250)	171 (169–180)	0.007
LW at first observed oestrus (kg)	312 ± 9.4	282 ± 8.1	0.012
LW at PSM (kg)	349 ± 6.6	354 ± 5.9	0.412
BCS at PSM	2.78 ± 0.037	2.86 ± 0.037	0.095
Conception rate to first service (%)	54.1 ± 15.92	50.0 ± 16.33	0.730
Conception rate to first and second service (%)	91.2 ± 7.68	73.7 ± 8.05	0.044
Serves per pregnancy	1.5 ± 0.09	2.0 ± 0.23	0.027
PSM to conception[b] (days)	120 (89–136)	110 (81–144)	0.826

Abbreviations: BCS, body condition score; Cu, copper; DM, dry matter; GGT, gamma-glutamyl transferase; GLDH, glutamate dehydrogenase; H, higher; LW, liveweight; PSM, planned start of mating; R, recommended.
[a]Mean of blood samples taken throughout the duration of the study.
[b]Presented as the median and associated 95% confidence interval.

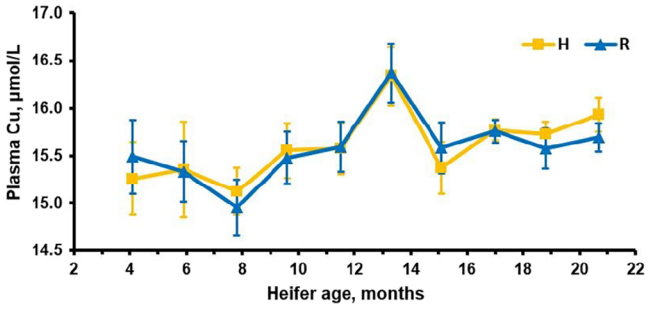

FIGURE 1 Plasma copper concentration of replacement dairy heifers fed either a recommended (R) or a high (H) dietary copper concentration from 4.1 to 20.7 months of age

ing H at 5.9 months of age, before decreasing until the end of the study in animals receiving either treatment (Figure 2a,b). There was also a treatment × time interaction ($p = 0.014$) for serum GLDH, with activities decreasing for heifers on treatment R over the duration of the study, while activities increased at the beginning of the study (4.1–5.9 months of age) for heifers on treatment H with a subsequent decrease thereafter.

Growth performance and fertility

There was no effect of treatment on initial ($p = 0.593$) or final ($p = 0.154$) heifer liveweight, with mean values across treatments of 137 ± 2.4 kg and 532 ± 5.3 kg, respectively (Table 1). In contrast, heifers on treatment H had a 0.03 kg/day higher ($p = 0.033$) growth rate over the duration of the study (0.76 ± 0.019 kg/day for R vs. 0.79 ± 0.015 kg/day for H). Heifers on treatment H also had a BCS that was 0.14 higher by the end of the study ($p = 0.022$; 3.16 ± 0.048 vs. 3.02 ± 0.046) and a 0.17 higher ($p = 0.025$) BCS gain compared to those on R over the duration of the study. In contrast, there was no effect of treatment on heifer liveweight ($p = 0.412$; mean = 352 ± 4.4 kg) and body condition score ($p = 0.095$) at the start of the planned mating period. First oestrus was observed to occur 38 days earlier ($p = 0.007$) for treatment H than for treatment R, with heifers being 30 kg lighter ($p = 0.012$). Conception rate to first service was unaffected ($p = 0.730$) by dietary treatment, with a mean of 50.0 ± 16.33% for H and 54.1 ± 15.92% for R. However, heifers on treatment H

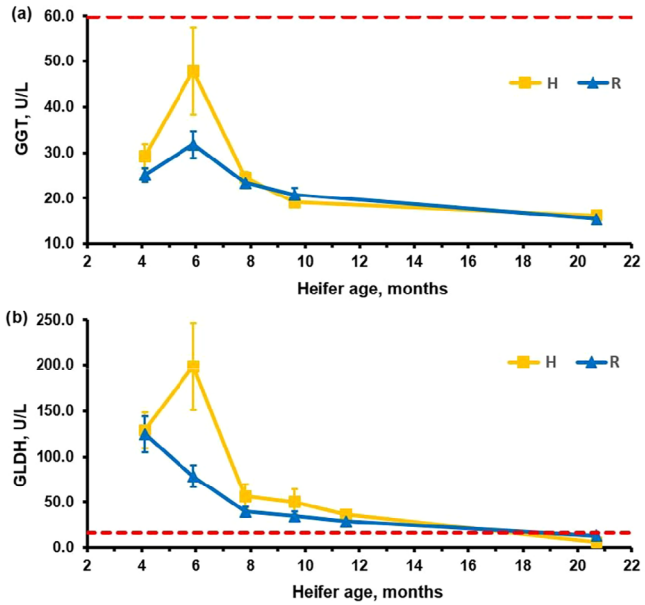

FIGURE 2 Plasma gamma-glutamyl transferase (GGT; a) and serum glutamate dehydrogenase (GLDH; b) of replacement dairy heifers fed either a recommended (R) or a high (H) dietary copper concentration from 4.1 to 20.7 months of age. For GGT, pooled standard error of differences of means = 4.68. Time, $p < 0.001$; copper × time, $p = 0.091$. Upper normal limits (- - -) for GGT and GLDH are presented according to Hunter et al.[23]

had a lower conception rate ($p = 0.044$) to first and second service (73.7 ± 8.05% for H vs. 91.2 ± 7.68% for R). Heifers on H also required 0.5 more ($p = 0.027$) serves per conception compared to those on R; however, there was no effect ($p = 0.826$) of treatment on the interval between the PSM and conception, with a mean of 110 (95% confidence interval [CI] 81–144) and 120 (95% CI 89–136) days for H and R, respectively.

DISCUSSION

This study is the first to determine the long-term implications of feeding excessive dietary Cu on the liver function, performance and fertility of replacement Holstein–Friesian dairy heifers during the rearing period. The liver is generally thought to be the primary organ of Cu storage in cattle, and one of the first biochemical changes to occur when dietary Cu is supplied above the animal's requirements is an increase in hepatic Cu concentration.[1] The ability of Cu supplied in the form of an intraruminal CuO bolus to increase hepatic Cu concentration has been well defined in the literature,[20] so it is unsurprising that heifers on treatment H had higher hepatic Cu concentrations throughout the duration of the current study. However, it was surprising that the highest hepatic Cu concentrations were observed in heifers on either treatment at 6.9 months of age, given the apparent lack of clinical disease. The most likely explanation is that these high liver Cu concentrations were caused by a large proportion of the heifers' intake prior to commencing the study being composed of a commercial pelleted concentrate, which contained a Cu concentration well in excess of the animals' requirements.[6] This high level of supplementation will have been further compounded by the increased absorption of Cu prior to the rumen fully developing.[21] Indeed, Suttle[22] reported a decrease in apparent Cu availability from 74.2% preweaning to 10.8% postweaning in artificially reared lambs. Hepatic Cu concentrations of the magnitude observed at the beginning of this study were well above the 508 mg/kg DM threshold used for the diagnosis of chronic Cu toxicity in cattle,[9] although others have suggested a range of 350–1500 mg/kg DM.[1] Hunter et al.[23] reported clinical signs of ill-thrift, low-grade diarrhoea and eventual death prior to weaning in 28% of Jersey heifer calves in a UK dairy herd that was exposed to excessive levels of Cu supplementation across all stages of production. The hepatic concentrations observed by Hunter et al.[23] were comparable to those observed at 6.9 months of age in the current study, but the lack of obvious clinical signs of toxicity and/or ill-thrift in our study may have been a result of the greater resistance to Cu toxicity in Holstein–Friesian cattle compared to Jersey cattle.[24] It is, however, difficult to draw conclusions on the short- or long-term implications of the initial Cu loading.

Similar to other studies that have examined the effects of dietary Cu concentration on Cu status,[12,20] plasma Cu concentration was unaffected, with a mean value of 15.5 µmol/L for both treatments. This value was well in excess of the 9 µmol/L threshold considered to denote adequacy,[25] but below the 19 µmol/L limit above which animals are hypothesised to be at risk of toxicity.[9] The current findings are consistent with those of other authors who suggest that plasma Cu concentration is a relatively poor indicator of hepatic Cu status[17,26,27] and may only be useful for diagnostic purposes when animals experience either very low or high hepatic concentrations with clinical manifestation of toxicity.[28] The accumulation of Cu at elevated concentrations within the liver can result in damage to hepatocytes with a subsequent release of liver function enzymes into the bloodstream.[29] High hepatic Cu concentrations at 6.9 months of age were associated with elevated serum activities of GLDH, indicating that heifers on both treatments were subjected to considerable liver damage at the beginning of the study when they were fed a standard creep pellet.[23] The discrepancy in treatment GLDH activity profiles between 4.1 and 5.9 months of age may be a function of rumen development and dietary Cu supply, which resulted in an earlier decrease in hepatic Cu status for heifers on R relative to those on H.[22,29] The subsequent decline in enzyme activities for heifers on either treatment by 12.4 months of age corresponded with declining hepatic Cu concentrations during the same timeframe and would appear to signify reduced hepatocellular damage as a result of decreased Cu absorption postweaning.[30] Of the biochemical changes demonstrated during this timeframe, GLDH was the only liver function enzyme that increased above its upper threshold for normality,[23] indicating that it is a more sensitive indicator of hepatopathy in cattle than GGT. Blood GLDH

activity is usually considered an indicator of hepatic damage, while GGT is associated with cholestasis[31]; therefore, it can be concluded that excessive Cu accumulation in the liver is more likely to result in hepatocyte damage than cholestasis. The initial blood results demonstrated that raising calves on a standard commercial diet can lead to liver damage, although further anecdotal evidence suggests that high levels of Cu supplementation to calves is surprisingly common.

The reasons for the increased growth rate of heifers on treatment H in the current study are unclear but may be related to increased feed digestibility and/or intake,[32] although it should be borne in mind that the differences were biologically small. Durand and Kawashima[33] reviewed the effects of dietary Cu concentration on ruminal fermentation both in vitro and in vivo and concluded that Cu supplementation in excess of the animal's requirements can have both positive or negative effects on cellulolysis, with these effects varying depending on the basal diet and inclusion rates of other minerals. The greater increase in BCS over the study period for heifers on treatment H is perhaps not surprising given that Holstein heifers have been shown to increase their ratio of fat deposition to lean tissue formation at increased growth rates.[34] For example, Atkins et al.[35] reported a BCS increase in replacement heifers of 0.08 units over a 3-month period when grown at 1.24 kg/day, compared to a BCS decrease of −0.06 when grown at 0.95 kg/day. However, there may be other factors contributing to the difference in BCS observed in the current study,[36,37] including the elevated hepatic Cu concentration of heifers on treatment H.[38,39]

It is generally hypothesised that the onset of puberty in cattle is closely related to body development or liveweight rather than chronological age.[40] Therefore, it was somewhat surprising that the first observed oestrus for heifers on treatment H not only occurred earlier but also at a reduced liveweight relative to those on R. It has been identified that insulin-like growth factor-1 (IGF-1) may serve to promote early follicular development,[41] and as such, its administration has been associated with the earlier onset of puberty.[42] This may also partially explain the decreased age and liveweight at first observed oestrus for heifers on treatment H, as higher hepatic Cu concentrations may have increased circulating IGF-1 concentrations, thereby removing oestradiol inhibition.[39] An increased hepatic secretion of IGF-1[39] may also have decreased circulating growth hormone (GH) concentrations and subsequently increased the subcutaneous adipose tissue deposition observed in heifers receiving H.[38,43]

This study is not the first to identify decreased conception rates as a result of Cu supplementation. Hawkins[44] reported a decrease in 21-day pregnancy rates (47% vs. 43% for control and treatment cows, respectively) when New Zealand Friesian × Jersey cows received 200 mg of Cu as a Ca Cu EDTA injection 10 days before their PSM, although the basal dietary Cu concentration and/or indicators of Cu status were not monitored. Starbuck et al.[45] also observed that the treatment of Holstein and Ayrshire dairy cows with a single dose of GH increased conception rates from 25.8% to 64.3% for animals in excess of 100 days in milk. It is thought that GH increases progesterone concentrations in dairy cattle after insemination, with a subsequent increase in the chances of successful implantation and pregnancy.[39,46] If circulating IGF-1 concentrations were increased due to higher hepatic Cu concentrations in heifers on treatment H,[39] the concurrent reduction in GH may have resulted in decreased progesterone concentrations and lower conception rates for these animals.[38,46] Neither circulating IGF-1, GH nor plasma progesterone concentrations were monitored in the current study, but they warrant further investigation.

Limitations

Retrospective analysis of serum GLDH identified liver damage when the animals commenced the study, and it would have been useful to determine the liver Cu concentration at this point. This elevation in serum GLDH came as a surprise to the authors as all heifers were reared in a standard commercial manner and did not demonstrate any ill-thrift or clinical signs of toxicity. As all heifers were subjected to this initial Cu loading, it is not possible to determine if the results would have differed if the pre-study Cu intake was lower and serum GLDH and liver Cu concentrations were initially within the normal range. In light of fertility parameter differences, it would have been useful to have paired the animals specifically on their predicted transmitting ability for fertility index, although there was no significant difference between treatment groups when analysed retrospectively. Additionally, genomic testing could have been employed to improve the reliability of allocation.

CONCLUSIONS

Concentrations of Cu that are commonly fed on UK farms during the early life of cattle were associated with excessive hepatic Cu accumulation in ranges used for the diagnosis of chronic Cu toxicity, along with concurrent evidence of liver damage in Holstein–Friesian heifer calves at 6.9 months of age. Feeding a Cu concentration that was above requirements throughout the rearing period resulted in a higher hepatic Cu concentration and a marginally increased growth rate and BCS. Overfeeding Cu also resulted in an earlier onset of first observed oestrus, but the conception rate to first and second services was significantly reduced. The reasons for the reduction in conception rate when heifers are fed a dietary Cu concentration above their requirements throughout the rearing period are not clear and warrant further investigation, but these findings highlight the need

to consider dietary Cu supply in relation to animal requirements.

AUTHOR CONTRIBUTIONS

Study conception, design and planning were undertaken by Liam Sinclair, James McCaughern and Alexander Mackenzie. Analysis of the data was undertaken by James McCaughern and Emma Bleach. All of the authors participated in the discussions and subsequent revisions surrounding the manuscript. The final manuscript was approved by all authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All the procedures that involved animals received local ethical approval (103-201512) and were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 (amended 2012; PPL 70/7636).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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