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Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Zinc, Copper, and Manganese Homeostasis and Potential Trace Metal Accumulation in Dairy Cows: Longitudinal Study from Late Lactation to Subsequent Mid-Lactation

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A B S T R A C T

Background: Trace metals are supplemented in cattle to prevent nutrient deficiencies. Levels supplemented to mitigate worst-case basal supply and availability scenarios can, however, result in trace metal intakes far above the nutritional requirements of dairy cows with high feed intakes.

Objectives: We evaluated Zn, Mn, and Cu balance in dairy cows from late lactation through the subsequent mid-lactation, a period of 24 wk characterized by large changes in dry matter intake.

Methods: Twelve Holstein dairy cows were housed in a tie-stall from 10 wk before to 16 wk after parturition and fed 1 unique lactation diet when lactating and a dry cow diet otherwise. After 2 wk of adaptation to the facility and diet, Zn, Mn, and Cu balances were determined at weekly intervals, by calculating the difference between total intakes and complete fecal, urinary, and milk outputs, with the latter 3 fluxes quantified over a 48-h period. Repeated measure mixed models were used to evaluate the effects on trace mineral balances over time.

Results: The Mn and Cu balances of cows were not significantly different from 0 mg/d between 8 wk prepartum and calving ($P \geq 0.54$), when dietary intake was the lowest of the period evaluated. However, when dietary intake was highest, between wk 6 and 16 postpartum, positive Mn and Cu balances were observed (80 and 20 mg/d, respectively, $P \leq 0.05$). Cows were in positive Zn balance throughout the study except during the first 3 wk after calving during which the Zn balance was negative.

Conclusions: Large adaptations occur in trace metal homeostasis in transition cows in response to changes in dietary intake. High dry matter intakes, associated with high milk production of dairy cows, combined with current Zn, Mn, and Cu supplementation practices may exceed regulatory homeostatic mechanisms resulting in potential body accumulation of Zn, Mn, and Cu.

Keywords: health, environment, accumulation, regulation, dietary supplementation

Introduction

Trace nutrients such as Zn, Cu, and Mn are essential for fundamental cellular metabolic processes. Deficiency negatively impacts the health and productivity of farm animals [1,2], but excess supply can also be detrimental to biological functions [3]. To prevent both deficiency and toxicity, animals have evolved highly regulated homeostatic mechanisms for Zn, Cu, and Mn. These mechanisms have been elegantly illustrated with radioisotope studies using rodents for Zn [4], Mn [5], and Cu [6]. In these studies, homeostasis was shown to be mainly governed by 2 key processes, the first being secretion into the gastrointestinal tract from endogenous sources via the pancreas, bile, and mucosa, and the second being regulated absorption of minerals from dietary and endogenous origins. The difference between these 2 opposite fluxes results in a positive or negative net balance of these trace metals, which usually leads to homeostasis. In this way, when dietary intake increases, endogenous secretion increases and fractional absorption decreases, as demonstrated in rodents and humans [4–7]. These concerted regulatory processes

Abbreviations: BW, body weight; DM, dry matter; ICP-MS, inductively coupled plasma mass spectrometry; TMR, total mixed ration.

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allow the maintenance of a net transfer of metal into the body to match net requirements over a broad range of dietary intakes.

In ruminants, data illustrating these regulatory mechanisms for Zn, Cu, and Mn are scarce, and it is often assumed that those regulatory processes are analogous to those in other species. The few studies investigating the relationship between Zn intake and fractional apparent Zn absorption in dairy cows appear to confirm observations from rodent studies [8–10]. In primiparous cows fed a diet containing Zn at 16.6 mg/kg dry matter (DM) versus 39.5 mg/kg DM for 6 wk, fractional apparent Zn absorption increased from 30% to 45% and resulted in minimal impact on whole-body Zn analyzed in the carcass, although milk Zn excretion was reduced, in relative terms, by 45% [8]. In contrast to rodents, positive relationships were reported between intake of Mn and Cu and their respective fractional apparent absorption in cattle [11,12]. Our insufficient understanding of how Zn, Mn, and Cu are regulated in cattle, as well as attempts to mitigate any possibility of deficiency, have resulted in generous supplementation of these trace metals in modern dairy production systems. Studies demonstrating that supplemental Mo, S, and/or Fe negatively affect Cu availability in ruminants have further supported these supplemental practices [13–17], despite the limited amount of well-documented reports of trace metal shortages in intensively fed dairy cattle. However, there is a growing concern about current trace metal supplementation practices. These concerns are particularly motivated by reports of high hepatic Cu content in culled cattle [18,19], suggesting that supplemental Cu levels were too high [20]. Excessive supplementation of Zn, Mn, and Cu is a concern because it leads to higher excretion of these elements in the environment [21], but also because it could negatively impact animal health [3]. In addition, the European Chemical Society listed Cu and Mn deposits worldwide as under risk for future supply shortage and Zn deposits even being under threat of supply shortage within the next 100 y [22].

Data evaluating how cattle cope with the fluctuations in Zn, Mn, and Cu intake along the lactation cycle are warranted to better substantiate and eventually revise feeding recommendations. The objective of our study was to quantify Zn, Mn, and Cu balance in the transitions from late lactation through the dry period, and to the subsequent lactation in dairy cows. This is a period characterized by changes in nutrient outputs linked to gestation and lactation, as well as by large changes in DM intake and dietary composition, and consequently Zn, Mn, and Cu intakes.

Methods

This paper focuses on quantitative Zn, Mn, and Cu homeostasis throughout lactation and the dry period in dairy cows. Other results from this experiment about DM intake, milk performance, change in body mass, digestibility of major feed components, and nitrogen metabolism can be found in Daniel et al. [23].

The study was conducted between September 2019 and April 2020 at the Dairy Research Facility of Trouw Nutrition Research & Development (Kempenshof, Boxmeer, the Netherlands). All experimental procedures were approved by the Central Authority for Scientific Procedures on Animals and conducted under the Dutch Act on Animal Experiments, which complies with the European Directive 2010/63/EU.

Animal management

Twelve Holstein Friesian dairy cows were used in this study. At the start of the study, 6 cows were primiparous and 6 cows were multiparous (i.e., 2 cows in lactation 2, 2 cows in lactation 3, 1 cow in lactation 4, and 1 cow in lactation 5). Mean body weight (BW) was 723 (± SD 66) kg, and mean milk production of the ongoing lactation (305 d) was 10,330 (± 1940) kg, with a mean milk fat of 4.6% (± 0.4%) and mean milk protein of 3.5% (± 0.2%). Further details on the cows and management can be found in Daniel et al. [23]. Approximately 10 wk before the expected calving date, cows were moved from a free-stall barn to a tie-stall barn with individual feed troughs and free access to water. The first 2 wk in the tie-stall facility were considered as an adaptation period to the diet and housing. Cows remained in the tie-stall barn until the study ended, 16 wk after calving. Cows were dried-off 6 wk prior to the expected calving date, resulting in a median dry period length of 43 d (range of 35 to 54 d).

Diets and feeding

In this study, no dietary treatments were evaluated. Instead, the aim was to quantify Zn, Mn, and Cu balance throughout lactation and the dry period in dairy cows fed the same diets. For that purpose, 2 diets were formulated and were fed as a total mixed ration (TMR). One TMR was for the dry period and 1 TMR for the lactation period. The compositions of the TMR are shown in Supplemental Table 1. The respective TMRs were offered ad libitum during the dry and lactation periods. Orts were removed daily between 09:30 and 10:00 and subtracted from the TMR offered the previous day to calculate daily TMR intake. Immediately after removal of the orts, the fresh TMR was weighed and allocated into the individual feed troughs. Analyzed chemical composition of forages and mash concentrates and calculated compositions of the TMRs are shown in Supplemental Table 2. The dry cow TMR provided 61, 37, and 11 mg/kg DM of Zn, Mn, and Cu, respectively. These levels correspond to 244%, 142%, and 76% of the calculated requirement for Zn, Mn, and Cu, respectively, for a cow of 700 kg eating 14 kg/d of DM [24]. In lactation, the TMR provided 132, 90, and 23 mg/kg DM of Zn, Mn, and Cu, respectively. For a cow of 700 kg producing 40 kg/d of milk and eating 24 kg/d of DM, these levels correspond to 225%, 346%, and 229% of the calculated requirement for Zn, Mn, and Cu, respectively [24].

Sampling of feces and urine

Feces and urine were quantitatively collected over a 48-h period each week, from 8 wk before the expected calving date until 16 wk after calving. Each collection period started at 10:00 on Monday and ended at 10:00 on Wednesday, coinciding with feeding. Full details are given in Daniel et al. [23].

Sampling of feed, milk, and serum

Feedstuffs were sampled every other week while fed. Cows were milked in place twice daily at 06:00 and 17:00, and milk yield was recorded at each milking. Milk samples were collected on Monday and Tuesday evenings and Tuesday and Wednesday mornings. Blood samples were collected from the coccygeal vein every Monday at 12:00. Further details on feed, milk, and serum sample processing are given in Daniel et al. [23].

Sampling of liver tissue

Liver biopsy was collected 4 times for each cow: 9 d before dry-off, 6 d after dry-off, between 5 and 10 d before calving, and between 10 and 20 d after calving. Biopsies were performed on the Wednesday afternoon following the 48-h collection period of urine and feces so that the biopsy procedure would have minimal impact on the next collection period. Briefly, the cow was sedated by intramuscular administration of 0.5 mL of a solution containing 20 mg/mL of xylazine (Sedazine, Produlab Pharma B.V.). The surgical site, previously shaved, was thoroughly cleansed with 3 alternating rounds of povidone-iodine scrubbing followed by a 70% isopropyl alcohol rinse. Upon disinfecting the surgical site, 6 mL of a solution containing 20 mg/mL of procaine hydrochloride (Procamidor, Richter Pharma AG) was administered to the subcutaneous space and intercostal muscles. The surgical site was disinfected again, as described previously. After a 2-cm incision of the skin, a stainless-steel hepatic biopsy tool was introduced. The site of the incision was between the 10th and 11th ribs at the intersection of the line made from the right hip down to the point of the elbow of the front right leg of the animal. Approximately 1 g of liver tissue was collected, and the incision site was closed using staples, which were removed at least 10 d later. The tissue was placed in a Petri dish, rinsed with sterile saline, sliced into 3 sections, transferred into 3 separate cryovials, snap-frozen in liquid N_2, and stored at −80°C until analyses. After the procedure, 3 mL per 100 kg BW of a nonsteroidal anti-inflammatory drug containing 100 mg/mL of ketoprofen (Ceva Santé Animale B.V.) was administered intramuscularly.

Chemical analysis

Methods of analysis of feces samples for DM, and feed samples for DM, gross energy, crude ash, crude fat, neutral detergent fiber, acid detergent fiber, acid detergent lignin, starch, sugar, and minerals are given in Daniel et al. [23]. A dry aliquot of each feed and feces sample was analyzed for moisture and trace element concentrations at the University of Nottingham (NUVetNA laboratory). Samples were dried at 70°C until no further weight loss. Approximately 0.1 to 0.2 g of dry material was weighed into a high-pressure digestion vessel (HVT50, Anton Paar) and to this 3 mL 68% nitric acid (Primar plus, Fisher Scientific), 2 2 mL 30% hydrogen peroxide (Analar, VWR Ltd), and 3 mL deionized water (17 MΩ, Purite HP 160, Suez) was added before being run on a digest microwave (Multiwavepro, Anton Parr) with a 10-min ramp to 140°C, 20 min hold at 140°C, and subsequent cooling to 55°C. Digested contents were transferred to a 25-mL universal tube (Sarstedt) with 7 mL deionized water. Blanks and appropriate standards/certified reference material were included with each batch run. Sera and acidified urine were diluted 0.5 mL in 10 mL with a diluent containing 0.5% HNO_3, 4% methanol, and internal standards: Sc (25 μg/L), Ge (10 μg/L), Rh (5 μg/L), and Ir (2.5 μg/L) into 14 mL (105 mm × 16.8 mm). Trace element concentrations in samples were determined with inductively coupled plasma mass spectrometry (ICP-MS) (Thermo-Fisher iCAP-Q, Thermo-Fisher Scientific) with a 'Flatopole collision cell' (charged with helium gas for all elements except selenium where it is charged with hydrogen – changes within sample) upstream of the analytical quadrupole to reduce polyatomic interferences. External calibration standards were all in the range of 0–100 μg/L for trace elements and 0–100 mg/L for macroelements. Samples were introduced on a covered autosampler (Cetac ASX-520) through a 1317090 PFA-ST nebulizer (ESI, Thermo-Fisher Scientific). Sample processing was undertaken using Qtegra software (Thermo-Fisher Scientific).

Milk and liver samples were analyzed at the veterinary laboratory of Royal GD. Trace element concentrations in samples were determined with ICP-MS using an Agilent ICP-MS 7700x (Agilent Technologies Netherlands B.V.). The quantification was performed after calibration with external standards for Cu, Mn, and Zn (Inorganic Ventures). Milk samples were diluted with a proprietary diluent, and internal standards germanium and scandium (NIST traceable, Inorganic Ventures) were added prior to analysis. Liver samples were digested with 65% nitric acid using a microwave oven at peak temperature 200°C, maximum pressure 40 MPa, 4 min ramp time, 5 min hold time, and medium stirring (Discover SP Microwave Synthesizer, CEM Microwave Technologies). Samples were analyzed after the addition of internal standards germanium and scandium (NIST traceable, Inorganic Ventures) and dilution with water to a volume of 25 mL. Liver results were reported on a DM basis, assuming a water fraction of 73% based on the mean DM content of liver samples reported in the study by Counotte et al. [19].

Calculations

Variability in gestation length meant that the dry period duration ranged from 35–54 d. To present the data on the same figures, data near dry-off were adjusted. Wk −8, −7, −6, and −5 from calving, as shown in all reported figures, correspond to the actual wk −2, −1, +1, and +2 from dry-off, while wk −4 to 16 corresponded to the actual weeks from calving.

Apparent absorption of Zn, Mn, and Cu was calculated as the difference between mean daily intake, measured over 7 d, and mean daily fecal output of each trace metal measured over each 48-h period and expressed relative to mean daily intake (%). The balance of Zn, Mn, and Cu were estimated by the difference between mean daily intake, measured over 7 d, and mean daily outputs (in feces, milk, and urine) of each trace metal measured over each 48-h period.

Statistical analysis

Weekly cow data were analyzed with PROC MIXED of SAS 9.4 (SAS Institute Inc) according to the following model:

$$Y_{ij} = \mu + W_i(P_j) + P_k + \varepsilon_{ij}, \quad [1]$$

in which Y_{ij} is the dependent variable, μ is the overall mean, and ε_{ij} is the random residual. The model included the fixed effect of week relative to calving (W_i, i ∈ [-8; 16]) nested in periods (P_j, i ∈ [1; 5]) and the fixed effect of P_j. The cow was considered as the experimental unit, and W was included as a repeated statement using the autoregressive (1) covariance matrix. The 5 periods corresponded to the late lactation period (wk −8 and −7), the dry period (wk −6 to −1), and 3 periods of early lactation (wk 1 to 5, wk 6 to 10, and wk 11 to 16). For weekly least square means of Zn, Mn, and Cu balance, statistical differences from 0 are also reported in this manuscript. Weekly mean, median, IQR, and outliers are illustrated in all figures. For hepatic Zn, Mn, and Cu concentrations, the 4 measurements per

cow (−9 d before dry-off, 6 d after dry-off, 10 to 5 d before calving, and 10 to 20 d after calving) were used as fixed effects instead of wk relative to calving. Residuals for urinary Zn, Mn, and Cu excretions were not normally distributed. Thus, data were log transformed to comply with the model assumption.

Results

Intake and output of Zn, Mn, and Cu

Weekly dietary intake and fecal outputs of Zn, Mn, and Cu are presented in Figures 1, 2, and 3. Over the 24 wk studied, large fluctuations in intakes of Zn, Mn, and Cu were observed, mainly due to large changes in intake and to supplemental levels being lower during the dry period as compared to the lactation periods. In the last 2 wk of lactation, intake of Zn, Mn, and Cu were 2560, 1750, and 439 mg/d, respectively. During the dry period, intakes of Zn, Mn, and Cu dropped to 833, 505, and 146 mg/d, respectively, representing approximately a 70% decrease for all 3 metals. From wk 11 to 16 after calving, when DM intake was highest (24.4 kg/d, Table 1), intakes of Zn, Mn, and Cu were 3220, 2190, and 551 mg/d, respectively, about 4 times higher than in the dry period. Feces were by far the dominant output route for Zn, Mn, and Cu. Across the study (excluding wk −6 and wk 1), fecal outputs of Zn, Mn, and Cu equated to 91.3%, 99.4%, and 98.6% of dietary intake of Zn, Mn, and Cu, respectively. Urinary excretions (Figure 4) and milk outputs (Supplemental Figures 1, 2, and 3) were very small in comparison to intakes. Milk Zn outputs varied between 3.7% and 10.6% of dietary Zn intake, but milk outputs of Mn and Cu were below 0.07% and 1.30%, respectively. Urinary excretions of Zn, Mn, and Cu were all below 0.2% of the dietary intake. Although low, changes in urinary Zn and Mn excretions did occur (Figure 4). During the dry period, urinary Cu excretion was 0.44 mg/d but increased to 0.71 mg/d at wk 11 to 16 of lactation ($P < 0.01$). For Mn, urinary excretion was 0.02 mg/d during the dry period but increased 4- to 6-fold during lactation (i.e., from 0.06 to 0.12 mg/d, $P < 0.01$). For Zn, urinary excretions were higher between wk −8 to 5 compared to wk 6 to 16 with a peak, numerically highest, at wk 2 after calving (median = 3.00 mg/d; IQR = 1.95–5.61, Figure 4). Concentrations and daily export of Zn, Mn, and Cu in milk are shown in Supplemental Figures 1, 2, and 3, respectively. Within the period investigated, milk Zn concentration was highest in the first week after calving (median = 7.9 mg/L; IQR = 6.3–8.7). For milk Mn and Cu, the concentrations were lowest and highest, respectively, in early lactation (Supplemental Figures 2 and 3). After calving milk Mn concentration increased ($P < 0.01$) between wk 1 and 16 from 7 (IQR = 5–9) to 32 (IQR = 25-37) μg/L, whereas milk Cu concentration decreased ($P < 0.01$) from 122 (IQR = 79–156) to 74 (IQR = 60–88) μg/L. Milk Zn, Mn, and Cu concentrations at the end of previous lactation (last 2 wk) were similar to values reported in wk 11 to 16 after calving (Supplemental Figures 1–3, Table 1, $P \geq 0.57$).

Zn, Mn, and Cu balance

Zn, Mn, and Cu balance are presented in Figures 1, 2, and 3, respectively, as well as in Table 1. The first week after the introduction of a new diet (wk −6 and wk 1), marked effects were observed in Zn, Mn, and Cu balance. The decrease in intake of Zn, Mn and Cu at dry-off resulted in a large decrease in Zn, Mn, and Cu balance (7.2-, 13.4- and 74.6-fold decreased compared to the last 2 wk of lactation). These negative balances were, however, short-lived as in wk −5, Zn, Mn, and Cu balance returned to similar values as observed in the last 2 wk of lactation (Figure 1, 2, and 3). In addition to the potential release of metals from the body, these negative metal balances could be explained by a bias in the calculation, since the new diet was only introduced 72 h prior to the start of the 48-h collection period, and the dietary trace metal concentrations used may not be representative. Apart from these 2 transition weeks, Cu and Mn balances were relatively constant from wk −8 to wk 3, and not different from 0 mg/d ($P > 0.33$), with balances varying between −12.5 and 8.3 mg/d for Cu (median, Figure 3), and between −33.4 and 35.4 mg/d for Mn (median, Figure 2). From 4 wk after calving, Cu and Mn balance increased substantially, with several weeks having Cu balance (6 out of 13 wk, $P < 0.05$) and Mn balance (8 out of 13 wk, $P < 0.05$) greater than 0 mg/d. For Zn, positive balances were observed during the dry period, when Zn intake was the lowest of all the periods investigated (Table 1). In wk 2 and 3 after calving, the Zn

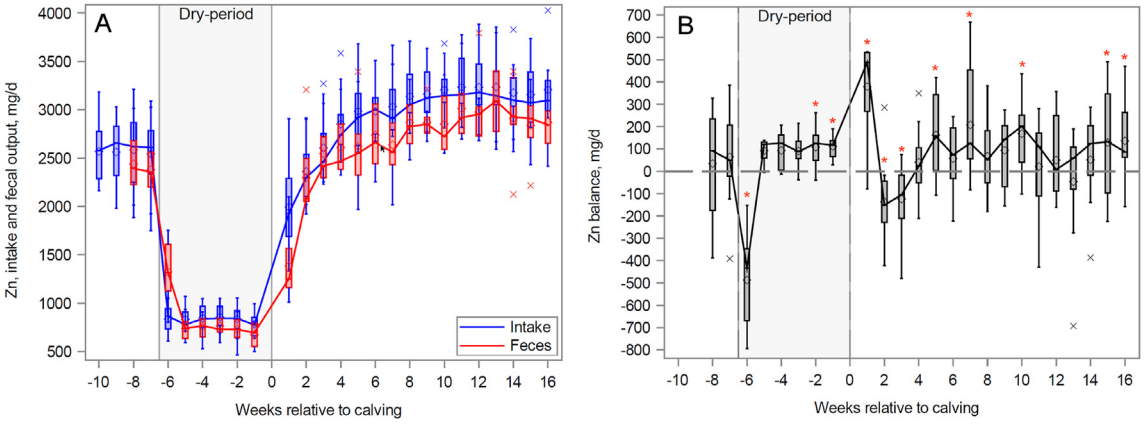

FIGURE 1. Zn intake and Zn output in feces (A), and Zn balance (B), relative to calving in dairy cows (from wk −10 to wk 4: $n = 12$, for wk > 4: $n = 11$). Boxes represent the interquartile range, diamonds represent mean, and whiskers include all values within ± 1.5 × interquartile range. Values represented with an × are outside that range. Lines connect boxes at their median. * Weekly mean differs from 0, $P < 0.05$.

TABLE 1
Zn, Mn, and Cu fluxes and balance relative to calving in dairy cows[1]

	Late lactation	Dry period	Early lactation			SEM
	Wk −8 to −7	Wk −6 to −1	Wk 1 to 5	Wk 6 to 10	Wk 11 to 16	
Dry matter intake, kg/d	19.4^{b}	13.7^{c}	19.4^{b}	23.5^{a}	24.4^{a}	0.66
Intake, mg/d						
Zn	2560^{b}	833^{c}	2560^{b}	3110^{a}	3220^{a}	83.1
Mn	1750^{b}	505^{c}	1740^{b}	2120^{a}	2190^{a}	56.3
Cu	439^{b}	146^{c}	438^{b}	532^{a}	551^{a}	14.2
Fecal output[2], mg/d						
Zn	2410^{b}	739^{c}	2510^{b}	2790^{a}	2930^{a}	68.2
Mn	1730^{b}	522^{c}	1780^{b}	2010^{a}	2120^{a}	46.9
Cu	440^{b}	147^{c}	452^{b}	502^{a}	530^{a}	12.5
Apparent absorption[2], %						
Zn	5.5^{b}	12.0^{a}	6.5^{b}	10.1^{ab}	8.3^{ab}	1.19
Mn	0.3^{ab}	-2.4^{b}	2.9^{a}	5.1^{a}	2.5^{a}	1.32
Cu	−0.7^{b}	0.1^{b}	2.0^{ab}	5.6^{a}	3.2^{ab}	1.37
Urinary excretion[3], mg/d						
Zn	1.69^{a}	1.44^{a}	1.94^{a}	0.90^{b}	0.86^{b}	0.092
Mn	0.12^{a}	0.02^{b}	0.06^{a}	0.09^{a}	0.08^{a}	0.168
Cu	0.45^{b}	0.44^{b}	0.55^{ab}	0.54^{ab}	0.71^{a}	0.084
Milk output						
Zn, mg/d	103^{b}	-	199^{a}	194^{a}	200^{a}	9.9
Mn, µg/d	668^{b}	-	433^{b}	1090^{a}	1270^{a}	65.8
Cu, µg/d	1440^{c}	-	4200^{a}	3450^{b}	3140^{b}	259.2
Milk concentration						
Zn, mg/L	4.76^{ab}	-	5.38^{a}	4.36^{b}	4.75^{ab}	0.222
Mn, µg/L	32.5^{a}	-	10.8^{c}	24.1^{b}	29.9^{a}	1.78
Cu, µg/L	70.2^{c}	-	112.4^{a}	78.2^{b}	74.0^{bc}	7.18
Balance[2], mg/d						
Zn	49.4^{ab}	98.0^{a}	-14.7^{b}	119.7^{a}	60.9^{ab}	30.22
Mn	4.5^{b}	−11.6^{b}	53.0^{ab}	106.5^{a}	54.1^{ab}	20.61
Cu	−3.1^{b}	−0.6^{b}	5.0^{b}	25.3^{a}	13.9^{ab}	5.34
Serum concentration						
Zn, µmol/L	18.2^{bc}	19.6^{a}	17.1^{c}	17.9^{bc}	18.8^{ab}	0.33
Mn, nmol/L	44.3^{b}	42.7^{b}	44.5^{b}	49.5^{a}	50.4^{a}	1.18
Cu, µmol/L	11.6^{c}	12.4^{b}	15.0^{a}	13.9^{ab}	12.7^{bc}	0.54

[1] Twelve Holstein dairy cows were housed in a tie-stall from 10 wk before to 16 wk after parturition and fed 1 unique lactation diet when lactating (i.e., late lactation and early lactation) and 1 unique dry cow diet otherwise. [a,b,c] Labeled means in a row without a common letter differ ($P < 0.05$).
[2] Data from wk −6 and wk 1 were not used because fecal excretion was still influenced by a previous diet.
[3] Values were log transformed prior to analysis, and the results were back transformed. SEMs are expressed as log.

balance was negative ($P < 0.05$) with medians of −152.2 and −105.3 mg/d, respectively. In a manner similar to Mn and Cu, an increase in Zn balance was observed between wk 2 and 5 (Figure 1, wk 2 versus wk 5 with $P = 0.01$).

Fractional apparent absorption of Zn, Mn, and Cu

The mean fractional apparent Mn absorption was lowest during the dry period as compared to periods in early lactation ($P < 0.05$, Table 1, Supplemental Figure 4). In a similar fashion, apparent fractional Cu absorption increased with dietary Cu supply, from a mean of -0.7% during late lactation to 5.6% during wk 6 to 10 ($P < 0.05$, Table 1, Supplemental Figure 4). For Zn, fractional apparent absorption (Supplemental Figure 4) was highest during the dry period compared to late lactation and the first 5 wk of early lactation ($P < 0.01$), whereas it was intermediate between wk 6 and 16. Overall, fractional apparent Zn absorption was positively correlated with Zn intake during lactation ($P < 0.01$).

Serum Zn, Mn, and Cu concentrations

Serum concentrations of Zn, Mn, and Cu are shown in Figure 5 and mean per period in Table 1. The highest serum Zn concentration was observed during the dry period and during the last 5 wk of the early lactation period ($P < 0.01$). In lactation, serum Zn was the lowest the first 5 wk after calving and increased linearly ($P < 0.01$) until the end of the study, to a level similar to previous late lactation ($P = 0.75$). In contrast, serum Cu was the highest after parturition and decreased linearly thereafter ($P < 0.01$) to a concentration not significantly different from late lactation ($P = 0.52$, Figure 5). Compared with serum Cu and Zn concentrations, serum Mn concentrations were much lower (from 42.7 to 50.4 nmol/L). Higher concentrations (12%–17% higher) were observed in wk 6–16 compared to the rest of the study ($P < 0.01$, Table 1, Figure 5).

Hepatic Zn, Mn, and Cu concentrations

Hepatic Zn, Mn, and Cu concentrations are shown in Supplemental Figure 5. The concentration of Zn was 40% higher after calving than at the end of the previous lactation ($P = 0.03$), whereas concentrations during the dry period were intermediate. Greater variability between animals was observed after calving. For Cu, the hepatic concentration decreased linearly from 9 d before dry-off to 10–20 d after calving ($P = 0.03$). Hepatic Mn concentration was not significantly affected within the period studied ($P = 0.13$).

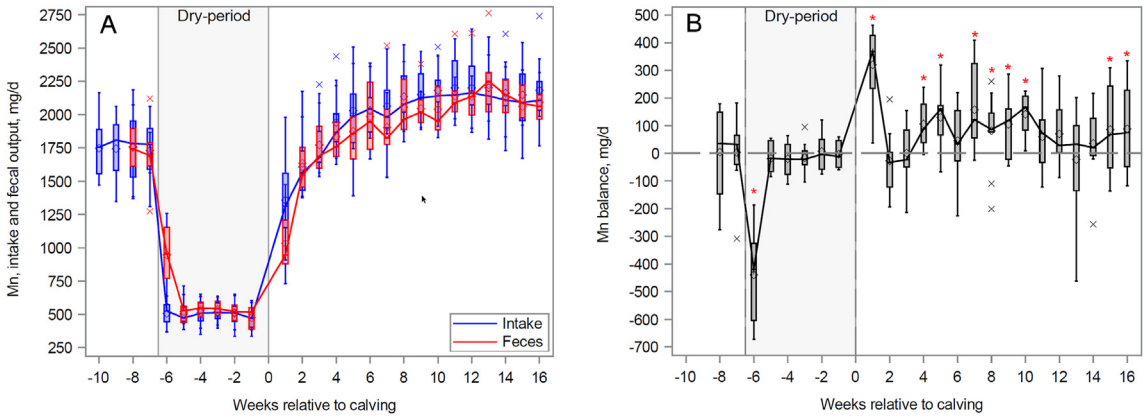

FIGURE 2. Mn intake and Mn output in feces (A), and Mn balance (B), relative to calving in dairy cows (from wk −10 to wk 4: $n = 12$, for wk > 4: $n = 11$). Boxes represent the interquartile range, diamonds represent mean, and whiskers include all values within ± 1.5 × interquartile range. Values represented with an × are outside that range. Lines connect boxes at their median. * Weekly mean differs from 0, $P < 0.05$.

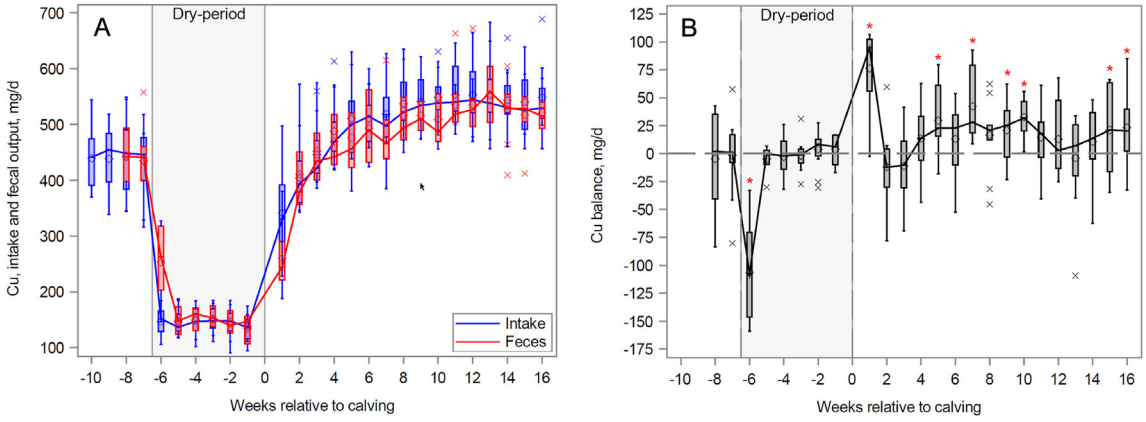

FIGURE 3. Cu intake and Cu output in feces (A), and Cu balance (B), relative to calving in dairy cows (from wk −10 to wk 4: $n = 12$, for wk > 4: $n = 11$). Boxes represent the interquartile range, diamonds represent mean, and whiskers include all values within ± 1.5 × interquartile range. Values represented with an × are outside that range. Lines connect boxes at their median. * Weekly mean differs from 0, $P < 0.05$.

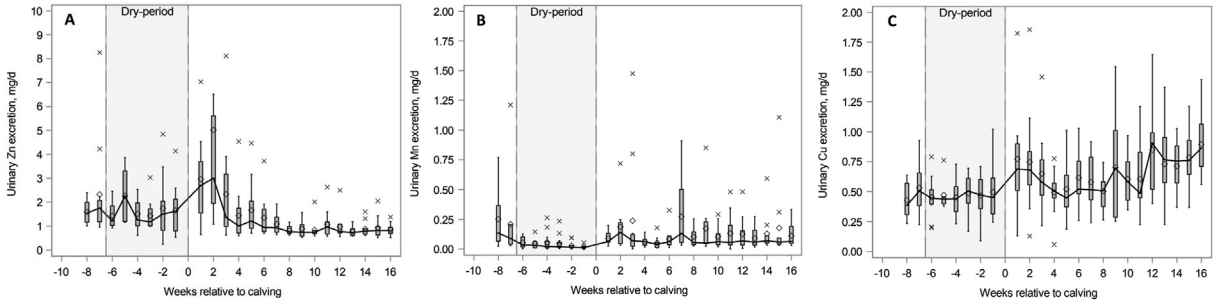

FIGURE 4. Urinary Zn (A), Mn (B), and Cu (C) excretions, relative to calving in dairy cows (from wk −10 to wk 4: $n = 12$, for wk > 4: $n = 11$). Boxes represent the interquartile range, diamonds represent mean, and whiskers include all values within ± 1.5 × interquartile range. Values represented with an × are outside that range. Lines connect boxes at their median.

Discussion

Intakes of Zn, Mn, and Cu were influenced by the large fluctuations of DM intake through the production cycle (13.7 kg/d in the dry period versus 24.4 kg/d at wk 11 to 16) but also by the supplementation strategy applied. In the lactation period, 57%, 60%, and 63% of the total dietary intakes of Zn, Mn, and Cu, respectively, were from the supplements. During the dry period, these fractions were smaller, with 34%, 28%, and 38%, for Zn, Mn, and Cu, respectively. A lower level of supplementation during the dry period is not an uncommon practice in dairy cattle nutrition [25,26], as it is considered that in the absence of lactation requirements, as well as milder physiological challenges in this period, lower supply may be justified.

It is however known by factorial prediction of the net requirements of Zn, Mn, and Cu [24] across the study period that

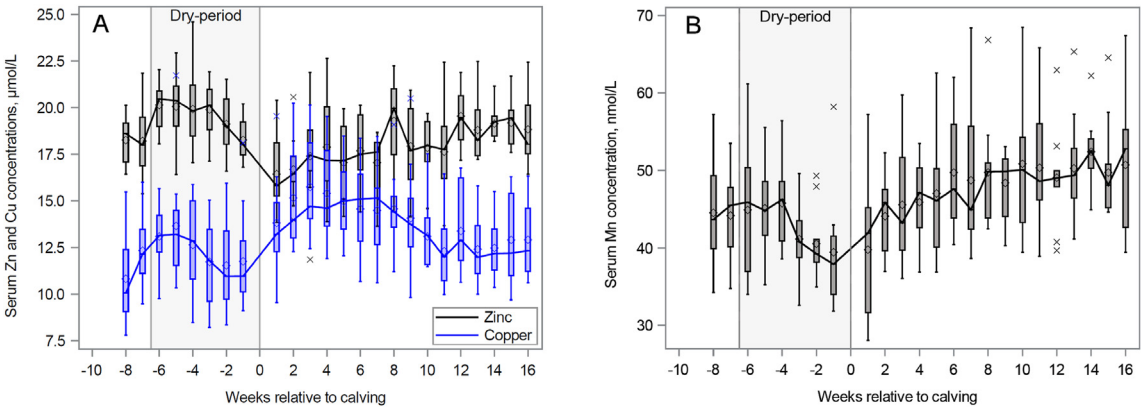

FIGURE 5. Serum Zn, Cu (A), and Mn (B) concentrations, relative to calving in dairy cows (from wk −10 to wk 4: $n = 12$, for wk > 4: $n = 11$). Boxes represent the interquartile range, diamonds represent mean, and whiskers include all values within ± 1.5 × interquartile range. Values represented with an × are outside that range. Lines connect boxes at their median.

the changes in net requirements would be much smaller than the changes in mineral intakes. For Zn, the net requirement of a 700-kg cow is predicted [24] to increase from 79 to 300 mg/d between late gestation (i.e., dry period) and the peak of milk production of 45 kg/d (i.e., wk 8 to 16). This additional requirement of 221 mg/d represents only 9% of the additional Zn intake in the present study (i.e., increasing from 833 mg/d, when in the dry period, to 3221 mg/d, between wk 11 and 16). This disproportionate change in supply as compared to net requirements is even greater for Mn and Cu, as export through the milk of these trace elements is low. Differences in net requirements are predicted to be rather small: 11.8 to 12.0 mg/d for Cu and 2.1 to 3.2 mg/d for Mn [24] between the dry period and peak of milk production. Such an increase in net requirements represents less than 0.1% of the additional intake of these minerals reported in this study. Animals in general are capable of tolerating chronic and transient excess supplies of these minerals by absorptive and excretory regulation [4–7]. However, the total supply level, the relatively sudden changes in supply, and the mismatch with changes in net requirements may represent a challenge for cows to maintain trace metal homeostasis, which could eventually affect their health.

Zn, Mn, and Cu balance before calving

Despite the large decrease in the supply of Cu and Mn from late lactation into the dry period, cows were able to maintain a constant balance of Cu and Mn, proving a very efficient regulatory control upon reduced supply. For Cu, this occurred even though the mean dietary intake during the dry period (146 mg/d) was well below the requirement of 270 mg/d for 700-kg late gestating cows [24]. Before calving, the Cu and Mn balance did not differ from 0, in agreement with the expected low net requirement from the fetus and conceptus of 3.1 mg/d for Cu and 0.58 mg/d for Mn [27].

In contrast to Mn and Cu, sizable Zn retention would be expected from gestation, as well as from any fluctuation in body protein mass. Based on the slaughter data of House and Bell [27], daily Zn accumulation of 22 mg associated with the growth of the gravid uterus would be expected. In addition, as reported by Daniel et al. [23], the nitrogen balance of cows prior to calving was positive in this study, with a mean of 16 g/d. This increase in body protein mass is also expected to be associated with Zn retention. Using Zn content of muscle as reference, around 25 mg/kg wet weight [28], it was calculated that a positive nitrogen balance of 16 g/d would be associated with additional Zn retention of 12 mg/d of Zn. This, together with Zn accretion in the gravid uterus, would be expected to result in a Zn retention of 34 mg/d, a number below the mean Zn balance reported here before calving (98 mg/d), suggesting that additional Zn may have been retained elsewhere in the body. The homeorhetic effect of lactation on changes in the body Zn content of cows is not sufficiently understood or described. Some data using lactating rodents indicate that during gestation, the whole-body Zn of the mother is increased and then decreased during lactation [29,30]. Whether this is also the case in dairy cattle and to what extent gestation and lactation affect the whole-body Zn content of the mother is unknown and deserves further investigation. Interestingly, the Zn balance was negative in wk 2 and 3 after calving (−152.2 and −105.3 mg/d, respectively) when the nitrogen balance was also negative [23].

Zn, Mn, and Cu balance after calving

The large increase in Zn, Mn, and Cu balances observed around wk4 to 5 suggests that homeostasis could not be sustained in this study. When Mn and Cu balances were plotted against their dietary intake (Supplemental Figure 6), the slopes of the regressions during the lactation phase were 0.16 (SE ± 0.06; $r^2 = 0.38$, $P = 0.02$) and 0.18 (± 0.06; $r^2 = 0.33$, $P < 0.01$), respectively. These linear responses could indicate that 16% of the excess Mn and 18% of excess Cu are retained in the body. A similar linear and positive relationship (slope of 0.26 ± 0.04) was also reported between apparent Mn retention and Mn intake using data from both dry and lactating dairy cows [11], although this finding was not interpreted as unregulated retention. Furthermore, work from Sansom and colleagues [31], in which a high amount of Mn (about 12.5 g/d) was fed to 2 cows, suggested, based on Mn appearance in the portal circulation, that only 0.54% of increasingly supplied Mn was absorbed. As a result of the high amounts fed, such low fractional absorption still resulted in a net transfer into the body of 67.5 mg/d. In a different study, determining the effects of short-term infusions of large amounts of Mn into the blood of steers, it was concluded that the capacity of the liver to remove Mn from the blood and excrete it into bile is very high, with a maximum excretion rate of

1210 ± 130 μg/min of Mn, equivalent to 1742 mg/d [32]. Based on this conclusion, an increase in Mn retention upon high dietary supply would not be expected. However, the data from this balance study and the other Mn balance study of Weiss and Socha [11] suggest otherwise.

Fractional apparent absorption of Zn, Mn, and Cu

In contrast to results from rodent studies on trace metal regulation [4–6], the present study with dairy cattle illustrates a directionally opposite relationship between intake of Zn, Mn, and Cu and their respective fractional apparent absorption rates. This increased apparent absorption rates with increased intakes result in an estimated positive net retention for the 3 trace metals studied. Our observations are, however, consistent with the few studies evaluating these relationships in ruminants. An increase in dietary Cu content from 10 to 35 to 50 mg/kg DM in nonlactating, nongestating adult Holstein cows resulted in a linear increase in apparent absorption from 5.5% to 10.9% to 17.1%, respectively [12]. Similarly, an increase in the apparent fractional absorption of Mn from 2.3% to 6.1% was reported in late gestation cows when Mn intake increased from 505 mg/d to 744 mg/d [11].

However, the scarce Zn retention data in ruminants would indicate that as dietary Zn intake increases, albeit at a much lower level than in the current study (from 17 to 62 mg/kg DM), apparent Zn absorption decreases [8,33]. Absorption of trace metals is known to be governed by 2 phases, a highly regulated and saturable phase mediated by specific transporter proteins and an unregulated phase [34]. The second phase is likely to predominate at high metal concentrations, providing a plausible explanation for the results found in this study.

Fate of Zn, Mn, and Cu was retained in the body

As outlined previously, whole-body retention of Zn, Mn, and Cu calculated from balance data measured between wk 4 to 16 suggested relevant body accumulation of all 3 metals. If the calculated daily retentions over the 12-wk period are summed (i.e., by summing all weekly balance from wk 4 to 16, and multiplying the sum by 7), whole-body Zn, Mn, and Cu would have increased by 8.4 g, 6.7 g, and 1.7 g, respectively. To put these numbers in perspective, the total amount of Zn, Mn, and Cu in a cow of 730 kg is estimated to be around 15 g, 0.3 g, and 1.7g, respectively (i.e., estimated from analyzed carcass of grazing sheep [35]). Thus, these theoretical accumulations would result in 1.6 times more Zn, 23 times more Mn, and 2 times more Cu in the whole body, respectively. It is however noteworthy, that the retention of nutrients calculated from balance data is potentially subject to error. To this respect, in the present study, nitrogen balance, reported by Daniel et al. [23], was however in good agreement with changes in BW, suggesting a rather accurate collection, sampling, and analysis of excretions. Nevertheless, further studies are warranted to confirm the potentially very relevant findings of this study.

Ruminants are more prone to hepatic Cu accumulation than nonruminant species, as they are less able to adapt to excess dietary supply [36]. As a result, a wide variation in the hepatic Cu concentration is observed across dairy cows [18,19,37]. If the calculated increase of 1.7 g of whole-body Cu (i.e., over the last 12 wk of this study) would entirely accumulate in the liver, hepatic Cu content would increase by 600 mg/kg DM by the end of the study, assuming a liver DM size of 3 kg [38]. This increase in concentration is large, but physiologically possible, as indicated by the ranges of hepatic Cu concentrations reported for dairy cows [18,19,37]. Additionally, studies evaluating hepatic Cu concentrations at different ages in growing cattle [39,40] show that daily increases of 4.1, 6.0, and 7.5 mg/kg DM were achieved in the liver with Cu supplies of 0.66, 1.05, and 1.17 mg/d/kg BW, respectively. An increase in the hepatic Cu content of 600 mg/kg DM over 12 wk, as predicted from the results of our study, would be equivalent to a daily increase of 7.1 mg/kg DM in the liver. Unfortunately, hepatic Cu content was not determined at the end of the study to confirm these estimates. Nevertheless, the high hepatic Cu concentration (median of 473 mg/kg DM) reported during late lactation confirms a large amount of Cu was stored in the liver of these dairy cows. This concentration is comparable to the median previously reported of 443 mg/kg DM [37] and is not far from the threshold indicative of toxicity (150 mg/kg wet, or 555 mg/kg DM assuming a liver DM content of 73%) reported in the literature [20]. The decrease hepatic Cu concentration observed during the dry period (Supplementary Figure 5), at a time Cu supply was the lowest among the periods evaluated, most likely indicated that animals were able to remove excess Cu from the liver at a greater rate than true Cu absorption.

Whole-body Mn and Zn content, on the other hand, have been reported to vary little in lambs: 0.7–1.2 and 20.8–25.6 mg/kg of Mn and Zn, respectively, in fresh carcass weight [41], 0.69–1.04 and 25.7–30.2 mg/kg of Mn and Zn, respectively, in empty BW [42], or in grazing goats, 0.86–1.09 and 23.7–27.8 mg/kg of Mn and Zn, respectively, in empty BW [43]. The mean body Zn content reported for lactating cows were also in agreement with these ranges: 22.1–23.1 mg/kg empty BW [8]. However, Zn and Mn can accumulate in the body tissues of ruminants when fed at high levels. A study in sheep [44], for example, demonstrated that the Mn concentration in the bone increased linearly from 3 to 59 mg/kg ash when the dietary Mn content was increased from 31 mg/kg DM to an extreme level of 8 g/kg DM of Mn for 12 wk. Similarly, liver, kidney, and bone Mn were all increased when lambs were fed high levels of Mn at 0.9–4.5 g/kg DM [45,46]. In growing beef cattle, Zn concentrations in the liver (from 41 to 326 mg/kg wet weight), kidney (from 22 to 479 mg/kg wet weight), pancreas (from 49 to 249 mg/kg wet weight), and bone (from 72 to 198 mg/kg DM, marrow-free) linearly increased when Zn supply increased from 0.9 to 1.6 g/kg DM [47]. Nevertheless, these increases were achieved with much greater dietary Mn and Zn concentrations than in our study. Interestingly, when rats were injected with Mn, 40% of the Mn retained in the body (9.3% of the injected dose) was in the skin, 22% in the bone, and 13% in the liver [48]. In sheep, 55% of the total body Mn was found to be in wool and skin [35]. Variations in the Mn content in dairy cattle skin are unknown, but an unreasonably large increase in Mn concentrations, and/or loss via sloughed skin cells and secretions, would be necessary to match the estimated apparent retention in this experiment. Studies evaluating the variability in trace metal content of skin and bone from dairy cattle would be of value to further understand their response to changes in trace metal supply.

Consequences for animal health

Increased urinary excretions indicate an excess of minerals in the blood circulation. Such an excess may arise either from high dietary intakes together with the overflow of storage capacities or alternatively from increased mobilization from endogenous stores. The rise in urinary Zn reported after calving may be explained by mobilization of body protein [23], leading to a release of accompanying Zn. Trace metals, including Cu, Mn, and Zn, are important components of antioxidant systems. However, when concentrations rise above optimal levels, trace metals can cause oxidative stress [49]. Among the 3 metals evaluated, Cu is considered the most toxic, and excess can induce liver lesions and ultimately a hemolytic crisis that may lead to death [50]. Nevertheless, the susceptibility to Cu poisoning varies between individual animals. The role of metallothionein, an intracellular protein, is believed to be crucial for the safe storage of Cu in the cell [51]. However, in cattle, the fraction of Cu bound to metallothionein in the liver decreases as the hepatic Cu content increased, from about 50% to less than 10% [52]. This indicates that cattle have limited capacity to synthesize metallothionein in response to increased Cu intake, as was reported for sheep [53]. In a study that compared liver Cu concentrations to histological evidence of hepatocellular damage, a consistent relationship between the amount of Cu stored in the liver of dairy cows and the presence of hepatic lesions could not be established [54]. Using a limited number of animals, García-Vaquero et al. [55] demonstrated that a supplementation of $CuSO_4$ as low as 15 mg/kg Cu·DM to growing cattle resulted in increased oxidative damage in the liver containing 136 mg/kg of Cu·wet weight. Evidence of hepatic oxidative stress correlated with the Cu concentrations in the liver of cows was also presented by Strickland et al. [54]. Although a toxic threshold cannot be easily defined due to variability in susceptibility when comparing cows to sheep, it is clear that oversupplementation of Cu to dairy cows increases hepatic Cu and that this represents a poisoning risk [19] or a risk of increased hepatic oxidative stress [54].

Mitigation of environmental impact

These data indicate potential limitations in the homeostasis of Zn, Mn, and Cu in dairy cows when continuously exposed to a high, but in line with common practice, supply of trace metals. Nevertheless, our study also proves the resilience of the homeostatic system when faced with a temporary low dietary supply of trace metals. Earlier studies have reported no detrimental effects on performance in cows temporarily fed metal levels below the recommendation. As an example, lactating cows fed a diet with 16.6 mg/kg DM of Zn for 6 wk were able to maintain milk productivity with marginal impact on whole-body Zn content [8]. Similarly, 60-d-old wether lambs fed a diet containing 2.2 mg/kg DM of Cu for 120 d maintained hepatic Cu concentrations at a constant level from 55.9 to 60.9 mg/kg fresh weight [56]. The effectiveness of metal homeostasis to prevent deficiency in ruminants should be acknowledged when making recommendations on trace metal feeding.

A major and immediate benefit of supplying a more moderate amount of trace metals to dairy cows will be a lower excretion in the environment [21]. In fact, the current practice in commercial dairy herds largely exceeds feeding recommendations for Zn, Mn, and Cu [25,26,57,58], indicating that reductions of trace metals in manure is achievable. There is an opportunity to critically revise current recommendations and nutritional practices in trace metal feeding of dairy cows, given the opportunity to better guarantee the sufficiency and tolerance suggested by this data, but also for the immediate economic and environmental benefits of optimizing the supply of these nutrients.

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Author contribution

The authors' responsibilities were as follows—JBD, JHD, DB, JMT: designed research; JBD, JHD: conducted research; JBD, DB, SVDD, DVDM, NK, WW, JMT: analyzed and interpreted data; JBD: wrote the paper; JMT: had the primary responsibility for the final content; and all authors: read and approved the final manuscript.

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Author disclosures

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2023.02.022.

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