Diagnostic findings of copper, selenium and manganese deficiency in dairy and beef cattle submitted to the California Animal Health and Food Safety Laboratory

***David J Schaeffer,**[1] MS, PhD; **Robert Poppenga,**[2] DVM, PhD, DABVT; **David Villar,**[3] DVM, MS, PhD, DABVT

[1]College of Veterinary Medicine, University of Illinois, Urbana, IL 61802
[2]Section of Toxicology, California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616
[3]David Villar, Grupo CIBAC, Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellin, Colombia (retired)
***Corresponding author**: Dr. David Schaeffer, **dschaeff@illinois.edu**

Abstract

Copper (Cu), selenium (Se) and manganese (Mn) concentrations in 1,490 beef and dairy cattle livers submitted to the California Animal Health & Food Safety Lab System (CAHFS) laboratory between 2012 and 2021 were analyzed. The aim was to compare the correlation patterns of hepatic Cu, Se and Mn in neonates, adolescents and adults for selected pathologies and diseases. Deficiencies were more common in beef than dairy cattle, accounting for 33.5% (287/855) and 5.2% (33/635) for Cu, 46% (385/836), and 9.8% (62/630) for Se. Conditions in beef that were rare or absent in dairy were anaplasmosis, histophilosis, nutritional myopathy, Foothill abortion, Johne's disease and sudden death attributed to severely deficient copper. Bovine respiratory disease (BRD) was diagnosed in beef and dairy cattle, with 14.4% (123/855) and 14.5% (92/635) cases, respectively. However, the number of BRD cases deficient in Cu or Se was 68.3% (84/123) and 10.8% (10/92) for beef and dairy cattle, respectively. Salmonellosis and scours were rare in beef cattle, but were the 2 most common diagnoses in dairy cattle, with 21.1% (134/635) cases (111 in group D1, 22 in groups B and C), and 16.4% (104/635), respectively. All dairy cases diagnosed with *Salmonella* Group D1 (most likely *S.* Dublin) ranged between 5 and 240 days of age. Copper and selenium deficiencies were extensive in beef but not in dairy cattle. Conditions, such as BRD in adult beef and poor BRD vaccination response in beef calves, were more likely to occur when Cu and/or Se were deficient.

Key words: micromineral deficiency, copper, selenium, bovine

Introduction

Micromineral (MM) deficiencies are pervasive challenges for livestock producers and veterinarians. Subclinical deficiencies might be a more extensive problem because specific clinical symptoms are not evident enough to recognize the deficiency, which probably increases economic losses.[1] The requirements for minerals and vitamins for optimizing immune function are compromised first, followed by a reduction in growth and reproductive performance. The latter decreases before apparent signs of mineral deficiency.[1,2,3,4] Consequently, it can be reasoned that classical clinical signs might represent only a small part of the overall features of micromineral deficiencies. The "iceberg concept" in epidemiology describes the situation of micromineral deficiencies, where a large percentage of the problems (immunity, growth, reproduction, uptake and metabolism) are subclinical or otherwise hidden from view, and only a few individuals ("tip of the iceberg") are apparent to the producer and veterinarian.[5,6]

Many combinations of microminerals and macrominerals affect nutrient deficiencies, disease susceptibility, fertility, gestation abnormalities, scours, weight gain, toxicity and other problems in cattle and other ruminants.[7] Most have been statistically inadequate in determining interactions due to low animal numbers and confounders, such as the lack of individuals' micromineral intakes or samples from inappropriate tissues, like urine or serum. For example, concentrations of Cu, Se and Mn were inadequate in a high percentage of sera, although serum levels are maintained until liver reserves are exhausted.[8,9] A survey of 555 beef cattle across California found that serum Cu (28%), Se (12%), Mn (92%), zinc (Zn, 32%) and iron (Fe, 52%) concentrations were below their critical concentration; yet, "copper levels were probably elevated"[9] over liver depleted by maintaining serum levels. Excess liver levels of one micromineral (MM) can cause a deficiency of others, such as antagonism of copper by excessive dietary Fe,[9] molybdenum (Mo), and sulfur (S).[10,11,12,13] Organ responses differ, such as a high correlation between Cu and Mn in the kidney, but not in the liver.[11]

Given the potential for adverse health consequences, producers need to know the extent of deficiencies in a herd. This study focuses on the magnitudes, directions and patterns of correlations for selected hepatic microminerals. We interrogated a diagnostic database to identify conditions with a strong micromineral relationship. This sequence reverses the usual epidemiological order of determining MM concentrations associated with a specific disease.

Our interest in micromineral correlations arose from studies proposing that gestational deficiency of Mn, with or without other microminerals, caused gestational death and fetal abnormalities, such as congenital joint laxity and dwarfism (CJLD).[14,15] Some studies reported concentrations without correlations.[1,9,16,17] Some correlations for non-normal (skewed) data were misinterpreted as Pearson correlations when they were Spearman rank correlations. Most studies did not acknowledge that significant correlations between two minerals, symbolically *i* and *j* (Table 1, for example, $M_{i=Cu}$ and $M_{j=Se}$), implied that both contributed to the disease.[10,12,13,16,17,18]

Our primary objective, based on a qualitative assumption of micromineral homeostasis, was to determine if the correlation patterns of hepatic Cu, Se and Mn for diseases in the California Animal Health & Food Safety Lab System (CAHFS) diagnostic database at different ages would be similar for beef and dairy cattle. Secondary hypotheses conjectured about the CAHFS data from published descriptive statistics were: 1) not

Table 1: Descriptive statistics* of hepatic copper (Cu), selenium (Se) and manganese (Mn) in all beef and all dairy cattle in the CAHFS data.

	Age (days)	Cu_d	Cu_n	Cu_h	Se_d	Se_n	Se_h	Mn_d	Mn_n	Cu (all)	Se (all)	Mn (all)
Beef: Age > 0												
N	749	276	414	57	353	259	133	260	391	747	745	651
Range	1-6935	0.85-42	16-120	110-360	0.02-0.34	0-0.52	0.5-9.9	0-2	0.6-13.5	0.85-360	0-9.9	0-13.5
Mean ± SD	717 ± 992	10.77 ± 7.17	53.03 ± 21.63	143.2 ± 42.43	0.15 ± 0.06	0.33 ± 0.1	1.2 ± 1.3	1.4 ± 0.41	2.84 ± 1.23	44.29 ± 40.23	0.4 ± 0.67	2.26 ± 1.21
Age not reported												
N	38	4	27	7	9	21	8	19	7	38	38	26
Range		2.6-20	23-97	120-170	0.083-0.19	0-0.48	0.36-1	0.4-1.7	0.3-5.7	2.6-170	0-1	0.3-5.7
Mean ± SD		9.9 ± 8.16	59.07 ± 22.66	145.7 ± 18.13	0.16 ± 0.03	0.34 ± 0.13	0.64 ± 0.25	0.92 ± 0.43	2.5 ± 2.14	69.86 ± 44.52	0.36 ± 0.22	1.35 ± 1.32
Dairy: Age > 0												
N	602	30	374	195	63	324	211	209	323	599	598	532
Range	1-4745	0.95-24	0-100	110-480	0.05-0.48	0-0.54	0.44-78	0.4-2	0.5-14	0-480	0-78	0.4-14
Mean ± SD	278 ± 516	10.38 ± 6.73	65.22 ± 21.25	156 ± 49.95	0.19 ± 0.09	0.38 ± 0.09	1.11 ± 5.33	1.48 ± 0.35	2.92 ± 1.31	92.01 ± 56.65	0.62 ± 3.18	2.35 ± 1.26
Age not reported												
N	4	2	28	6	3	11	21	21	4	36	35	25
Range		14-22	25-110	110-320	0.1-0.22	0.26-0.47	0.52-1.9	0.3-1.9	0.3-3.5	14-320	0.1-1.9	0.3-3.5
Mean ± SD		18 ± 5.66	54.64 ± 22.76	186.7 ± 77.89	0.18 ± 0.07	0.39 ± 0.09	0.8 ± 0.38	0.85 ± 0.45	2.17 ± 1.35	74.61 ± 62.62	0.62 ± 0.38	1.06 ± 0.8

* N = total cases and hepatic concentration deficient (_d), normal (_n) or high (_h).

all covariances or correlations could be equal or zero, 2) differences in the metabolic requirements with age would result in dissimilar patterns between some age classes in the same production class, 3) binary correlations of the deficiency status of two microminerals would be small, i.e., a deficiency of one micromineral did not presage deficiencies in others.

Materials and methods

One author (DV) had assembled a library on microminerals from the CAHFS diagnostic data during a stay as a visiting scholar invited by Dr. Poppenga. The others' compilation of statistics included clinical and environmental health in pets, production and wildlife species. The collections were developed using statistics and physiology monographs, cited references, commercial search services that predated the internet, government documents, and statistical software documentation (from 1970 onwards), as well as Google, Google Scholar, PubMed, CABI Digital Library and Chemical Abstracts. We searched the collections for *covariance matrix*, *correlation matrix*, *matrix pattern* or *structure*, *microminera*, and *trace minerals*; refining terms were *equality, ruminant*, *cattle*, *liver, hepatic, blood, serum, plasma* and specific elements in various combinations. Most discovered papers were irrelevant, involving genetic correlations or diet, for example. Specific coding questions used focused searches of examples for SAS (Statistical Analysis System, Cary, NC) documentation.

Animal care

This research used the CAHFS database and published data and did not involve animals.

CAHFS data

We downloaded 855 beef and 635 dairy cattle cases from the CAHFS diagnostic database, which contains tissues submitted between 2012 and 2021 by owners or veterinarians for pathology and micromineral analysis. The CAHFS laboratory employed standardized protocols and quality control measures. The criteria for inclusion in this research were a whole animal necropsy or a complete set of tissues, diagnosis, age, production class (beef or dairy), breed, hepatic Cu, Se and Mn concentrations (ppm ww), pathology diagnosis, and supplemental pathology comments. Exclusion criteria included an advanced stage of autolysis that precluded postmortem examinations or a history of multivitamin administration. The records did not include diet, housing or management. All variables *not* explicitly specified in an ANOVA model (e.g., condition, breed, diet, housing) are implicitly represented in the covariances and correlations.

Puls' criteria were followed to establish deficient, normal and high liver concentrations.[20] Copper ≤ 24 ppm ww was deficient, 25-100 ppm ww was normal, and ≥ 101 ppm ww was high. Selenium ≤ 0.24 ppm ww was deficient, normal was 0.25-0.50 ppm ww, and high was ≥ 0.51 ppm ww. Manganese < 2 ppm was deficient.

Statistical methods

File creation and basic statistics

The CAHFS data was imported into SAS 9.4 (SAS Institute Inc., Cary, NC). The minimum, maximum, mean, median, standard deviation, skewness, kurtosis and Shapiro-Wilk and Anderson-Darling normality tests were used as descriptive statistics for each production class, age class and all cases. Binary-coded liver concentrations (deficient = 1, normal + high = 0) were cross-tabulated. Age distribution (days) comparisons for all beef and dairy used normal and lognormal probability plots. Spearman and Pearson correlations of age, Cu, Se and Mn (ppm ww) were determined for all dairy and beef cattle. Micromineral (MM) concentrations were on different scales, and the subsets being analyzed were standardized within their product class to have a mean of 0 and a standard deviation of 1. We labeled the absolute correlation values as follows: 0 to 0.19, "very weak"; 0.2 to 0.39, "weak"; 0.40 to 0.59, "moderate"; 0.6 to 0.79, "strong"; and 0.8 to 1.0, "very strong". Most correlations were "very weak" or "weak". Venn diagrams of standardized MM concentrations visualized micromineral relationships for selected pathologies. Odds ratios for a pathology compared dairy vs. beef and each MM as deficient or normal-high within each product class.

Results

The leading beef breeds were Angus (n = 431) and crosses (n = 388), while the dairy breeds were Holsteins (n = 432) and Jerseys (n = 189; Table 1). Concentrations of Cu, Se and Mn, production class (beef, dairy), and age class were available for 1,297/1,490 cases. Age was missing for 192 of 857 (22%) beef cattle and 88 of 638 (13.8%) dairy cattle. Average Mn concentrations (ppm) were similar for all deficient and normal cattle: BRD (8.7, 9.2), undetermined (3.6, 5.2), scours (3.1, 4.3), septicemia (2.1, 2.7), and salmonellosis (1.9, 5.0). Frequencies of deficiency for combinations of Mn, Cu and Se for beef and dairy cattle are shown in Table 2.

We did not identify any papers that compared the correlation patterns of microminerals in ruminants. Most correlations of the CAHFS data were "very weak" or "weak," and Spearman correlations slightly exceeded Pearson. The Spearman and Pearson correlation patterns differed between (e.g., all cases or conditions) or within product classes (e.g., age). The correlations between BRD counts in beef and dairy were Spearman R = 0.46 and Pearson R = 0.81 (Figure 1). Spearman correlation matrices differed between beef with no deficiencies and those with at least one deficiency. The adolescent and adult correlation matrices were similar and differed from the neonate matrix ($P_{\chi 2} = 0.0049$). The Spearman correlation coefficients for beef age were Se (-0.12), Cu (-0.26) and Mn (0.28), and for dairy, they were Se (-0.030), Cu (-0.013) and Mn (no data). Our random sampling study and correlations for BRD in beef and dairy cattle supported the hypothesis that the MM matrix for a disease was reproducible for samples of more than 50 per group. The numbers of beef and dairy cattle with their respective liver Cu and Se concentrations, according to their mineral status (deficient, normal, excess), are shown in Figures 2 and 3. The number of deficient Cu and Se beef cases is probably underestimated if we consider that pre-ruminant calves rely on their liver birth stores to supply MM in the first months of life, while they are nursing and diluting stores due to rapid weight gains.

The classical signs of deficiency in beef cattle were rare: sudden death due to low copper levels (< 10 ppm ww) occurred in 14 cattle, nutritional myopathy due to low selenium levels in 21, or congenital defects resulting from low manganese (Mn) levels in 12. Primary diagnoses for 645 beef cattle were identified in 34%, 50% and 16% of neonates ≥ 1 day old, juveniles (≤ 370 days) and adults ($P_{\chi 2} < 0.01$). The primary diagnoses for 534 dairy cattle were 15%, 46% and 39%, respectively. Causes of death were "undetermined" in 25.3% (168/664) of beef and 18.8% (89/474) of dairy cases.

Table 2: The number of beef and dairy livers deficient in 0, 1, 2 or 3 microminerals (copper (Cu), selenium (Se) and manganese (Mn)).

Micromineral deficiencies	Beef (n = 857)	Dairy (n = 638)	All (n = 1,495)
No microminerals are deficient	231	350	581
At least one micromineral is deficient	626	288	914
All deficiencies by micromineral			
Cu	285*	32	317
Se	390	66	456
Mn	331	235	566
Only specified micromineral deficient			
Cu	45*	8	53
Se	130	34	164
Mn	147	203	350
Cu & Se	120	11	131
Cu & Mn	44	11	55
Se & Mn	64	19	83
Cu & Se & Mn	76	2	78

*Of the 285 beef cattle with copper deficiencies, 45 were deficient only in Cu, while others were also deficient in Se and/or Mn.

Figure 1: Venn diagram for BRD in 113 beef and 85 dairy cattle without missing concentrations. Each MM was standardized by product class to have a mean of 0 and a standard deviation of 1. The distribution of standardized concentrations in overlapping segments is similar for both dairy and beef cases, as indicated by Pearson's correlation coefficient of 0.81 and Spearman's correlation coefficient of 0.46.

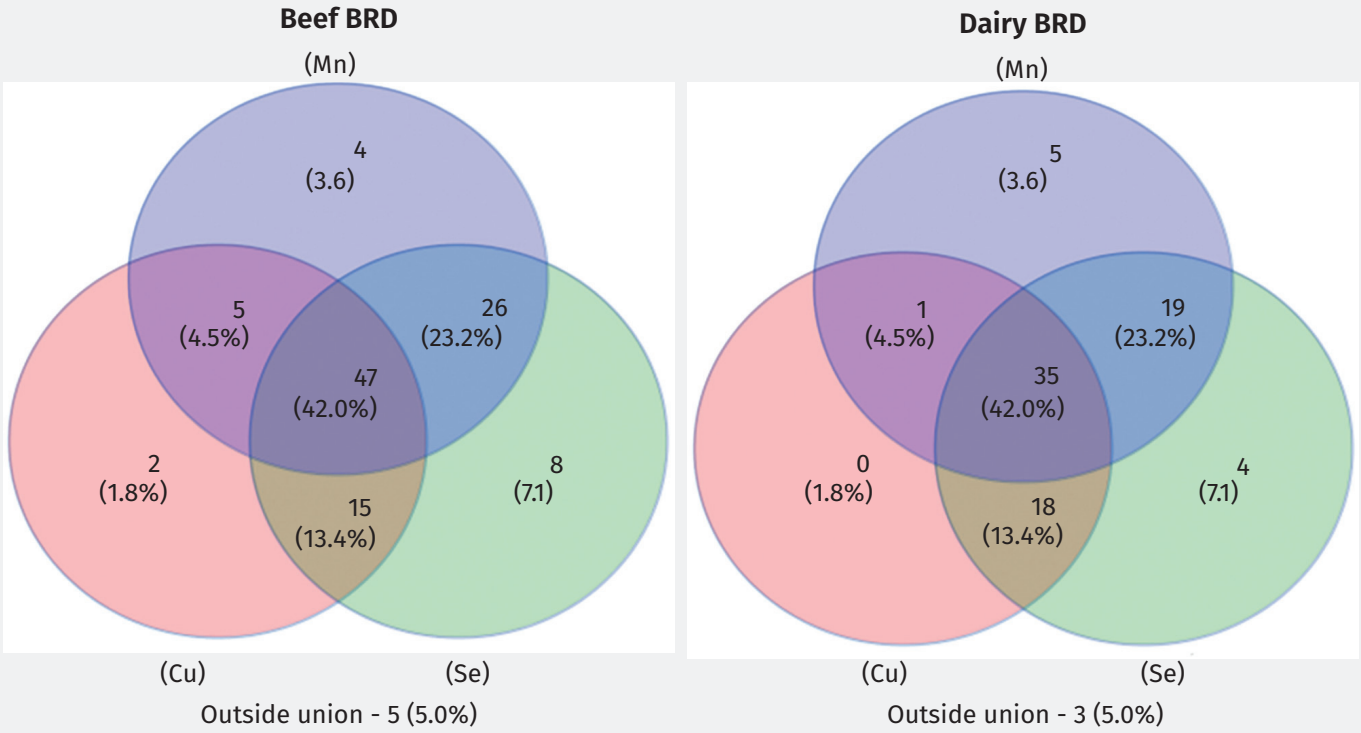
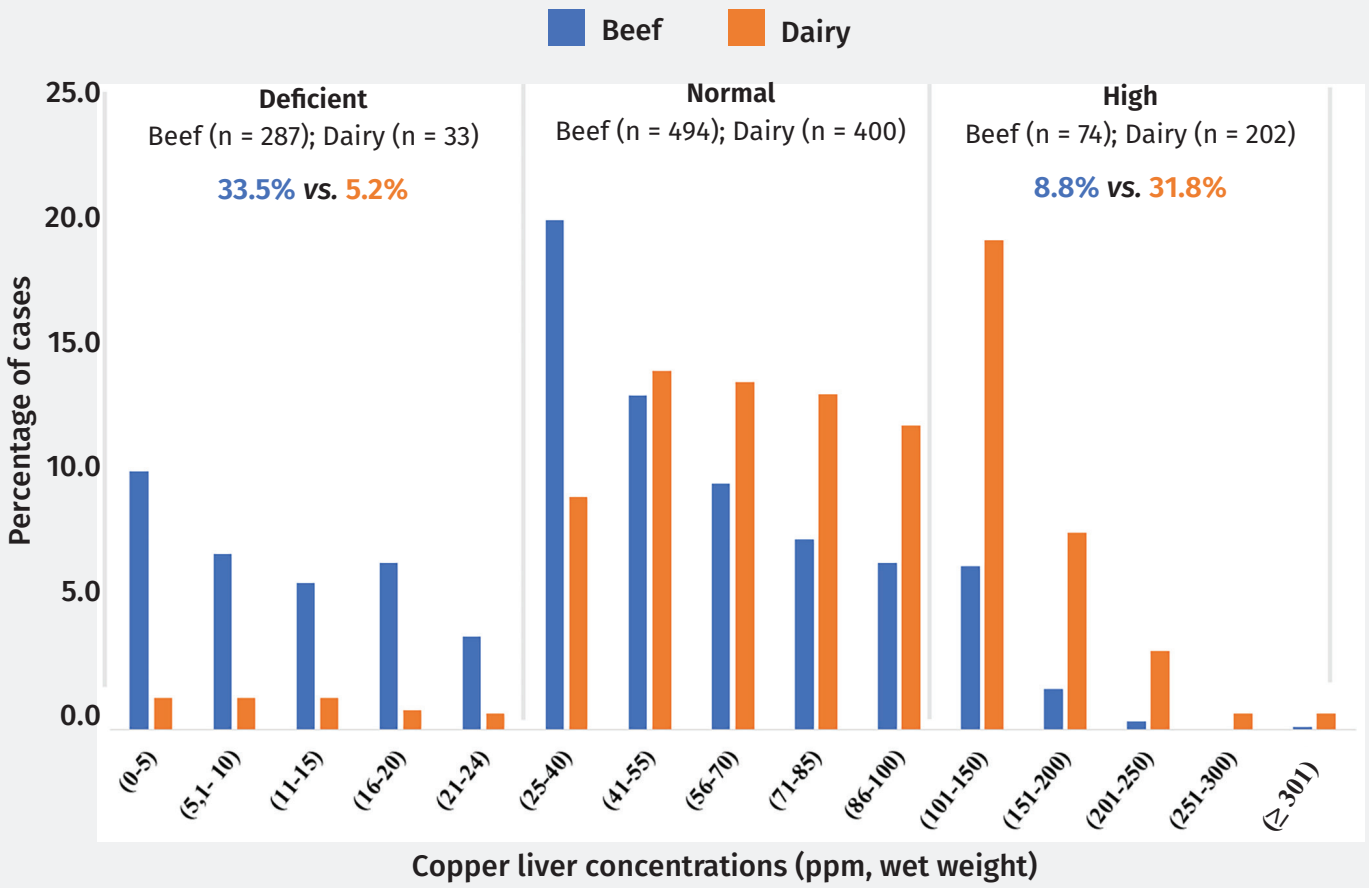


Figure 2: Copper liver concentrations in beef and dairy cattle submitted for diagnosis to the California Animal Health and Food Safety Laboratory System between 2012 and 2020 (n = 855 beef, n = 635 dairy).



BRD was the most common cause of mortality in beef (14.4%, 123/855) and the third most common cause in dairy (14.5%, 92/635). The type of pathogens identified and number of cases of BRD for beef and dairy BRD are shown in Table 3 in decreasing order of frequency. Two differences were a median age of 8 months for beef and 3 months for dairy. The number of BRD cases deficient in Cu and Se was 68.3% (84/123) for beef and 10.8% (10/92) for dairy cattle. There were 25 beef cases with BRD in adults (≥ 730 days of age), and most were deficient in one or more of the following nutrients: copper (4), selenium (4), and both copper and selenium (11). Vaccination history against BRD pathogens was provided in 16 beef cases aged 150 to 390 days, there was severe deficieny in both Cu and Se in 10 animals, and 6 others had either a Cu or Se deficiency. Conditions diagnosed in beef (number in parenthesis), but rare or absent in dairy, were histophilosis (n = 30), anaplasmosis (n = 29), white muscle disease due to Se deficiency (n = 21), Johne's disease (n = 16), sudden death with low copper (n = 14), and Foothill abortion (n = 12). Scours were rarely diagnosed in beef cattle, with 2.3% (20/855) of cases, but was the second most common condition in dairy cattle, at 16.4% (104/635) of cases. The median age of neonatal calves affected with scouring pathogens was 10 days for dairy and 5.5 days for beef. When analysis for post-colostrum IgG intake was performed, partial or total failure of passive transfer was diagnosed in 21 of 34 dairy calves and 19 of 27 beef calves. In total, a failure of passive transfer was found in 65.5% (40/61) neonatal calves that died from scours or bacterial septicemia in the first weeks of life. Salmonellosis was the most frequent diagnosis in dairy cattle (107 in group D1, 21 in groups B and C), but it was only diagnosed in 8 beef cases (5 in group B, 2 in group D1, and 1 in group C1). The median age of dairy calves affected with *Salmonella* Group D1 (most likely *S.* dublin) was 60 days, and no cases were older than 240 days, except for one adult cow that was severely deficient in copper and selenium. The most common diagnosis of *Salmonella* Group D in dairy calves was septicemia, characterized by severe systemic and diffuse necrosuppurative and fibrinous inflammatory lesions in multiple organs and intravascular fibrin thrombi. When the case history was provided, high rectal temperatures, death in 24 hours with or without diarrhea and respiratory signs, and sudden death were the most common signs.

Of the 244 dairy cattle with one deficiency (Table 2), the most frequent diagnoses were BRD (n = 55, 22.5%), *Salmonella* (n = 34, 13.9%), scours (n = 40, 16.4%), septicemia (n = 15, 6.1%), and unknown + undetermined (n = 35, 14.3%). Of the 353 dairy cattle without deficiencies, the most frequent diagnoses were BRD (n = 67, 19.0%), *Salmonella* (n = 72, 21.5%), scours (n = 61, 17.3%), septicemia (n = 22, 6.2%), and undetermined or unknown (n = 50, 14.2%). Scouring pathogens were diagnosed in 1 juvenile and 103 neonatal dairy cattle. Some conditions with a statistically significant odds ratio of deficient MM are listed in Table 4, and the frequency (%) of conditions with excess Cu and Se is presented in Table 5.

Figure 3: Selenium liver concentrations in beef and dairy cattle submitted for diagnosis to the California Animal Health and Food Safety Laboratory System between 2012 and 2020 (n = 836 beef, n = 630 dairy).

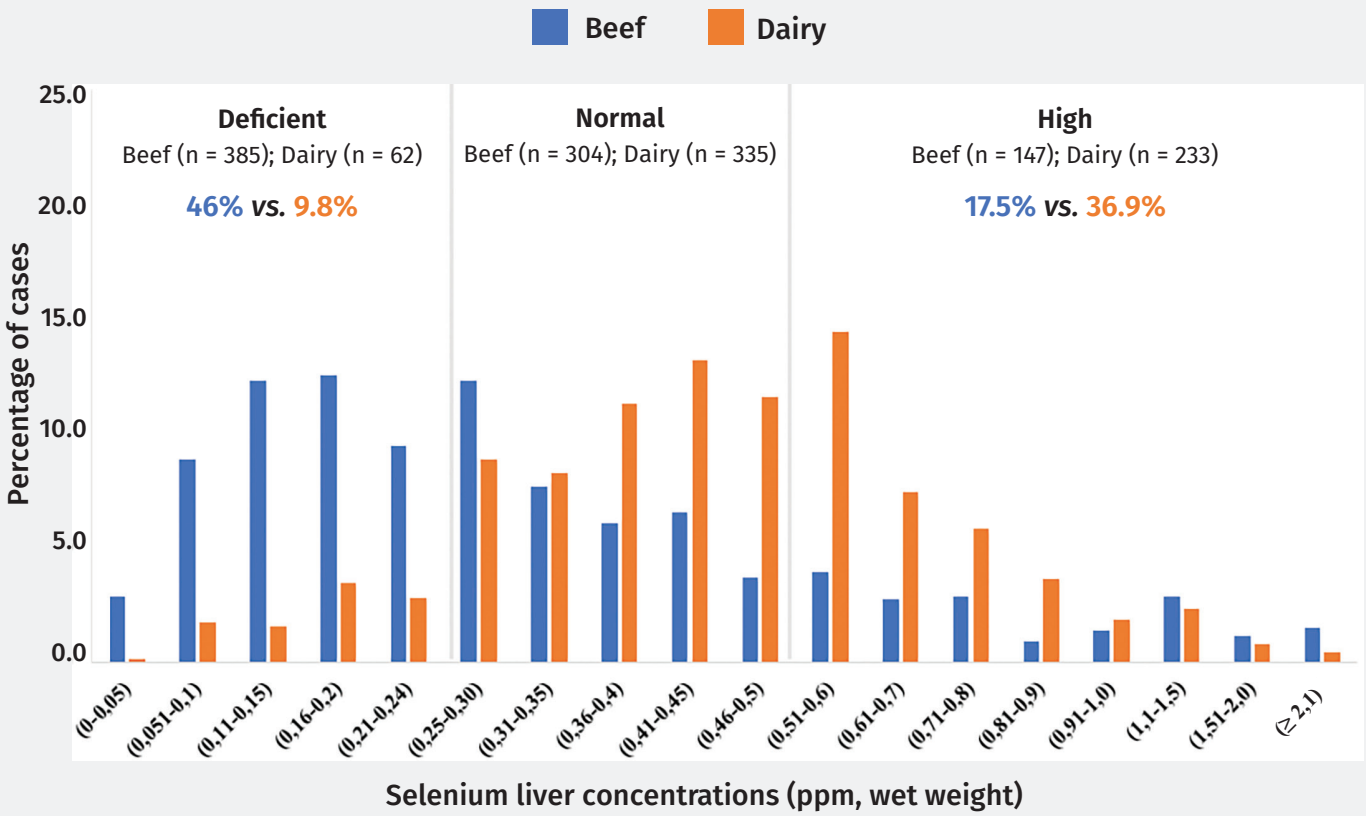


Table 3: The number of cases with bovine respiratory disease complex pathogens identified in California cattle (n = 168 beef, n = 130 dairy†).

Pathogen	Number of cases in beef*	Number of cases in dairy*	Total
Mannheimia haemolytica	73	50	123
Mycoplasma bovis	54	27	81
Pasteurella multocida	34	21	55
Histophilus somni	28	14	42
BRSV	27	16	43
BVD	14	3	17
Trueperella pyogenes	12	22	34
Streptococcus spp.	5	4	9
Mycoplasma bovirhinis	4	2	6
Salmonella Group B	2	0	2
Salmonella Group D1	0	3	3
Parainfluenza 1	1	0	1
IBR	0	2	2

* Note: Combinations of all the above pathogens were present in all cases, so the numbers for each pathogen cannot be added together.

† The overall number of cases of BRD is slightly higher than reported in the text because it includes other comorbidities not associated with respiratory pathogens (i.e., heavy parasitism by coccidiosis and trichostrongylosis).

Table 4: Odds ratios (OR) for deficient versus normal + high for hepatic copper (Cu), selenium (Se) and manganese (Mn) in beef vs. dairy cattle for selective diagnoses.*

Diagnosis	Odds ratios (OR) for normal vs. deficient						Odds ratios for all beef vs. dairy		
	Beef			Dairy			Beef vs. Dairy		
	Cu	Se	Mn	Cu	Se	Mn	Cu	Se	Mn
Anaplasmosis	0.67	3.2	0.54	No cases			Most or all are beef		
Bloat	2.3	2.3		No cases			Most or all are beef		
BRD alone	0.37*	0.72	0.65	1.67	1.26	0.72	0.026*	0.086*	†
BRD (± other conditions)	0.32	0.53*	0.67*	1.85	0.96	0.86	0.029*	0.070*	0.6
Coccidiosis	0.38&	0.95	2.21	†	0.57	0.9	†	0.225‡	2.14
Congenital defects	1.51	1.42	0.13**	†	†	0.35	†	†	0.33
Myocardiopathy with low selenium	2.78		0.92	0.10&		1.41	2.75	†	0.58
Omphalitis	†	5.17	3.21	†	0.7	†			
Scours	†	0.20*	0.92	0.16&	0.68	9.99	†	0.47	0.96
Septicemia (*E. coli*)	0.42	0.36	8.71	1.36		1.07	0.33		0.76
Sudden death with low copper	†	2.16	0.37	†	8.71	1.42	†	0.56	3.33

* Significant ($P < 0.05$)

† One or more counts = 0

‡ $P < 0.1$

Table 5: Percentage of total cases with excess copper, selenium or both (ppm) with the disease. The high concentration of Mn is above 1,000 ppm, which is 1-3 orders of magnitude greater than the tissue.

Frequent high concentrations	Copper			Selenium			Copper and Selenium		
	Beef	Dairy	Total	Beef	Dairy	Total	Beef	Dairy	Total
Total	74	201	275	149	232	381	34	139	173
Scours	5	40	45	8	35	43	4	26	30
Bovine respiratory disease	4	36	40	22	35	57	2	24	26
Salmonellosis	0	13	13	0	20	20	0	11	11
Septicemia *E. coli*	6	7	13	5	5	10	1	4	5
Coccidiosis	1	8	9	2	9	11	1	6	7

Comorbidities were implicated in 117 deaths. At least 10 cases for all cattle (beef and dairy combined) were reported for BRD and salmonellosis (n = 20), BRD and BVD (n = 16), BRD and coccidiosis (n = 15), BRD and trichostrongylosis (n = 13), and salmonellosis and scouring pathogens (n = 11).

Discussion

Nutritional diseases have a complex, multifactorial etiology.[21,22,23,24,25] We infer from epidemiology that if specific microminerals are "necessary and sufficient"[17,22] to cause a specific disease, the matrix pattern should be dissimilar for healthy and diagnostic cases. Disentangling correlated risk factors in managed herds can be complex, and diagnostic cases often require additional assumptions to account for unknown histories, diets and husbandry practices. Furthermore, the interpretation of ANOVA differs for transformed data; for example, the analysis of logarithms involves the multiplication (not addition) of the arithmetic values. Production and research herd studies often use non-probability "convenience samples" to demonstrate that deficiency rates are high for cases (sensitivity) and low for noncases (specificity). However, a herd is often smaller than is statistically required to conclude that most cases, but few noncases, have both mineral deficiencies and the associated disease.

Correlation matrices of MM-associated diseases and mineral status (deficient or normal/high) differ for beef and dairy cattle. The CAHFS data did not include healthy cattle. Tissue interactions include a secondary tissue deficiency of Cu and Se, reported to be caused by decreased dietary availability with increasing dietary Mo.[10,11,12,26] Tissue Cu levels are very low when dietary Zn levels are high and accumulate to toxic levels when dietary Zn is very low.[11,12,13] High dietary Cu can be attenuated with added Se.[11,12,27] Contrarily, metabolic interactions between Se and Cu decrease the toxicity of selenium alone.[27] Some interactions are tissue-specific, such as Cu with Se (R = 0.43), Zn (R = 0.33) and Mn (R = 0.46) in the kidney (R > 0.3) but not the liver, and copper with lead only in the liver (R = 0.88).[12]

Correlations are not causal relationships. Epidemiological causality studies compare micromineral concentrations and correlations in sick and healthy herds using a tolerance threshold for the condition.[16,17] The limitation is that most diseases are multifactorial, in which environmental, infectious agents, nutrition (not just micronutrients) and management factors predispose individuals to disease. Nonetheless, these studies are commonly used to associate subclinical micromineral deficiencies with increased pathologies at the herd level compared to healthy herds. One study reported that inadequate copper status was not associated with adult disorders in pregnant females but was a significant risk factor for poor calf performance (e.g., growth retardation, OR 10.9) or health (perinatal mortality, OR 4.0; diarrhea, OR 3.6; vaccination failure, OR 5.0; heart failure, OR 9.4).[16] Se deficiency was associated with adult female disorders (e.g., lower fertility, OR 2.5; abortion, OR 6.3; retained placenta OR 5.9; metritis, OR 2.7) and calf disorders (growth retardation, OR 5.3; perinatal mortality, OR 30.8; diarrhea, OR 13.8; vaccination failure, OR 15.4; nutritional myopathy, OR 77.5).[16,17]

The extent to which deficiency compromises vaccine efficacy has been rarely investigated.[28,29] Sixteen beef animals vaccinated for BRD pathogens developed BRD. Selenium and copper were severely deficient in 10 animals, and 6 others had either a Cu or Se deficiency. Twenty-five adult beef (> 730 days old) developed BRD; 19 had severe Cu and/or Se deficiency. Injecting MM improved BRD vaccination responses.[29] Severe scours in dairy cattle were attributed to deficient Se,[29] Mo toxicosis,[11,12] and "inadequate" Cu in newborn calves.[30,31] The CAHFS data supported a link between scours and deficient Mn (87%; 45/52 cases) but not deficient Cu (1/104 dairy cases) or Se (11/104 dairy cases). Besides, only 3 out of 20 scouring beef calves were Se deficient.

The association between subclinical trace mineral deficiencies and pathological disorders is not easily determined, as disorders can have a multifactorial origin.[21,22,23,24,25] For example, although BRD is ultimately an infectious disease, it is a multifaceted problem with other predisposing factors also at play.[22,24,29] The stresses due to weaning, marketing, transportation and the plane of nutrition are more likely to affect the immune system when commingling exposes animals to infectious agents.[22,29] "These stressors may be 'necessary, but not sufficient' components, thus requiring an additive effect to manifest as disease."[22,29]

An initial step in any investigation should rule out factors that could enhance the disease risk. In a study of best management practices, low-risk calves originated from a single source and had undergone a preconditioning program that included vaccination, castration, dehorning, weaning and adaptation to feed bunk.[23]

Two factors contributed to scours and BRD in the CAHFS cattle. A failure of passive transfer was found in 65.5% (40/61) of neonatal calves that died from scours or bacterial septicemia in the first weeks of life. For beef calves that developed BRD, a history of recent weaning, transport and commingling was frequently mentioned in the submission forms. Typical management factors in the beef industry would also suggest that no preconditioning was implemented in these calves, making them more vulnerable to developing BRD. Despite the multiple factors predisposing calves to BRD, administering injectable trace minerals to highly stressed heifers at initial processing improved weight gains and feed efficiency during the receiving period and, most important, reduced the rate of BRD morbidity and costs of antibiotic treatment.[24] The percentage of dairy cattle diagnosed with BRD (14.5%) was similar to the BRD mortality (19.3%) in pre-weaned dairy calves from 5 large dairy herds across California.[25] The high rates of BRD accompanied by Cu or Se deficiency in beef (68.3%) compared to dairy (10.8%) cattle in the CAHFS data suggest that MM deficiencies predispose beef cattle to BRD pathogens.

Epidemiologists distinguish between internal validity, which applies to the study itself, and external validity, which pertains to other populations or the characteristics of a specific measure.[32] Whether the biological basis is known, internal validity should always be in the protocol. We examined the internal validity of the CAHFS data using graphs and tabulations. Most pathologies were diagnosed infrequently, as were associations with nominal concentrations (deficient, normal, high). Frequent pathologies were examined for product class and age subsets, and correlations were used to identify micromineral associations of tissue concentrations. Frequencies of BRD in beef and dairy cattle, as well as scours in dairy cattle, were sufficient to evaluate associations with multiple deficiencies of Cu, Se and Mn. Fetal deaths and skeletal malformations that are often attributed to deficient Mn during gestation were rare.[14,15,33,34,35]

We assessed external validity by comparing CAHFS data with a similar published dataset of hepatic Cu, Se and Mn concentrations, which lacked information on pathology and production class.[36] Also, a laboratory study important to our internal and external validity reported microminerals from 6 sections of the livers from 10 Holstein-Friesian (HF), 10 Galician Blond (GB), and 10 GB ´ HF crosses (all aged 10 months).[37] "The distribution of all trace elements, except cobalt and zinc, varied significantly across the liver."[37] The specific liver section(s) analyzed were not reported in the CAHFS data and were not specified in most other studies.

To plan a successful intervention program, it is essential to identify the steps in the causal chain that can be manipulated, which requires a deeper understanding of disease etiology beyond simple concepts, such as agent causality.[38] Our research used micromineral concentrations to illuminate cattle health reported in a diagnostic database. Cited studies support that low or high micromineral concentrations increased the risk of some diseases in the CAHFS cattle (Tables 4 and 5).

Conclusion

Establishing a herd's mineral status starts with authoritative diet recommendations,[20,39,40,41] and is confirmed by liver concentrations from statistically random-sampled cattle. Because our data set did not include healthy individuals from the same herds, limited conclusions can be made on the true Cu and Se status of the bovine population in California cattle. Based on postmortem liver samples, we can conclude there is a big difference between the percentage of beef and dairy cattle that were deficient in Cu and/or Se, allowing us to infer that beef, but not dairy cattle, continue to be largely deficient in both elements. By contrast, the high percentage of dairy cattle with Cu and Se concentration above the normal range suggests that dairy herds were over-supplemented in both elements. It was striking to find such a big difference between the type of pathologies diagnosed in dairy and beef cattle, with salmonellosis and scours being the two primary diseases affecting dairy but not beef cattle. By contrast, the main disease affecting beef cattle was BRD that was accompanied by Cu and/or Se deficiency in 68.3% of cases. However, because MM concentrations in the remainder of the herd were not obtained, this retrospective study cannot establish causal relationships between Cu and Se deficiencies with the identified pathologies.

Our primary motivation was to determine whether common structural patterns in correlation matrices reproducibly differentiated disease subsets based on production class, age and diagnosis. The predominant subset correlation patterns were unstructured (UN), meaning all variances and covariances (or correlations) differed within a subset. Patterns for different subsets also differed. Depending on the hypothesis, analysis of micromineral correlation patterns could be informative of internal validity or question the external validity of one's understanding.

Acknowledgments

We thank Dr. Diego Luna and Dr. Marta Miranda for data from healthy calves we used in unreported simulation studies. The reviewers and editors provided valuable advice on organization and content. Additional grammar corrections were identified using Grammarly.

Funding

The authors received no financial support for the research, authorship or publication of this article.

Conflicts of interest

The authors declare that they have no potential conflicts of interest regarding this article's research, authorship or publication.

Author contributions

DV conceived the study and collected the data during a self-funded trip to the University of California, Davis (2022). He compiled and reviewed publications on selenium, copper and manganese deficiency. He wrote the initial draft, edited all subsequent drafts, and approved the final version. DJS conducted literature searches to identify statistical relationships and correlation patterns in bovine (and other species) tissues, performed all statistical analyses, prepared the figures, and created the data tables. He wrote all the subsequent drafts and approved the final version.

References

1. Kegley EB, Ball JJ, Beck PA. Impact of mineral and vitamin status on beef cattle immune function and health. *J Anim Sci.* 2016;94:5401-5413. **https://doi:10.2527/jas.2016-0720**

2. Larson CK. Role of trace minerals in animal production. What do I need to know about trace minerals for beef and dairy cattle, horses, sheep and goats? Presented at the 2005 Nutrition Conference sponsored by the Department of Animal Science, UT Extension, and University Professional and Personal Development, The University of Tennessee. **http://animalrange.montana.edu/documents/courses/ANSC320/ConnieLarsenTraceminerals.pdf**

3. Wikse S. The relationship of trace element deficiencies to infectious diseases of beef calves. In: Proceedings of the Texas Beef Cattle Short Horn Course. College Station (TX): Texas A&M University; 1992, p 8.

4. Olson KC. Management of mineral supplementation programs for cow-calf operations. *Vet Clin North Am Food Anim Pract*. 2007;23(1): 69-90. **https://doi:10.1016/j.cvfa.2006.11.005**

5. Last JM. Commentary: The Iceberg revisited. *Int J Epidemiol.* 2013;42(6):1613–1615, **https://doi:10.1093/ije/dyt112**

6. Last JM, Adelaide DPH. The Iceberg: 'completing the clinical picture' in general practice, *Int J Epidemiol.* 2013;42(6):1608-1613. **https://doi:10.1093/ije/dyt113**

7. Johnson B. The Mineral Wheel PD1611. **https://www.abcplus.biz/Site_Free_Choice_ Minerals**

8. Tessman RK, Lakritz J, Tyler JW, Casteel SW, Williams JE, Dew RK. Sensitivity and specificity of serum copper determination for detection of copper deficiency in feeder calves. *J Am Vet Med Assoc*. 2001; 218:756-760. **https://doi:10.2460/javma.2001.218.756**

9. Davy JS, Forero LC, Shapero MWK, Rao DR, Becchetti TA, Rivers CK, Stackhouse JW, DeAtley KL, McNabb BR. Mineral status of California beef cattle. *Transl Anim Sci.* 2019;3:66-73. **https://doi:10.1093/tas/txy114**

10. Miller WJ. New concepts and developments in metabolism and homeostasis of inorganic elements in dairy cattle. A review. *J Dairy Sci*. 1975;58:1549-1560. **https://doi:10.3168/jds.s0022-0302(75)84751-5**

11. Gawthorne JM: Copper interactions. In Howell JM, Gawthorne JM (eds): *Copper in Animals and Man, Volume 1.* Boca Raton, FL, CRC Press, 1987, 79-99.

12. Thompson LJ, Hall JO, Meerdink GL. Toxic effects of trace element excess. *Vet Clin North Am Food Anim Pract*. 1991;7(1):277-306. **https://doi:10.1016/s0749-0720(15)30818-5**

13. Blanco-Penedo I, Cruz JM, López-Alonso M, Miranda M, Castillo C, Hernández J, Benedito JL. Influence of copper status on the accumulation of toxic and essential metals in cattle. *Environ Internat.* 2006;32(7):901-906. **https://doi:10.1016/j.envint.2006.05.012**

14. Valero G, Alley MR, Bidco LM, Manktelow BW, Merrall M, Lawes GS. Chondrodystrophy in calves associated with manganese deficiency. *NZ Vet J.* 1990;38(4):161-167. **https://doi:10.1080/00480169.1990.35645**

15. Schaeffer D, Villar D. Evidence for and against manganese deficiency as causal for congenital joint deficiency disease or death in fetal and neonatal cattle. *J Vet Diagn Invest.* 2024;36(6):827-831. **https://doi:10.1177/10406387241257672**

16. Enjalbert F, Lebreton P, Salta O. Effects of copper, zinc, and selenium status on performance in commercial dairy and beef herds: retrospective study. *J Anim Physiol Anim Nutr* (Berl). 2006;90(11-12):459-466. **https://doi:10.1111/j.1439-0396.2006.00627.x**

17. Guyot H, Saegerman C, Lebreton P, Sandersen C, Rollin F. Epidemiology of trace elements deficiencies in Belgian beef and dairy cattle herds. *J Trace Elem Med Biol.* 2009;23(2):116-123. **https://doi:10.1016/j.jtemb.2009.02.006**

18. Herdt TH, Wisnieski L, Buchweitz J. Random-effects linear model application to herd-level assessment of bovine hepatic trace mineral concentrations. *J Vet Diag Invest.* 2021;33(3):469-478. **https://doi:10.1177/10406387219993**

19. Conover WJ, Iman RL. Rank transformations as a bridge between parametric and nonparametric statistics. *Am Stat*. 1981;35(3):124-130. **https://doi:10.2307/2683975**

20. Puls R. *Mineral Levels in Animal Health. Diagnostic Data*. Clearbrook, British Columbia: Sherpa International, 1994

21. Schinwald M, Creutzinger K, Keunen A, Winder CB, Haley D, Renaud DL. Predictors of diarrhea, mortality, and weight gain in male dairy calves. *J Dairy Sci.* 2022;105(6):5296-5309. **https://doi:10.3168/jds.2021-21667**

22. Taylor JD, Fulton RW, Lehenbauer TW, Step DL, Confer AW. The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors? *Can Vet J*. 2010;51(10):1095-1102.

23. Wilson BK, Richards CJ, Step DL, Krehbiel CR. Best management practices for newly weaned calves for improved health and well-being. *J Anim Sci.* 2017;95(5):2170-2182. **https://doi:10.2527/jas.2016.1006**

24. Richeson JT, Kegley EB. Effect of supplemental trace mineral and performance of highly stressed, newly received beef heifers. *Prof Anim Sci*. 2011;27(5):461-466. **https://doi:10.15232/S1080-7446(15)30519-2**

25. Dubrowsky SA, Eenennaam ALV, Karle BM, Rossito PV, Lehenbauer TW, Aly SS. Bovine respiratory disease (BRD) causes specific and overall mortality in preweaned calves in California dairies. The BRD 10K Study. *J Dairy Sci.* 2019;102:7320-7328. **https://doi:10.3168/jds.2018-15463**

26. Hill CH. Interrelationships of selenium with other trace elements. *Fed Proc*. 1975;34(11):2096-2100. PMID: 1175797

27. Perdrizet U, Blakley B, Al Dissi A. Concentrations and deficiencies of minerals in cattle submitted to a diagnostic laboratory in Saskatchewan from 2003-2012: A retrospective study. *Can Vet J*. 2020; 61(1):57-63.

28. Cerone S, Sansinanea A, Auza N. Copper deficiency alters the immune response of bovine. *Nutr Res*. 1995;15(9):1333-1341. **https://doi:10.1016/0271-5317(95)02008-J**

29. Palomares RA. Trace minerals supplementation with great impact on beef cattle immunity and health. *Animals.* 2022;12, 2839. **https://doi.org/10.3390/ani12202839**

30. Corbett RB. Trace mineral nutrition in confinement dairy cattle. *Vet Clin North Am Food Anim Pract.* 2023;39(3):425-438. **https://doi:10.1016/j.cvfa.2023.06.004**

31. Naylor JM, Kasaru TR, Blakley BR, Townsend HGG. Diagnosis of copper deficiency and effects of supplementation in beef cows. *Can J Vet Res.* 1989;53(3):343-348. PMID: 2766156

32. Foxman B. Chapter 8 – Determining the reliability and validity and interpretation of a measure in the study populations. In: Foxman B, ed. *Molecular Tools and Infectious Disease Epidemiology*. Academic Press. 2012:117-132. **https://doi:10.1016/B978-0-12-374133-2.00008-3**

33. Howes AD, Dyer IA. Diet and supplemental mineral effects on manganese metabolism in newborn calves. *J Anim Sci.* 1971;32(1):141-145. **https://doi:10.2527/jas1971.321141x**

34. Rojas MA, Dyer IA, Cassatt WA. Manganese deficiency in the bovine. *J Anim Sci.* 1965;24:664-667. **https://doi:10.2527/jas1965.243664x**

35. Staley GP, van der Lugt JJ, Axsel G, Loock AH. Congenital skeletal malformations in Holstein calves associated with putative manganese deficiency. *J S Afr Vet Assoc*. 1994;65(2):73-78. PMID: 7776338

36. Counotte G, Holzhauer M, Dijken SC van, Muskens J, Merwe DV der. Levels of trace elements and potentially toxic elements in bovine livers: A trend analysis from 2007 to 2018. *PLoS One.* 2019;14(4): e0214584. Database version 1.

37. Luna D, Miranda M, Pereira V, López-Alonso M. Variation in trace element content between liver lobes in cattle. How important is sampling site? *J Trace Elem Miner.* 2019; 2:53-57. **https://doi:10.1016/j.jtemb.2018.11.008**

38. Hancock DD, Wikse SE. Investigation planning and data gathering. *Vet Clin North Am Food Anim Pract.* 1988;4(1):1-15. **https://doi:10.1016/S0749-0720(15)31072-0**

39. National Research Council (NRC). Nutrient Requirements of Beef Cattle. Eighth Revised Edition. Washington, DC: The National Academies Press. 2016. **https://doi:10.17226/19014**

40. National Research Council. Nutrient Requirements of Dairy Cattle, Eighth Revised Edition. Washington, DC: The National Academies Press. 2021. **https://doi:10.17226/25806**

41. Olson KC. Management of mineral supplementation programs for cow-calf operations. *Vet Clin North Am Food Anim Pract.* 2007;23(1):69-90. **https://doi:10.1016/j.cvfa.2006.11.005**

