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Correlations of milk and serum element concentrations with production and management traits in dairy cows

S. J. Denholm,[1]* T. N. McNeilly,[2] S. Bashir,[3] M. C. Mitchell,[2] E. Wall,[1] and A. A. Sneddon[3]
[1]Scotland's Rural College, Peter Wilson Building, King's Buildings, Edinburgh, EH9 3JG, Scotland
[2]Moredun Research Institute, Pentlands Science Park, Midlothian, EH26 0PZ, Scotland
[3]The Rowett Institute, University of Aberdeen, Aberdeen, AB25 2ZD, Scotland

ABSTRACT

The present study investigated the potential consequences, positive or negative, that selection for favorable production-related traits may have on concentrations of vitamin B_{12} and key chemical elements in dairy cow milk and serum and the possible impact on milk healthiness, and associated benefits, for the dairy product consumer. Milk and serum samples (950 and 755, respectively) were collected from Holstein-Friesian dairy cows (n = 479) on 19 occasions over a 59-mo period, generating 34,258 individual records, and analyzed for concentrations of key trace and quantity elements, heavy metals, and milk vitamin B_{12}. These data were then matched to economically important production data (milk, fat, and protein yield) and management data (dry matter intake, liveweight, and body condition score). Multivariate animal models, including full pedigree information, were used to analyze data and investigate relationships between traits of interest. Results highlighted negative genetic correlations between many quantity and trace elements in both milk and serum with production and management traits. Milk yield was strongly negatively correlated with the milk quantity elements Mg and Ca (genetic correlation between traits, $r_a = -0.58$ and -0.63, respectively) as well as the trace elements Mn, Fe, Ni, Cu, Zn, and Mo ($r_a = -0.32$, -0.58, -0.52, -0.40, -0.34, and -0.96, respectively); and in serum, Mg, Ca, Co, Fe, and Zn ($r_a = -0.50$, -0.36, -0.68, -0.54, and -0.90, respectively). Strong genetic correlations were noted between dry matter intake with V ($r_a = 0.97$), Fe ($r_a = -0.69$), Ni ($r_a = -0.81$), and Zn ($r_a = -0.75$), and in serum, strong negative genetic correlations were observed between dry matter intake with Ca and Se ($r_a = -0.95$ and -0.88, respectively). Body condition score was negatively correlated with serum P, Cu, Se, and Pb ($r_a = -0.45$, -0.35, -0.51, and -0.64, respectively) and positively correlated with Mn, Fe, and Zn ($r_a = 0.40$, 0.71, and 0.55, respectively). Our results suggest that breeding strategies aimed at improving economically important production-related traits would most likely result in a negative impact on levels of beneficial nutrients within milk for human consumption (such as Mg, Ca, Fe, Zn, and Se).
Key words: micronutrient, heavy metal, dairy cow, production, management data

INTRODUCTION

Milk and milk-derived products (e.g., butter, cheese, yogurt, and ice cream) are a major source of micronutrients, vital dietary components required by humans to maintain normal body function, prevent deficiencies, and remain healthy (FAO and WHO, 2004; Gernand et al., 2016). In Western diets, dairy products have been shown to be the main source of calcium (Ca) intake and contribute 54% to the daily global dietary intake of Ca (Devriese et al., 2006). In the United Kingdom, dairy products provide 60%, 60%, 55%, 10%, and 150% of Reference Nutrient Intake (i.e., the minimum amounts of micronutrient required in a healthy diet; WHO, 1996) for Ca, phosphorus (P), iodine (I), magnesium (Mg), and vitamin B_{12}, respectively (Henderson et al., 2003a,b; Kliem and Givens, 2011). It is also important to highlight that micronutrients are not only key for human nutrition and health, but they also have economic importance and value in downstream processing procedures. Magnesium, P, and Ca play a crucial role in milk coagulation, a key component in cheese production and other high-value dairy products (Lucey and Fox, 1993; Malacarne et al., 2006; Toffanin et al., 2015a). Milk is an extremely valuable agricultural commodity, contributing 27% and 10% to the global value added (i.e., gross income) of livestock and agriculture, respectively. Statistics published by the Food and Agriculture Organization of the United Nations (**FAO**) show that dairy cows provide the main source of milk in the world (estimated 83% of global milk production), followed by buffalo (13%), goats (2%), sheep (1%), and camels (<1%) (FAO, 2019). Thus, a program aimed

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*Corresponding author: scott.denholm@sruc.ac.uk

at increasing the levels of nutrients in such products would not only benefit the health of dairy consumers, it would also be likely to have economic benefits.

Previous work has highlighted that element levels in dairy cow milk and serum are significantly influenced by dietary intake (Rooke et al., 2010; Denholm et al., 2019). Furthermore, many element concentrations have been shown to be significantly heritable in both milk (van Hulzen et al., 2009; Buitenhuis et al., 2015; Visentin et al., 2019) and serum (Morris et al., 2006; Tsiamadis et al., 2016; Denholm et al., 2019).

This study builds on previous analyses with the objective of investigating the relationships between micronutrient and heavy metal concentrations in dairy cow milk and serum with economically important production and management traits. Exploring such correlations will highlight any potential consequences, positive or negative, that selection for enhanced production may have on milk healthiness for the dairy product consumer. For the purposes of the present study, we focus on the quantity elements sodium (Na), Mg, P, potassium (K), and Ca; the trace elements vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo), and I; the heavy metals cadmium (Cd), mercury (Hg), and lead (Pb); and the vitamin cobalamin, more commonly known as vitamin B_{12}.

MATERIALS AND METHODS

Animals

Dairy cows were Holstein-Friesian (n = 479) and involved in an ongoing, long-term selection experiment (selected since 1973) for genotype × environment, simulating the variety of UK dairy systems (Veerkamp et al., 1994). Cows were from the Langhill pedigree herd housed at the Dairy Research Centre of Scotland's Rural College, Crichton Royal Farm, Dumfries, Scotland, between 2012 and 2016 and were within their first to fifth lactation (inclusive). Cows were equally distributed across 2 genetic lines (control and select) and were either daughters of sires selected for producing milk in line with the UK national average genetic merit for kilograms of fat and protein or daughters of sires selected for producing milk with the highest genetic merit available in the United Kingdom (top 5%) for fat and protein (Veerkamp et al., 1994). For the present study, 229 cows were from the control line and 250 from the select. Within each genetic line, cows were equally assigned 1 of 2 divergent diets: a low-forage, high-energy ration based on by-products and minimal land use, simulating high-input commercial systems; and a high-forage, lower-energy ration based on homegrown components and using the maximum amount of land available, thus simulating low-input grazing systems (Pryce et al., 1999; Roberts and March, 2013). The UK average milk yield per cow/lactation is approximately 7,956 L (Uberoi, 2020); for comparison, the high-merit cows have a target milk yield of 7,500 and 13,000 L per lactation for cows on the low- and high-energy diet, respectively.

Ethics Statement

Blood sample collection was conducted in accordance with UK Home Office regulations (PPL No: 60/4278 Dairy Systems, Environment and Nutrition), and procedures were approved by the Animal Experimentation Committee of Scotland's Rural College. Otherwise, the study was restricted to routine on-farm observations and measurements that did not inconvenience or stress the animals.

Milk and Blood Sampling

Protocol. Cows were routinely milked 3 times per day in the morning (**AM**), afternoon (**MD**), and evening (**PM**). Whole milk samples were collected at 16 sample points between June 2012 and January 2015 with repeated sampling of individual cows. These samples were selected from AM milks to coincide with any blood sampling taking place at that time. Blood samples were collected at 13 sample points between April 2013 and May 2016. Both sampling windows included summer and winter periods and were distributed throughout the 305-d lactation. When possible, milk and blood samples were collected on the same day. In total, 950 milk samples and 766 serum samples were collected such that they accounted for genotype (47% and 53% from control and select cows, respectively) and management (49% and 51% for by-product and homegrown diets, respectively) of cows to give a balanced representation of the herd. Milk production (AM only) ranged from 0 kg to 23.1 kg (average 10.1 kg) on the day of sampling. Samples were evenly distributed across the early, middle, and late stages of lactation (31%, 35%, and 34%, respectively). Further information regarding sample collection is presented in Table S2 of the supplementary information accompanying Denholm et al. (2019).

Preparation and Processing. A subset of milk samples collected as part of a previous project (Denholm et al., 2017; Denholm et al., 2018) were analyzed for concentrations of vitamin B_{12}. In summary, 256 samples were collected over 11 sample points from 64 cows (50% control, 50% select; 48% by-product feed, 52% homegrown) and evenly distributed across lactation (31%, 32%, and 37% for early, middle, and late lactation, respectively). These samples were centrifuged at 3,000 × g for 30 min at 4°C and the skim milk fraction retained from below the fat layer using a fine-tipped Pastette. Whole blood samples were collected into plain Vacutainers (BD) with blood allowed to coagulate before centrifugation at 2,000 × g for 10 min at 18°C and the serum retained. All milk and serum samples were stored at −20°C before analysis.

Analysis of Quantity Elements, Trace Elements, Heavy Metals, and Vitamin B_{12}. Inductively coupled plasma mass spectrometry analysis was used to quantify concentrations of quantity elements (Na, Mg, P, K, and Ca), essential trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, and I), and heavy metals (Cd, Hg, and Pb) in milk and serum. For measurement of vitamin B_{12} in undiluted milk, a commercial competitive assay (RIDAscreen, R-Biopharm) was used. Analyses were carried out according to the methods previously published by our group (Denholm et al., 2019).

Recording of Management Traits. Data relating to production and management were collected and matched to each cow's corresponding element profile at time of sampling base on week in milk. When possible, data were matched to element information on the same day, ± 1 d. These data included economically important traits such as milk yield and composition traits, which included total milk yield (kg), milk fat content (%), and protein content (%), as well as management-related traits, such as DMI (kg), liveweight (**LWT**, kg), and BCS.

Total milk yield was recorded each day throughout lactation and was the sum of 3 daily milkings (AM, MD, and PM); milk fat and protein content were recorded on a weekly basis on a Tuesday (±1 d)—this was, in general, the same day as any element sampling. Values obtained for milk fat and protein content were used to calculate total fat and protein yields (kg) by multiplying them by the corresponding daily milk yield. Feed was offered to animals via individual feed bins with daily individual feed intake (**FI**, kg) recorded using HOKO automatic feed measurement gates (HOKO-system, Hokofarm Group); DMI was calculated from daily FI as DMI = FI(DM/1,000), where DM (g/kg) was the dry matter content of the feed, determined in a 60°C oven; LWT was recorded daily using a gated weighing system and was the average of 3 weigh periods (AM, MD, and PM) postmilking. Finally, BCS was collected by the same farm technician on a weekly basis (Tuesday ± 1 d) for the entirety of each sampling period. Animals were scored 0 to 5 in 0.25 increments such that lower scores indicated thinner animals and higher scores indicated fatter animals (Lowman et al., 1976). For the purposes of analysis, weekly trait averages (per week in milk) were used for the final data set.

Data Preparation and Preprocessing. Element, vitamin B_{12}, and production data (traits) were combined with individual animal information, resulting in a data set with 36,789 individual trait data points (milk and serum elements, B_{12}, and production and management traits), before being subjected to quality-control measures. For the purposes of the present study, the interquartile range (**IQR**) was calculated for each trait with any values falling out with $Q_1 - 1.5 \times \text{IQR} < x < Q_3 + 1.5 \times IQR$ (where Q_1 and Q_3 are the first and third quartiles, respectively) considered an outlier and removed from the data set. Additionally, all null values were removed, as were values below the detection limit of the test used to quantify chemical elements and vitamin B_{12}. In total 2,531 individual data points were removed. This included 2,209 records from the micronutrient data, 158 records from milk yield and composition data, and 164 records from the DMI, LWT, and BCS data. To ensure normality, any skewed data were log-transformed before analysis. The final data set contained 34,258 individual trait records and is summarized in Table 1.

Regarding the milk yield and composition traits, milk yield averaged 28.06 kg/d, with fat and protein content averaging 3.76% (1.04 kg) and 3.18% (0.89 kg), respectively. Variability was determined by the coefficient of variation (**CV**, %). Moderate to large CV were observed in the element traits ranging from 11% (K) to 156% (Co) and 7% (Na) to 133% (Mn) in milk and serum, respectively. In the production and management traits, CV were lower and ranged from 10% (protein %) to 40% (DMI).

Statistical Analyses

Investigation into correlations between element concentrations (including vitamin B_{12}) and production and management traits was carried out via a series of multivariate analyses of a mixed-effects linear animal model (1). Fixed effects included in the model were diet group, genetic group, lactation number, weeks in milk, year × season of calving interaction, and year × month of record interaction. Cow was fitted as a random effect to account for the additive genetic effect of the nth individual cow, with genetic relationships between individuals accounted for by fitting a pedigree relationship matrix (7 generations). The permanent environmental effect of the nth individual cow was also fitted as a

Table 1. Descriptive statistics of the element (including vitamin B_{12}) and production data[1]

Trait	Count[2]	Mean	SD	Minimum	Maximum	CV%	σ_p^2	h^2
Milk element								
Sodium[3] (Na, mg/L)	878 (946)	364.16	83.97	148.92	602.97	23.06	5,292.90	0.00
Magnesium[3] (Mg, mg/L)	908 (946)	113.19	15.58	69.74	155.98	13.76	197.65	0.31*
Phosphorus[3] (P, mg/L)	904 (946)	946.18	135.04	586.19	1,300.76	14.27	13,164.00	0.14*
Potassium[3] (K, mg/L)	892 (946)	1,774.39	191.81	1,239.38	2,301.43	10.81	28,720.00	0.12
Calcium[3] (Ca, mg/L)	900 (946)	1,192.18	181.14	679.46	1,684.86	15.19	20,639.00	0.20*
Vanadium[4] (V, µg/L)	186 (229)	2.70	1.96	0.06	7.22	72.43	3.69	0.37
Chromium[4] (Cr, µg/L)	726 (870)	25.54	20.14	0.29	104.68	78.87	327.60	0.07
Manganese[4] (Mn, µg/L)	889 (939)	38.08	14.49	1.06	83.46	38.05	156.58	0.25*
Iron[4] (Fe, µg/L)	848 (938)	1,077.02	886.26	41.22	4,017.03	82.29	606,640.00	0.02
Cobalt[4] (Co, µg/L)	411 (485)	4.30	6.71	0.06	30.40	156.12	27.24	0.01
Nickel[4] (Ni, µg/L)	321 (387)	427.28	463.02	0.86	2,113.01	108.37	163,940.00	0.05
Copper[4] (Cu, µg/L)	916 (920)	120.77	78.20	0.96	386.82	64.75	2,768.70	0.19*
Zinc[4] (Zn, µg/L)	919 (946)	4,348.23	938.97	1,916.91	6,833.17	21.59	831,030.00	0.28*
Selenium[4] (Se, µg/L)	916 (946)	19.10	4.67	6.83	31.81	24.46	10.46	0.15*
Molybdenum[4] (Mo, µg/L)	888 (943)	37.20	12.14	6.26	71.86	32.63	73.85	0.23*
Iodine[4] (I, µg/L)	448 (473)	1,448.35	610.52	161.20	3,290.90	42.15	269,630.00	0.21
Cadmium[5] (Cd, µg/L)	733 (800)	0.16	0.10	0.01	0.45	61.80	0.01	0.00
Mercury[5] (Hg, µg/L)	417 (526)	0.31	0.26	0.01	1.04	83.35	0.06	0.14
Lead[5] (Pb, µg/L)	829 (897)	4.82	3.17	0.02	15.72	65.75	7.85	0.00
Vitamin B_{12} (B_{12}, µg/L)	247 (253)	0.82	0.38	0.50	1.75	47.08	0.09	0.00
Serum element								
Sodium[3] (Na, mg/L)	169 (177)	3,133.18	210.64	2,533.07	3,757.49	6.72	17,740.00	0.17
Magnesium[3] (Mg, mg/L)	734 (754)	23.01	5.11	9.79	36.62	22.22	20.81	0.17*
Phosphorus[3] (P, mg/L)	743 (754)	139.07	44.97	20.75	260.98	32.33	967.81	0.10
Potassium[3] (K, mg/L)	732 (754)	206.02	35.54	105.38	302.45	17.25	926.37	0.23*
Calcium[3] (Ca, mg/L)	707 (754)	105.37	17.76	58.03	153.36	16.86	258.83	0.12
Vanadium[4] (V, µg/L)	584 (589)	0.68	0.21	0.15	1.25	31.15	0.03	0.06
Chromium[4] (Cr, µg/L)	221 (405)	0.89	0.76	0.00	4.23	85.71	0.52	0.00
Manganese[4] (Mn, µg/L)	626 (737)	6.46	8.61	0.07	36.70	133.22	12.08	0.05
Iron[4] (Fe, µg/L)	654 (754)	2,179.44	1,144.67	292.15	6,157.91	52.52	614,420.00	0.07
Cobalt[4] (Co, µg/L)	610 (754)	1.34	0.68	0.11	4.39	50.73	0.21	0.03
Nickel[4] (Ni, µg/L)	392 (439)	3.85	2.71	0.01	12.14	70.31	7.23	0.00
Copper[4] (Cu, µg/L)	726 (754)	601.02	195.51	108.52	1,113.46	32.53	25,133.00	0.33*
Zinc[4] (Zn, µg/L)	626 (754)	910.90	330.06	201.57	2,109.51	36.23	46,066.00	0.11
Selenium[4] (Se, µg/L)	717 (754)	75.96	18.58	26.11	124.59	24.45	261.75	0.07
Molybdenum[4] (Mo, µg/L)	662 (754)	14.92	13.62	1.26	62.88	91.30	90.72	0.00
Cadmium[5] (Cd, µg/L)	666 (741)	0.11	0.11	0.00	0.46	100.76	0.00	0.23*
Mercury[5] (Hg, µg/L)	265 (309)	3.43	3.22	0.00	14.89	93.97	10.79	0.46
Lead[5] (Pb, µg/L)	738 (738)	83.23	81.74	0.01	367.56	98.20	969.16	0.00
Production and management								
Milk yield (kg)	1,631 (1,643)	28.06	8.33	4.50	50.59	29.70	33.800	0.10
Fat yield (kg)	1,573 (1,643)	1.04	0.30	0.19	2.26	28.65	0.053	0.07
Protein yield (kg)	1,567 (1,643)	0.89	0.23	0.17	1.59	26.25	0.028	0.02
DMI (kg/d)	1,626 (1,646)	15.76	6.32	1.16	33.48	40.11	11.281	0.07
Liveweight (kg)	1,623 (1,762)	599.46	69.37	406.00	794.00	11.57	2358.100	0.34*
BCS[6] (0–5)	1,490 (1,495)	2.04	0.36	1.25	2.75	17.79	0.107	0.23*

[1]Phenotypic variances and heritability estimates for element concentrations are also presented. σ_p^2 = total phenotypic SD; h^2 = heritability.

[2]Total numbers of records before outlier removal given in parentheses.

[3]Quantity element.

[4]Trace element.

[5]Heavy metal.

[6]Scored in 0.25 increments, where higher value represents fatter animals.

*Significantly different from zero at $P < 0.05$.

random effect to account for repeated sampling of the same animal.

$$\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_{1i}\mathbf{a}_i + \mathbf{Z}_{2i}\mathbf{pe}_i + \mathbf{e}_i, \quad [1]$$

where $\mathbf{y}_i$ is a vector of observations for trait i; $\mathbf{b}_i$ is a vector of fixed effects associated with trait i; $\mathbf{a}_i$ is a vector of random additive genetic effects associated with trait i; $\mathbf{pe}_i$ is a vector of permanent environmental effects associated with trait i; $\mathbf{X}_i$, $\mathbf{Z}_{1i}$, and $\mathbf{Z}_{2i}$ are incidence matrices linking phenotypic records to fixed, additive genetic, and permanent environmental effects for trait i, respectively; and $\mathbf{e}_i$ is a vector of random residual effects associated with trait i.

Variance Components and Correlations

Variance components (additive genetic, permanent environmental, residual, and total phenotypic variance) were estimated (along with corresponding standard errors) from (1) using a restricted maximum likelihood (**REML**) approach (a form maximum likelihood estimation) and the software package ASReml version 3 (Gilmour et al., 2009). In contrast to maximum likelihood, REML produces unbiased estimates of both variance and covariance parameters (Patterson and Thompson, 1971).

Correlations (phenotypic, r_p; genetic, r_a; and permanent environmental, r_{pe}) between traits were calculated according to

$$r_{i,j} = \frac{\mathrm{cov}(i,j)}{\sqrt{\sigma_i^2 + \sigma_j^2}},$$

where i and j are traits of interest; $cov(i, j)$ is the covariance of traits i and j; σ_i^2 is the variance of trait i; and σ_j^2 is the variance of trait j. Furthermore, to capture the uncertainty when estimating correlation coefficients, confidence intervals (CI, 95%) were calculated to provided limits within which the true (population) value is likely to lie:

$$CI = \left(\frac{e^{2L}-1}{e^{2L}+1}, \frac{e^{2U}-1}{e^{2U}+1}\right),$$

$$L = z_r - \frac{z_{1-\alpha/2}}{\sqrt{n-3}},$$

$$U = z_r + \frac{z_{1-\alpha/2}}{\sqrt{n-3}},$$

$$z_r = 0.5 \times \ln\left(\frac{1+r}{1-r}\right),$$

where r is the correlation coefficient, α is the significance level, n is the sample size, L is the lower confidence limit, U is the upper confidence limit, and z_r is the z-score transformation of the correlation coefficient r.

In total, 684 correlations were calculated; as such, the Bonferroni correction method was used to adjust confidence intervals to account for multiple testing. Bonferroni-corrected confidence intervals (0.99994%) that contained zero were considered noninformative.

RESULTS

Correlations Between Element and Production and Management Traits

Additive genetic correlations of element concentrations with milk, fat, and protein yield and DMI, LWT, and BCS are presented in Table 2. Corresponding phenotypic and permanent environmental correlations are presented in Tables 3 and 4, respectively. Correlations between residuals are given for information in Supplemental Table S1 (https://doi.org/10.6084/m9.figshare.21108847.v1; Denholm et al., 2022). No significant correlations (genetic or phenotypic) were observed between production and management traits and vitamin B_{12}.

Milk Yield

Milk yield was observed to have a strong negative genetic correlation with Mg and Ca as well as Mn, Fe, Ni, Cu, Zn, and Mo. Strong positive genetic correlations between milk yield and milk V, Cr, and Co were also observed. Phenotypic correlations were observed with Na, K, and V. Permanent environmental correlations included K, Ca, and Zn.

In serum, milk yield was observed to have a similarly negative genetic correlation with Mg, Ca, Fe, and Zn. In contrast to milk Co, serum Co was also found to be negatively genetically correlated with milk yield. Phenotypic correlations included P and Pb. Permanent environmental correlations included P, Co, Ni, Zn, Hg, and Pb.

Fat Yield

Fat yield was negatively genetically correlated with Fe, Mo, and Hg; a moderate positive genetic correlation with milk I was also observed. No significant phenotypic correlations with fat yield and any of the milk elements were observed. A permanent environmental correlation was noted with Zn.

In serum, negative genetic correlations with fat yield were observed with Fe and Zn. Additionally, both Na and P were found to be positively genetically correlated with fat yield, as were Cu and Pb. No significant phenotypic correlations with fat yield and any of the serum elements were observed. Permanent environmental correlations included V and Ni.

Protein Yield

In contrast to fat, I was found to be negatively genetically correlated with protein yield and Fe positively

Table 2. Additive genetic correlations between element concentrations (including vitamin B_{12}) and milk, fat, and protein yield and DMI, liveweight, and BCS[1]

Trait	Milk yield (kg)	Fat yield (kg)	Protein yield (kg)	DMI (kg/d)	Liveweight (kg)	BCS (0–5)
Milk						
Sodium[2] (Na, mg/L)	—	—	—	—	—	—
Magnesium[2] (Mg, mg/L)	−0.58 (−0.69, −0.44)*	−0.18 (−0.35, 0.01)	—	0.03 (−0.15, 0.22)	−0.11 (−0.29, 0.08)	0.07 (−0.12, 0.25)
Phosphorus[2] (P, mg/L)	—	−0.04 (−0.22, 0.14)	—	−0.22 (−0.39, −0.04)*	0.21 (0.03, 0.38)*	0.02 (−0.17, 0.20)
Potassium[2] (K, mg/L)	0.11 (−0.07, 0.29)	0.09 (−0.09, 0.27)	—	0.28 (0.10, 0.44)*	−0.35 (−0.50, −0.18)*	0.08 (−0.10, 0.26)
Calcium[2] (Ca, mg/L)	−0.63 (−0.73, −0.51)*	0.14 (−0.04, 0.32)	—	0.20 (0.02, 0.37)*	−0.28 (−0.44, −0.11)*	−0.21 (−0.38, −0.03)*
Vanadium[3] (V, µg/L)	0.25 (0.07, 0.41)*	−0.26 (−0.42, −0.08)*	—	0.97 (0.96, 0.98)*	0.65 (0.53, 0.75)*	0.35 (0.18, 0.50)*
Chromium[3] (Cr, µg/L)	0.49 (0.33, 0.62)*	0.27 (0.09, 0.43)*	0.50 (0.35, 0.63)*	−0.25 (−0.41, −0.07)*	−0.16 (−0.34, 0.02)	−0.27 (−0.43, −0.09)*
Manganese[3] (Mn, µg/L)	−0.32 (−0.47, −0.14)*	−0.06 (−0.24, 0.13)	0.00 (−0.18, 0.18)	−0.39 (−0.54, −0.22)*	0.17 (−0.02, 0.34)	0.07 (−0.12, 0.25)
Iron[3] (Fe, µg/L)	−0.58 (−0.69, −0.45)*	−0.67 (−0.76, −0.56)*	0.77 (0.68, 0.84)*	−0.69 (−0.78, −0.58)*	—	—
Cobalt[3] (Co, µg/L)	0.86 (0.81, 0.90)*	—	—	—	—	—
Nickel[3] (Ni, µg/L)	−0.52 (−0.65, −0.38)*	—	—	−0.81 (−0.87, −0.74)*	−0.73 (−0.80, −0.63)*	−0.27 (−0.43, −0.09)*
Copper[3] (Cu, µg/L)	−0.40 (−0.54, −0.23)*	−0.13 (−0.30, 0.06)	−0.63 (−0.73, −0.51)*	−0.53 (−0.65, −0.39)*	−0.04 (−0.22, 0.15)	0.12 (−0.06, 0.30)
Zinc[3] (Zn, µg/L)	−0.34 (−0.49, −0.17)*	−0.19 (−0.36, −0.01)*	−0.22 (−0.39, −0.04)*	−0.75 (−0.82, −0.66)*	−0.24 (−0.41, −0.06)*	−0.20 (−0.37, −0.02)*
Selenium[3] (Se, µg/L)	—	−0.02 (−0.20, 0.16)	—	0.16 (−0.02, 0.33)	0.32 (0.15, 0.48)*	0.08 (−0.11, 0.26)
Molybdenum[3] (Mo, µg/L)	−0.96 (−0.97, −0.95)*	−0.44 (−0.57, −0.27)*	—	−0.04 (−0.22, 0.15)	0.52 (0.37, 0.64)*	0.26 (0.08, 0.43)*
Iodine[3] (I, µg/L)	−0.17 (−0.35, 0.01)	0.33 (0.16, 0.49)*	−0.34 (−0.49, −0.17)*	0.17 (−0.01, 0.34)	−0.00 (−0.19, 0.18)	−0.13 (−0.31, 0.05)
Cadmium[4] (Cd, µg/L)	—	—	—	—	—	—
Mercury[4] (Hg, µg/L)	0.04 (−0.14, 0.22)	−0.49 (−0.62, −0.34)*	0.12 (−0.06, 0.30)	−0.15 (−0.33, 0.03)	0.62 (0.49, 0.72)*	0.08 (−0.11, 0.26)
Lead[4] (Pb, µg/L)	—	—	—	—	—	—
Vitamin B_{12} (B_{12}, µg/L)	—	—	—	—	—	—
Serum						
Sodium[2] (Na, mg/L)	0.32 (0.14, 0.47)*	0.31 (0.14, 0.47)*	—	−0.41 (−0.55, −0.25)*	—	−0.66 (−0.75, −0.54)*
Magnesium[2] (Mg, mg/L)	−0.50 (−0.62, −0.34)*	−0.03 (−0.21, 0.15)	—	−0.12 (−0.30, 0.06)	−0.49 (−0.62, −0.33)*	−0.11 (−0.29, 0.08)
Phosphorus[2] (P, mg/L)	−0.06 (−0.24, 0.13)	0.30 (0.12, 0.46)*	−0.80 (−0.86, −0.72)*	−0.18 (−0.35, 0.01)	−0.78 (−0.84, −0.70)*	−0.45 (−0.59, −0.29)*
Potassium[2] (K, mg/L)	−0.15 (−0.32, 0.04)	−0.07 (−0.25, 0.12)	−0.85 (−0.89, −0.78)*	−0.36 (−0.51, −0.19)*	−0.63 (−0.73, −0.50)*	−0.27 (−0.43, −0.10)*
Calcium[2] (Ca, mg/L)	−0.36 (−0.51, −0.19)*	−0.14 (−0.32, 0.04)	−0.37 (−0.52, −0.21)*	−0.95 (−0.96, −0.93)*	−0.36 (−0.51, −0.19)*	−0.09 (−0.27, 0.10)
Vanadium[3] (V, µg/L)	0.08 (−0.10, 0.26)	0.10 (−0.09, 0.27)	—	−0.06 (−0.24, 0.12)	0.21 (0.03, 0.38)*	0.26 (0.08, 0.42)*
Chromium[3] (Cr, µg/L)	—	—	—	—	—	—
Manganese[3] (Mn, µg/L)	—	−0.05 (−0.23, 0.13)	−0.27 (−0.43, −0.10)*	0.02 (−0.17, 0.20)	0.16 (−0.02, 0.33)	0.40 (0.24, 0.55)*
Iron[3] (Fe, µg/L)	−0.54 (−0.66, −0.39)*	−0.52 (−0.64, −0.38)*	−0.99 (−0.99, −0.98)*	−0.44 (−0.57, −0.28)*	0.16 (−0.02, 0.33)	0.71 (0.61, 0.79)*
Cobalt[3] (Co, µg/L)	−0.68 (−0.76, −0.56)*	—	—	—	—	—
Nickel[3] (Ni, µg/L)	—	—	—	—	0.45 (0.29, 0.58)*	0.17 (−0.02, 0.34)
Copper[3] (Cu, µg/L)	0.08 (−0.10, 0.26)	0.30 (0.12, 0.46)*	0.24 (0.06, 0.40)*	0.11 (−0.08, 0.28)	−0.13 (−0.31, 0.05)	−0.35 (−0.50, −0.18)*
Zinc[3] (Zn, µg/L)	−0.90 (−0.93, −0.86)*	−0.64 (−0.74, −0.52)*	—	0.21 (0.03, 0.38)*	0.23 (0.05, 0.40)*	0.55 (0.40, 0.66)*
Selenium[3] (Se, µg/L)	0.12 (−0.06, 0.30)	0.00 (−0.18, 0.18)	0.17 (−0.02, 0.34)	−0.88 (−0.91, −0.83)*	−0.38 (−0.53, −0.21)*	−0.51 (−0.63, −0.36)*
Molybdenum[3] (Mo, µg/L)	—	—	—	—	—	—
Cadmium[4] (Cd, µg/L)	0.25 (0.07, 0.42)*	0.09 (−0.10, 0.27)	0.56 (0.42, 0.67)*	0.04 (−0.15, 0.22)	0.11 (−0.07, 0.29)	0.14 (−0.05, 0.31)
Mercury[4] (Hg, µg/L)	0.32 (0.15, 0.48)*	0.09 (−0.10, 0.26)	0.38 (0.21, 0.52)*	−0.01 (−0.20, 0.17)	−0.11 (−0.29, 0.07)	0.26 (0.08, 0.42)*
Lead[4] (Pb, µg/L)	—	0.28 (0.10, 0.44)*	—	—	—	−0.64 (−0.74, −0.52)*

[1]Corresponding 95% Bonferroni-corrected confidence intervals are given in parentheses. Confidence intervals containing zero are considered noninformative. Em dashes indicate the result is not estimable due to components in the calculation being at, or close to, zero.

[2]Quantity element.

[3]Trace element.

[4]Heavy metal.

*Results significant at $P < 0.05$.

Table 3. Phenotypic correlations between element concentrations (including vitamin B_{12}) and milk, fat, and protein yield and DMI, liveweight, and BCS[1]

Trait	Milk yield (kg)	Fat yield (kg)	Protein yield (kg)	DMI (kg/d)	Liveweight (kg)	BCS (1–5)
Milk						
Sodium[2] (Na, mg/L)	−0.19 (−0.36, −0.01)*	−0.17 (−0.34, 0.01)	−0.20 (−0.37, −0.02)*	−0.07 (−0.25, 0.11)	0.09 (−0.10, 0.27)	0.15 (−0.03, 0.32)
Magnesium[2] (Mg, mg/L)	−0.05 (−0.24, 0.13)	0.10 (−0.08, 0.28)	0.05 (−0.14, 0.23)	−0.01 (−0.20, 0.17)	0.08 (−0.11, 0.26)	0.13 (−0.05, 0.31)
Phosphorus[2] (P, mg/L)	−0.08 (−0.26, 0.10)	0.15 (−0.03, 0.32)	0.04 (−0.14, 0.22)	0.07 (−0.11, 0.25)	0.08 (−0.11, 0.26)	0.10 (−0.08, 0.28)
Potassium[2] (K, mg/L)	0.28 (0.10, 0.44)*	0.18 (−0.01, 0.35)	0.25 (0.08, 0.42)*	0.12 (−0.07, 0.29)	−0.09 (−0.27, 0.09)	−0.05 (−0.23, 0.14)
Calcium[2] (Ca, mg/L)	−0.08 (−0.26, 0.10)	0.13 (−0.05, 0.31)	0.03 (−0.16, 0.21)	0.05 (−0.13, 0.23)	−0.02 (−0.20, 0.17)	−0.01 (−0.19, 0.18)
Vanadium[3] (V, µg/L)	0.19 (0.01, 0.36)*	0.06 (−0.13, 0.24)	0.16 (−0.02, 0.34)	0.29 (0.12, 0.45)*	0.02 (−0.16, 0.20)	−0.13 (−0.30, 0.06)
Chromium[3] (Cr, µg/L)	−0.01 (−0.19, 0.18)	0.04 (−0.15, 0.22)	0.01 (−0.17, 0.19)	−0.02 (−0.20, 0.16)	0.00 (−0.18, 0.18)	0.05 (−0.14, 0.23)
Manganese[3] (Mn, µg/L)	−0.07 (−0.25, 0.11)	0.01 (−0.18, 0.19)	−0.01 (−0.19, 0.17)	0.04 (−0.14, 0.22)	0.06 (−0.13, 0.24)	0.04 (−0.14, 0.22)
Iron[3] (Fe, µg/L)	0.01 (−0.17, 0.19)	0.04 (−0.15, 0.22)	0.03 (−0.16, 0.21)	−0.00 (−0.19, 0.18)	0.07 (−0.11, 0.25)	0.07 (−0.11, 0.25)
Cobalt[3] (Co, µg/L)	0.14 (−0.04, 0.32)	0.08 (−0.11, 0.26)	0.15 (−0.04, 0.32)	0.07 (−0.12, 0.25)	0.10 (−0.08, 0.28)	0.04 (−0.15, 0.22)
Nickel[3] (Ni, µg/L)	0.09 (−0.09, 0.27)	0.13 (−0.05, 0.31)	0.08 (−0.11, 0.26)	0.06 (−0.12, 0.24)	−0.01 (−0.19, 0.18)	−0.06 (−0.24, 0.12)
Copper[3] (Cu, µg/L)	−0.10 (−0.27, 0.09)	−0.04 (−0.22, 0.14)	−0.08 (−0.26, 0.11)	0.01 (−0.18, 0.19)	−0.01 (−0.19, 0.17)	−0.02 (−0.20, 0.16)
Zinc[3] (Zn, µg/L)	0.01 (−0.17, 0.20)	0.13 (−0.06, 0.30)	0.09 (−0.10, 0.27)	0.03 (−0.16, 0.21)	0.00 (−0.18, 0.18)	−0.04 (−0.22, 0.14)
Selenium[3] (Se, µg/L)	−0.10 (−0.28, 0.08)	0.06 (−0.12, 0.24)	0.03 (−0.16, 0.21)	0.05 (−0.13, 0.23)	0.09 (−0.10, 0.27)	−0.03 (−0.22, 0.15)
Molybdenum[3] (Mo, µg/L)	−0.10 (−0.28, 0.08)	0.05 (−0.13, 0.23)	0.01 (−0.18, 0.19)	0.14 (−0.04, 0.31)	0.07 (−0.12, 0.25)	0.03 (−0.15, 0.21)
Iodine[3] (I, µg/L)	0.03 (−0.16, 0.21)	0.03 (−0.15, 0.21)	0.04 (−0.15, 0.22)	0.11 (−0.07, 0.29)	−0.11 (−0.29, 0.07)	−0.14 (−0.31, 0.05)
Cadmium[4] (Cd, µg/L)	0.02 (−0.16, 0.20)	0.06 (−0.13, 0.24)	0.07 (−0.11, 0.25)	0.03 (−0.15, 0.21)	0.00 (−0.18, 0.19)	−0.00 (−0.18, 0.18)
Mercury[4] (Hg, µg/L)	−0.01 (−0.19, 0.18)	0.03 (−0.16, 0.21)	−0.02 (−0.20, 0.17)	0.00 (−0.18, 0.19)	0.01 (−0.18, 0.19)	0.04 (−0.15, 0.22)
Lead[4] (Pb, µg/L)	0.09 (−0.09, 0.27)	0.06 (−0.13, 0.24)	0.06 (−0.12, 0.24)	0.02 (−0.16, 0.20)	−0.10 (−0.28, 0.08)	−0.13 (−0.30, 0.06)
Vitamin B_{12} (B_{12}, µg/L)	−0.11 (−0.29, 0.07)	−0.05 (−0.23, 0.13)	−0.07 (−0.25, 0.12)	0.05 (−0.13, 0.23)	0.05 (−0.13, 0.23)	0.09 (−0.09, 0.27)
Serum						
Sodium[2] (Na, mg/L)	0.03 (−0.15, 0.21)	0.05 (−0.13, 0.23)	0.06 (−0.12, 0.24)	0.03 (−0.16, 0.21)	0.03 (−0.15, 0.21)	0.01 (−0.17, 0.20)
Magnesium[2] (Mg, mg/L)	0.09 (−0.09, 0.27)	0.09 (−0.10, 0.26)	0.10 (−0.08, 0.28)	0.13 (−0.05, 0.31)	0.07 (−0.11, 0.25)	0.04 (−0.14, 0.22)
Phosphorus[2] (P, mg/L)	0.21 (0.03, 0.38)*	0.14 (−0.04, 0.32)	0.21 (0.03, 0.38)*	0.10 (−0.09, 0.27)	−0.02 (−0.21, 0.16)	−0.04 (−0.23, 0.14)
Potassium[2] (K, mg/L)	0.04 (−0.14, 0.22)	0.02 (−0.16, 0.20)	0.05 (−0.13, 0.23)	−0.00 (−0.18, 0.18)	−0.00 (−0.18, 0.18)	−0.02 (−0.20, 0.16)
Calcium[2] (Ca, mg/L)	0.06 (−0.12, 0.24)	0.04 (−0.15, 0.22)	0.09 (−0.09, 0.27)	−0.06 (−0.24, 0.13)	0.03 (−0.15, 0.22)	0.01 (−0.17, 0.19)
Vanadium[3] (V, µg/L)	0.05 (−0.14, 0.23)	0.01 (−0.17, 0.19)	0.08 (−0.10, 0.26)	−0.14 (−0.31, 0.05)	0.01 (−0.17, 0.19)	−0.01 (−0.20, 0.17)
Chromium[3] (Cr, µg/L)	−0.10 (−0.27, 0.09)	−0.07 (−0.25, 0.11)	−0.06 (−0.24, 0.12)	−0.11 (−0.28, 0.08)	−0.06 (−0.24, 0.13)	−0.01 (−0.19, 0.17)
Manganese[3] (Mn, µg/L)	−0.01 (−0.19, 0.17)	−0.04 (−0.22, 0.14)	0.01 (−0.17, 0.19)	−0.03 (−0.21, 0.16)	−0.01 (−0.19, 0.17)	0.10 (−0.09, 0.27)
Iron[3] (Fe, µg/L)	0.01 (−0.17, 0.20)	0.01 (−0.18, 0.19)	0.04 (−0.15, 0.22)	−0.03 (−0.21, 0.16)	0.13 (−0.05, 0.31)	0.11 (−0.08, 0.29)
Cobalt[3] (Co, µg/L)	0.17 (−0.01, 0.35)	0.15 (−0.03, 0.33)	0.17 (−0.01, 0.34)	0.24 (0.06, 0.40)	0.03 (−0.16, 0.21)	−0.02 (−0.20, 0.17)
Nickel[3] (Ni, µg/L)	−0.01 (−0.19, 0.18)	−0.04 (−0.22, 0.15)	−0.04 (−0.22, 0.14)	−0.06 (−0.24, 0.13)	−0.08 (−0.26, 0.10)	−0.05 (−0.23, 0.14)
Copper[3] (Cu, µg/L)	−0.07 (−0.25, 0.12)	−0.04 (−0.22, 0.14)	−0.07 (−0.25, 0.12)	−0.06 (−0.24, 0.12)	−0.04 (−0.22, 0.14)	−0.01 (−0.19, 0.17)
Zinc[3] (Zn, µg/L)	0.02 (−0.16, 0.20)	0.03 (−0.16, 0.21)	0.05 (−0.14, 0.23)	0.01 (−0.18, 0.19)	0.11 (−0.08, 0.29)	0.05 (−0.13, 0.23)
Selenium[3] (Se, µg/L)	0.12 (−0.06, 0.30)	0.10 (−0.09, 0.27)	0.12 (−0.06, 0.30)	0.04 (−0.14, 0.22)	0.03 (−0.16, 0.21)	−0.05 (−0.23, 0.13)
Molybdenum[3] (Mo, µg/L)	0.16 (−0.02, 0.34)	0.08 (−0.10, 0.26)	0.13 (−0.06, 0.30)	0.12 (−0.07, 0.29)	−0.03 (−0.21, 0.16)	0.07 (−0.11, 0.25)
Cadmium[4] (Cd, µg/L)	0.10 (−0.09, 0.27)	0.08 (−0.11, 0.26)	0.11 (−0.08, 0.28)	0.12 (−0.06, 0.30)	−0.02 (−0.20, 0.16)	−0.04 (−0.22, 0.14)
Mercury[4] (Hg, µg/L)	−0.05 (−0.23, 0.13)	−0.08 (−0.26, 0.10)	−0.02 (−0.20, 0.17)	−0.01 (−0.19, 0.17)	0.13 (−0.05, 0.31)	0.13 (−0.05, 0.31)
Lead[4] (Pb, µg/L)	0.25 (0.07, 0.41)*	0.14 (−0.05, 0.31)	0.24 (0.06, 0.41)*	−0.04 (−0.23, 0.14)	−0.01 (−0.19, 0.17)	0.02 (−0.16, 0.20)

[1]Corresponding 95% Bonferroni-corrected confidence intervals are given in parentheses. Confidence intervals containing zero are considered noninformative.

[2]Quantity element.

[3]Trace element.

[4]Heavy metal.

*Results significant at $P < 0.05$.

Table 4. Permanent environmental correlations between element concentrations (including vitamin B_{12}) and milk, fat, and protein yield and DMI, liveweight, and BCS[1]

Trait	Milk yield (kg)	Fat yield (kg)	Protein yield (kg)	DMI (kg/d)	Liveweight (kg)	BCS (1–5)
Milk						
Sodium[2] (Na, mg/L)	−0.23 (−0.40, −0.05)*	−0.27 (−0.43, −0.09)*	−0.37 (−0.52, −0.20)*	−0.06 (−0.24, 0.13)	0.10 (−0.08, 0.28)	0.32 (0.14, 0.47)*
Magnesium[2] (Mg, mg/L)	—	—	—	—	—	—
Phosphorus[2] (P, mg/L)	0.11 (−0.07, 0.29)	0.23 (0.04, 0.39)*	0.25 (0.07, 0.41)*	0.92 (0.89, 0.95)*	0.13 (−0.06, 0.30)	0.45 (0.30, 0.59)*
Potassium[2] (K, mg/L)	0.37 (0.20, 0.52)*	0.02 (−0.17, 0.20)	0.38 (0.21, 0.52)*	−0.49 (−0.62, −0.34)*	−0.03 (−0.21, 0.15)	−0.29 (−0.45, −0.11)*
Calcium[2] (Ca, mg/L)	−0.29 (−0.45, −0.11)*	−0.09 (−0.27, 0.09)	−0.01 (−0.19, 0.17)	−0.60 (−0.70, −0.47)*	0.38 (0.21, 0.53)	0.81 (0.73, 0.86)*
Vanadium[3] (V, µg/L)	—	—	—	—	—	—
Chromium[3] (Cr, µg/L)	—	—	—	—	—	—
Manganese[3] (Mn, µg/L)	—	—	—	—	—	—
Iron[3] (Fe, µg/L)	—	—	—	—	—	—
Cobalt[3] (Co, µg/L)	0.20 (0.02, 0.37)*	0.28 (0.11, 0.44)*	0.24 (0.06, 0.40)*	−0.13 (−0.31, 0.05)	−0.24 (−0.41, −0.06)*	−0.73 (−0.81, −0.63)*
Nickel[3] (Ni, µg/L)	—	—	—	—	—	—
Copper[3] (Cu, µg/L)	—	—	—	—	—	—
Zinc[3] (Zn, µg/L)	0.31 (0.13, 0.46)*	0.50 (0.35, 0.63)*	0.23 (0.05, 0.40)*	—	0.29 (0.11, 0.45)*	0.09 (−0.09, 0.27)
Selenium[3] (Se, µg/L)	—	—	—	—	—	—
Molybdenum[3] (Mo, µg/L)	—	—	—	—	—	—
Iodine[3] (I, µg/L)	—	—	—	—	—	−0.54 (−0.66, −0.40)
Cadmium[4] (Cd, µg/L)	—	—	—	—	−0.60 (−0.70, −0.47)*	—
Mercury[4] (Hg, µg/L)	—	—	—	—	—	—
Lead[4] (Pb, µg/L)	0.58 (0.44, 0.69)*	−0.12 (−0.30, 0.06)	0.18 (−0.00, 0.35)	0.07 (−0.12, 0.25)	−0.17 (−0.35, 0.01)	—
Vitamin B_{12} (B_{12}, µg/L)	0.34 (0.16, 0.49)*	0.51 (0.36, 0.63)*	0.45 (0.29, 0.58)*	—	0.77 (0.68, 0.83)*	—
Serum						
Sodium[2] (Na, mg/L)	−0.07 (−0.25, 0.11)	0.10 (−0.09, 0.27)	−0.08 (−0.26, 0.10)	0.87 (0.82, 0.91)*	0.72 (0.62, 0.80)*	0.39 (0.22, 0.53)*
Magnesium[2] (Mg, mg/L)	—	—	—	—	—	—
Phosphorus[2] (P, mg/L)	0.42 (0.25, 0.56)*	0.13 (−0.06, 0.30)	0.51 (0.36, 0.63)*	0.55 (0.41, 0.66)*	0.32 (0.15, 0.47)*	0.06 (−0.13, 0.24)
Potassium[2] (K, mg/L)	—	—	—	—	—	—
Calcium[2] (Ca, mg/L)	—	—	—	—	—	—
Vanadium[3] (V, µg/L)	−0.22 (−0.38, −0.03)*	−0.37 (−0.51, −0.20)*	−0.26 (−0.43, −0.08)*	−0.19 (−0.36, −0.01)*	−0.11 (−0.29, 0.07)	−0.17 (−0.35, 0.01)
Chromium[3] (Cr, µg/L)	—	—	—	0.52 (0.38, 0.64)*	—	—
Manganese[3] (Mn, µg/L)	—	—	—	—	—	—
Iron[3] (Fe, µg/L)	—	—	—	—	—	—
Cobalt[3] (Co, µg/L)	0.36 (0.19, 0.51)*	0.24 (0.06, 0.40)*	0.27 (0.09, 0.43)*	−0.15 (−0.32, 0.03)	0.42 (0.25, 0.56)*	−0.36 (−0.51, −0.19)*
Nickel[3] (Ni, µg/L)	0.73 (0.63, 0.80)*	0.64 (0.52, 0.74)*	0.63 (0.50, 0.73)*	0.87 (0.82, 0.91)*	−0.16 (−0.33, 0.02)	−0.15 (−0.32, 0.03)
Copper[3] (Cu, µg/L)	—	—	—	—	—	—
Zinc[3] (Zn, µg/L)	0.40 (0.23, 0.54)*	0.23 (0.04, 0.39)*	0.29 (0.11, 0.45)*	−0.51 (−0.64, −0.37)	0.10 (−0.08, 0.28)	−0.38 (−0.53, −0.21)*
Selenium[3] (Se, µg/L)	−0.27 (−0.43, −0.09)*	−0.15 (−0.33, 0.03)	−0.08 (−0.26, 0.10)	—	0.27 (0.09, 0.43)*	0.19 (0.01, 0.36)*
Molybdenum[3] (Mo, µg/L)	0.20 (0.02, 0.37)*	−0.44 (−0.58, −0.28)*	0.01 (−0.17, 0.20)	−0.91 (−0.93, −0.87)	0.12 (−0.06, 0.30)	−0.17 (−0.35, 0.01)
Cadmium[4] (Cd, µg/L)	—	—	—	—	—	—
Mercury[4] (Hg, µg/L)	−0.37 (−0.51, −0.20)*	−0.25 (−0.42, −0.07)*	−0.13 (−0.31, 0.05)	−0.13 (−0.31, 0.05)	0.19 (0.01, 0.36)*	0.01 (−0.18, 0.19)
Lead[4] (Pb, µg/L)	0.52 (0.38, 0.65)*	0.02 (−0.16, 0.21)	0.53 (0.38, 0.65)*	0.06 (−0.12, 0.24)	0.09 (−0.10, 0.26)	−0.02 (−0.21, 0.16)

[1]Corresponding 95% Bonferroni-corrected confidence intervals are given in parentheses. Confidence intervals containing zero are considered noninformative. Em dashes indicate the result is not estimable due to components in the calculation being at, or close to, zero.

[2]Quantity element.

[3]Trace element.

[4]Heavy metal.

*Results significant at $P < 0.05$.

correlated. Other correlations between milk elements and protein yield included genetic correlations with Cr and Cu, as well as a phenotypic correlation with K. Permanent environmental correlations included Na and K.

In serum, P was found to be negatively genetically correlated with protein yield, as were K and Ca. As with fat yield, a (strong) negative genetic correlation between protein yield and Fe was obtained; Mn was also negatively genetically correlated with protein yield. Positive genetic correlations observed with protein yield included Cu, Cd, and Hg. Phenotypic correlations with protein yield were observed with P and Pb. A permanent environmental correlation was noted with P.

Dry Matter Intake

In milk, a moderate negative correlation between DMI and P was observed. Strong negative genetic correlations were also noted between DMI and Fe, Ni, and Zn. Additional moderate (negative) genetic correlations with DMI included P, Mn, and Cu, with a positive correlation observed with V. The only significant phenotypic correlation was with V; however, moderate to strong permanent environmental correlations were observed with P, K, and Ca.

In serum, strong negative genetic correlations were observed between DMI and Ca and Se. Other moderate genetic correlations included P, Fe, and Zn. No significant phenotypic correlations were observed between serum elements and DMI. Permanent environmental correlations were observed with both P and Zn.

Liveweight

In milk, negative genetic correlations were observed between LWT and milk K, Ca, and Ni. Additional positive genetic correlations with LWT included V, Se, Mo, and Hg. No significant phenotypic correlations were observed between milk elements and LWT. Permanent environmental correlations with LWT included Ca and Zn.

In serum, strong negative genetic correlations were noted between LWT and Mg, P, K, Ca, and Se. No significant phenotypic correlations were observed between serum elements and LWT. Permanent environmental correlations included Na, P, Co, Se, and Hg.

Body Condition Score

In milk, a moderate positive genetic correlation between BCS and V was noted. No significant phenotypic correlations were observed between milk elements and BCS. Permanent environmental correlations with BCS included P, K, Ca, and I.

In serum, BCS was negatively genetically correlated with P, Cu, Se, and Pb and positively correlated with Mn, Fe, and Zn. No significant phenotypic correlations were observed between serum elements and BCS. Permanent environmental correlations included Co and Zn.

DISCUSSION

The present study aimed to highlight potential consequences, positive or negative, that selection for enhanced production may have on milk healthiness, and associated benefits, for the dairy product consumer. This was carried out by exploring correlations (genetic and phenotypic) between milk and serum micronutrient concentrations (and heavy metals) and milk, fat, and protein yield and DMI, LWT, and BCS. Many nutrients important in the diet of humans, such as Mg, Ca, Fe, and Zn, were found to be negatively correlated with our production and management traits of interest. In our previous study, carried out using the same herd, we noted that genetic group had no significant effect on concentrations of quantity or trace elements, vitamin B_{12}, or heavy metals, the only exception being Hg (Denholm et al., 2019). Considering results from the present study, this suggests that the impact of selection on micronutrient concentrations is perhaps being mitigated by selection for other traits included in the index as part of the breeding program. This is an area that warrants further study.

Quantity Elements

Magnesium, P, and Ca are crucial to human health and (in the case of P and Ca) have also been shown to provide valuable indicators of milk quality (Lucey and Fox, 1993).

Genetic variation in the milk quantity elements Mg, P, and Ca in dairy cows has been observed previously, with varying heritability estimates reported in the ranges 0.08–0.60, 0.29–0.62, and 0.54–0.72, respectively (van Hulzen et al., 2009; Buitenhuis et al., 2015; Visentin et al., 2019). Furthermore, Toffanin et al. (2015b) obtained heritability estimates of 0.10 and 0.12 for Ca and P predicted from mid-infrared spectral data, and more recently our group obtained heritability estimates of 0.30 and 0.20 for Mg and Ca, respectively, from milk samples collected from 479 Holstein-Friesians (Denholm et al., 2019). Moreover, previous results obtained by our group highlighted significant positive genetic correlations between quantity and trace elements in milk such that selection for increased levels of one milk element might be expected to also increase the levels of other important elements (Denholm et al., 2019). Specifically, selection for increased milk Ca would likely boost P, Zn, and Se levels, thus bringing about multiple potential improvements in levels of milk quantity and trace elements for dairy consumers.

Results from the present study showed significant negative relationships between quantity elements, in both milk and serum, and economically important traits. Significant genetic correlations were observed between Mg and Ca and milk yield. Concentrations of both elements (milk and serum) were found to be negatively correlated with milk yield, a relationship consistent with previous findings for Ca (Toffanin et al., 2015b). Serum P, K, and Ca were also negatively correlated with protein yield. These results suggest an unfavorable negative correlation with milk yield such that higher yields would be expected to result in lower concentrations of Mg and Ca (as well as Fe and Zn) in the milk, a relationship consistent with the literature. Toffanin et al. (2015b) hypothesized that the observed lower mineral content may be due to dilution resulting from older cows producing greater amounts of milk during lactation; we did observe a significant effect of parity on model results in the cases of both Mg and Ca.

Based on the data we collected and analyzed, we observed evidence that selection for increased serum quantity elements would most likely have a negative impact on DMI, LWT, and BCS. This would similarly be the case with milk P, K, and Ca, suggesting that selecting for increased quantity elements in milk or serum may result in thinner, lighter cows.

Trace Elements

Iron deficiency in humans is one of the main causes of anemia, a nutritional problem affecting some 2 billion people worldwide (WHO, 2005). We observed a negative relationship between Fe (milk and serum) and milk yield, fat yield, and DMI (a strong positive correlation between milk Fe and protein yield was also observed) such that increased yields and DMI would most likely result in lower concentrations of Fe in milk (for human consumption) as well as in the blood of the individual cow. Consequently, trying to increase the Fe content of milk could have a negative impact on production as well as dairy cow health.

It has been shown that genetic variation of Zn levels in cow milk exists (van Hulzen et al., 2009; Buitenhuis et al., 2015; Denholm et al., 2019). In the present study we observed a negative genetic relationship between milk Zn concentrations and milk, fat, and protein yield; moreover, serum Zn was also strongly negatively correlated with milk and fat yield.

As with Zn, genetic variation has been observed in Se levels in milk (van Hulzen et al., 2009; Buitenhuis et al., 2015; Denholm et al., 2019). Positive genetic correlations have been reported between Se and Mg, P, Ca, Mn, and Zn (Denholm et al., 2019), and results from the present study show milk Se levels were positively correlated with liveweight, whereas serum Se was negatively correlated with DMI, BCS, and LWT. Selenium, Cu, and Co are considered important trace elements of cattle that affect performance (Graham, 1991); milk Cu was also found to be negatively correlated with milk yield and DMI.

Iodine was found to have a negative genetic correlation with protein yield and a positive correlation with fat yield; however, it is known that concentrations of I within milk are affected by several other external factors—for example, use of iodine-containing udder sanitizers, levels of goitrogenic compounds in feeds, and milk pasteurization (Flachowsky et al., 2014).

Heavy Metals

In addition to the elements present in milk that are beneficial to human health, it is important to consider those that are potentially detrimental. Heavy metals, including Cd, Pb, and Hg, are toxic elements sometimes present in milk but at very low concentrations (Gaucheron, 2013). Some studies have shown that environmental factors can significantly increase milk levels of heavy metals—for example, in lactating cows raised near industrial plants or in cities (Krelowska-Kulas, 1990; Patra et al., 2008; Maas et al., 2011). Breeding strategies aimed at improving levels of beneficial elements within milk should seek to minimize levels of toxic heavy metals.

In the present study, we observed positive correlations between serum concentrations of Cd and Hg and milk and protein yield, and Pb and fat yield, suggesting concentrations of these heavy metals in dairy cow blood may be elevated with increased selection for production. Serum Hg and Pb were also correlated with BCS. In milk, concentrations of Hg were negatively correlated with fat yield, suggesting there should be no unwanted increase in heavy metal concentrations in milk for human consumption due to selection for increased production. Moreover, previous results by our group showed no significant genetic correlations of heavy metals with micronutrient levels, suggesting genetic selection programs aimed at increasing micronutrient levels would not inadvertently increase levels of toxic heavy metals such as Cd, Pb, or Hg (Denholm et al., 2019).

Vitamin B_{12}

Vitamin B_{12} is an essential vitamin in humans for the purpose of DNA synthesis and cellular energy production. Deficiency can result in serious clinical consequences such as disruption of DNA and cell metabolism (Green, 2013). Concentrated in animal tissues, B_{12} can only be obtained from absorption via the consumption of animal products—for example, dairy, meat, and fish (Hunt et al., 2014), with milk and dairy products contributing significantly to B_{12} intake in humans (150% of Reference Nutrient Intake; Henderson et al., 2003a,b; Kliem and Givens, 2011). This presents a potentially attractive breeding target in terms of enhancing nutrient quality for the consumer. Results from the data collected and analyzed in the present study failed to highlight any notable relationships between production (or management) traits and milk vitamin B_{12}. This may, in part, be due to the size of the B_{12} data available for analysis (247 records from 63 cows). Although no significant results were obtained relating to B_{12}, we did observe strong genetic correlations between milk yield and milk and serum Co (vitamin B_{12} contains Co), as well as protein yield and milk. Cobalt is required in the diet of cattle to synthesize vitamin B_{12} endogenously by rumen bacteria (Stemme et al., 2008); thus, this may present an alternative avenue for increasing milk B_{12} content for dairy consumers.

CONCLUSIONS

Milk and milk-derived products have great potential to be used as a route to improve micronutrient intakes and reduce micronutrient deficiencies in populations worldwide. However, results from the data gathered and analyzed in the present study suggest that breeding strategies aimed at improving production and condition would most likely result in a negative impact on the levels of beneficial elements within milk (such as Mg, Ca, Fe, Zn, and Se) for human consumption.

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ORCIDS

S. J. Denholm https://orcid.org/0000-0002-6291-8269
T. N. McNeilly https://orcid.org/0000-0001-6469-0512
E. Wall https://orcid.org/0000-0002-7072-5758
A. A. Sneddon https://orcid.org/0000-0001-9620-2085