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Phenotypic and genetic analysis of milk and serum element concentrations in dairy cows

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ABSTRACT

Enhancing micronutrient (i.e., mineral and vitamin) concentrations within milk and serum from dairy cows is important for both the health of the cow and the nutritive value of the milk for human consumption. However, a good understanding of the genetics underlying the micronutrient content in dairy cattle is needed to facilitate such enhancements through feeding or breeding practices. In this study, milk (n = 950) and serum (n = 766) samples were collected from Holstein-Friesian dairy cows (n = 479) on 19 occasions over a 59-mo period and analyzed for concentrations of important elements. Additionally, a subset of 256 milk samples was analyzed for concentrations of vitamin B_{12}. Cows belonged to 2 genetic lines (average and highest genetic merit for milk fat plus protein yield) and were assigned to 1 of 2 diets based on either a by-product or homegrown ration. Univariate models accounting for repeated records were used to analyze element and vitamin B_{12} data and investigate the effect of genotype and feeding system as well as derive estimates of variance components and genetic parameters. Bivariate models were used to study correlations both within and between milk and serum. Only concentrations of Hg in milk were seen to be affected by genotype, with higher concentrations in cows with high genetic merit. In contrast, element concentrations were influenced by feeding system such that cows fed the homegrown diet had increased milk concentrations of Ca, Cu, I, Mn, Mo, P, and K and increased serum concentrations of Cd, Cu, Fe, Mo, and V. Cows on the by-product diet had increased milk concentrations of Mg, Se, and Na and increased serum concentrations of P and Se. Heritability (h^2) estimates were obtained for 6 milk and 4 serum elements, including Mg ($h^2_{milk} = 0.30$), K ($h^2_{serum} = 0.18$), Ca ($h^2_{milk} = 0.20$; $h^2_{serum} = 0.12$), Mn ($h^2_{milk} = 0.14$), Cu ($h^2_{serum} = 0.22$), Zn ($h^2_{milk} = 0.24$), Se ($h^2_{milk} = 0.15$; $h^2_{serum} = 0.10$), and Mo ($h^2_{milk} = 0.19$). Significant estimates of repeatability were observed in all milk and serum quantity elements (Na, Mg, P, K, and Ca) as well as 5 milk and 7 serum trace elements. Only K in milk and serum was found to have a significant positive genetic and phenotypic correlation (0.52 and 0.22, respectively). Significant phenotypic associations were noted between milk and serum Ca (0.17), Mo (0.19), and Na (−0.79). Additional multivariate analyses between measures within sample type (i.e., milk or serum) revealed significant positive associations, both phenotypic and genetic, between some of the elements. In milk, Se was genetically correlated with Ca (0.63), Mg (0.59), Mn (0.40), P (0.53), and Zn (0.52), whereas in serum, V showed strong genetic associations with Cd (0.71), Ca (0.53), Mn (0.63), Mo (0.57), P (0.42), K (0.45), and Hg (−0.44). These results provide evidence that element concentrations in milk and blood of dairy cows are significantly influenced by both diet and genetics and demonstrate the potential for genetic selection and dietary manipulation to alter nutrient concentration to improve both cow health and the healthfulness of milk for human consumption.
Key words: micronutrient, heavy metal, dairy cow, heritability, correlation

INTRODUCTION

Micronutrients are required throughout life and consist of vitamins and minerals that are essential for maintaining normal body function and health in humans and other animals (FAO/WHO, 2004; Gernand et al., 2016). Neither humans nor other animals can synthesize micronutrients within the body; therefore, micronutrients must be obtained from the diet. Suboptimal intakes of these vitamins and minerals can affect normal growth and development, reducing performance

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as well as increasing susceptibility to, for example, Keshan disease in humans and white muscle disease in cattle due to Se deficiency (Muth et al., 1958; Yu, 1982).

Whereas vitamins are organic molecules, minerals such as P, Ca, Fe, Zn, Se, and I are inorganic but are required only in very small amounts. Minerals can be further classified into trace elements (e.g., Fe, Zn, Se, and I) that are required in low amounts and quantity elements (e.g., Mg, P, K, and Ca) that are required in larger amounts. When intakes of quantity or trace elements or vitamins from the diet are insufficient, deficiencies can arise that can compromise animal and human health and increase the risk of disease. Indeed, concern is currently growing that a sizable portion of the human population does not meet micronutrient reference nutrient intake (**RNI**) values (i.e., the amount of nutrient required to prevent deficiency; Rooke et al., 2010; Givens et al., 2014).

Dairy products, such as milk and cheese, are important sources of minerals and vitamins and contribute substantially to dietary intakes of Ca, P, I, Zn, and Mg (60, 60, 55, 18, and 10% of RNI, respectively) as well as vitamins A, B_2, and B_{12} (26, 52, and 150% of RNI, respectively; Kliem and Givens, 2011). Importantly, Mg and Ca are increasingly significant factors in bone development, especially in children (Givens et al., 2014). Furthermore, the circulating concentrations of these minerals and vitamins in the blood and milk of dairy cows likely relate to the fitness of the animal given their important roles in numerous physiological and immunological processes (Percival, 1998; Doherty, 2007; Maggini et al., 2007; Hoffmann and Berry, 2008; Prasad, 2008; Alpert, 2017). Therefore, identifying breeding strategies within the cow to increase milk micronutrient concentrations as well as optimizing micronutrient concentrations within the cow herself should be of ultimate benefit to both the cow and the human dairy product consumer. Moreover, because heavy metals such as Pb, Hg, and Cd, which have potential adverse effects on health, can also be found in milk (Rey-Crespo et al., 2013), breeding strategies should also be commensurate with reducing, or at least not increasing, concentrations of these metals where possible. Dietary manipulation of mineral concentrations in livestock has been demonstrated, yet there is a relative lack of knowledge concerning the contribution of cow genetics to variation in concentrations of elements (including heavy metals) and vitamins within the blood and milk of dairy cows (Rooke et al., 2010).

The aim of this study was to carry out a phenotypic and genetic analysis of mineral, vitamin, and heavy metal concentrations in dairy cow milk and serum to determine (1) the effect of genotype and diet on individual element and vitamin B_{12} concentrations, (2) whether relationships exist between element concentrations (including vitamin B_{12}) in milk and serum, and (3) whether variation between animals exists that would permit selection for optimized element and vitamin B_{12} concentrations that would benefit the health of both the cow and the human consumer.

MATERIALS AND METHODS

Animals

Animals involved in this study were from the Langhill pedigree herd of Holstein-Friesian dairy cows (n = 479) housed at the Scotland's Rural College Dairy Research Centre in Dumfries, Scotland, between 2012 and 2016. All cows were part of a long-term (ongoing) selection experiment for genotype × environment following a 2 × 2 approach (Veerkamp et al., 1994). Briefly, the herd has been divided equally between 2 distinct genetic lines (control and select) selected since 1970 and assigned to 1 of 2 diets based on differing rations. The control line has been bred to bulls of UK national average genetic merit for kilograms of fat plus protein yield. In contrast, the high genetic merit select line (top 5% genetic merit) has been bred from bulls with the highest genetic merit for kilograms of fat plus protein yield. The 2 diet groups consist of 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 homegrown (**HG**) ration consisted of components grown exclusively on farm and included grazed grass, grass silage, red clover silage, forage maize, lucerne silage, crimped wheat, and beans. Additionally, the HG ration was balanced with purchased minerals. In contrast, the by-product (**BP**) ration consisted of biscuit meal, sugar beet pulp, chopped straw, breakfast cereal, wheat distillers dark grains, soybean meal (Hipro 50%, Tarff Valley Ltd., Ringford, UK), Vitagold (KW Feeds, Ayrshire, UK), protected fat (Megalac, Volac International Ltd., Hertfordshire, UK), molasses, and minerals. Mineral compositions of the HG and BP diets are presented in Supplemental Table S1 (https://doi.org/10.3168/jds.2019-16960). Target milk yields of select cows on the low- and high-energy diets are 7,500 and 13,000 L/lactation, respectively; the UK average per cow per lactation is approximately 7,557 (AHDB Dairy, 2017).

Ethics Statement

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

Sampling Protocol

Samples used in the present study were collected across several years and seasons from the same ongoing experimental system; 385 (of 479) cows had 2 or more samples. Furthermore, samples were selected such that they accounted for genotype and management of cows to give a balanced representation of the herd. In total, 950 milk samples and 766 serum samples were collected for analysis of element and vitamin B_{12} (milk only) concentrations. Further information regarding sample collection is presented in Supplemental Table S2 (https://doi.org/10.3168/jds.2019-16960).

Milk Samples. Cows in the Langhill herd are milked 3 times daily (morning, midday, and evening), and for the present study milk samples were taken from the morning milking (at the same time as any blood sampling). Milk samples were collected on 16 separate occasions between June 2012 and January 2015 and included summer and winter periods. All milk samples were whole milk except for 256 samples that were from skim milk collected as part of a previous project (Denholm et al., 2017, 2018). For these latter samples, milk was first centrifuged at 3,000 × *g* for 30 min at 4°C and the skim milk fraction was retained from below the fat layer using a fine-tipped pastette. All samples were stored at −20°C before analysis.

Blood Samples. Whole-blood samples were collected on 13 separate occasions between April 2013 and May 2016 and included summer and winter periods. Samples were collected into plain Vacutainers (BD, Reading, UK); blood was allowed to coagulate before centrifugation at 2,000 × *g* for 10 min at 18°C (using a refrigerated centrifuge), and the serum was retained. All samples were stored at −20°C before analysis.

Analysis of Quantity and Trace Element and Heavy Metal Concentrations

All milk and serum samples were analyzed, and concentrations of circulating quantity elements (Na, Mg, P, K, and Ca), trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, and I), and heavy metals (Cd, Hg, and Pb) were recorded. Milk samples (1.0 mL) were digested in nitric acid (8.0 mL; 65% vol/vol) using the MARS 6 digestion system (CEM Corp., Matthews, NC) and then stored overnight at room temperature. The temperature of the samples was increased from room temperature to 210°C and then held for 10 min before cooling.

Serum samples (50 μL) were added to hydrogen peroxide (10 μL; 30% wt/wt) and nitric acid (40 μL; 65% vol/vol) and then digested at 85°C for 40 min. Digested samples were diluted in decomposition matrix before inductively coupled plasma MS (**ICP-MS**) analysis. The decomposition matrix was nitric acid (2% vol/vol) and hydrochloric acid (0.5% vol/vol) in distilled deionized water (Millipore UK, Watford, UK), which was used for preparation of all solutions.

The measured isotopes analyzed by ICP-MS were ^{23}Na, ^{24}Mg, ^{31}P, ^{39}K, ^{44}Ca, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{78}Se, ^{95}Mo, ^{127}I, ^{111}Cd, ^{202}Hg, and ^{208}Pb. All element standards were used in stock solutions of 1,000 mg/L, which served for preparation of calibration solutions and internal standard solution. The ICP-MS measurements were carried out using an Agilent 7700X spectrometer (Agilent Technologies UK, Wokingham, UK) equipped with a MicroMist nebulizer and nickel sampler and skimmer cones. The flow of mineral standards (National Institute of Standards and Technology, Gaithersburg, MD) and samples was joined together with a flow of erbium internal standard solution (1 mg/L). The mixed flow (~500 μL/min) was delivered by the peristaltic pump to the nebulizer of the ICP-MS setup. The duration of the ICP-MS analysis was 3.0 min. Data acquisition was 1 point, 5 replicates, 100 sweeps per replicate. Milk and mussel reference materials were obtained from LGC Group (Middlesex, UK).

For the quantification of I in milk samples, these were first digested at 90°C in 5% tetramethylammonium hydroxide (**TMAH**) for 3 h and then cooled. The TMAH (≥97%; Sigma-Aldrich, Dorset, UK) was diluted to 5% using ultrapure water (18.2 MΩ × cm; Elga, Pure-Flex, Cheshire, UK). The I content in the milk samples was then determined by ICP-MS (7700X, Agilent Technologies) in standard analysis mode using external calibration. The stock standard solution was gravimetrically prepared in-house from high-purity potassium iodide (+99.99%; Thermo Fisher Scientific, Altrincham, UK) in 5% TMAH. The calibration standards were prepared by serial dilution of this stock using 5% TMAH, and a tellurium internal standard was added at the same level as in the samples to a final concentration of 150 ng/mL. The method accuracy was monitored using ERM-BD150 skim milk powder certified reference material (LGC Standards, Middlesex, UK) with a certified I content of 1,730 ± 140 μg/kg (dry weight basis).

Analysis of Vitamin B_{12}

Milk samples collected between April 2013 and January 2015 (11 sample points yielding 256 samples; n = 64) were analyzed for vitamin B_{12} concentrations. Vitamin B_{12} in undiluted milk was measured using a commercial competitive assay (RIDAscreen, R-Biopharm, Pfungstadt, Germany). Absorbance was measured at 450 nm, where values are inversely related to vitamin B_{12} concentration using standards in the range of 0 to 30 μg/L. The detection limit of the assay was 0.5 μg/L.

Data Preparation and Preprocessing

Element and vitamin B_{12} data were combined with individual animal information before being subjected to quality control measures. For the purposes of the present study, the interquartile range (IQR) was calculated for each trait (x) with any concentrations falling out, with $Q_1 - 1.5 \times \text{IQR} < x < Q_3 - 1.5 \times \text{IQR}$ (where Q_1 and Q_3 are the first and third quartiles, respectively) considered an outlier and removed from the data set. To ensure normality, all data were log-transformed before analysis. The final data set consisted of 938 milk and 754 serum records, with 385 (of 479) cows having more than 1 record.

Statistical Analyses

Data were analyzed using a mixed-effects linear animal model (Equation 1). Genetic relationships between individuals within the data set were accounted for by fitting a pedigree relationship matrix. Full pedigree information spanning 7 generations was available for all cows in the study:

$$\mathbf{y} = \mathbf{Xa} + \mathbf{Z_1b} + \mathbf{Z_2c} + \mathbf{e}. \quad [1]$$

Here, $\mathbf{y}$ is a vector of trait observations (i.e., mineral, vitamin, heavy metal); $\mathbf{a}$ is a vector of fixed effects; $\mathbf{b}$ is a vector of random additive genetic effects; $\mathbf{c}$ is a vector of permanent environmental effects; $\mathbf{e}$ is a vector of random residual effects; and $\mathbf{X}$, $\mathbf{Z_1}$, and $\mathbf{Z_2}$ are incidence matrices linking phenotypic records to fixed and additive genetic and permanent environmental effects, respectively.

Fixed effects included diet group, genetic group, lactation number, week 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 (pedigree data for 2,793 animals were included). To account for repeated observations per cow, the permanent environmental effect of the nth individual cow was also fitted as a random effect. All analyses were carried out using ASReml version 3 (Gilmour et al., 2009).

RESULTS

Summary Statistics

Tables 1 and 2 summarize the element and vitamin B_{12} data generated from milk and serum samples, respectively. Trait variability was determined by calculating the coefficient of determination (%). Within-milk variability was in the range of 22% (Zn) to 156% (Co) for trace elements and 11% (K) to 23% (Na) for quantity elements. In serum, variability ranged from 24% (Se) to 133% (Mn) for trace elements and 7% (Na) to 32% (P) for quantity elements. Variability was greater in serum compared with milk and in trace elements compared with quantity elements.

Effect of Genotype and Diet on Element Concentrations

The effect of both genetic line and diet group on concentrations of micronutrients and heavy metals in milk and serum is summarized in Table 3, with P-values representing whether a significant difference was observed between predicted mean concentrations. Analyses revealed a predisposition for increased concentrations of Hg in the milk of select cows (mean = 0.19 μg/L, $P = 0.01$). No significant effect of genotype on the concentration of any other element in either milk or serum was observed. In contrast, diet was found to have a significant and varying effect on the concentration of 10 milk and 7 serum minerals. Cows on the HG diet had higher milk concentrations of Ca (mean = 1,257.02 mg/L, $P < 0.001$), Cu (mean = 61.39 μg/L, $P = 0.01$), I (mean = 1,346.82 μg/L, $P < 0.001$), K (mean = 1,739.23 mg/L, $P < 0.001$), Mn (mean = 36.82 μg/L, $P < 0.001$), Mo (mean = 41.89 μg/L, $P < 0.001$), and P (mean = 954.42 mg/L, $P < 0.001$) compared with those on the BP diet (Table 3). Conversely, cows on the BP diet had higher milk concentrations of Mg (mean = 116.50 mg/L, $P < 0.001$), Na (mean = 375.40 mg/L, $P < 0.001$), and Se (mean = 20.09 μg/L, $P < 0.001$). Regarding serum elements, cows on the HG diet showed higher concentrations of Cd (mean = 0.09 μg/L, $P < 0.001$), Cu (mean = 484.69 μg/L, $P < 0.001$), Fe (mean = 1,787.19 μg/L, $P < 0.02$), and Mo (mean = 17.49 μg/L, $P < 0.001$) compared with BP-fed cows, which showed higher serum concentrations of P (mean = 154.04 mg/L, $P < 0.001$), Se (mean = 73.03 μg/L, $P < 0.001$), and V (mean = 0.53 μg/L, $P < 0.001$).

Table 1. Descriptive statistics of the milk element and vitamin B_{12} data

Item	Count	Cows[1]	Mean	SD	Minimum	Maximum	CV (%)
Quantity elements (mg/L)							
Na	878	202/318	364.16	83.97	148.92	602.97	23.06
Mg	908	206/320	113.19	15.58	69.74	155.98	13.76
P	904	205/318	946.18	135.04	586.19	1,300.76	14.27
K	892	202/317	1,774.39	191.81	1,239.38	2,301.43	10.81
Ca	900	203/318	1,192.18	181.14	679.46	1,684.86	15.19
Trace elements (μg/L)							
V	186	103/184	2.70	1.96	0.06	7.22	72.43
Cr	726	188/298	25.54	20.14	0.29	104.68	78.87
Mn	889	204/320	38.08	14.49	1.06	83.46	38.05
Fe	848	194/312	1,077.02	886.26	41.22	4,017.03	82.29
Co	411	80/164	4.30	6.71	0.06	30.40	156.12
Ni	321	115/232	427.28	463.02	0.86	2,113.01	108.37
Cu	916	210/324	120.77	78.20	0.96	386.82	64.75
Zn	919	208/321	4,348.23	938.97	1,916.91	6,833.17	21.59
Se	916	208/323	19.10	4.67	6.83	31.81	24.46
Mo	888	200/314	37.20	12.14	6.26	71.86	32.63
I	448	131/267	1,448.35	610.52	161.20	3,290.90	42.15
Heavy metals (μg/L)							
Cd	733	205/311	0.16	0.10	0.01	0.45	61.80
Hg	417	188/301	0.31	0.26	0.01	1.04	83.35
Pb	829	203/316	4.82	3.17	0.02	15.72	65.75
Vitamin B_{12} (μg/L)	247	63/64	0.82	0.38	0.50	1.75	47.08

[1]Number of cows/total cows with at least 2 observations of trait.

Variance Components

Variance components of the milk and serum elements are presented in Tables 4 and 5, respectively. Additive genetic variance was small for both milk and serum traits, and in most cases genetic variance was higher in serum traits compared with those in milk. Heritability ($\boldsymbol{h^2}$) estimates were obtained for 17 of the 20 milk traits, 6 of which were significant (Mg, Ca, Mn, Zn, Se, and I). In serum, heritability estimates were obtained for 16 of 18 traits, of which 4 were significant (K, Ca, Cu, and Se). Significant element heritabilities appeared to be greater in milk traits compared with their corresponding serum trait. The highest heritability in milk and serum was observed in Mg ($h^2 = 0.30$, $P = 0.002$) and Cu ($h^2 = 0.22$, $P < 0.001$), respectively. Milk and

Table 2. Descriptive statistics of the serum element data

Item	Count	Cows[1]	Mean	SD	Minimum	Maximum	CV (%)
Quantity elements (mg/L)							
Na	169	1/168	3,133.18	210.64	2,533.07	3,757.49	6.72
Mg	734	215/322	23.01	5.11	9.79	36.62	22.22
P	743	214/323	139.07	44.97	20.75	260.98	32.33
K	732	215/323	206.02	35.54	105.38	302.45	17.25
Ca	707	214/318	105.37	17.76	58.03	153.36	16.86
Trace elements (μg/L)							
V	584	213/214	0.68	0.21	0.15	1.25	31.15
Cr	221	63/214	0.89	0.76	0.00	4.23	85.71
Mn	626	215/259	6.46	8.61	0.07	36.70	133.22
Fe	654	213/263	2,179.44	1,144.67	292.15	6,157.91	52.52
Co	610	214/230	1.34	0.68	0.11	4.39	50.73
Ni	392	201/212	3.85	2.71	0.01	12.14	70.31
Cu	726	215/315	601.02	195.51	108.52	1,113.46	32.53
Zn	626	214/258	910.90	330.06	201.57	2,109.51	36.23
Se	717	214/322	75.96	18.58	26.11	124.59	24.45
Mo	662	165/321	14.92	13.62	1.26	62.88	91.30
Heavy metals (μg/L)							
Cd	666	215/283	0.11	0.11	0.00	0.46	100.76
Hg	265	139/151	3.43	3.22	0.00	14.89	93.97
Pb	738	215/325	83.23	81.74	0.01	367.56	98.20

[1]Number of cows/total cows with at least 2 observations of trait.

serum quantity elements were all moderately to highly repeatable, and we observed significant repeatability in 5 milk and 10 serum trace elements.

Associations Within Milk or Serum Elements

Correlations between element concentrations within milk are presented in Table 6, and those within serum are presented in Table 7. Strong positive genetic correlations (significantly different from zero at $P < 0.05$) were observed within milk between the quantity elements, in particular Ca, Mg, and P (Table 6). Magnesium was positively associated with both Ca (r = 0.45, $P < 0.001$) and P (r = 0.49, $P < 0.001$); a positive association between P and Ca was also observed (r = 0.61, $P < 0.001$). Moreover, strong positive genetic correlations were observed for Se with Ca (r = 0.63, $P < 0.001$), Mg (r = 0.59, $P < 0.001$), Mn (r = 0.40, $P = 0.034$), P (r = 0.53, $P < 0.001$), and Zn (r = 0.52, $P < 0.001$). Consistent phenotypic relationships were also observed between the milk micronutrients and are presented in full in Table 6.

Within serum, a similar set of genetic relationships was observed between the quantity elements, although no significant genetic relationships were observed with Se (Table 7). The most genetically correlated nutrient in serum was V, which showed strong associations with Cd (r = 0.71, $P = 0.003$), Ca (r = 0.53, $P < 0.001$), Mn (r = 0.63, $P = 0.039$), Hg (r = −0.44, $P = 0.003$), Mo (r = 0.57, $P < 0.001$), P (r = 0.42, $P = 0.006$), and K (r

Table 3. Effect of diet and genotype on element concentrations in milk and serum[1]

	Predicted mean[2]			
Item	By-product	Homegrown	SED[3]	*P*-value
Milk				
Quantity elements (mg/L)				
Na	375.40	338.32	1.02	<0.001
Mg	116.50	110.81	1.01	<0.001
P	878.40	954.42	1.01	<0.001
K	1,661.21	1,739.23	1.01	<0.001
Ca	1,107.32	1,257.02	1.01	<0.001
Trace elements (μg/L)				
Mn	27.49	36.81	1.03	<0.001
Cu	54.55	61.39	1.05	0.009
Se	20.09	15.14	1.01	<0.001
Mo	32.90	41.89	1.02	<0.001
I	1,082.79	1,346.82	1.04	<0.001
Serum				
Quantity element (mg/L)				
P	154.04	108.80	1.02	<0.001
Trace elements (μg/L)				
V	0.53	0.42	1.03	<0.001
Fe	1,663.20	1,787.19	1.03	0.019
Cu	418.01	484.69	1.02	<0.001
Se	73.02	64.65	1.02	<0.001
Mo	5.46	17.49	1.05	<0.001
Heavy metal (μg/L)				
Cd	0.04	0.09	1.09	<0.001
Milk	Control line	Select line		
Heavy metal (μg/L)				
Hg	0.14	0.19	1.11	0.010

[1]Predicted mean values were obtained via univariate models (accounting for all other sources of systematic variation). Only predicted mean concentrations that were significantly different ($P < 0.05$) are presented.

[2]The homegrown ration consisted of components grown exclusively on farm and included grazed grass, grass silage, red clover silage, forage maize, lucerne silage, crimped wheat, and beans. Additionally, it was balanced with purchased minerals. In contrast, the by-product ration consisted of biscuit meal, sugar beet pulp, chopped straw, breakfast cereal, wheat distillers dark grains, soybean meal (Hipro 50%, Tarff Valley Ltd., Ringford, UK), Vitagold (KW Feeds, Ayrshire, UK), protected fat (Megalac, Volac International Ltd., Hertfordshire, UK), molasses, and minerals. The control line had been bred to bulls of UK national average genetic merit for kilograms of fat plus protein yield. In contrast, the high genetic merit select line (top 5% genetic merit) had been bred from bulls with the highest genetic merit for kilograms of fat plus protein yield.

[3]Standard error of the difference.

Table 4. Variance components and heritability (h^2) estimates of the milk elements and vitamin B_{12} data

Item	SD			h^2 (SE)	Repeatability (SE)
	Additive genetic	Permanent environment	Total phenotypic		
Quantity elements (mg/L)					
Na	0.000	0.006	0.039	NE[1]	0.16* (0.040)
Mg	0.005	0.000	0.016	0.30* (0.090)	0.30* (0.044)
P	0.002	0.002	0.016	0.12 (0.070)	0.22* (0.042)
K	0.001	0.002	0.010	0.11 (0.078)	0.27* (0.045)
Ca	0.003	0.000	0.015	0.20* (0.078)	0.22* (0.043)
Trace elements (μg/L)					
V	0.063	0.000	0.978	0.06 (0.161)	0.06 (0.161)
Cr	0.013	0.000	0.450	0.03 (0.035)	0.03 (0.035)
Mn	0.020	0.000	0.139	0.14* (0.039)	0.14* (0.039)
Fe	0.006	0.000	0.531	0.01 (0.028)	0.01 (0.028)
Co	0.039	0.000	0.963	0.04 (0.056)	0.04 (0.056)
Ni	0.049	0.000	1.247	0.04 (0.093)	0.04 (0.093)
Cu	0.018	0.000	0.413	0.04 (0.028)	0.04 (0.028)
Zn	0.011	0.009	0.047	0.24* (0.116)	0.43* (0.042)
Se	0.005	0.001	0.033	0.15* (0.072)	0.18* (0.041)
Mo	0.011	0.000	0.060	0.19* (0.041)	0.19* (0.041)
I	0.031	0.003	0.140	0.22 (0.123)	0.24* (0.081)
Heavy metals (μg/L)					
Cd	0.000	0.000	0.511	NE	NE
Pb	0.000	0.000	0.577	NE	NE
Hg	0.038	0.000	1.009	0.04 (0.064)	0.04 (0.064)
Vitamin B_{12} (μg/L)	0.034	0.008	0.346	0.10 (0.226)	0.12 (0.095)

[1]Not estimable due to additive genetic variance >0.
*Significantly different from zero at $P < 0.05$.

Table 5. Variance components and heritability (h^2) estimates of the serum elements data

Item	SD			h^2 (SE)	Repeatability (SE)
	Additive genetic	Permanent environment	Total phenotypic		
Quantity elements (mg/L)					
Na	0.001	0.003	0.004	0.34 (0.234)	1.00* (0.002)
Mg	0.007	0.000	0.047	0.14 (0.083)	0.14* (0.050)
P	0.006	0.007	0.062	0.09 (0.079)	0.20* (0.046)
K	0.004	0.000	0.024	0.18* (0.051)	0.18* (0.051)
Ca	0.003	0.000	0.025	0.12* (0.049)	0.12* (0.049)
Trace elements (μg/L)					
V	0.007	0.017	0.073	0.09 (0.121)	0.33* (0.059)
Cr	0.000	0.000	1.215	NE[1]	NE
Mn	0.012	0.000	0.324	0.04 (0.044)	0.04 (0.044)
Fe	0.012	0.000	0.130	0.09 (0.046)	0.09 (0.046)
Co	0.009	0.007	0.124	0.07 (0.100)	0.14* (0.055)
Ni	0.245	0.113	0.830	0.30 (0.183)	0.43* (0.076)
Cu	0.018	0.000	0.084	0.22* (0.051)	0.22* (0.051)
Zn	0.007	0.005	0.067	0.11 (0.095)	0.18* (0.054)
Se	0.005	0.000	0.053	0.10* (0.047)	0.10* (0.047)
Mo	0.000	0.093	0.335	NE	0.28* (0.057)
Heavy metals (μg/L)					
Cd	0.044	0.182	0.909	0.05 (0.086)	0.25* (0.059)
Pb	0.062	0.153	0.546	0.11 (0.103)	0.39* (0.051)
Hg	0.709	0.613	1.433	0.49 (0.347)	0.92* (0.017)

[1]Not estimable due to additive genetic variance >0.
*Significantly different from zero at $P < 0.05$.

Table 6. Correlations between element concentrations and vitamin B_{12} within milk[1]

Item	Na	Mg	P	K	Ca	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	I	Hg	B_{12}
Na		0.16	−0.13	−0.26	0.03	0.56	−0.60	−0.04	−0.02	0.32	—[2]	−0.27	−0.15	0.09	−0.06	−0.21	0.78	0.17
		(0.16)	(0.21)	(0.20)	(0.19)	(0.88)	(0.47)	(0.22)	(0.58)	(0.55)	—	(0.36)	(0.16)	(0.20)	(0.19)	(0.24)	(0.63)	(0.40)
Mg	0.36*		0.49*	0.20	0.45*	−0.47	−0.84	0.20	0.05	−0.12	0.56	−0.24	0.17	0.59*	0.13	0.10	−0.01	−0.05
	(0.04)		(0.11)	(0.13)	(0.12)	(0.91)	(0.59)	(0.17)	(0.45)	(0.38)	(0.85)	(0.26)	(0.11)	(0.11)	(0.15)	(0.19)	(0.44)	(0.31)
P	0.39*	0.56*		−0.04	0.61*	−0.61	−0.11	0.55*	0.25	0.31	—	0.31	0.23	0.53*	0.22	−0.27	−0.07	0.21
	(0.04)	(0.03)		(0.17)	(0.09)	(1.28)	(0.37)	(0.16)	(0.53)	(0.44)	—	(0.30)	(0.12)	(0.13)	(0.16)	(0.20)	(0.50)	(0.34)
K	0.19*	0.35*	0.54*		0.02	−0.57	0.27	0.29	0.31	0.46	0.15	−0.19	−0.05	−0.09	0.02	0.12	0.13	0.18
	(0.04)	(0.04)	(0.03)		(0.16)	(0.75)	(0.38)	(0.17)	(0.58)	(0.47)	(0.53)	(0.27)	(0.13)	(0.17)	(0.16)	(0.20)	(0.49)	(0.37)
Ca	0.30*	0.57*	0.75*	0.43*		−0.29	0.01	0.57*	−0.07	−0.15	0.93	0.37	0.25*	0.63*	0.14	−0.05	0.22	0.15
	(0.04)	(0.03)	(0.02)	(0.04)		(0.79)	(0.39)	(0.17)	(0.54)	(0.43)	(0.89)	(0.29)	(0.12)	(0.12)	(0.16)	(0.21)	(0.70)	(0.36)
V	−0.19	0.01	−0.23*	−0.07	0.02		0.70	0.72	0.84	0.42	—	—	−0.08	0.32	−0.29	0.46	0.36	—
	(0.10)	(0.10)	(0.10)	(0.11)	(0.11)		(1.44)	(1.10)	(2.11)	(1.42)	—	—	(0.52)	(0.80)	(0.97)	(1.04)	(1.37)	—
Cr	0.07	−0.02	0.01	0.02	−0.05	0.02		−0.04	−0.62	−0.76	—	—	−0.16	−0.62	0.03	−0.85	0.07	—
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.11)		(0.40)	(1.65)	(1.55)	—	—	(0.30)	(0.44)	(0.35)	(0.70)	(1.03)	—
Mn	0.19*	0.17*	0.22*	0.08	0.16*	−0.08	0.21*		0.01	0.13	0.55	0.24	0.41*	0.40*	0.26	−0.02	−0.42	0.33
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.09)	(0.04)		(0.69)	(0.37)	(0.52)	(0.31)	(0.14)	(0.18)	(0.18)	(0.24)	(0.53)	(0.40)
Fe	0.10*	0.04	0.07	0.05	−0.02	−0.06	0.40*	0.42*		—	0.83	0.66	0.05	0.24	0.30	−0.34	—	—
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.10)	(0.04)	(0.03)		—	(1.04)	(1.05)	(0.43)	(0.50)	(0.59)	(0.74)	—	—
Co	0.07	0.11	0.14*	0.04	−0.04	−0.00	0.32*	0.37*	0.59*		—	−0.42	0.30	0.26	−0.76	−0.44	—	−0.82
	(0.06)	(0.06)	(0.06)	(0.06)	(0.06)	(0.12)	(0.06)	(0.05)	(0.03)		—	(0.69)	(0.51)	(0.40)	(1.14)	(0.57)	—	(1.19)
Ni	−0.23*	−0.11	0.04	−0.00	0.10	—	0.09	0.17*	0.50*	—		—	0.01	−0.05	0.38	—	0.99	—
	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	—	(0.07)	(0.07)	(0.05)	—		—	(0.39)	(0.43)	(0.89)	—	(2.42)	—
Cu	0.04	0.02	0.01	−0.09*	0.04	0.09	—	0.03	−0.09*	−0.24*	—		0.20	0.27	−0.21	−0.64	0.19	—
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.10)	—	(0.04)	(0.04)	(0.05)	—		(0.24)	(0.29)	(0.29)	(0.35)	(0.78)	—
Zn	0.22*	0.37*	0.38*	0.15*	0.35*	0.00	0.13*	0.25*	0.15*	0.22*	−0.13	0.11*		0.52*	0.16	−0.04	−0.28	0.28
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.10)	(0.04)	(0.04)	(0.04)	(0.06)	(0.07)	(0.04)		(0.10)	(0.13)	(0.17)	(0.48)	(0.30)
Se	0.32*	0.49*	0.43*	0.15*	0.42*	−0.03	0.05	0.22*	0.10*	0.23*	−0.19*	0.03	0.48*		0.25	0.08	−0.11	−0.01
	(0.04)	(0.03)	(0.03)	(0.04)	(0.03)	(0.10)	(0.04)	(0.04)	(0.04)	(0.05)	(0.07)	(0.04)	(0.03)		(0.17)	(0.22)	(0.47)	(0.36)
Mo	0.11*	0.20*	0.26*	0.07	0.23*	−0.00	0.14*	0.29*	0.25*	0.37*	0.14*	0.04	0.23*	0.21*		−0.12	−0.47	−0.14
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.10)	(0.04)	(0.04)	(0.04)	(0.05)	(0.07)	(0.04)	(0.04)	(0.04)		(0.22)	(0.44)	(0.34)
I	−0.06	0.01	−0.05	0.08	−0.02	0.01	0.03	−0.05	0.03	−0.05	−0.01	−0.17*	−0.04	−0.00	0.14*		−0.40	—
	(0.05)	(0.06)	(0.06)	(0.06)	(0.06)	(0.17)	(0.07)	(0.06)	(0.06)	(0.12)	(0.09)	(0.06)	(0.06)	(0.06)	(0.06)		(0.50)	—
Hg	0.07	−0.05	−0.16*	−0.11	−0.14*	0.04	0.13*	−0.04	—	0.07	0.06	0.10	0.01	0.00	0.03	−0.22*		−0.93
	(0.06)	(0.06)	(0.06)	(0.06)	(0.06)	(0.15)	(0.06)	(0.05)	—	(0.08)	(0.11)	(0.05)	(0.06)	(0.06)	(0.06)	(0.09)		(0.95)
B_{12}	0.13	−0.00	0.04	0.07	0.06	−0.01	—	0.05	0.03	0.19	—	—	0.02	0.16*	0.08	—	−0.10	
	(0.09)	(0.08)	(0.09)	(0.09)	(0.09)	(0.17)	—	(0.08)	(0.10)	(0.10)	—	—	(0.06)	(0.08)	(0.07)	—	(0.09)	

[1]Additive genetic correlations are presented above the diagonal, and phenotypic correlations are presented below. Corresponding SE are given in parentheses.

[2]Not estimable.

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

Table 7. Correlations between element concentrations within serum[1]

Item	Na	Mg	P	K	Ca	V	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	Cd	Hg	Pb
Na		0.60*	—[2]	0.58*	0.57	0.24	0.50	0.45	0.27	0.40	−0.15	0.18	0.59	0.14	0.67	—	0.98*
		(0.37)	—	(0.24)	(0.56)	(0.29)	(0.66)	(0.40)	(0.39)	(0.35)	(0.34)	(0.36)	(0.337)	(0.33)	(0.35)	—	(0.07)
Mg	0.68*		0.43*	0.53*	0.49*	0.28	0.01	0.77*	−0.08	0.14	0.19	0.51*	−0.26	0.36	−0.18	0.05	−0.01
	(0.05)		(0.16)	(0.17)	(0.20)	(0.19)	(0.49)	0.17)	(0.29)	(0.22)	(0.20)	(0.17)	(0.31)	(0.20)	(0.25)	(0.21)	(0.19)
P	—	0.68*		0.66*	0.67*	0.42*	−0.38	0.32	−0.05	−0.01	0.01	0.03	0.32	−0.02	0.03	−0.12	0.00
	—	(0.03)		(0.11)	(0.11)	(0.15)	(1.04)	(0.24)	(0.24)	(0.19)	(0.18)	(0.21)	(0.18)	(0.20)	(0.22)	(0.18)	(0.16)
K	0.71*	0.64*	0.71*		0.63*	0.45*	−0.22	0.39	0.05	0.10	0.17	0.23	0.20	0.55*	0.42	−0.20	0.05
	(0.05)	(0.03)	(0.02)		(0.14)	(0.16)	(0.49)	(0.25)	(0.25)	(0.20)	(0.19)	(0.20)	(0.24)	(0.18)	(0.21)	(0.18)	(0.17)
Ca	0.63*	0.70*	0.82*	0.77*		0.53*	−0.21	0.49	−0.06	0.07	0.26	0.34	0.08	0.48*	0.59*	−0.22	0.09
	(0.06)	(0.02)	(0.02)	(0.02)		(0.17)	(0.70)	(0.25)	(0.30)	(0.24)	(0.19)	(0.17)	(0.31)	(0.22)	(0.25)	(0.22)	(0.21)
V	0.09	0.37*	0.46*	0.42*	0.43*		0.63*	0.18	−0.05	0.31	0.03	0.21	0.21	0.57*	0.71*	−0.44*	−0.09
	(0.25)	(0.04)	(0.04)	(0.04)	(0.04)		(0.29)	(0.24)	(0.23)	(0.18)	(0.18)	(0.18)	(0.21)	(0.14)	(0.14)	(0.14)	(0.15)
Mn	0.34*	0.26*	0.30*	0.23*	0.26*	0.35*		0.02	0.23	−0.15	−0.03	0.50	−0.17	−0.20	0.28	0.08	−0.40
	(0.17)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)		(0.60)	(0.49)	(0.45)	(0.43)	(0.43)	(0.58)	(0.48)	(0.44)	(0.39)	(0.44)
Fe	0.23	0.61*	0.55*	0.42*	0.55*	0.34*	0.29*		0.11	−0.21	0.17	0.43	−0.04	0.37	−0.52	−0.15	−0.12
	(0.15)	(0.03)	(0.03)	(0.04)	(0.03)	(0.04)	(0.04)		(0.34)	(0.27)	(0.25)	(0.24)	(0.34)	(0.24)	(0.30)	(0.25)	(0.24)
Co	0.33	0.57*	0.59*	0.45*	0.47*	0.32*	0.31*	0.54*		0.43	0.08	−0.16	0.10	−0.47	−0.26	−0.67*	−0.20
	(0.19)	(0.03)	(0.03)	(0.04)	(0.04)	(0.05)	(0.04)	(0.03)		(0.24)	(0.22)	(0.30)	(0.29)	(0.28)	(0.28)	(0.22)	(0.20)
Ni	−0.33	−0.01	−0.02	−0.03	0.02	0.07	0.10	0.01	0.05		0.11	0.10	0.41	0.21	0.22	−0.35*	0.29
	(0.50)	(0.06)	(0.06)	(0.06)	(0.06)	(0.07)	(0.06)	(0.06)	(0.06)		(0.18)	(0.21)	(0.25)	(0.21)	(0.21)	(0.14)	(0.16)
Cu	0.37*	0.61*	0.53*	0.56*	0.67*	0.35*	0.26*	0.47*	0.49*	0.06		0.22	0.11	0.19	−0.07	−0.30	−0.01
	(0.10)	(0.03)	(0.03)	(0.03)	(0.03)	(0.05)	(0.04)	(0.04)	(0.04)	(0.06)		(0.18)	(0.23)	(0.19)	(0.21)	(0.18)	(0.16)
Zn	−0.28	0.70*	0.62*	0.54*	0.70*	0.39*	0.34*	0.61*	0.55*	0.06	0.59*		−0.07	0.21	−0.04	−0.15	0.51*
	(0.19)	(0.02)	(0.03)	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)	(0.04)	(0.06)	(0.03)		(0.24)	(0.22)	(0.24)	(0.19)	(0.17)
Se	0.71*	0.58*	0.73*	0.60*	0.75*	0.37*	0.24*	0.48*	0.58*	−0.01	0.66*	0.59*		0.31	−0.20	−0.19	0.32
	(0.05)	(0.03)	(0.02)	(0.03)	(0.02)	(0.04)	(0.04)	(0.04)	(0.03)	(0.06)	(0.03)	(0.03)		(0.26)	(0.28)	(0.24)	(0.21)
Mo	0.32*	0.33*	0.18*	0.23*	0.16*	0.35*	0.10	0.32*	0.24*	0.05	0.31*	0.30*	0.14*		0.10	−0.21	0.49*
	(0.10)	(0.04)	(0.05)	(0.04)	(0.05)	(0.05)	(0.05)	(0.04)	(0.05)	(0.07)	(0.04)	(0.05)	(0.05)		(0.22)	(0.19)	(0.15)
Cd	0.02	0.14*	0.09*	0.16*	0.14*	0.34*	0.16*	0.08	0.13*	0.11	0.10*	0.15*	0.05	0.23*		−0.23	−0.24
	(0.16)	(0.00)	(0.05)	(0.04)	(0.05)	(0.04)	(0.04)	(0.05)	(0.05)	(0.07)	(0.05)	(0.05)	(0.05)	(0.05)		(0.17)	(0.18)
Hg	—	0.05	0.11	0.04	−0.01	−0.20*	0.03	0.01	−0.17*	−0.19*	−0.05	−0.04	−0.01	−0.10	−0.10		−0.11
	—	(0.08)	(0.08)	(0.07)	(0.07)	(0.08)	(0.07)	(0.07)	(0.07)	(0.09)	(0.08)	(0.08)	(0.07)	(0.09)	(0.07)		(0.13)
Pb	0.86*	0.11*	0.21*	0.13*	0.16*	0.13*	0.10*	0.14*	0.11*	0.19*	0.10*	0.24*	0.19*	0.26*	−0.01	−0.04	
	(0.03)	(0.05)	(0.05)	(0.05)	(0.04)	(0.05)	(0.05)	(0.05)	(0.05)	(0.06)	(0.05)	(0.05)	(0.04)	(0.05)	(0.05)	(0.08)	

[1]Additive genetic correlations are presented above the diagonal, and phenotypic correlations are presented below. Corresponding SE are given in parentheses.

[2]Not estimable.

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

= 0.45, $P = 0.006$). Phenotypically, V showed moderate to strong correlations with all other serum nutrients except for Cr, Hg, Ni, and Na.

Associations Between Milk and Serum Elements

Genetic correlations of elements between milk and serum are presented in Table 8, and phenotypic correlations are shown in Table 9 (results from the full analysis are available in Supplemental Table S3, https://doi.org/10.3168/jds.2019-16960). Significant additive genetic correlations (significantly different from zero at $P < 0.05$) were found to exist between several of the milk and serum elements, with most being positive (Table 8). The strongest negative associations were observed between serum Ni with milk V (r = −0.98, $P = 0.008$), Co (r = −0.86, $P = 0.011$), and Na (r = −0.62, $P = 0.017$). Potassium was the only element that was found to have a significant correlation between concentrations recorded in milk and serum (r = 0.45, $P = 0.025$). Further, milk K was found to be significantly positively correlated with serum Mg (r = 0.53, $P = 0.008$), Co (r = 0.48, $P = 0.039$), Mo (r = 0.45, $P = 0.020$), and Cd (r = 0.43, $P = 0.035$). Moreover, serum Mg was highly correlated with milk Ca (r = 0.54, $P = 0.014$), Mn (r = 0.57, $P = 0.028$), and P (r = 0.49, $P = 0.029$). Associations involving heavy metals were observed only between serum Cd and milk Se, K, and Mn.

All phenotypic correlations obtained between milk and serum element concentrations are presented in Table 9. Statistically significant correlations were obtained for Ca (r = 0.17, $P = 0.019$), Mo (r = 0.19, $P = 0.009$), K (r = 0.19, $P = 0.006$), and Na (r = –0.79, $P < 0.001$). Serum Na was also found to be strongly positively correlated with milk Ca (r = 0.81, $P < 0.001$), Zn (r = 0.74, $P < 0.001$), K (r = 0.64, $P < 0.001$), and Mg (r = 0.49, $P = 0.024$). The majority of associations observed were positive, but negative correlations were noted for milk Cr with serum Se (r = −0.16, $P = 0.029$), milk Fe with serum Pb (r = −0.62, $P < 0.001$) and serum Cd (r = −0.288, $P = 0.002$), milk Hg with serum Cu (r = −0.23, $P = 0.003$) and serum Mo (r = −0.20, $P = 0.034$), milk Zn with serum Co (r = −0.15, $P = 0.033$), and milk B_{12} with serum Ni (r = −0.38, $P < 0.001$; Table 9). It was noted that Cd and Pb in milk

Table 8. Additive genetic correlations (r) between milk and serum element concentrations with corresponding SE and *P*-values[1]

Milk	Serum	r (SE)	*P*-value
Na[2] (mg/L)	Ca[2] (mg/L)	0.56 (0.275)	0.049
Na[2] (mg/L)	Ni[3] (μg/L)	−0.62 (0.244)	0.017
Mg[2] (mg/L)	Fe[3] (μg/L)	0.65 (0.266)	0.021
P[2] (mg/L)	Mg[2] (mg/L)	0.49 (0.215)	0.029
K[2] (mg/L)	Cd[4] (μg/L)	0.43 (0.194)	0.035
K[2] (mg/L)	Co[3] (μg/L)	0.48 (0.222)	0.039
K[2] (mg/L)	Mg[2] (mg/L)	0.53 (0.189)	0.008
K[2] (mg/L)	Mo[3] (μg/L)	0.45 (0.182)	0.020
K[2] (mg/L)	K[2] (mg/L)	0.45 (0.192)	0.025
Ca[2] (mg/L)	Mg[2] (mg/L)	0.54 (0.210)	0.014
V[3] (μg/L)	Ni[3] (μg/L)	−0.98 (0.354)	0.008
Mn[3] (μg/L)	Cd[4] (μg/L)	0.77 (0.219)	<0.001
Mn[3] (μg/L)	Mg[2] (mg/L)	0.57 (0.247)	0.028
Co[3] (μg/L)	Ni[3] (μg/L)	−0.86 (0.319)	0.011
Se[3] (μg/L)	Cd[4] (μg/L)	−0.46 (0.210)	0.036
Mo[3] (μg/L)	Fe[3] (μg/L)	0.68 (0.302)	0.031

[1]Only correlations significantly different from zero at $P < 0.05$ are presented.
[2]Quantity element.
[3]Trace element.
[4]Heavy metal.

Table 9. Phenotypic correlations (r) between milk and serum elements and vitamin B_{12} with corresponding SE and *P*-values[1]

Milk	Serum	r (SE)	*P*-value
Na[2] (mg/L)	Cd[4] (μg/L)	0.39 (0.070)	<0.001
Na[2] (mg/L)	Pb[4] (μg/L)	0.36 (0.074)	<0.001
Na[2] (mg/L)	Na[2] (mg/L)	−0.79 (0.175)	<0.001
Mg[2] (mg/L)	Fe[3] (μg/L)	0.19 (0.064)	0.004
Mg[2] (mg/L)	Na[2] (mg/L)	0.49 (0.208)	0.024
P[2] (mg/L)	Cd[4] (μg/L)	0.32 (0.072)	<0.001
P[2] (mg/L)	Ca[2] (mg/L)	0.15 (0.071)	0.043
P[2] (mg/L)	Fe[3] (μg/L)	0.15 (0.069)	0.037
P[2] (mg/L)	Mg[2] (mg/L)	0.15 (0.070)	0.037
K[2] (mg/L)	Cd[4] (μg/L)	0.30 (0.074)	<0.001
K[2] (mg/L)	Ca[2] (mg/L)	0.18 (0.069)	0.014
K[2] (mg/L)	Co[3] (μg/L)	0.21 (0.066)	0.002
K[2] (mg/L)	Fe[3] (μg/L)	0.15 (0.068)	0.037
K[2] (mg/L)	Mg[2] (mg/L)	0.20 (0.067)	0.005
K[2] (mg/L)	Mo[3] (μg/L)	0.18 (0.076)	0.027
K[2] (mg/L)	P[2] (mg/L)	0.16 (0.064)	0.019
K[2] (mg/L)	K[2] (mg/L)	0.19 (0.067)	0.006
K[2] (mg/L)	Na[2] (mg/L)	0.64 (0.173)	<0.001
Ca[2] (mg/L)	Cd[4] (μg/L)	0.22 (0.078)	0.006
Ca[2] (mg/L)	Ca[2] (mg/L)	0.17 (0.070)	0.019
Ca[2] (mg/L)	Fe[3] (μg/L)	0.16 (0.068)	0.024
Ca[2] (mg/L)	Mg[2] (mg/L)	0.14 (0.069)	0.047
Ca[2] (mg/L)	Na[2] (mg/L)	0.81 (0.118)	<0.001
V[3] (μg/L)	Pb[4] (μg/L)	0.53 (0.107)	<0.001
Cr[3] (μg/L)	Se[3] (μg/L)	−0.16 (0.070)	0.029
Fe[3] (μg/L)	Cd[4] (μg/L)	−0.28 (0.086)	0.002
Fe[3] (μg/L)	Pb[4] (μg/L)	−0.62 (0.046)	<0.001
Co[3] (μg/L)	Cd[4] (μg/L)	0.56 (0.063)	<0.001
Zn[3] (μg/L)	Co[3] (μg/L)	−0.15 (0.066)	0.033
Zn[3] (μg/L)	Na[2] (mg/L)	0.74 (0.142)	<0.001
Se[3] (μg/L)	Ca[2] (mg/L)	0.15 (0.068)	0.037
Mo[3] (μg/L)	Cd[4] (μg/L)	0.22 (0.072)	0.004
Mo[3] (μg/L)	Fe[3] (μg/L)	0.17 (0.063)	0.011
Mo[3] (μg/L)	Mo[3] (μg/L)	0.19 (0.070)	0.009
Hg[4] (μg/L)	Cu[3] (μg/L)	−0.23 (0.074)	0.003
Hg[4] (μg/L)	Mo[3] (μg/L)	−0.20 (0.089)	0.034
Vitamin B_{12} (μg/L)	Cd[4] (μg/L)	0.21 (0.092)	0.028
Vitamin B_{12} (μg/L)	Ni[3] (μg/L)	−0.38 (0.096)	<0.001

[1]Only correlations significantly different from zero at $P < 0.05$ are presented.
[2]Quantity element.
[3]Trace element.
[4]Heavy metal.

as well as Cr in serum showed no associations with any other nutrient, whether in milk or serum.

DISCUSSION

The main aim of this study was to estimate (co)variance components of important dairy cattle milk and serum elements (minerals, heavy metals), as well as milk vitamins B_{12}, to explore potential selection strategies for optimizing concentrations for the benefit of both the cow and the human dairy product consumer. Significant heritability estimates were obtained for 6 milk and 4 serum minerals in addition to repeatability estimates for 10 milk and 15 serum elements (Tables 4 and 5). From the literature, the majority of genetic analyses previously reported correspond to quantity elements in milk (a summary of h^2 values in the literature can be found in Supplemental Table S4, https://doi.org/10.3168/jds.2019-16960). Milk Ca, Mg, P, K, and Na have been shown to have heritabilities ranging from 0.10 (Toffanin et al., 2015) to 0.72 (Buitenhuis et al., 2015), 0.08 (Buitenhuis et al., 2015) to 0.60 (van Hulzen et al., 2009), 0.12 (Toffanin et al., 2015) to 0.62 (van Hulzen et al., 2009), 0.19 (Visentin et al., 2019) to 0.46 (van Hulzen et al., 2009), and 0.20 (Buitenhuis et al., 2015) to 0.24 (Visentin et al., 2019), respectively. Heritability estimates for some milk trace elements have also been reported, including Cu (0.28, Buitenhuis et al., 2015), Fe (0.15, Buitenhuis et al., 2015), Mn (0.13, Buitenhuis et al., 2015), Se (0.20, van Hulzen et al., 2009; 0.20, Buitenhuis et al., 2015), and Zn (0.41, van Hulzen et al., 2009; 0.57, Buitenhuis et al., 2015). Regarding serum, a genetic analysis of Ca, Mg, P, and K carried out by Tsiamadis et al. (2016) reported heritabilities of 0.20, 0.21, 0.25, and 0.10, respectively. Furthermore, heritabilities of serum Cu and Zn have been reported as 0.22 (Morris et al., 2006). The results from the present study are within these ranges for these nutrients. We also investigated several milk and serum elements (including heavy metals) that we believe have not yet been reported in dairy cows. As such, we believe that this is the first study to estimate heritability of the milk trace element Mo (0.19) as well as repeatability estimates for milk and serum trace elements and heavy metals.

Concentrations of I in milk are known to be affected by several factors, including dietary I level, the presence of I antagonists (e.g., glucosinolates) in the feed, farm management practices, teat dipping with I-containing substances, and milk processing (Flachowsky et al., 2014). In the present study, milk I was influenced by diet type and was significantly repeatable (0.24, $P = 0.005$); this should be important given the importance of UK intakes of I for milk and dairy products (Kliem and Givens, 2011). Although mean milk I concentrations were much higher than those listed in the current UK Food Composition Database (Food Standards Agency, 2015), it is important to note that the current study analyzed raw milk and that pasteurization is known to substantially reduce I concentrations in milk (Nazeri et al., 2015).

Sodium is another essential quantity element that has been shown to be an important factor in milk production (Derrig et al., 1974; Spek et al., 2013) and is lost though milk, urine, saliva, and feces (Renkema et al., 1962). We observed a strong negative phenotypic association between concentrations of Na in milk and serum ($r = -0.79$, $P < 0.001$), suggesting that increased milk Na concentrations correspond to a decrease in serum concentrations. During lactation, Na concentrations of milk have been shown to increase (Gueguen et al., 1961; Safwate et al., 1981), whereas in blood large variations (dependent on physiological condition or age) have been observed (Skrzypczak et al., 2013). Moreover, it has been hypothesized that decreased Na concentrations in early lactation may be due to decreased plasma rennin activity postcalving (Ożgo et al., 2008).

Milk is also an excellent source of vitamin B_{12}, and milk and dairy products contribute significantly to vitamin B_{12} intakes in humans (150% of RNI; Henderson et al., 2003a,b; Kliem and Givens, 2011), making it an attractive breeding target in terms of enhancing nutrient quality for the consumer. Vitamin B_{12} contains Co, and Co is required in the diet of cattle in order for this vitamin to be synthesized endogenously by rumen bacteria (Stemme et al., 2008). Although the concentration of Co in serum was repeatable (0.14, $P = 0.019$), we did not observe significant repeatability in milk Co or vitamin B_{12}. The estimated heritability of vitamin B_{12} in milk was not significant ($h^2 = 0.12$, $P = 0.18$); this was also true for milk and serum Co ($h^2 = 0.04$, $P = 0.31$ and $h^2 = 0.07$, $P = 0.30$, respectively). Furthermore, we found no significant associations between milk vitamin B_{12} and Co in either milk or serum.

Genetic line (average or highest genetic merit for milk fat plus protein yield) had no significant effect on circulating element or vitamin B_{12} concentrations in either milk or blood serum with the exception of the heavy metal Hg, which showed higher concentrations in the milk of select-line cows. Moreover, because the cows were part of an experimental research herd, any biases in management between the genetic lines were nonexistent such that cows within the same line but on diverging diets were consequently unaffected by management decisions (Pryce et al., 1999). Concentrations of elements in both milk and serum were affected depending on whether the cow was fed the homegrown or by-product ration. This has potential benefits for

manipulation of nutrient content through changes in management alone, a benefit that could be complemented or improved through selection and breeding. It also suggests that selection for higher milk fat and protein is independent of blood or milk micronutrient concentrations.

Given the mostly positive genetic correlations among the milk minerals examined in the present study, selection alone for a single milk mineral might be expected to also increase the concentrations of other minerals. For example, selection for milk Ca would likely boost P, Zn, and Se concentrations, leading to multiple improvements in milk mineral concentrations for the benefit of the human dairy consumer.

Furthermore, because heavy metals have adverse effects on health, any breeding objectives should also be directed toward minimizing concentrations of these metals or at least not increasing concentrations. The findings of this study identified few significant genetic associations of heavy metals with micronutrient concentrations, and in cases where a significant association was found, these tended to be negative. This suggests that genetic selection programs aimed at increasing micronutrient concentrations should not inadvertently increase concentrations of toxic heavy metals. The minimum risk level has not been established for Cd or Hg in milk, but the minimum risk level for Pb in EU milk is 20 μg/kg (European Union, 2001). The Pb concentrations as found in milk in this study were below levels of food safety concern in the European Union.

It is interesting to note that although significant phenotypic relationships were observed between some milk and corresponding serum element measurements, only 1 genetic association was identified (between milk and serum K). Moreover, we observed stronger and additional relationships between differing nutrients between milk and serum. Results from the present study agree with those of Wang et al. (2014) in that concentrations of Cu, Fe, and Zn in milk do not reflect corresponding serum concentrations. Additionally, our findings suggest that the same is true of all elements examined in the present study with the exception of Na, K, Ca, and Mo.

CONCLUSIONS

The present study established that circulating concentrations of elements in both the milk and serum of dairy cows are significantly influenced by genetics and feeding system. As expected, diet had a significant effect on mineral concentrations, especially in milk, and as such provided a potential route for manipulation via changes in rations. The results presented provide clear evidence that many of such traits are heritable, indicating that selection for desired element concentrations in both milk and serum is possible. This work will help inform industry solutions to better improve both genetics and management practices for the benefit of not only the cow but also the healthfulness of the milk for the consumer.

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