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

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

3 *By Denholm et al.* Elements are essential dietary components in human health. Enhancing
4 nutritious element concentrations within dairy cow milk and serum, whilst ensuring
5 concentrations of toxic elements such as heavy metals are minimized, is important both from
6 the perspective of the health of the cow and the nutritional value of her milk for human
7 consumption. Our results suggest element concentrations in dairy cow milk and serum are
8 significantly influenced by both diet and genetics, and that a combination of genetic selection
9 and dietary manipulation could be employed to alter such concentrations to improve both
10 cow health and the healthiness of milk for human consumption.

11

12 **ANALYSIS OF MILK AND SERUM ELEMENT CONCENTRATIONS**

13

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

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ABSTRACT

29 Enhancing micronutrient (*i.e.*, mineral and vitamin) concentrations within milk and serum
30 from dairy cows is important both from the perspective of the health of the cow and the
31 nutritive value of the milk for human consumption. However, a good understanding of the
32 genetics underlying micronutrient content in dairy cattle is needed to facilitate such
33 enhancements through feeding or breeding practices. In this study, milk ($n=950$) and serum
34 ($n=766$) samples were collected from Holstein-Friesian dairy cows ($n=479$) on 19 occasions
35 over a 59 month period and analyzed for concentrations of important elements. Additionally,
36 a subset of 256 milk samples was also analyzed for concentrations of vitamin B₁₂. Cows
37 belonged to 2 genetic lines (average and highest genetic merit for milk fat plus protein yield)
38 and were assigned one of 2 diets based on either a by-product (BP) or home-grown (HG)
39 ration. Univariate models accounting for repeated records were used to analyze element and
40 vitamin B₁₂ data and investigate the impact of genotype and feeding system as well as derive
41 estimates of variance components and genetic parameters. Bivariate models were used to
42 study correlations both within and between milk and serum. Only concentrations of Hg in
43 milk were seen to be affected by genotype, with higher concentrations in high genetic merit
44 cows. In contrast, element concentrations were influenced by feeding system such that cows
45 fed the HG diet had increased milk concentrations of Ca, Cu, I, Mn, Mo, P and K, and
46 increased serum concentrations of Cd, Cu, Fe, Mo and V. Cows on the BP diet saw increased
47 milk concentrations of Mg, Se and Na and serum concentrations of P and Se. Heritability
48 estimates were obtained for 6 milk and 4 serum elements, including Mg ($h^2_{milk} = 0.30$), Ca
49 ($h^2_{milk} = 0.20$; $h^2_{serum} = 0.12$), Mn ($h^2_{milk} = 0.14$), Cu ($h^2_{serum} = 0.22$), Zn ($h^2_{milk} = 0.24$), Se
50 ($h^2_{milk} = 0.15$; $h^2_{serum} = 0.10$) and Mo ($h^2_{milk} = 0.19$). Significant estimates of repeatability

51 were observed in all milk and serum quantity elements (Na, Mg, P, K, Ca) as well as 5 milk
52 and 7 serum trace elements. Only K in milk and serum was found to have a significant
53 positive genetic and phenotypic correlation (0.52 and 0.22, respectively). Significant
54 phenotypic associations were noted between milk and serum Ca (0.17), Mo (0.19) and Na (-
55 0.79). Additional multi-variate analyses between measures within sample type (i.e., milk or
56 serum) revealed significant positive associations, both phenotypic and genetic, between some
57 of the elements; in milk Se was genetically correlated with Ca (0.63), Mg (0.59), Mn (0.40),
58 P (0.53) and Zn (0.52); whereas in serum V showed strong genetic associations with Cd
59 (0.71), Ca (0.53), Mn (0.63), Mo (0.57), P (0.42), K (0.45) and Hg (-0.44). These results
60 provide evidence that element concentrations in milk and blood of dairy cows are
61 significantly influenced by both diet and genetics, and demonstrate the potential for genetic
62 selection and dietary manipulation to alter nutrient concentration to improve both cow health
63 and the healthiness of milk for human consumption.

64 **Keywords:** micronutrient; heavy metal; dairy cow; heritability; correlation

65

66

INTRODUCTION

67 Micronutrients are required throughout life and consist of vitamins and minerals that
68 are essential for maintaining normal body function and health in humans and other animals
69 (FAO and WHO, 2004; Gernand et al., 2016). Neither humans nor other animals can
70 synthesize micronutrients within the body and therefore micronutrients must be obtained
71 from the diet. When intakes of these vitamins and minerals are suboptimal this can affect
72 normal growth and development, reducing performance as well as increasing susceptibility
73 to, for example, Keshan disease in humans and white muscle disease in cattle due to selenium
74 (Se) deficiency (Muth et al., 1958; Yu, 1982).

75 Whereas vitamins are organic molecules, minerals such as phosphorus (P), calcium
76 (Ca), iron (Fe), zinc (Zn), Se, and iodine (I) are inorganic but are required only in very small
77 amounts. Minerals can be further classified into trace elements (e.g. Fe, Zn, Se, I) that are
78 required in low amounts and quantity elements (e.g. Mg, P, K, Ca) which are required in
79 larger amounts. When intakes of quantity/trace elements or vitamins from the diet are
80 insufficient, deficiencies can arise that can compromise animal and human health and
81 increase the risk of disease. Indeed, there is a growing concern currently that sizable
82 proportions of the human population do not meet micronutrient Reference Nutrient Intake
83 (RNI) values, i.e., the amount of nutrient required to prevent deficiency (Rooke et al., 2010;
84 Givens et al., 2014).

85 Dairy products, such as milk and cheese, are important sources of minerals and
86 vitamins and contribute substantially to dietary intakes of Ca, P, I, Zn and Mg (60%, 60%,
87 55%, 18% and 10% of RNI, respectively) as well as vitamins A, B₂ and B₁₂ (26%, 52% and
88 150% of RNI, respectively) (Kliem and Givens, 2011). Importantly, Mg and Ca are
89 increasingly significant factors in bone development, especially in children (Givens et al.,
90 2014). Furthermore, the circulating concentrations of these minerals and vitamins in the
91 blood and milk of dairy cows likely relate to the fitness of the animal given their important
92 roles in numerous physiological and immunological processes (Percival, 1998; Doherty,
93 2007; Maggini et al., 2007; Hoffmann and Berry, 2008; Prasad, 2008; Alpert, 2017).
94 Therefore, identifying breeding strategies within the cow to increase milk micronutrient
95 concentrations as well as optimizing micronutrient concentrations within the cow herself
96 should be of ultimate benefit to both the cow and the human dairy product consumer.
97 Moreover, as heavy metals such as lead (Pb), mercury (Hg), and cadmium (Cd) which have
98 potential adverse effects on health can also be found in milk (Rey-Crespo et al., 2013),
99 breeding strategies should also be commensurate with reducing, or at least not increasing,

100 concentrations of these metals where possible. Dietary manipulation of mineral
101 concentrations in livestock has been demonstrated yet there is a relative lack of knowledge
102 concerning the contribution of cow genetics to variation in concentrations of elements
103 (including heavy metals) and vitamins within the blood and milk of dairy cows (Rooke et al.,
104 2010).

105 The aim of this study was to carry out a phenotypic and genetic analysis of mineral,
106 vitamin and heavy metal concentrations in dairy cow milk and serum in order to determine;
107 1) the effect genotype and diet on individual element and vitamin B₁₂ concentrations; 2) if
108 relationships exist between element concentrations (including vitamin B₁₂) in milk and
109 serum; and 3) if variation between animals exists that would permit selection for optimized
110 element and vitamin B₁₂ concentrations that would be of benefit to both the health of the cow
111 and that of the human consumer.

112

113

MATERIAL AND METHODS

114 *Animals*

115 Animals involved in this study were from the Langhill pedigree herd of Holstein-
116 Friesian dairy cows ($n=479$) housed at the SRUC Dairy Research Centre in Dumfries,
117 Scotland, between 2012 and 2016. All cows were part of a long-term (on-going) selection
118 experiment for genotype x environment following a 2 by 2 approach (Veerkamp et al.,
119 1994). Briefly, the herd has been divided equally between two distinct genetic lines (Control
120 and Select) selected since 1970, and assigned one of two diets based on differing rations. The
121 Control line has been bred to bulls of UK national average genetic merit for kilogram fat plus
122 protein yield (**kg F + P**). In contrast, the high genetic merit Select line (top 5% genetic merit)
123 has been bred from bulls with the highest genetic merit for kg F + P. The two diet groups
124 consist of a low forage, high energy ration based on by-products and minimal land use,

125 simulating high-input commercial systems, and a high forage, lower energy ration based on
126 home-grown components and using the maximum amount of land available, thus simulating
127 low-input grazing systems (Pryce et al., 1999; Roberts and March, 2013).

128 The Home-grown ration (**HG**) consisted of components grown exclusively on farm
129 and included grazed grass, grass silage, red clover silage, forage maize, lucerne silage,
130 crimped wheat and beans. Additionally, the HG ration was balanced with purchased minerals.
131 In contrast the By-product ration (**BP**) consisted of biscuit meal, sugar beet pulp, chopped
132 straw, breakfast cereal, wheat distillers dark grains, soya bean meal (Hipro 50%), Vitagold,
133 protected Fat (Megalac), molasses and minerals. Mineral compositions of both HG and BP
134 diets are presented in Table S1 of the accompanying Supplementary Material. Target milk
135 yields of Select cows on the low and high energy diets are 7,500 and 13,000 liters per
136 lactation, respectively, the UK average per cow/lactation is approx. 7,557 (AHDB Dairy,
137 2017).

138

139 *Ethics Statement*

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

145

146 *Sampling Protocol*

147 Samples used in the present study were collected across several years and seasons
148 from the same on-going experimental system; 385 (of 479) cows having 2 or more samples.
149 Furthermore, samples were selected such that they accounted for genotype and management

150 of cows in order to give a balanced representation of the herd. In total, 950 milk samples and
151 766 serum samples were collected for analysis of element and vitamin B₁₂ (milk only)
152 concentrations. Further information regarding sample collection is presented in Table S2 of
153 the accompanying Supplementary Material.

154 **Milk Samples.** Cows in the Langhill herd are milked three times daily (AM, MD, PM)
155 and for the present study milk samples were taken from the AM milking (at the same time as
156 any blood sampling). Milk samples were collected on 16 separate occasions between June
157 2012 and January 2015 and included summer and winter periods. All milk samples were
158 whole milk except for 256 samples which were from skimmed milk collected as part of a
159 previous project (Denholm et al., 2017, 2018). For these latter samples, milk was first
160 centrifuged at 3,000 × g for 30 mins at 4°C and the skimmed milk fraction retained from
161 below the fat layer using a fine tipped pastette. All samples were stored at -20°C prior to
162 analysis.

163 **Blood Samples.** Whole blood samples were collected on 13 separate occasions
164 between April 2013 and May 2016 and included summer and winter periods. Samples were
165 collected into plain Vacutainers (BD, Reading, UK) with blood allowed to coagulate before
166 centrifugation at 2,000 × g for 10 min and the serum retained. All samples were stored at -
167 20°C prior to analysis.

168

169 ***Analysis of Quantity and Trace Element and Heavy Metal Concentrations***

170 All milk and serum samples were analyzed and concentrations of circulating quantity
171 elements (Na, Mg, P, K, Ca), trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, I) and
172 heavy metals (Cd, Hg, Pb) were recorded. Milk samples (1.0ml) were digested in nitric acid
173 (8.0ml, (65% (v/v))) using the MARS 6 digestion system (CEM, Matthews, USA) and then

174 stored overnight at room temperature. Samples were ramped from room temperature to
175 210°C and then held at this temperature for 10 min before being cooled.

176 Serum samples (50µl) were added to hydrogen peroxide (10µl (30% (w/w))) and nitric
177 acid (40µl (65% (v/v))) and then digested at 85°C for 40 mins. Digested samples were diluted
178 in decomposition matrix prior to inductively-coupled plasma mass spectrometry (ICP-MS)
179 analysis. The decomposition matrix was nitric acid (2% (v/v)) and hydrochloric acid (0.5%
180 (v/v)) in distilled deionized water (Millipore, UK), which was used for preparation of all
181 solutions.

182 The measured isotopes analyzed by ICP-MS were ²³Na, ²⁴Mg, ³¹P, ³⁹K, ⁴⁴Ca, ⁵¹V,
183 ⁵²Cr, ⁵⁵Mn, ⁵⁶Fe, ⁵⁹Co, ⁶⁰Ni, ⁶³Cu, ⁶⁶Zn, ⁷⁸Se, ⁹⁵Mo, ¹²⁷I, ¹¹¹Cd, ²⁰²Hg, and ²⁰⁸Pb. All element
184 standards were used in stock solutions of 1000mg/L, which served for preparation of
185 calibration solutions and internal standard solution. The ICP-MS measurements were carried
186 out using the Agilent 7700X spectrometer (Agilent Technologies, UK) equipped with a
187 MicroMist nebulizer and nickel sampler and skimmer cones. The flow of mineral standards
188 (NIST, USA) and samples was joined together with a flow of erbium internal standard
189 solution (1mg/L). The mixed flow (approximately 500µL /min) was delivered by the
190 peristaltic pump to the nebulizer of the ICP-MS setup. Duration of ICP-MS analysis was 3.0
191 min. Data acquisition was one point, five replicates, 100 sweeps per replicate. Milk and
192 mussel reference materials were obtained from LGC (UK).

193 For the quantification of Iodine in milk samples, these were first digested at 90⁰C in
194 5% Tetramethylammonium hydroxide (TMAH) for 3h and then cooled. The TMAH (≥97%;
195 Sigma Aldrich, Gillingham, Dorset, UK) was diluted to 5% using ultrapure water (18.2 MΩ
196 cm, Elga PureFlex, UK). The iodine content in the milk samples was then determined by
197 ICP-MS (7700x, Agilent Technologies, UK) in standard analysis mode using external
198 calibration. The stock standard solution was gravimetrically prepared in-house from high

199 purity potassium iodide (+99.99%, Thermo Fisher Scientific, UK) in 5% TMAH. The
200 calibration standards were prepared by serial dilution of this stock, using 5% TMAH, and a
201 Tellurium internal standard also added at the same level as in the samples to a final
202 concentration of 150ng/ml. The method accuracy was monitored using ERM-BD150
203 Skimmed Milk Powder certified reference material (LGC Standards, UK) with a certified
204 iodine content of 1730 ± 140 $\mu\text{g}/\text{kg}$ (dry weight basis).

205

206 *Analysis of Vitamin B₁₂*

207 Milk samples collected between April 2013 and January 2015 (11 sample points
208 yielding 256 samples, $n=64$) were analyzed for vitamin B₁₂ concentrations. Vitamin B₁₂ in
209 undiluted milk was measured using a commercial competitive assay (RIDAscreen, R-Bio-
210 pharm, Germany). Absorbance was measured at 450nm where values are inversely related to
211 vitamin B₁₂ concentration using standards in the range 0 - 30 $\mu\text{g}/\text{L}$. The detection limit of the
212 assay was 0.5 $\mu\text{g}/\text{L}$.

213

214 *Data Preparation and Pre-Processing*

215 Element and vitamin B₁₂ data were combined with individual animal information
216 before being subjected to quality control measures. For the purposes of the present study the
217 interquartile range (IQR) was calculated for each trait with any concentrations falling out
218 with $Q_1 - 1.5 \times \text{IQR} < x < Q_3 - 1.5 \times \text{IQR}$ (where Q_1 and Q_3 are the 1st and 3rd quartiles,
219 respectively) considered an outlier and removed from the dataset. To ensure normality, all
220 data were log-transformed prior to analysis. The final dataset consisted of 938 milk and 754
221 serum records, with 385 (of 479) cows having more than 1 record.

222

223 *Statistical Analyses*

224 Data were analyzed using a mixed effects linear animal model (1). Genetic
225 relationships between individuals within the dataset were accounted for by fitting a pedigree
226 relationship matrix. Full pedigree information spanning seven generations was available for
227 all cows in the study.

228

$$y = Xa + Z_1b + Z_2c + e \quad (1)$$

229 Here y is a vector of trait observations (i.e., mineral/vitamin/heavy metal); a is a vector of
230 fixed effects; b is a vector of random additive genetic effects; c is a vector of permanent
231 environmental effects; e is a vector of random residual effects; X , Z_1 and Z_2 are incidence
232 matrices linking phenotypic records to fixed and additive genetic, and permanent
233 environmental effects, respectively.

234 Fixed effects included: diet group; genetic group; lactation number; week in milk;
235 year \times season of calving interaction; and, year \times month of record interaction. Cow was fitted
236 as a random effect to account for the additive genetic effect of the n^{th} individual cow
237 (pedigree data for 2,793 animals was included). To account for repeated observations per cow
238 the permanent environmental effect of the n^{th} individual cow was also fitted as a random
239 effect. All analyses were carried out using ASReml version 3 (Gilmour et al., 2009).

240

241

RESULTS

Summary Statistics

243 Tables 1 and 2 summarize the element and vitamin B₁₂ data generated from milk and
244 serum samples, respectively. Trait variability was determined by calculation of the coefficient
245 of determination (CV, %). Within milk variability was in the range 22% (Zn) to 156% (Co)
246 and 11% (K) to 23% (Na) for trace and quantity elements, respectively. In serum, variability
247 ranged from 24% (Se) to 133% (Mn) for trace and from 7% (Na) to 32% (P) for quantity

248 elements. Variability was greater in serum compared to milk and in trace elements compared
249 to quantity elements.

250

251 ***Impact of Genotype and Diet on Element Concentrations***

252 The effect of both genetic line and diet group on concentrations of micronutrients and
253 heavy metals in milk and serum are summarized in Table 3 with *P*-values representing
254 whether or not there was a significant difference observed between predicted mean
255 concentrations. Analyses revealed a predisposition for increased concentrations of Hg in the
256 milk of Select cows ($\bar{x} = 0.19 \mu\text{g/L}$, $P=0.01$). No significant impact of genotype on the
257 concentration of any other element in either milk or serum was observed. In contrast, diet was
258 found to have a significant and varying effect on the concentration of 10 milk and 7 serum
259 minerals. Cows on the HG diet had higher milk concentrations of Ca ($\bar{x} = 1257.02 \text{ mg/L}$,
260 $P<0.001$), Cu ($\bar{x} = 61.39 \mu\text{g/L}$, $P=0.01$), I ($\bar{x} = 1346.82 \mu\text{g/L}$, $P<0.001$), K ($\bar{x} = 1739.23$
261 mg/L , $P<0.001$), Mn ($\bar{x} = 36.82 \mu\text{g/L}$, $P<0.001$), Mo ($\bar{x} = 41.89 \mu\text{g/L}$, $P<0.001$) and P ($\bar{x} =$
262 954.42 mg/L , $P<0.001$) compared to those on the BP diet (Table 3). Conversely, cows on the
263 BP diet had higher milk concentrations of Mg ($\bar{x} = 116.50 \text{ mg/L}$, $P<0.001$), Na ($\bar{x} = 375.40$
264 mg/L , $P<0.001$) and Se ($\bar{x} = 20.09 \mu\text{g/L}$, $P<0.001$). Regarding serum elements, cows on the
265 HG diet showed higher concentrations of Cd ($\bar{x} = 0.09 \mu\text{g/L}$, $P<0.001$), Cu ($\bar{x} = 484.69 \mu\text{g/L}$,
266 $P<0.001$), Fe ($\bar{x} = 1787.19 \mu\text{g/L}$, $P<0.02$) and Mo ($\bar{x} = 17.49 \mu\text{g/L}$, $P<0.001$) in comparison
267 to BP fed cows who showed higher serum concentrations of P ($\bar{x} = 154.04 \text{ mg/L}$, $P<0.001$),
268 Se ($\bar{x} = 73.03 \mu\text{g/L}$, $P<0.001$) and V ($\bar{x} = 0.53 \mu\text{g/L}$, $P<0.001$).

269

270 ***Variance Components***

271 Variance components of the milk and serum elements are presented in Tables 4 and 5,
272 respectively. Additive genetic variance was small for both milk and serum traits and in most

273 cases genetic variance was higher in serum traits compared to those in milk. Heritability
274 estimates were obtained for 17 of the 20 milk traits, 6 of which were significant (Mg, Ca, Mn,
275 Zn, Se, I). In serum, heritability estimates were obtained for 16 of 18 traits, of which 4 were
276 significant (K, Ca, Cu, Se). Significant element heritabilities appeared to be greater in milk
277 traits compared to their corresponding serum trait. The highest heritability in milk and serum
278 was observed in Mg ($h^2 = 0.30, P=0.002$) and Cu ($h^2 = 0.22, P<0.001$), respectively. Milk
279 and serum quantity elements were all moderately to highly repeatable, and we also observed
280 significant repeatability in 5 milk and 10 serum trace elements.

281

282 *Associations Within Milk or Within Serum Elements*

283 Correlations between element concentrations within milk are presented in Table 6 and
284 within serum in Table 7. Strong positive genetic correlations (significantly different from
285 zero at $P < 0.05$) were observed within milk between the quantity elements, in particular Ca,
286 Mg and P (Table 6). Mg was positively associated with both Ca ($r = 0.45, P < 0.001$) and P (r
287 $= 0.49, P < 0.001$); a positive association between P and Ca was also observed ($r = 0.61, P$
288 < 0.001). Moreover, strong positive genetic correlations were also observed between Se with
289 Ca ($r = 0.63, P < 0.001$), Mg ($r = 0.59, P < 0.001$), Mn ($r = 0.40, P = 0.034$), P ($r = 0.53, P$
290 < 0.001) and Zn ($r = 0.52, P < 0.001$). Consistent phenotypic relationships were also observed
291 between the milk micronutrients and are presented in full in Table 6.

292 Within serum, a similar set of genetic relationships were observed between the
293 quantity elements although no significant genetic relationships were observed with Se (Table
294 7). The most genetically correlated nutrient in serum was V, which showed strong
295 associations with Cd ($r = 0.71, P = 0.003$), Ca ($r = 0.53, P < 0.001$), Mn ($r = 0.63, P = 0.039$),
296 Hg ($r = -0.44, P = 0.003$), Mo ($r = 0.57, P < 0.001$), P ($r = 0.42, P = 0.006$) and K ($r = 0.45, P$

297 =0.006). Phenotypically, V showed moderate to strong correlations with all other serum
298 nutrients except for Cr, Hg, Ni and Na.

299

300 *Associations Between Milk and Serum Elements*

301 Genetic correlations of elements between milk and serum are presented in Table 8
302 with phenotypic correlations shown in Table 9 (Results from the full analysis are available in
303 Table S3 of the accompanying Supplementary Material). Significant additive genetic
304 correlations (significantly different from zero at $P < 0.05$) were found to exist between a
305 number of the milk and serum elements with most being positive (Table 8). The strongest
306 negative associations were observed between serum Ni with milk V ($r = -0.98$, $P = 0.008$),
307 Co ($r = -0.86$, $P = 0.011$) and Na ($r = -0.62$, $P = 0.017$). Potassium (K) was the only element
308 that was found to have a significant correlation between concentrations recorded in milk and
309 serum ($r = 0.45$, $P = 0.025$). Further, milk K was found to be significantly positively
310 correlated with serum Mg ($r = 0.53$, $P = 0.008$), Co ($r = 0.48$, $P = 0.039$), Mo ($r = 0.45$, $P =$
311 0.020), and Cd ($r = 0.43$, $P = 0.035$). Moreover, serum Mg was highly correlated with milk
312 Ca ($r = 0.54$, $P = 0.014$), Mn ($r = 0.57$, $P = 0.028$) and P ($r = 0.49$, $P = 0.029$). Associations
313 involving heavy metals were only observed between serum Cd and milk Se, K and Mn.

314 All phenotypic correlations obtained between milk and serum element concentrations
315 are presented in Table 9. Statistically significant correlations were obtained for Ca ($r = 0.17$,
316 $P = 0.019$), Mo ($r = 0.19$, $P = 0.009$), K ($r = 0.19$, $P = 0.006$) and Na ($r = -0.79$, $P <$
317 0.001). Serum Na was also found to be strongly positively correlated with milk Ca ($r = 0.81$,
318 $P < 0.001$), Zn ($r = 0.74$, $P < 0.001$), K ($r = 0.64$, $P < 0.001$) and Mg ($r = 0.49$, $P = 0.024$).
319 The majority of associations observed were positive but negative correlations were noted
320 between milk Cr and serum Se ($r = -0.16$, $P = 0.029$); milk Fe with serum Pb ($r = -0.62$, $P <$
321 0.001) and serum Cd ($r = -0.288$, $P = 0.002$); milk Hg with serum Cu ($r = -0.23$, $P = 0.003$)

322 and serum Mo ($r = -0.20$, $P = 0.034$); milk Zn with serum Co ($r = -0.15$, $P = 0.033$); and
323 milk B₁₂ with serum Ni ($r = -0.38$, $P < 0.001$) (Table 9). It was noted that Cd and Pb in milk
324 as well as Cr in serum showed no associations with any other nutrient whether in milk or
325 serum.

326

327

DISCUSSION

328 The main aim of this study was to estimate (co)variance components of important
329 dairy cattle milk and serum elements (minerals, heavy metals), as well as milk vitamins B₁₂,
330 in order to explore potential selection strategies for optimizing concentrations both for the
331 benefit the cow and the human dairy product consumer. Significant heritability estimates
332 were obtained for 6 milk and 4 serum minerals in addition to repeatability estimates for 10
333 milk and 15 serum elements (Tables 4 and 5). From the literature, the majority of genetic
334 analyses previously reported correspond to quantity elements in milk (a summary of h^2 values
335 found in the literature can be found in Table S4 of the accompanying Supplementary
336 Material). Milk Ca, Mg, P, K and Na have been shown to have heritabilities ranging from
337 0.10 (Toffanin et al., 2015) to 0.72 (Buitenhuis et al., 2015), 0.08 (Buitenhuis et al., 2015) to
338 0.60 (van Hulzen et al., 2009), 0.12 (Toffanin et al., 2015) to 0.62 (van Hulzen et al., 2009),
339 0.19 (Visentin et al., 2018) to 0.46 (van Hulzen et al., 2009), and 0.20 (Buitenhuis et al.,
340 2015) to 0.24 (Visentin et al., 2018), respectively. Heritability estimates for some milk trace
341 elements have also been reported including Cu (0.28, Buitenhuis et al., 2015), Fe (0.15,
342 Buitenhuis et al., 2015), Mn (0.13, Buitenhuis et al., 2015), Se (0.20, van Hulzen et al., 2009;
343 0.20, Buitenhuis et al., 2015) and Zn (0.41, van Hulzen et al., 2009; 0.57, Buitenhuis et al.,
344 2015). Regarding serum, a genetic analysis of Ca, Mg, P and K carried out by Tsiamadis et
345 al. (2016) reported heritabilities of 0.20, 0.21, 0.25 and 0.10, respectively. Furthermore,
346 heritabilities of serum Cu and Zn have both been reported as 0.22 (Morris et al., 2006). The

347 results from the present study are within these ranges for these nutrients and we also
348 investigated a number of milk and serum elements (including heavy metals) that we believe
349 have not yet been reported in dairy cows. As such, we believe this is the first study to
350 estimate heritability of the milk trace element Mo (0.19) as well as repeatability estimates for
351 milk and serum trace elements and heavy metals.

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

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

372 Milk is also an excellent source of vitamin B₁₂ and milk and dairy products contribute
373 significantly to vitamin B₁₂ intakes in humans (150% of RNI, Henderson et al., 2003a; b;
374 Kliem and Givens, 2011) making it an attractive breeding target in terms of enhancing
375 nutrient quality for the consumer. Vitamin B₁₂ contains Co and Co is required in the diet of
376 cattle in order that this vitamin is synthesized endogenously by rumen bacteria (Stemme et
377 al., 2008). Although the concentration of Co in serum was repeatable (0.14, $P=0.019$), we did
378 not observe significant repeatability in milk Co or vitamin B₁₂. The estimated heritability for
379 vitamin B₁₂ in milk was not significant ($h^2=0.12$, $P=0.18$) and this was also true for milk and
380 serum Co ($h^2=0.04$, $P=0.31$; $h^2=0.07$, $P=0.30$, respectively). Furthermore, we found no
381 significant associations between milk vitamin B₁₂ and Co in either milk or serum.

382 Genetic line (average or highest genetic merit for milk fat plus protein yield) had no
383 significant effect on circulating element or vitamin B₁₂ concentrations in either milk or blood
384 serum with the exception of the heavy metal Hg which showed higher concentrations in the
385 milk of Select line cows. Moreover, due to cows being part of an experimental research herd
386 any biases in management between the genetic lines were non-existent such that cows within
387 the same line but on diverging diets were consequently unaffected by management decisions
388 (Pryce et al., 1999). Concentrations of elements in both milk and serum were effected
389 depending on whether the cow was fed the Home-grown or By-product ration. This has
390 potential benefits for manipulation of nutrient content through changes in management alone,
391 a benefit that could be complemented/improved through selection and breeding. It also
392 suggests that selection for higher milk fat and protein is independent of blood or milk
393 micronutrient concentrations.

394 Given the mostly positive genetic correlations among the milk minerals examined in
395 the present study, selection alone for one milk mineral might be expected to also increase the
396 concentrations of other minerals. Therefore, selection for milk Ca would likely boost P, Zn

397 and Se concentrations for example, leading to multiple improvements in milk mineral
398 concentrations for the benefit of the human dairy consumer.

399 Furthermore, since heavy metals have adverse effects on health, any breeding
400 objectives should also be directed towards minimizing concentrations of these metals or at
401 least, not to increase concentrations. The findings of this study identified few significant
402 genetic associations of heavy metals with micronutrient concentrations and, in cases where a
403 significant association was found, these tended to be negative. This suggests that genetic
404 selection programs aimed at increasing micronutrient concentrations should not inadvertently
405 increase concentrations of toxic heavy metals. The minimum risk level (MRL) has not been
406 established for Cd or Hg in milk, but the MRL for Pb in EU milk is $20 \mu\text{g kg}^{-1}$ (CE
407 Regulation no. 2001/466). The Pb concentrations as found in milk in this study were below
408 levels of food safety concern in the EU.

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

417

418

CONCLUSIONS

419 Through the present study we have established that circulating concentrations of
420 elements in both the milk and serum of dairy cows are significantly influenced by genetics
421 and feeding system. As expected, diet had a significant effect on mineral concentrations,

422 especially in milk, and as such provided a potential route for manipulation via changes in
423 rations. The results presented provide clear evidence that many of such traits are heritable
424 indicating that selection for desired element concentrations in both milk and serum is
425 possible. This work will help inform industry solutions to better improve both genetics and
426 management practices for the benefit of not only the cow but also the healthiness of the milk
427 for the consumer.

428

429

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551

552 **Table 1.** Descriptive statistics of the milk element and vitamin B₁₂ data

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

553 ¹ Number of cows (/ total cows) with at least 2 observations of trait

554 [†] Coefficient of variation

555 ^q Quantity element

556 ^t Trace element

557 ^h Heavy metal

558

559

560 **Table 2.** Descriptive statistics of the serum element data

Nutrient	Coun t	Cows ¹	Mean	SD	Minimu m	Maximu m	CV%†
Sodium (Na, mg/L) ^q	169	1/168	3,133.1 8	210.64	2,533.07	3,757.49	6.72
Magnesium (Mg, mg/L) ^q	734	215/32 2	23.01	5.11	9.79	36.62	22.22
Phosphorus (P, mg/L) ^q	743	214/32 3	139.07	44.97	20.75	260.98	32.33
Potassium (K, mg/L) ^q	732	215/32 3	206.02	35.54	105.38	302.45	17.25
Calcium (Ca, mg/L) ^q	707	214/31 8	105.37	17.76	58.03	153.36	16.86
Vanadium (V, µg/L) ^t	584	213/21 4	0.68	0.21	0.15	1.25	31.15
Chromium (Cr, µg/L) ^t	221	63/214	0.89	0.76	0.00	4.23	85.71
Manganese (Mn, µg/L) ^t	626	215/25 9	6.46	8.61	0.07	36.70	133.2 2
Iron (Fe, µg/L) ^t	654	213/26 3	2,179.4 4	1,144.6 7	292.15	6,157.91	52.52
Cobalt (Co, µg/L) ^t	610	214/23 0	1.34	0.68	0.11	4.39	50.73
Nickel (Ni, µg/L) ^t	392	201/21 2	3.85	2.71	0.01	12.14	70.31
Copper (Cu, µg/L) ^t	726	215/31 5	601.02	195.51	108.52	1,113.46	32.53
Zinc (Zn, µg/L) ^t	626	214/25 8	910.90	330.06	201.57	2,109.51	36.23
Selenium (Se, µg/L) ^t	717	214/32 2	75.96	18.58	26.11	124.59	24.45
Molybdenum (Mo, µg/L) ^t	662	165/32 1	14.92	13.62	1.26	62.88	91.30
Cadmium (Cd, µg/L) ^h	666	215/28 3	0.11	0.11	0.00	0.46	100.7 6
Mercury (Hg, µg/L) ^h	265	139/15 1	3.43	3.22	0.00	14.89	93.97
Lead (Pb, µg/L) ^h	738	215/32 5	83.23	81.74	0.01	367.56	98.20

561 1 Number of cows (/ total cows) with at least 2 observations of trait

562 † Coefficient of variation

563 q Quantity element

564 t Trace element

565 h Heavy metal

566

567 **Table 3.** Impact of diet and genotype on element concentrations in milk and serum. Predicted
 568 mean values for the By-product and Home-grown diets, as well as for Control and Select
 569 genetic lines, obtained via univariate models (accounting for all other sources of systematic
 570 variation). Only predicted mean concentrations that were significantly different ($P < 0.05$) are
 571 presented

Nutrient	\bar{x}_{BP}	\bar{x}_{HG}	SED [‡]	<i>P</i>
Milk				
Sodium (Na, mg/L) ^q	375.40	338.32	1.02	<0.001
Magnesium (Mg, mg/L) ^q	116.50	110.81	1.01	<0.001
Phosphorus (P, mg/L) ^q	878.40	954.42	1.01	<0.001
Potassium (K, mg/L) ^q	1,661.21	1,739.23	1.01	<0.001
Calcium (Ca, mg/L) ^q	1,107.32	1,257.02	1.01	<0.001
Manganese (Mn, µg/L) ^t	27.49	36.81	1.03	<0.001
Copper (Cu, µg/L) ^t	54.55	61.39	1.05	0.009
Selenium (Se, µg/L) ^t	20.09	15.14	1.01	<0.001
Molybdenum (Mo, µg/L) ^t	32.90	41.89	1.02	<0.001
Iodine (I, µg/L) ^t	1,082.79	1,346.82	1.04	<0.001
Serum				
Phosphorus (P, mg/L) ^q	154.04	108.80	1.02	<0.001
Vanadium (V, µg/L) ^t	0.53	0.42	1.03	<0.001
Iron (Fe, µg/L) ^t	1,663.20	1,787.19	1.03	0.019
Copper (Cu, µg/L) ^t	418.01	484.69	1.02	<0.001
Selenium (Se, µg/L) ^t	73.02	64.65	1.02	<0.001
Molybdenum (Mo, µg/L) ^t	5.46	17.49	1.05	<0.001
Cadmium (Cd, µg/L) ^h	0.04	0.09	1.09	<0.001
	\bar{x}_C	\bar{x}_S	SED [‡]	<i>P</i>
Milk				
Mercury (Hg, mg/L) ^h	0.14	0.19	1.11	0.010

572 \bar{x}_{BP} Predicted By-product mean
 573 \bar{x}_{HG} predicted Home-grown mean
 574 \bar{x}_C Predicted Control line mean
 575 \bar{x}_S Predicted Select line mean
 576 ‡ Standard error difference
 577 q Quantity element
 578 t Trace element
 579 h Heavy metal
 580

581 **Table 4.** Variance Components and heritability (h^2) estimates of the milk elements and
 582 vitamin B₁₂ data

Nutrient	σ_a	σ_{pe}	σ_p	h^2 (S.E.)	Repeatability
Sodium (Na, mg/L) ^q	0.000	0.006	0.039	N.E.	0.16* (0.040)
Magnesium (Mg, mg/L) ^q	0.005	0.000	0.016	0.30* (0.090)	0.30* (0.044)
Phosphorus (P, mg/L) ^q	0.002	0.002	0.016	0.12 (0.070)	0.22* (0.042)
Potassium (K, mg/L) ^q	0.001	0.002	0.010	0.11 (0.078)	0.27* (0.045)
Calcium (Ca, mg/L) ^q	0.003	0.000	0.015	0.20* (0.078)	0.22* (0.043)
Vanadium (V, µg/L) ^t	0.063	0.000	0.978	0.06 (0.161)	0.06 (0.161)
Chromium (Cr, µg/L) ^t	0.013	0.000	0.450	0.03 (0.035)	0.03 (0.035)
Manganese (Mn, µg/L) ^t	0.020	0.000	0.139	0.14* (0.039)	0.14* (0.039)
Iron (Fe, µg/L) ^t	0.006	0.000	0.531	0.01 (0.028)	0.01 (0.028)
Cobalt (Co, µg/L) ^t	0.039	0.000	0.963	0.04 (0.056)	0.04 (0.056)
Nickel (Ni, µg/L) ^t	0.049	0.000	1.247	0.04 (0.093)	0.04 (0.093)
Copper (Cu, µg/L) ^t	0.018	0.000	0.413	0.04 (0.028)	0.04 (0.028)
Zinc (Zn, µg/L) ^t	0.011	0.009	0.047	0.24* (0.116)	0.43* (0.042)
Selenium (Se, µg/L) ^t	0.005	0.001	0.033	0.15* (0.072)	0.18* (0.041)
Molybdenum (Mo, µg/L) ^t	0.011	0.000	0.060	0.19* (0.041)	0.19* (0.041)
Iodine (I, µg/L) ^t	0.031	0.003	0.140	0.22 (0.123)	0.24* (0.081)
Cadmium (Cd, µg/L) ^h	0.000	0.000	0.511	N.E.	N.E.
Lead (Pb, µg/L) ^h	0.000	0.000	0.577	N.E.	N.E.
Mercury (Hg, µg/L) ^h	0.038	0.000	1.009	0.04 (0.064)	0.04 (0.064)
Vitamin B ₁₂ (B ₁₂ , µg/L)	0.034	0.008	0.346	0.10 (0.226)	0.12 (0.095)

583 σ_a Additive genetic SD

584 σ_{pe} Permanent environment SD

585 σ_p Total phenotypic SD

586 q Quantity element

587 t Trace element

588 h Heavy metal

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

590 N.E. Not estimable due to additive genetic variance $\rightarrow 0$

591

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

Nutrient	σ_a	σ_{pe}	σ_p	h^2 (S.E.)	Repeatability
Sodium (Na, mg/L) ^q	0.001	0.003	0.004	0.34 (0.234)	1.00* (0.002)
Magnesium (Mg, mg/L) ^q	0.007	0.000	0.047	0.14 (0.083)	0.14* (0.050)
Phosphorus (P, mg/L) ^q	0.006	0.007	0.062	0.09 (0.079)	0.20* (0.046)
Potassium (K, mg/L) ^q	0.004	0.000	0.024	0.18* (0.051)	0.18* (0.051)
Calcium (Ca, mg/L) ^q	0.003	0.000	0.025	0.12* (0.049)	0.12* (0.049)
Vanadium (V, $\mu\text{g/L}$) ^t	0.007	0.017	0.073	0.09 (0.121)	0.33* (0.059)
Chromium (Cr, $\mu\text{g/L}$) ^t	0.000	0.000	1.215	N.E.	N.E.
Manganese (Mn, $\mu\text{g/L}$) ^t	0.012	0.000	0.324	0.04 (0.044)	0.04 (0.044)
Iron (Fe, $\mu\text{g/L}$) ^t	0.012	0.000	0.130	0.09 (0.046)	0.09 (0.046)
Cobalt (Co, $\mu\text{g/L}$) ^t	0.009	0.007	0.124	0.07 (0.100)	0.14* (0.055)
Nickel (Ni, $\mu\text{g/L}$) ^t	0.245	0.113	0.830	0.30 (0.183)	0.43* (0.076)
Copper (Cu, $\mu\text{g/L}$) ^t	0.018	0.000	0.084	0.22* (0.051)	0.22* (0.051)
Zinc (Zn, $\mu\text{g/L}$) ^t	0.007	0.005	0.067	0.11 (0.095)	0.18* (0.054)
Selenium (Se, $\mu\text{g/L}$) ^t	0.005	0.000	0.053	0.10* (0.047)	0.10* (0.047)
Molybdenum (Mo, $\mu\text{g/L}$) ^t	0.000	0.093	0.335	N.E.	0.28* (0.057)
Cadmium (Cd, $\mu\text{g/L}$) ^h	0.044	0.182	0.909	0.05 (0.086)	0.25* (0.059)
Lead (Pb, $\mu\text{g/L}$) ^h	0.062	0.153	0.546	0.11 (0.103)	0.39* (0.051)
Mercury (Hg, $\mu\text{g/L}$) ^h	0.709	0.613	1.433	0.49 (0.347)	0.92* (0.017)

593 σ_a Additive genetic SD

594 σ_{pe} Permanent environment SD

595 σ_p Total phenotypic SD

596 q Quantity element

597 t Trace element

598 h Heavy metal

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

600 N.E. Not estimable due to additive genetic variance $\rightarrow 0$

601

602
603**Table 6.** Correlations between element concentrations and vitamin B₁₂ within milk. Additive genetic correlations are presented above the diagonal with phenotypic below. Corresponding standard errors are given in parenthesis

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

604

- Not estimable

605

* Significantly different from zero at $P < 0.05$

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Table 7. Correlations between element concentrations within serum. Additive genetic correlations are presented above the diagonal with phenotypic below. Corresponding standard errors are given in parenthesis

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

609

- Not estimable

610

* Significantly different from zero at $P < 0.05$

611 **Table 8.** Additive genetic correlations (r) between milk and serum element concentrations
612 with corresponding standard errors (S.E.) and P-values (P). Only correlations significantly
613 different from zero (at $P < 0.05$) results are presented

Milk	Serum	r (S.E.)	P
Sodium (Na, mg/L) ^q	Calcium (Ca, mg/L) ^q	0.56 (0.275)	0.049
Sodium (Na, mg/L) ^q	Nickel (Ni, µg/L) ^t	-0.62 (0.244)	0.017
Magnesium (Mg, mg/L) ^q	Iron (Fe, µg/L) ^t	0.65 (0.266)	0.021
Phosphorus (P, mg/L) ^q	Magnesium (Mg, mg/L) ^q	0.49 (0.215)	0.029
Potassium (K, mg/L) ^q	Cadmium (Cd, µg/L) ^h	0.43 (0.194)	0.035
Potassium (K, mg/L) ^q	Cobalt (Co, µg/L) ^t	0.48 (0.222)	0.039
Potassium (K, mg/L) ^q	Magnesium (Mg, mg/L) ^q	0.53 (0.189)	0.008
Potassium (K, mg/L) ^q	Molybdenum (Mo, µg/L) ^t	0.45 (0.182)	0.020
Potassium (K, mg/L) ^q	Potassium (K, mg/L) ^q	0.45 (0.192)	0.025
Calcium (Ca, mg/L) ^q	Magnesium (Mg, mg/L) ^q	0.54 (0.210)	0.014
Vanadium (V, µg/L) ^t	Nickel (Ni, µg/L) ^t	-0.98 (0.354)	0.008
Manganese (Mn, µg/L) ^t	Cadmium (Cd, µg/L) ^h	0.77 (0.219)	<0.001
Manganese (Mn, µg/L) ^t	Magnesium (Mg, mg/L) ^q	0.57 (0.247)	0.028
Cobalt (Co, µg/L) ^t	Nickel (Ni, µg/L) ^t	-0.86 (0.319)	0.011
Selenium (Se, µg/L) ^t	Cadmium (Cd, µg/L) ^h	-0.46 (0.210)	0.036
Molybdenum (Mo, µg/L) ^t	Iron (Fe, µg/L) ^t	0.68 (0.302)	0.031

614 q Quantity element

615 t Trace element

616 h Heavy metal

617

618 **Table 9.** Phenotypic correlations (*r*) between milk and serum elements and vitamin B₁₂ with
 619 corresponding standard errors (S.E.) and P-values (*P*). Only correlations significantly
 620 different from zero (at *P*<0.05) results are presented

Milk	Serum	<i>r</i> (S.E.)	<i>P</i>
Sodium (Na, mg/L) ^q	Cadmium (Cd, µg/L) ^h	0.39 (0.070)	<0.001
Sodium (Na, mg/L) ^q	Lead (Pb, µg/L) ^h	0.36 (0.074)	<0.001
Sodium (Na, mg/L) ^q	Sodium (Na, mg/L) ^q	-0.79 (0.175)	<0.001
Magnesium (Mg, mg/L) ^q	Iron (Fe, µg/L) ^t	0.19 (0.064)	0.004
Magnesium (Mg, mg/L) ^q	Sodium (Na, mg/L) ^q	0.49 (0.208)	0.024
Phosphorus (P, mg/L) ^q	Cadmium (Cd, µg/L) ^h	0.32 (0.072)	<0.001
Phosphorus (P, mg/L) ^q	Calcium (Ca, mg/L) ^q	0.15 (0.071)	0.043
Phosphorus (P, mg/L) ^q	Iron (Fe, µg/L) ^t	0.15 (0.069)	0.037
Phosphorus (P, mg/L) ^q	Magnesium (Mg, mg/L) ^q	0.15 (0.070)	0.037
Potassium (K, mg/L) ^q	Cadmium (Cd, µg/L) ^h	0.30 (0.074)	<0.001
Potassium (K, mg/L) ^q	Calcium (Ca, mg/L) ^q	0.18 (0.069)	0.014
Potassium (K, mg/L) ^q	Cobalt (Co, µg/L) ^t	0.21 (0.066)	0.002
Potassium (K, mg/L) ^q	Iron (Fe, µg/L) ^t	0.15 (0.068)	0.037
Potassium (K, mg/L) ^q	Magnesium (Mg, mg/L) ^q	0.20 (0.067)	0.005
Potassium (K, mg/L) ^q	Molybdenum (Mo, µg/L) ^t	0.18 (0.076)	0.027
Potassium (K, mg/L) ^q	Phosphorus (P, mg/L) ^q	0.16 (0.064)	0.019
Potassium (K, mg/L) ^q	Potassium (K, mg/L) ^q	0.19 (0.067)	0.006
Potassium (K, mg/L) ^q	Sodium (Na, mg/L) ^q	0.64 (0.173)	<0.001
Calcium (Ca, mg/L) ^q	Cadmium (Cd, µg/L) ^h	0.22 (0.078)	0.006
Calcium (Ca, mg/L) ^q	Calcium (Ca, mg/L) ^q	0.17 (0.070)	0.019
Calcium (Ca, mg/L) ^q	Iron (Fe, µg/L) ^t	0.16 (0.068)	0.024
Calcium (Ca, mg/L) ^q	Magnesium (Mg, mg/L) ^q	0.14 (0.069)	0.047
Calcium (Ca, mg/L) ^q	Sodium (Na, mg/L) ^q	0.81 (0.118)	<0.001
Vanadium (V, µg/L) ^t	Lead (Pb, µg/L) ^h	0.53 (0.107)	<0.001
Chromium (Cr, µg/L) ^t	Selenium (Se, µg/L) ^t	-0.16 (0.070)	0.029
Iron (Fe, µg/L) ^t	Cadmium (Cd, µg/L) ^h	-0.28 (0.086)	0.002
Iron (Fe, µg/L) ^t	Lead (Pb, µg/L) ^h	-0.62 (0.046)	<0.001
Cobalt (Co, µg/L) ^t	Cadmium (Cd, µg/L) ^h	0.56 (0.063)	<0.001
Zinc (Zn, µg/L) ^t	Cobalt (Co, µg/L) ^t	-0.15 (0.066)	0.033
Zinc (Zn, µg/L) ^t	Sodium (Na, mg/L) ^q	0.74 (0.142)	<0.001
Selenium (Se, µg/L) ^t	Calcium (Ca, mg/L) ^q	0.15 (0.068)	0.037
Molybdenum (Mo, µg/L) ^t	Cadmium (Cd, µg/L) ^h	0.22 (0.072)	0.004
Molybdenum (Mo, µg/L) ^t	Iron (Fe, µg/L) ^t	0.17 (0.063)	0.011
Molybdenum (Mo, µg/L) ^t	Molybdenum (Mo, µg/L) ^t	0.19 (0.070)	0.009
Mercury (Hg, µg/L) ^h	Copper (Cu, µg/L) ^t	-0.23 (0.074)	0.003
Mercury (Hg, µg/L) ^h	Molybdenum (Mo, µg/L) ^t	-0.20 (0.089)	0.034
Vitamin B ₁₂ (B ₁₂ , µg/L)	Cadmium (Cd, µg/L) ^h	0.21 (0.092)	0.028
Vitamin B ₁₂ (B ₁₂ , µg/L)	Nickel (Ni, µg/L) ^t	-0.38 (0.096)	<0.001

621 q Quantity element

622 t Trace element

623 h Heavy metal