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Genetic Parameters of Subclinical Macromineral Disorders and Major Clinical Diseases in Post Parturient Holstein Cows

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Interpretive Summary

The genetic parameters of subclinical hypocalcemia, hypophosphatemia, subclinical hypomagnesemia, hypokalemia and hyperphosphatemia during the first 8 days after calving were studied in Holstein dairy cows. Repeated measurements of calcium, phosphorus, magnesium and potassium serum concentrations together with recordings of clinical diseases during the same period were used in random regression model analyses. The heritability estimates of the associated health traits suggest that genetic selection is feasible and could help minimize health problems after calving.

ABSTRACT

The main objective of this study was to assess the genetic parameters of subclinical disorders associated with subclinical hypocalcemia (**SCHCa**), hypophosphatemia (**HypoP**), subclinical hypomagnesemia (**SCHMg**), hypokalemia (**HypoK**) and hyperphosphatemia (**HyperP**), as well as of major clinical diseases after calving in Holstein cows. The secondary objective was to estimate the associated genetic and phenotypic correlations among these subclinical and clinical conditions after calving in Holstein cows. The study was conducted in 9 dairy herds located in Northern Greece. None of the herds used any kind of preventive measures for milk fever (**MF**). A total of 1,021 Holstein cows with pedigree information were examined from November 2010 until November 2012. The distribution across parities was 466 (parity 1), 242 (parity 2), 165 (parity 3) and 148 (parity 4 and above) cows. All cows were subjected to a detailed clinical examination and blood sampled on the 1st, 2nd, 4th and 8th day after calving. Serum concentrations of Ca, P, Mg and K were measured in all samples, while β-hydroxybutyrate acid (BHBA) was measured only for day 8. The final data set included 4,064 clinical and 16,848 biochemical records (4,020 Ca, 4,019 P, 4,020 Mg and 3,792 K and 997 BHBA). Data of 1,988 observations of Body Condition Score (BCS) at days 1 and 8, were also available. All health traits were analyzed with a univariate random regression model. The genetic analysis for macromineral-related disorders included 986 cows with no obvious signs of MF (35 cows with MF were excluded).Analysis for other health traits included all 1,021 cows. A similar single record model was used for the analysis of BHBA. Genetic correlations among traits were estimated with a series of bivariate analyses. Statistically significant daily heritabilities of SCHCa (0.13 – 0.25), HypoP (0.18 – 0.33), SCHMg (0.11 – 0.38) and HyperP (0.14 – 0.22) were low to moderate, while that of HypoK was low (0.08 – 0.10). The heritability of BCS was 0.20±0.10. Statistically

significant daily heritabilities of clinical diseases were those of MF (0.07 – 0.11), left displaced abomasum (0.19 – 0.31) and mastitis (0.15 – 0.41). Results suggest that these health disorders are heritable traits and could be minimized with proper genetic selection. Statistically significant phenotypic correlations were estimated for the first time between macromineral concentrations and almost all transition cow metabolic and infectious health disorders.

Key words: subclinical macromineral disorders, postpartum diseases, genetic parameters

INTRODUCTION

During the transition period (3 weeks before to 3-4 weeks after calving) the modern high producing dairy cow is at increased risk of encountering a multitude of interrelated health disorders (Larsen et al., 2001; Lean et al., 2013). In a study that included 151,000 records, Ingvartsen et al. (2003) clearly demonstrated that disease incidence is highest during the first 10 days after calving. Negative energy balance, macromineral-related disorders and reduced immunity are the three major causes of transition period diseases (Goff, 2006a). Prevention of health disorders around calving is based on the implementation of various managerial and nutritional strategies; for example, body condition score (BCS) evaluation and post calving β-hydroxybutyric acid (BHBA) serum concentration are proposed to be routinely used as energy balance indicators (Oikonomou et al., 2008a; LeBlanc, 2010).

Macromineral serum concentration changes are mainly caused by increased cow requirements at the onset of lactation combined with reduced feed intake and possibly delayed homeostatic mechanisms (Goff, 2006a). Macromineral-related disorders, relating to calcium (Ca), phosphorus

71 (P), magnesium (Mg) and potassium (K) concentrations, are at the center of the disease cascade
72 that dairy cows experience during the transition period (Goff, 2004), in either clinical or
73 subclinical form (Goff, 2006b).
74
75 Subclinical hypocalcemia (SCHCa, serum Ca concentration < 8.3 mg/dL) is by far the most
76 common macromineral-related health disorder associated with calving (Horst and Goff, 2003;
77 Goff, 2008; Peek and Divers, 2008). Clinical hypocalcemia (parturient paresis – "milk fever",
78 MF) has a detrimental role in major post-calving clinical disease incidence, since it is associated
79 with: retained fetal membranes (RFM), metritis (MET), mastitis (MAST), displaced abomasum
80 (left or right, LDA and RDA, respectively), ketosis (KET) and uterine prolapse (UP) (Correa et
81 al., 1990; Gröhn and Bruss, 1990; DeGaris and Lean, 2008). Subclinical hypocalcemia is
82 assumed to have the same negative effects but relevant literature is lacking.
83
84 Lower than normal P concentrations (HypoP, P < 4.2 mg/dL) are common at the onset of
85 lactation; recumbent MF dairy cows often have very low P concentration (P < 2.0 mg/dL) (Goff,
86 2004). Elevated P concentrations (HyperP, P > 7.80 mg/dL) increase the risk of MF (Lean et al.,
87 2013; Grünberg, 2014). While clinical hypomagnesaemia ("grass tetany", serum Mg < 1.0
88 mg/dL) may still appear in grazing herds, it is not at all common in confined and TMR-fed cows
89 (Peek and Divers, 2008). On the other hand, subclinical hypomagnesemia (SCHMg, serum Mg <
90 1.8 mg/dL) is involved in the etiology of SCHCa and MF (Littledike et al., 1983; Rude, 1998;
91 Schonewille et al., 2008). Mild hypokalemia (serum K between 2.6 and 3.8 mmol/L) is common
92 in early lactation (Sattler and Fecteau, 2014), while severe hypokalemia (serum K < 2.5 mmol/L)

93 is very rare in dairy cattle, mostly associated with concurrent infectious disease (Sattler et al.
94 1998).
95
96 Macromineral-related disorders usually resolve by the end of the first week post-calving but their
97 effects are long-lasting, impairing milk production and reproductive efficiency of dairy cows
98 (Goff, 2006b). Despite the extensive knowledge regarding the pathophysiology of macromineral-
99 related disorders and the various management practices that may alleviate them (Thilsing-
100 Hansen et al., 2002; Goff, 2004; Mulligan et al., 2006), problems are still common. Disease
101 incidence rates, even in many well-managed herds, still remain unacceptably high (Mulligan and
102 Doherty, 2008). During the last decades, genetic selection for disease resistance enjoys increased
103 popularity because genetic progress, no matter how small, is permanent and cumulative (Eggen,
104 2012). Genetic parameters for various clinical diseases around calving have been estimated in
105 several large scale studies (Lin et al., 1989; Lyons et al., 1991; Heringstad et al., 2005).
106 Heritabilities of Ca, P, Mg and K serum concentrations have only recently been reported
107 (Tsiamadis et al., 2016); however, there is lack of information concerning subclinical
108 macromineral-related disorders.
109
110 The objectives of this study were to estimate: 1) the heritability of SCHCa, HypoP, HyperP,
111 SCHMg, HypoK, BHBA and BCS, 2) the heritability of major clinical health disorders (MF,
112 RFM, MET, MAST, LDA, RDA, KET and UP) and 3) relevant genetic and phenotypic
113 correlations, during the first 8 days after calving.
114

MATERIALS AND METHODS

The research was conducted in compliance with institutional guidelines and approved by the Research Committee of the Aristotle University of Thessaloniki, Thessaloniki, Greece. All farmers gave informed consent for the cows to be included in the study and to undergo the testing procedures.

Animals and Management

A total of 1,021 Holstein cows from 9 commercial free-stall dairy herds in Northern Greece were included in the study. The distribution across parities was 466, 242, 165 and 148 cows for parities 1, 2, 3 and 4 and above, respectively. Farms were visited regularly between November 2010 and November 2012 for data collection. No herd used any kind of preventive measures for hypocalcemia. Total mixed rations (TMR) were formulated to meet or exceed net energy and metabolizable protein requirements according to National Research Council recommendations (NRC, 2001).

Clinical Examination, Blood Sampling and Analyses

All animals were clinically examined and blood sampled by the first author on the 1st, 2nd, 4th and 8th day after calving. Body condition score was recorded on the 1st and 8th day after calving using the 1- to 5- point scale of Ferguson et al. (1994), in increments of 0.25. At this scale, 1 is for emaciated and 5 for obese animals.

Blood sampling was performed by coccygeal venipuncture into 10-ml vacuum glass tubes without anticoagulant (BD Vacutainer®, Plymouth, United Kingdom) for serum macromineral

138 measurements. Samples were placed in a cooler, transported to the Diagnostic Laboratory of the
139 Faculty of Veterinary Medicine and centrifuged immediately upon arrival (3,000 x g for 15 min,
140 room temperature 21^{o}C). Serum was transferred into polyethylene tubes and stored at -80^{o}C until
141 assay. All sera were analyzed for total Ca and Mg concentrations using flame atomic absorption
142 spectrophotometry (Perkin ElmerAAnalyst 100, Perkin Elmer Co, Norwalk, CT, USA),
143 according to manufacturer's instructions. Serum inorganic phosphorus concentrations were
144 determined photometrically using a Flexor E autoanalyzer (Vital Scientific, Spankeren, The
145 Netherlands), according to the procedure described by Daly and Ertingshausen (1972), with the
146 use of standard commercial reagents (Thermo Fisher Scientific Inc. USA). Potassium serum
147 concentrations were measured using an ion-selective electrode according to manufacturer's
148 instructions (Electrolyte Analyzer 9180, Roche Austria). The intra- and inter-assay coefficients
149 of variation for all the above analyses were less than 3%. Beta-hydroxybutyric acid was
150 measured only on the 8th day after calving by a spectrophotometric kinetic method (Bruss, 2008).
151 The intra-assay coefficient was 2 to 4%, while the inter-assay coefficient was 4 to 8%, both of
152 which are within the desirable range.
153
154 ***Disease Definitions and Cut-offs***
155 In our study, SCHCa, HypoP, HyperP, SCHMg and HypoK were defined based on threshold
156 values provided in relevant literature and were expressed as presence or absence of the condition
157 (binary traits). Animals with serum concentrations below or equal to 8.3 mg/dL for Ca, 4.2
158 mg/dL for P, 1.8 mg/dL for Mg, and 3.9 mmol/L for K, were considered as cases of SCHCa,
159 HypoP, SCHMg and HypoK, respectively (Goff, 2008; Divers and Peek, 2008; Horst and Goff,
160 2003). Moreover, animals with inorganic serum P concentration ≥ 7.80 mg/dL were considered

161 HyperP cases, while cows with serum BHBA ≥ 1,200 μmol/L were considered subclinically
162 ketotic (Divers and Peek, 2008).
163
164 Clinical diseases were defined as follows: a) MF, standing (showing mild ataxia, excitability,
165 muscle tremors and reduced ruminal motility) or recumbent cow (Kelton et al., 1998; Oetzel,
166 2011); b) RFM, fetal membranes were visible at the vulva or were identified in the uterus by
167 vaginal examination more than 12 hours after calving (Melendez et al., 2003); c) MET, fetid
168 uterine discharge, with or without fever (Sheldon et al., 2006); d) MAST, milk clots or abnormal
169 mammary discharge from one or more quarters (Kelton et al., 1998); e) KET, decreased appetite
170 together with elevated blood BHBA (> 2,000 μmol/L), in the absence of obvious concurrent
171 disease (Kelton et al., 1998; Duffield et al., 2009); f) LDA/RDA, decreased appetite
172 accompanied by a clearly audible "ping" sound, produced by percussion of the left/right
173 abdominal wall (between the 9^{th} and 12^{th} ribs), respectively (Kelton et al., 1998).
174
175 ***Data set***
176 Pedigree information was available for all 1,021 cows (332 common sires and 786 common
177 dams). The total population in the study increased to 4,262 animals, when all available pedigree
178 information included, spanning the last 5 (overlapping) generations. Calving date, parity number,
179 calving ease and twinning was recorded. From the 1,021 cows, 35 were diagnosed with MF
180 during the first 4 days after calving, treated appropriately with intravenous Ca and excluded from
181 the genetic analysis of macromineral-related health traits. Therefore, 986 cows were included in
182 the genetic analysis for SCHCa, HypoP, HyperP, SCHMg and HypoK. However, genetic

183 analysis for the other recorded clinical health traits (MF, RFM, MET, MAST, LDA, RDA, KET
184 and UP) included all 1,021 cows.
185
186 The final data set included 4,064 clinical observations for MF, RFM, MET, MAST, LDA, RDA,
187 KET and UP. Moreover, observations for death (DE) and involuntary culling (INVCULL) during
188 the same time-period were also included in the data set, as well as 1,988 BCS records. In total,
189 16,848 biochemical records were available, consisting of 4,020 Ca, 4,019 P, 4,020 Mg, 3,792 K
190 (days 1, 2, 4 and 8 after calving) and 997 BHBA (only on day 8) measurements. Changes of the
191 macrominerals concentrations between day 1 and day 4, as well as between day 1 and day 8 were
192 calculated as the regression slope of macromineral concentrations on time. Thus, these
193 measurements reflected the average daily change in said concentrations and were treated as
194 different traits.
195
196 ***Statistical Analysis***
197 Macromineral-related and disease-related health traits measured on days 1 through 8 were
198 analyzed with a random regression model which accounted for the covariance between
199 successive records of the same animal; each trait was analyzed separately:
200
202 $$Y_{ijkmn} = HYS_i + L_j + M_k + a_1 \cdot age + \sum_{m=0}^{2} b_m P_m D + \sum_{m=0}^{2} A_{nm} P_m D + e_{ijkmn}$$
201 (1)
203 where:
204 Y_{ijkmn} is the health trait record of cow *n*;
205 HYS_i is the fixed effect of herd-year-season of calving *i* (72 levels);

206 L_j the fixed effect of number of lactation j (4 levels);

207 M_k the fixed effect of calendar month k (12 levels);

208 a_1 the linear regression coefficient on age at calving (age);

209 P_m orthogonal polynomial of order m;

210 b_m the fixed regression coefficient on days from calving (D);

211 A_{nm} the random regression coefficient on days from calving associated with the additive
212 genetic effect of cow n including all pedigree data (4,262 animals spanning five
213 generations);

214 e_{ijkmn} the random residual term.

215 The fixed effects in the model including the polynomial order in the fixed regression were fitted
216 after preliminary analyses had confirmed their statistically significant effect ($P<0.05$) on the
217 traits based on the F-test. Further increasing the order of the polynomial did not have a
218 significant effect ($P>0.05$). Similarly, the final order of the random polynomial (third for either
219 trait) was determined with the use of the log-likelihood ratio test in sequential analyses of
220 gradually increasing orders. The final order choice was also confirmed with the Akaike
221 Information Criterion test. Four measurement error classes were defined for each the day from
222 calving (1^{st}, 2^{nd}, 4^{th} and 8^{th}). The definition of these classes, even at this small time span, aimed
223 to capture the day-to-day differences in health events at the beginning of lactation. Covariances
224 between the error classes were assumed to be zero.

225

226 A random permanent environment effect was also fitted to model (1) resulting in a practically
227 zero corresponding variance component estimate, possibly due to the short period our data

spanned (8 days). The log-likelihood ratio tests between the models including and excluding permanent environment were not significant (P>0.05) in all analyses.

There was also an effort to fit a Logit function in model (1) to account for the binary nature of the disease traits. However, this was proved unfeasible within the context of a random regression model.

Serum BHBA concentration for day 8 from calving and average estimates for BCS on days 1 and 8 and serum concentration changes between day 1 and day 4 (days 1-4), as well as day 1 and day 8 (days 1-8) after calving were analyzed using the following model:

$$Y_{ijkm} = HYS_i + L_j + a_1 \cdot age + A_k + e_{ijkm} \qquad (2)$$

where Y_{ijkm} is the log-transformed value for serum BHBA concentration or BCS or macromineral concentration change of cow k; A_k is the additive genetic effect of cow k and all effects are as in model 1.

Estimates of variance components from each model were used to calculate heritabilities for each trait, with the following equation:

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2}$$

where h^2 = the heritability estimate, σ_α^2= the additive genetic variance and σ_p^2= the phenotypic variance.

Genetic (r_α) and phenotypic (r_p) correlations among all traits analyzed with the above models were estimated based on co-variance components derived with a series of bivariate analyses based on the same model described for each trait, with the following equation:

$$r_{(\alpha,p)} = \frac{Cov_{(\alpha,p)}(X,Y)}{\sqrt{\sigma^2_{(\alpha,p)X} \times \sigma^2_{(\alpha,p)Y}}}$$

where $Cov_{(\alpha,p)}(X,Y)$= the additive genetic (Cov_α) or phenotypic (Cov_p) co-variance of traits X and Y and $\sigma^2_{(\alpha,p)X}$and$\sigma^2_{(\alpha,p)Y}$ are the genetic (σ_α^2) or phenotypic (σ_p^2) variances of relevant traits.

All analyses were conducted using the statistical software package ASREML (Gilmour and Gogel, 2006). In all cases, statistical significance was set at P<0.05.

RESULTS

Descriptive statistics for Ca, P, Mg, K and BHBA serum concentrations and BCS are presented in Table1. Average incidence of health disorders during the same time period after calving is presented in Table 2.

Random regression model was used for the generation of prevalence curves across all lactations for all health disorders during the first 8 days after calving. However, this was possible only for

SCHCa, HypoP, SCHMg, HypoK, and HyperP (Figure 1), and also for MF, LDA and MAST (Figure 2). The remaining health disorders had either low incidence (RDA, UP, INVCULL, DE), or were not present throughout the entire 8 day period (RFM: present only the first day; MET, KET: present mainly after the 4^{th} day), thus rendering it impossible to generate curves.

Day-to-day variances (phenotypic, genetic, and residual) and heritabilities for SCHCa, HypoP, SCHMg, HypoK, and HyperP are shown in Table 3 and for MF, LDA, and MAST in Table 4. All estimates presented were statistically greater than zero ($P < 0.05$). Day-to-day heritability estimates were low to moderate for SCHCa ($h^2 = 0.13 – 0.25$), HypoP ($h^2 = 0.18 – 0.33$), HyperP ($h^2 = 0.14 – 0.22$), SCHMg ($h^2 = 0.11 – 0.38$) and LDA ($h^2 = 0.19 – 0.31$), low for HypoK and MF ($h^2 = 0.07 – 0.11$), and moderate to high for MAST ($h^2 = 0.15 – 0.41$). Regarding serum BHBA, the heritability estimate was not statistically significant ($h^2 = 0.073 \pm 0.077$, $P = 0.12$), while for BCS was statistically significant ($h^2 = 0.20 \pm 0.10$, $P < 0.05$).

Significant genetic correlations: a) between serum Ca, P, Mg and K concentrations and health disorders, b) of macromineral concentration changes in days 1-4 and 1-8 after calving with health disorders, and c) among health disorders were not detected in the present study.

Statistically significant phenotypic correlations between overall serum Ca, P, Mg and K concentrations and health disorders during the first 8 days after calving are shown in Table 5. calcium, Mg and K concentrations had high negative correlations with the related subclinical disorders; this was not the case with P. Serum Ca concentrations had a low positive correlation with BCS and a low negative correlation with BHBA; moreover, correlations with most health

295 disorders were negative, either low (HypoP, HypoK, HyperP, LDA, RFM, MET and DE) or
296 moderate (MF, SCHMg). Correlations of Mg and K concentrations with health disorders were
297 similar with those of Ca. Magnesium (but not K) concentrations had a low positive correlation
298 with BCS. For those health disorders that significant correlations were detected, all were
299 negative albeit low. Regarding P, only a high positive correlation with HyperP and low ones with
300 MAST and UP were detected.
301
302 Statistically significant phenotypic correlations of serum macromineral concentrations on day 1
303 and their changes from day 1 to 4 and 1 to 8 after calving with health disorders during the first 8
304 days after calving are shown in Table 6. Calcium concentrations on day 1 and their changes had
305 similar correlations with the various health disorders as those presented in Table 5. Calcium
306 concentration on day 1 was mostly correlated with low concentrations of the other
307 macrominerals, with Ca-related disorders (SCHCa and MF) and MET, while Ca changes were
308 correlated with RFM, MET, KET,DE and INVCULL. Phosphorus concentration on day 1 had
309 similar correlations with the same health disorders as those presented in Table 5, as well.
310 Moreover, a negative correlation with BCS was detected. Phosphorus decrease over time was
311 negatively correlated with HyperP and positively correlated with MF, RFM and DE. High Mg
312 concentration on day 1 was again positively correlated with BCS and negatively with SCHMg
313 and MET. Magnesium changes were correlated with SCHCa, HypoP and BHBA, LDA, MET
314 and DE. Potassium concentrations on day 1 had also similar correlations with the same health
315 disorders as those presented in Table 5. Potassium changes were significantly correlated with
316 HyperP and SCHCa.
317

318 Statistically significant phenotypic correlations of MF, SCHCa, HypoP, HyperP, SCHMg and
319 HypoK with transition period health events are shown in Table 7. Correlations were low but
320 follow the same pattern as those of the respective macromineral serum concentrations, definitely
321 connecting these health conditions with each other.
322
323 **DISCUSSION**
324 This study aimed to estimate genetic parameters of subclinical and clinical diseases that occur
325 during the first 8 days after calving. Detailed records were obtained including day-to-day clinical
326 examination of cows by the same veterinarian.
327
328 Incidence of health disorders was estimated during the first 8 days after calving and a mixed
329 model was used for the estimation of the day-to-day prevalence, which was modeled as a third
330 polynomial fixed regression on days postpartum. The latter gave an accurate mapping of the
331 health status of the population in study.
332
333 Prevalence in this study is in agreement with those of Reinhardt et al. (2011) regarding SCHCa,
334 of Staufenbiel (2002) and Macrae et al. (2006) regarding HypoP and HyperP, of Masoero et al.
335 (2003) regarding SCHMg and of Peek and Divers (2008) regarding HypoK. Moreover, incidence
336 and prevalence of major clinical diseases recorded in this study were very similar with those
337 reported in the literature (Kelton et al., 1998; Heringstad et al., 2005; Melendez and Risco, 2005;
338 LeBlanc, 2008). Therefore, our estimations of various genetic parameters are concurrent with the
339 global Holstein population kept under similar management practices.
340

341 Heritabilities of Ca, P, Mg and K serum concentrations have only recently been reported
342 (Tsiamadis et al., 2016). Heritabilities of SCHCa, HypoP, HyperP, SCHMg and HypoK
343 estimated in this study, are reported for the first time in the literature. They were low to moderate
344 but generally within the range reported for other traits such as milk yield (h^2= 0.20 – 0.50
345 (Castillo-Juarez et al., 2000; Windig et al., 2006; Bastin et al., 2011)), somatic cell count (h^2 =
346 0.03 – 0.11 (Koeck et al., 2012; Heringstad et al., 2006)) and longevity (h^2 = 0.01 – 0.36
347 (Veerkamp and Brotherstone, 2001; Jamrozik et al., 2008)), which are already used in breeding
348 programs. Heringstad et al. (2007) reported that there is potential for selection against metabolic
349 disease resistance and there are several studies that investigate the genetic basis of non-infectious
350 disease resistance (Lin et al., 1989; Lyons et al., 1991; Abdel-Azim et al., 2005). Substantial and
351 statistically significant genetic variance estimates derived in the present study corroborate these
352 assertions.
353
354 At the same time, low heritability estimates suggest that environmental factors have a strong
355 influence in the etiology of the studied traits. Nutrition, management and housing of cows during
356 the transition period emerge as critical factors for prevention of these health disorders in the
357 short term. Nevertheless, genetic selection for resistance for these macromineral deficiency traits
358 could be effective and add permanent benefits to successfully address the problem in the long
359 term, thereby complementing management practices.
360
361 Heritability of BHBA in the present study was not statistically significant (h^2= 0.073±0.077).
362 Oikonomou et al. (2008b) also reported heritability estimates in primiparous Holstein cows
363 (h^2=0.25±0.18), which were not statistically significant. However, recently, van der Drift et al.

364 (2012) in a study of 1,772 Holstein cows of various parities between 5 and 60 days after calving
365 from 123 herds, using a similar animal model, reported a heritability estimate of 0.17±0.06
366 (P<0.001).This higher heritability estimate can be attributed to the much wider sampling period
367 (1 blood sample between 5 to 60 days after calving), which possibly resulted in a higher
368 incidence of hyperketonemia. The heritability estimate of BCS was statistically significant in the
369 present study (h^2=0.20±0.10). Koenen et al.(2001), Veerkamp and Brotherstone (2001) and
370 Oikonomou et al. (2008b) reported higher estimates (0.28 – 0.50) that were statistically
371 significant. Others (Jones et al., 1999; Dechow et al., 2001; Bastin et al., 2010) have reported
372 lower estimates (0.07 – 0.20), which are similar to our results. Heritability estimates of BCS tend
373 to be larger in mid to late lactation (Dechow et al., 2001) and it is likely that the focus of this
374 study on the first week after calving could have led to this moderate estimate.
375
376 The present study's estimates of MF heritability (h^2 =0.07 – 0.11) are in agreement with those of
377 Dyrendahl et al. (1972), Uribe et al. (1995), Pryce et al. (1997), Van Dorp et al. (1998) and
378 Heringstad et al. (2005). These, however, are generally lower than estimates reported by Lin et
379 al. (1989), Lyons et al. (1991) and Abdel-Azim et al. (2005) (h^2 = 0.30 – 0.40). Differences in
380 estimates can be attributed to methodology of statistical analysis, data collection (farm records),
381 and type and age of the population studied.
382
383 Our heritability estimates for LDA (h^2=0.18 – 0.31) are similar to those reported by Uribe et al.,
384 (1995) (h^2 = 0.304±0.005, across lactation with a threshold model). This is higher than other
385 estimates from linear models reported by Lyons et al. (1991), Appuhamy et al. (2009) and Koeck
386 et al. (2013). Moreover, Wolf (2001) and Hamann et al. (2004) with the use of threshold models

387 reported heritability estimates above 0.50. The moderate to high heritability estimates of the
388 present study can be attributed to a more accurate recording of the displacement made by the
389 veterinarian and to the binary nature of the trait that posed no ambiguity to the severity of the
390 disease and thus to the certainty of the diagnosis.
391
392 Heritability estimates for MAST vary across studies. Lin et al. (1989) reported heritabilities of
393 0.19±0.08, 0.31±0.10 and 0.18±0.09 for the 1st, 2nd and 3rd+ lactation, respectively. Uribe et al.
394 (1995) reported similar estimates for 1st lactation cows (h^2 = 0.15±0.05) but for all lactations
395 estimates were zero. Zwald et al. (2004) and Heringstad et al. (2005) reported much lower
396 estimates (h^2 = 0.09±0.01); more recently, Pérez-Cabal et al. (2009) and Vazquez et al. (2009)
397 also reported similar heritabilities (h^2= 0.09 and h^2 = 0.13, respectively), while Koeck et al.
398 (2013) estimated the heritability of clinical mastitis at 0.02±0.004. However, all these studies
399 estimated mastitis heritability across lactation. Our estimates (h^2 = 0.15 – 0.41) cover a small
400 portion of the entire lactation, only the first 8 days. Considering that clinical mastitis
401 immediately after calving is influenced by factors such as dry period management and
402 compromised immune status due to calving (Kimura et al., 2006; LeBlanc, 2010), this may well
403 be a different trait which, based on our results, could potentially respond to selection.
404
405 The present study did not detect any significant genetic correlation of Ca, P, Mg and K serum
406 concentrations and BCS with any postpartum health disorders. The absence of genetic
407 correlations could be attributed to the multifactorial etiology of most of these health events:
408 infectious agents may co-exist with metabolic and managerial deficiencies. Moreover, this lack
409 of genetic correlation may support the idea that these traits are controlled genetically by different

410 genes and individual selection should be applied. Contrary to expectations, this study did not find
411 a significant genetic correlation between SCHCa and MF. However, considering the disease
412 definitions, MF cases were defined as standing (showing mild ataxia, excitability, muscle
413 tremors and reduced ruminal motility) or recumbent cows; therefore, MF definition was solely
414 based on symptoms and not in any serum Ca measurement. Furthermore, this absence of genetic
415 correlation could also be attributed to the low incidence of MF. In this study, recumbent cows
416 were immediately treated with intravenous Ca solutions, rendering the measurement of serum Ca
417 concentrations meaningless. Moreover, it is known that there is no specific threshold of Ca
418 serum concentrations which always results in recumbent cows. Regarding the absence of any
419 genetic correlation of the remaining macrominerals with other health disorders, this may also be
420 attributed to the multifactorial etiology and to the low incidence of some of the health disorders
421 (e.g. metritis, mastitis and ketosis). On the other hand, the lack of any significant genetic
422 correlation in this study may be incidental. Therefore, as this is the first study of its kind, the
423 genetic analysis of other independent data sets may shed more light on this issue; more research
424 is needed in order to clarify these issues.
425
426 The reported phenotypic association of clinical and subclinical hypocalcemia with various
427 diseases after calving is based almost solely on pathophysiology, because of calcium's central
428 metabolic role; it is generally assumed that P and Mg serum concentrations are associated with
429 the same postpartum diseases through their relation with Ca metabolism (Rude, 1998; Goff,
430 2000; DeGaris and Lean, 2008). In a study of 2,190 cows from 33 herds, Curtis et al. (1983)
431 showed that cows with clinical hypocalcemia (MF) were at greater risk of developing dystocia
432 (6.5 times), RFM (3.2 times), KET (8.9 times) and MAST (8.1 times). Martinez et al. (2012)

found that cows with low serum Ca have higher BHBA concentrations. However, large scale research-based evidence for any association of subclinical macromineral-related disorders with postpartum cow health is lacking. In the present study, statistically significant phenotypic correlations of the four major macrominerals' serum concentrations and the corresponding subclinical disorders with the early postpartum disease cascade in dairy cows are reported for the first time. A strong association with energy metabolism is evident both at the KET and BHBA, as well as the BCS levels, with serious indirect and direct implications for future reproductive performance (RFM, MET and UP), MAST and replacement rates (LDA, INVCULL and DE). The correlation of HyperP with MAST is a novel finding and the exact mechanism of this association has to be further investigated. These results highlight not only the need for genetic selection against these subclinical disorders, which is feasible based on our heritability estimates, but also for enhanced implementation of pertinent management practices.

Herd management during early postpartum is a challenge for modern dairy farms. The ability of an animal to maintain normal serum macromineral concentrations is consistent with the successful management of the numerous health events after calving. Rapid metabolic changes of animals combined with stressors such as nutritional and grouping changes further compromise immunity status, favor metabolic and infectious diseases, and downgrade productivity and welfare. Postpartum health monitoring programs are implemented in many dairy farms worldwide since they greatly contribute to the early recognition and proper treatment of sick animals (Risco, 2011). Obviously, genetic selection can provide a valuable tool, as well. Standardized health monitoring programs across regions and countries could provide accurate phenotype information for novel functional traits, the discovery of their genetic markers and

finally, the creation of a new index ("disease resistance early postpartum"). This is, indeed, a very exciting prospect.

CONCLUSIONS

More research is needed on this issue, but results of the present study clearly indicate that subclinical Ca, P, Mg and K disorders during the first week after calving are heritable traits. Moreover, significant heritability estimates of BCS and MF, MAST and LDA during the same period were also derived. These genetic parameters can potentially be used to develop health indices for the selection of dairy cows that will effectively resist health challenges immediately after calving. Phenotypic correlations of high prevalence subclinical macromineral disorders with clinical diseases, reveal a deeper interrelationship among these traits and stresses the need for both innovative genetic selection and effective management practices.

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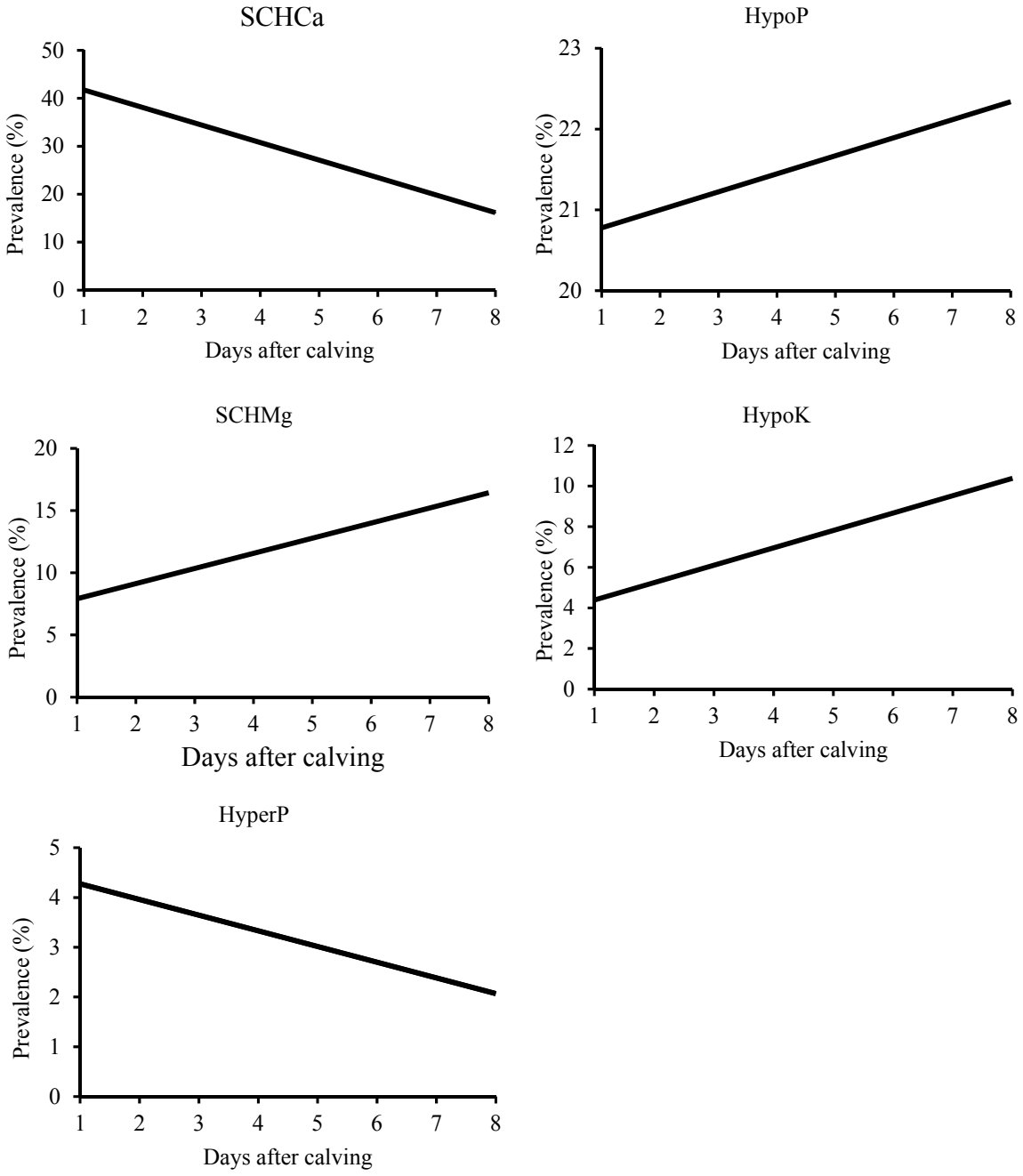
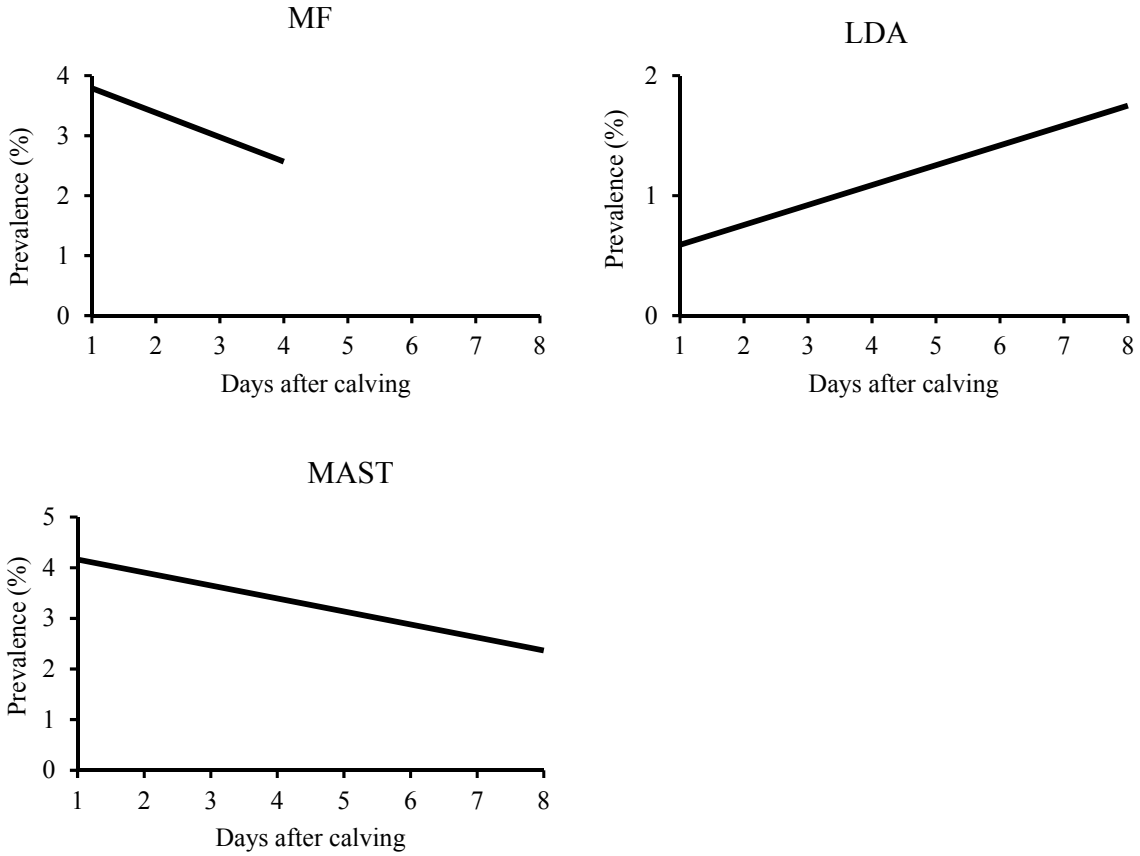


Tsiamadis Figure 1.



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682 **Tsiamadis Figure 2.**

Figure captures

Figure 1. Prevalence of subclinical hypocalcemia (SCHCa), hypophosphatemia (HypoP), hypomagnesemia (SCHMg), hypokalemia (HypoK) and hyperphosphatemia (HyperP) across all lactations, during the first 8 days after calving based on third order fixed regression polynomials.

Figure 2. Prevalence of milk fever (MF), left displacement of abomasum (LDA) and mastitis (MAST) across all lactations, during the first 8 days after calving based on third order fixed regression polynomials.

Table 1. Descriptive statistics of serum Calcium (Ca), Phosphorus (P), Magnesium (Mg), Potassium (K) and β-Hydroxybutyric acid (BHBA) concentrations, and Body Condition Score (BCS) during the first 8 days after calving

Trait	Mean	Std. Dev	Min	Max	No. Cows
Ca (mg/dL)	8.92	0.77	3.9	13.9	1,021
P (mg/dL)	5.21	0.89	1.1	10.5	1,021
Mg (mg/dL)	2.24	0.26	0.36	7.2	1,021
K (mmol/L)	4.59	0.39	2.4	6.3	1,018
BHBA (μmol/L)	829.03	602.29	160	4,870	997
BCS (1-5 scale)	3.12	0.41	1.75	4.75	977

Table 2. Average incidence of health disorders during the first 8 days after calving (1,021 cows; all traits are expressed as 0/1)

Trait	Average	Std. Dev
SCHCa	0.634	0.482
HypoP	0.498	0.500
SCHMg	0.323	0.468
HypoK	0.229	0.421
HyperP	0.090	0.286
MF	0.080	0.272
RFM	0.176	0.381
MET	0.337	0.473
MAST	0.099	0.299
LDA	0.035	0.185
RDA	0.001	0.031
KET	0.029	0.169
UP	0.003	0.054
INVCULL	0.005	0.070
DE	0.008	0.088

SCHCa: subclinical hypocalcemia, HypoP: hypophosphatemia, SCHMg: subclinical hypomagnesemia, HypoK: hypokalemia, HyperP: hyperphosphatemia, MF: milk fever, RFM: retained fetal membranes, MET: metritis, MAST: mastitis, LDA= left displacement of abomasum, RDA: right displacement of abomasum, KET: clinical ketosis, UP: uterine prolapse, INVCULL: involuntary culling, DE: death.

Table 3. Variances and heritability estimates of subclinical hypocalcemia (SCHCa), hypophosphatemia (HypoP), hypomagnesemia (SCHMg), hypokalemia (HypoK) and hyperphosphatemia (HyperP) by days after calving from random regression model analyses

Trait	Day after calving	σ_p^2	σ_a^2	σ_r^2	h^2
SCHCa	1st	0.22 (0.01)**	0.06 (0.01)**	0.16 (0.01)**	0.25 (0.03)**
	2nd	0.22 (0.01)**	0.04 (0.01)**	0.18 (0.01)**	0.20 (0.02)**
	4th	0.18 (0.01)**	0.03 (0.004)*	0.16 (0.01)**	0.15 (0.02)**
	8th	0.14 (0.01)**	0.02 (0.01)*	0.12 (0.01)**	0.13 (0.06)*
HypoP	1st	0.16 (0.01)**	0.03 (0.01)**	0.12 (0.01)**	0.21 (0.03)**
	2nd	0.16 (0.01)**	0.03 (0.004)**	0.14 (0.01)**	0.18 (0.02)**
	4th	0.16 (0.01)**	0.03 (0.003)**	0.13 (0.01)**	0.19 (0.02)**
	8th	0.18 (0.01)**	0.06 (0.01)***	0.12 (0.01)**	0.33 (0.06)**
SCHMg	1st	0.07 (0.003)**	0.03 (0.003)**	0.04 (0.003)**	0.38 (0.04)**
	2nd	0.09 (0.004)**	0.02 (0.002)**	0.06 (0.003)**	0.27 (0.03)**
	4th	0.14 (0.006)**	0.02 (0.002)**	0.13 (0.006)**	0.12 (0.01)**
	8th	0.13 (0.006)**	0.01 (0.006)*	0.11 (0.008)**	0.11 (0.05)*
HypoK	1st	0.05 (0.002)**	0.005 (0.002)*	0.05 (0.002)**	0.10 (0.03)*
	2nd	0.06 (0.003)**	0.005 (0.001)**	0.05 (0.003)**	0.08 (0.02)**
	4th	0.06 (0.003)**	0.005 (0.001)**	0.06 (0.003)**	0.08 (0.02)**
	8th	0.10 (0.004)**	0.010 (0.004)*	0.09 (0.005)**	0.10 (0.04)*
HyperP	1st	0.04 (0.002)**	0.01 (0.001)**	0.03 (0.002)**	0.22 (0.03)**
	2nd	0.03 (0.001)**	0.01 (0.001)**	0.03 (0.001)**	0.21 (0.03)**
	4th	0.03 (0.001)**	0.004 (0.001)**	0.02 (0.001)**	0.16 (0.02)**
	8th	0.02 (0.001)**	0.003 (0.001)*	0.02 (0.001)**	0.14 (0.06)*

Phenotypic (σ_p^2), genetic (σ_a^2), residual variances (σ_r^2) and heritability (h^2) estimations (standard errors in parentheses).

* P<0.05, ** P<0.001.

Table 4. Variances and heritability estimates of milk fever (MF), left displacement of abomasum (LDA), and mastitis (MAST) by days after calving from random regression model analyses

Trait	Day after calving	σ_p^2	σ_a^2	σ_r^2	h^2
MF	1st	0.046 (0.002)***	0.003 (0.001)***	0.043 (0.002)***	0.07 (0.02)***
	2nd	0.029 (0.001)***	0.002 (0.001)***	0.026 (0.001)***	0.08 (0.02)***
	4th	0.008 (0.000)***	0.001 (0.000)**	0.007 (0.000)***	0.11 (0.03)**
	8th	-	-	-	-
LDA	1st	0.01 (0.000)***	0.002 (0.000)***	0.01 (0.000)***	0.24 (0.03)***
	2nd	0.01 (0.000)***	0.002 (0.000)***	0.01 (0.000)***	0.19 (0.02)***
	4th	0.01 (0.000)***	0.003 (0.000)***	0.01 (0.000)***	0.26 (0.02)***
	8th	0.02 (0.001)***	0.006 (0.001)***	0.01 (0.001)***	0.31 (0.05)***
MAST	1st	0.04 (0.002)***	0.01 (0.001)***	0.02 (0.001)***	0.36 (0.03)***
	2nd	0.03 (0.001)***	0.01 (0.001)***	0.02 (0.001)***	0.41 (0.03)***
	4th	0.04 (0.002)***	0.01 (0.001)***	0.03 (0.001)***	0.18 (0.02)***
	8th	0.03 (0.001)***	0.004 (0.002)*	0.02 (0.002)***	0.15 (0.06)*

* Phenotypic (σ_p^2), genetic (σ_a^2), residual variances (σ_r^2) and heritability (h^2) estimations (standard errors in parentheses).

* P<0.05, ** P<0.01, *** P<0.001.

Table 5. Statistically significant phenotypic correlations of serum Calcium (Ca), Phosphorus (P), Magnesium (Mg) and Potassium (K) concentrations with Body Condition Score (BCS), β-Hydroxy-butyric acid (BHBA) and health disorders traits, during the first 8 days after calving (standard error in parentheses)

Trait	Ca	P	Mg	K
BCS	0.11 (0.03)**	-0.06 (0.03)^{t}	0.14 (0.03)***	-0.06 (0.03)^{t}
BHBA	-0.13 (0.03)***	NS	NS	-0.09 (0.03)*
SCHCa	-0.60 (0.02)***	NS	-0.09 (0.03)*	-0.10 (0.03)*
HypoP	-0.06 (0.03)*	NS	NS	NS
SCHMg	-0.22 (0.03)***	NS	-0.56 (0.02)***	-0.10 (0.03)*
HypoK	-0.17 (0.03)***	NS	-0.05 (0.03)^{t}	-0.48 (0.02)***
HyperP	-0.07 (0.3)*	0.46 (0.03)***	-0.08 (0.03)**	NS
MF	-0.32 (0.03)***	NS	NS	-0.11 (0.03)**
RFM	-0.14 (0.03)***	NS	-0.10 (0.03)*	-0.14 (0.03)***
MET	-0.18 (0.03)***	NS	-0.15 (0.03)***	-0.13 (0.03)***
MAST	NS	0.12 (0.03)***	-0.06 (0.03)^{t}	NS
LDA	-0.15 (0.03)***	NS	-0.07 (0.03)*	-0.06 (0.03)^{t}
RDA	NS	NS	NS	NS
KET	-0.05 (0.03)^{t}	NS	NS	NS
UP	NS	0.08 (0.03)*	NS	NS
INVCULL	NS	NS	NS	NS
DE	-0.09 (0.03)*	NS	0.06 (0.03)^{t}	-0.12 (0.03)***

SCHCa: subclinical hypocalcemia, HypoP: hypophosphatemia, SCHMg: subclinical hypomagnesemia, HypoK: hypokalemia, HyperP: hyperphosphatemia, MF: milk fever, RFM: retained fetal membranes, MET: metritis, MAST: mastitis, LDA= left displacement of abomasum, RDA: right displacement of abomasum, KET: clinical ketosis UP: uterine prolapse, INVCULL: involuntary culling, DE: death.

NS: non-significant.

* P<0.05, ** P<0.01, *** P<0.001.

^{t} 0.05≤P≤0.10

Single Record per animal, Bivariate analysis.

Table 6. Statistically significant phenotypic correlations of serum macromineral concentrations on day 1 after calving and their changes from days 1-4 and 1-8 after calving with Body Condition Score (BCS), β-Hydroxybutyric acid (BHBA) and health disorders traits, during the first 8 days after calving (standard error in parentheses)

	Ca_1	Ca Change_1-4	Ca Change_1-8	P_1	P Change_1-4	P Change_1-8	Mg_1	Mg Change_1-4	Mg Change_1-8	K_1	K Change_1-4	K Change_1-8
BCS	0.06 (0.03)[t]	NS	NS	-0.07 (0.03)*	NS	NS	0.11 (0.03)**	NS	NS	NS	NS	NS
BHBA	NS	NS	-0.06 (0.03)[t]	NS	NS	NS	NS	NS	-0.19 (0.03)***	-0.07 (0.03)*	NS	NS
SCHCa	-0.44 (0.03)***	0.06 (0.03)[t]	0.11 (0.03)**	NS	NS	NS	NS	NS	-0.08 (0.03)*	NS	NS	-0.07 (0.03)*
HypoP	-0.08 (0.03)*	NS	0.06 (0.03)[t]	-0.40 (0.03)***	NS	NS	NS	-0.07 (0.03)*	NS	-0.07 (0.03)*	0.07 (0.03)*	NS
SCHMg	-0.15 (0.03)***	NS	NS	NS	NS	NS	-0.25 (0.03)***	-0.13 (0.03)**	-0.11 (0.03)*	NS	NS	NS
HypoK	-0.12 (0.03)**	NS	NS	NS	0.06 (0.03)[t]	NS	NS	-0.06 (0.03)[t]	-0.06 (0.03)[t]	-0.25 (0.03)***	NS	-0.06 (0.03)[t]
HyperP	NS	NS	NS	0.36 (0.03)***	-0.13 (0.03)**	-0.22 (0.03)***	-0.05 (0.03)[t]	NS	NS	NS	NS	NS
MF	-0.26 (0.03)***	0.07 (0.03)*	NS	NS	0.06 (0.03)*	NS	NS	NS	NS	-0.09 (0.03)*	NS	NS
RFM	NS	-0.08 (0.03)*	-0.10 (0.03)**	NS	NS	0.07 (0.03)*	NS	NS	-0.06 (0.03)[t]	-0.08 (0.03)*	NS	NS
MET	-0.08 (0.03)*	-0.09 (0.03)*	-0.07 (0.03)*	NS	NS	NS	-0.07 (0.03)*	NS	-0.07 (0.03) *	NS	NS	NS
MAST	NS	NS	NS	0.07 (0.03)*	NS	NS	-0.05 (0.03)[t]	NS	NS	NS	NS	NS
LDA	-0.05 (0.03)[t]	NS	-0.06 (0.03)[t]	NS	NS	NS	NS	NS	-0.11 (0.03)**	NS	NS	NS
RDA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KET	NS	-0.07 (0.03)*	NS	NS	NS	NS	NS	NS	-0.06 (0.03)[t]	NS	NS	NS
UP	NS	NS	NS	0.07 (0.03)*	NS	NS	NS	NS	NS	NS	NS	NS
INVCULL	NS	-0.07 (0.03)*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DE	NS	-0.11 (0.03)**	-0.23 (0.03)***	NS	0.08 (0.03)*	0.15 (0.03)***	NS	0.06 (0.03)*	0.08 (0.03)**	-0.08 (0.03)*	NS	NS

Ca/P/Mg/K_1 = serum Calcium/ Phosphorus/ Magnesium/ Potassium concentration on day 1 after calving.

Ca/P/Mg/K Change_1-4 = serum Calcium/ Phosphorus/ Magnesium/ Potassium concentration change from days 1 to 4 after calving.

Ca/P/Mg/K Change_1-8 = serum Calcium/ Phosphorus/ Magnesium/ Potassium concentration change from days 1 to 8 after calving.

SCHCa: subclinical hypocalcemia, HypoP: hypophosphatemia, SCHMg: subclinical hypomagnesemia, HypoK: hypokalemia,
HyperP: hyperphosphatemia, MF: milk fever, RFM: retained fetal membranes, MET: metritis, MAST: mastitis, LDA= left displacement of abomasum, RDA: right displacement of abomasum, KET: clinical ketosis, UP: uterine prolapse, INVCLULL: involuntary culling, DE: death

NS: Non-significant

* P<0.05, ** P<0.01, *** P<0.001.

[t] 0.05≤P≤0.10

Single Record per animal, Bivariate analysis.

Table 7. Statistically significant phenotypic correlations of BHBA, SCHCa, HypoP, SCHMg and HypoK, HyperP and MF with transition period health events (standard error in parentheses)

	SCHCa	HypoP	SCHMg	HypoK	HyperP	MF	RFM	MET	MAST	LDA	RDA	KET	UP	INVCULL	DE
BHBA	0.07 (0.03)*	NS	0.07 (0.03)*	0.12 (0.03)**	-0.07 (0.03)*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SCHCa		0.08 (0.03)*	0.15 (0.03)**	0.11 (0.03)**	NS	0.12 (0.03)**	NS	0.11 (0.03)*	NS	0.08 (0.03)*	NS	NS	NS	NS	NS
HypoP			0.07 (0.03)*	NS	-0.09 (0.03)*	0.06 (0.03)*	NS	NS	-0.06 (0.03)[t]	NS	NS	NS	NS	NS	NS
SCHMg				0.08 (0.03)*	0.11 (0.03)**	NS	0.10 (0.03)*	0.13 (0.03)***	NS	NS	NS	NS	NS	NS	NS
HypoK						0.07 (0.03)*	0.12 (0.03)***	0.09 (0.03)*	NS	0.13 (0.03)***	0.06 (0.03)[t]	NS	NS	NS	NS
HyperP						NS	NS	NS	0.07 (0.03)*	NS	NS	NS	NS	NS	NS
MF							0.07 (0.03)*	0.06 (0.03)[t]	NS	0.11 (0.03)**	NS	-0.05 (0.03)[t]	0.19 (0.03)***	NS	0.18 (0.03)***

BHBA: β-hydroxybutyric acid, SCHCa: subclinical hypocalcemia, HypoP: hypophosphatemia, SCHMg: subclinical hypomagnesemia, HypoK: hypokalemia, HyperP: hyperphosphatemia, MF: milk fever, RFM: retained fetal membranes, MET: metritis, MAST: mastitis, LDA= left displacement of abomasum, RDA: right displacement of abomasum, KET: clinical ketosis, UP: uterine prolapse, INVCULL: involuntary culling, DE: death.

NS: Non-significant.

* P<0.05, ** P<0.01, *** P<0.001.

[t] 0.05≤P≤0.10

Single Record per animal, Bivariate analysis.