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## Genetic parameters of subclinical macromineral disorders and major clinical diseases in post parturient Holstein cows

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

3  
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16

17 **Interpretive Summary**

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

25 **ABSTRACT**

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

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

53

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

55

56

## INTRODUCTION

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

67

68 Macromineral serum concentration changes are mainly caused by increased cow requirements at  
69 the onset of lactation combined with reduced feed intake and possibly delayed homeostatic  
70 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.

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## 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 1<sup>st</sup>, 2<sup>nd</sup>, 4<sup>th</sup> and 8<sup>th</sup> day after calving. Body condition score was recorded on the 1<sup>st</sup> and 8<sup>th</sup> 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°C). Serum was transferred into polyethylene tubes and stored at -80°C until  
141 assay. All sera were analyzed for total Ca and Mg concentrations using flame atomic absorption  
142 spectrophotometry (Perkin ElmerAAAnalyst 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 8<sup>th</sup> 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  $\geq 7.80$  mg/dL were considered



161 HyperP cases, while cows with serum BHBA  $\geq 1,200$   $\mu\text{mol/L}$  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 ( $> 2,000$   $\mu\text{mol/L}$ ), 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<sup>th</sup> and 12<sup>th</sup> 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

$$Y_{ijkmn} = HYS_i + L_j + M_k + a_1 \cdot age + \sum_{m=0}^2 b_m P_m D + \sum_{m=0}^2 A_{nm} P_m D + e_{ijkmn}$$

201 (1)

202 where:

203  $Y_{ijkmn}$  is the health trait record of cow  $n$ ;

204  $HYS_i$  is the fixed effect of herd-year-season of calving  $i$  (72 levels);

205  $L_j$  the fixed effect of number of lactation  $j$  (4 levels);  
206  $M_k$  the fixed effect of calendar month  $k$  (12 levels);  
207  $a_1$  the linear regression coefficient on age at calving (age);  
208  $P_m$  orthogonal polynomial of order  $m$ ;  
209  $b_m$  the fixed regression coefficient on days from calving (D);  
210  $A_{nm}$  the random regression coefficient on days from calving associated with the additive  
211 genetic effect of cow  $n$  including all pedigree data (4,262 animals spanning five  
212 generations);  
213  $e_{ijkmn}$  the random residual term.

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

224

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

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

229

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

233

234 Serum BHBA concentration for day 8 from calving and average estimates for BCS on days 1 and  
235 8 and serum concentration changes between day 1 and day 4 (days 1-4), as well as day 1 and day  
236 8 (days 1-8) after calving were analyzed using the following model:

237

$$238 \quad Y_{ijkm} = HYS_i + L_j + a_1 \cdot age + A_k + e_{ijkm} \quad (2)$$

239

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

243

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

246

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

247

248 where  $h^2$  = the heritability estimate,  $\sigma_\alpha^2$ = the additive genetic variance and  $\sigma_p^2$ = the phenotypic  
249 variance.

250

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

254

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

255

256 where  $Cov_{(\alpha,p)}(X,Y)$ = the additive genetic ( $Cov_\alpha$ ) or phenotypic ( $Cov_p$ ) co-variance of traits  $X$   
257 and  $Y$  and  $\sigma_{(\alpha,p)X}^2$  and  $\sigma_{(\alpha,p)Y}^2$  are the genetic ( $\sigma_\alpha^2$ ) or phenotypic ( $\sigma_p^2$ ) variances of relevant traits.

258

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

261

262

## RESULTS

263 Descriptive statistics for Ca, P, Mg, K and BHBA serum concentrations and BCS are presented  
264 in Table 1. Average incidence of health disorders during the same time period after calving is  
265 presented in Table 2.

266

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

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

273

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

282

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

286

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

292 disorders were negative, either low (HypoP, HypoK, HyperP, LDA, RFM, MET and DE) or  
293 moderate (MF, SCHMg). Correlations of Mg and K concentrations with health disorders were  
294 similar with those of Ca. Magnesium (but not K) concentrations had a low positive correlation  
295 with BCS. For those health disorders that significant correlations were detected, all were  
296 negative albeit low. Regarding P, only a high positive correlation with HyperP and low ones with  
297 MAST and UP were detected.

298

299 Statistically significant phenotypic correlations of serum macromineral concentrations on day 1  
300 and their changes from day 1 to 4 and 1 to 8 after calving with health disorders during the first 8  
301 days after calving are shown in Table 6. Calcium concentrations on day 1 and their changes had  
302 similar correlations with the various health disorders as those presented in Table 5. Calcium  
303 concentration on day 1 was mostly correlated with low concentrations of the other  
304 macrominerals, with Ca-related disorders (SCHCa and MF) and MET, while Ca changes were  
305 correlated with RFM, MET, KET,DE and INVCULL. Phosphorus concentration on day 1 had  
306 similar correlations with the same health disorders as those presented in Table 5, as well.  
307 Moreover, a negative correlation with BCS was detected. Phosphorus decrease over time was  
308 negatively correlated with HyperP and positively correlated with MF, RFM and DE. High Mg  
309 concentration on day 1 was again positively correlated with BCS and negatively with SCHMg  
310 and MET. Magnesium changes were correlated with SCHCa, HypoP and BHBA, LDA, MET  
311 and DE. Potassium concentrations on day 1 had also similar correlations with the same health  
312 disorders as those presented in Table 5. Potassium changes were significantly correlated with  
313 HyperP and SCHCa.

314

315 Statistically significant phenotypic correlations of MF, SCHCa, HypoP, HyperP, SCHMg and  
316 HypoK with transition period health events are shown in Table 7. Correlations were low but  
317 follow the same pattern as those of the respective macromineral serum concentrations, definitely  
318 connecting these health conditions with each other.

319

320

## DISCUSSION

321 This study aimed to estimate genetic parameters of subclinical and clinical diseases that occur  
322 during the first 8 days after calving. Detailed records were obtained including day-to-day clinical  
323 examination of cows by the same veterinarian.

324

325 Incidence of health disorders was estimated during the first 8 days after calving and a mixed  
326 model was used for the estimation of the day-to-day prevalence, which was modeled as a third  
327 polynomial fixed regression on days postpartum. The latter gave an accurate mapping of the  
328 health status of the population in study.

329

330 Prevalence in this study is in agreement with those of Reinhardt et al. (2011) regarding SCHCa,  
331 of Staufenbiel (2002) and Macrae et al. (2006) regarding HypoP and HyperP, of Masoero et al.  
332 (2003) regarding SCHMg and of Peek and Divers (2008) regarding HypoK. Moreover, incidence  
333 and prevalence of major clinical diseases recorded in this study were very similar with those  
334 reported in the literature (Kelton et al., 1998; Heringstad et al., 2005; Melendez and Risco, 2005;  
335 LeBlanc, 2008). Therefore, our estimations of various genetic parameters are concurrent with the  
336 global Holstein population kept under similar management practices.

337



338 Heritabilities of Ca, P, Mg and K serum concentrations have only recently been reported  
339 (Tsiamadis et al., 2016). Heritabilities of SCHCa, HypoP, HyperP, SCHMg and HypoK  
340 estimated in this study, are reported for the first time in the literature. They were low to moderate  
341 but generally within the range reported for other traits such as milk yield ( $h^2 = 0.20 - 0.50$   
342 (Castillo-Juarez et al., 2000; Windig et al., 2006; Bastin et al., 2011)), somatic cell count ( $h^2 =$   
343  $0.03 - 0.11$  (Koeck et al., 2012; Heringstad et al., 2006)) and longevity ( $h^2 = 0.01 - 0.36$   
344 (Veerkamp and Brotherstone, 2001; Jamrozik et al., 2008)), which are already used in breeding  
345 programs. Heringstad et al. (2007) reported that there is potential for selection against metabolic  
346 disease resistance and there are several studies that investigate the genetic basis of non-infectious  
347 disease resistance (Lin et al., 1989; Lyons et al., 1991; Abdel-Azim et al., 2005). Substantial and  
348 statistically significant genetic variance estimates derived in the present study corroborate these  
349 assertions.

350

351 At the same time, low heritability estimates suggest that environmental factors have a strong  
352 influence in the etiology of the studied traits. Nutrition, management and housing of cows during  
353 the transition period emerge as critical factors for prevention of these health disorders in the  
354 short term. Nevertheless, genetic selection for resistance for these macromineral deficiency traits  
355 could be effective and add permanent benefits to successfully address the problem in the long  
356 term, thereby complementing management practices.

357

358 Heritability of BHBA in the present study was not statistically significant ( $h^2 = 0.073 \pm 0.077$ ).  
359 Oikonomou et al. (2008b) also reported heritability estimates in primiparous Holstein cows  
360 ( $h^2 = 0.25 \pm 0.18$ ), which were not statistically significant. However, recently, van der Drift et al.

361 (2012) in a study of 1,772 Holstein cows of various parities between 5 and 60 days after calving  
362 from 123 herds, using a similar animal model, reported a heritability estimate of  $0.17 \pm 0.06$   
363 ( $P < 0.001$ ). This higher heritability estimate can be attributed to the much wider sampling period  
364 (1 blood sample between 5 to 60 days after calving), which possibly resulted in a higher  
365 incidence of hyperketonemia. The heritability estimate of BCS was statistically significant in the  
366 present study ( $h^2 = 0.20 \pm 0.10$ ). Koenen et al. (2001), Veerkamp and Brotherstone (2001) and  
367 Oikonomou et al. (2008b) reported higher estimates (0.28 – 0.50) that were statistically  
368 significant. Others (Jones et al., 1999; Dechow et al., 2001; Bastin et al., 2010) have reported  
369 lower estimates (0.07 – 0.20), which are similar to our results. Heritability estimates of BCS tend  
370 to be larger in mid to late lactation (Dechow et al., 2001) and it is likely that the focus of this  
371 study on the first week after calving could have led to this moderate estimate.

372

373 The present study's estimates of MF heritability ( $h^2 = 0.07 - 0.11$ ) are in agreement with those of  
374 Dyrendahl et al. (1972), Uribe et al. (1995), Pryce et al. (1997), Van Dorp et al. (1998) and  
375 Heringstad et al. (2005). These, however, are generally lower than estimates reported by Lin et  
376 al. (1989), Lyons et al. (1991) and Abdel-Azim et al. (2005) ( $h^2 = 0.30 - 0.40$ ). Differences in  
377 estimates can be attributed to methodology of statistical analysis, data collection (farm records),  
378 and type and age of the population studied.

379

380 Our heritability estimates for LDA ( $h^2 = 0.18 - 0.31$ ) are similar to those reported by Uribe et al.,  
381 (1995) ( $h^2 = 0.304 \pm 0.005$ , across lactation with a threshold model). This is higher than other  
382 estimates from linear models reported by Lyons et al. (1991), Appuhamy et al. (2009) and Koeck  
383 et al. (2013). Moreover, Wolf (2001) and Hamann et al. (2004) with the use of threshold models

384 reported heritability estimates above 0.50. The moderate to high heritability estimates of the  
385 present study can be attributed to a more accurate recording of the displacement made by the  
386 veterinarian and to the binary nature of the trait that posed no ambiguity to the severity of the  
387 disease and thus to the certainty of the diagnosis.

388

389 Heritability estimates for MAST vary across studies. Lin et al. (1989) reported heritabilities of  
390  $0.19 \pm 0.08$ ,  $0.31 \pm 0.10$  and  $0.18 \pm 0.09$  for the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>+ lactation, respectively. Uribe et al.  
391 (1995) reported similar estimates for 1<sup>st</sup> lactation cows ( $h^2 = 0.15 \pm 0.05$ ) but for all lactations  
392 estimates were zero. Zwald et al. (2004) and Heringstad et al. (2005) reported much lower  
393 estimates ( $h^2 = 0.09 \pm 0.01$ ); more recently, Pérez-Cabal et al. (2009) and Vazquez et al. (2009)  
394 also reported similar heritabilities ( $h^2 = 0.09$  and  $h^2 = 0.13$ , respectively), while Koeck et al.  
395 (2013) estimated the heritability of clinical mastitis at  $0.02 \pm 0.004$ . However, all these studies  
396 estimated mastitis heritability across lactation. Our estimates ( $h^2 = 0.15 - 0.41$ ) cover a small  
397 portion of the entire lactation, only the first 8 days. Considering that clinical mastitis  
398 immediately after calving is influenced by factors such as dry period management and  
399 compromised immune status due to calving (Kimura et al., 2006; LeBlanc, 2010), this may well  
400 be a different trait which, based on our results, could potentially respond to selection.

401

402 The present study did not detect any significant genetic correlation of Ca, P, Mg and K serum  
403 concentrations and BCS with any postpartum health disorders. The absence of genetic  
404 correlations could be attributed to the multifactorial etiology of most of these health events:  
405 infectious agents may co-exist with metabolic and managerial deficiencies. Moreover, this lack  
406 of genetic correlation may support the idea that these traits are controlled genetically by different

407 genes and individual selection should be applied. Contrary to expectations, this study did not find  
408 a significant genetic correlation between SCHCa and MF. However, considering the disease  
409 definitions, MF cases were defined as standing (showing mild ataxia, excitability, muscle  
410 tremors and reduced ruminal motility) or recumbent cows; therefore, MF definition was solely  
411 based on symptoms and not in any serum Ca measurement. Furthermore, this absence of genetic  
412 correlation could also be attributed to the low incidence of MF. In this study, recumbent cows  
413 were immediately treated with intravenous Ca solutions, rendering the measurement of serum Ca  
414 concentrations meaningless. Moreover, it is known that there is no specific threshold of Ca  
415 serum concentrations which always results in recumbent cows. Regarding the absence of any  
416 genetic correlation of the remaining macrominerals with other health disorders, this may also be  
417 attributed to the multifactorial etiology and to the low incidence of some of the health disorders  
418 (e.g. metritis, mastitis and ketosis). On the other hand, the lack of any significant genetic  
419 correlation in this study may be incidental. Therefore, as this is the first study of its kind, the  
420 genetic analysis of other independent data sets may shed more light on this issue; more research  
421 is needed in order to clarify these issues.

422

423 The reported phenotypic association of clinical and subclinical hypocalcemia with various  
424 diseases after calving is based almost solely on pathophysiology, because of calcium's central  
425 metabolic role; it is generally assumed that P and Mg serum concentrations are associated with  
426 the same postpartum diseases through their relation with Ca metabolism (Rude, 1998; Goff,  
427 2000; DeGaris and Lean, 2008). In a study of 2,190 cows from 33 herds, Curtis et al. (1983)  
428 showed that cows with clinical hypocalcemia (MF) were at greater risk of developing dystocia  
429 (6.5 times), RFM (3.2 times), KET (8.9 times) and MAST (8.1 times). Martinez et al. (2012)

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

442

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

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

455

456

## CONCLUSIONS

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

465

466

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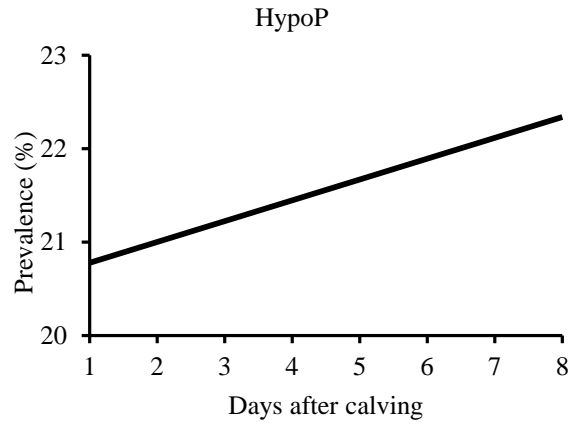
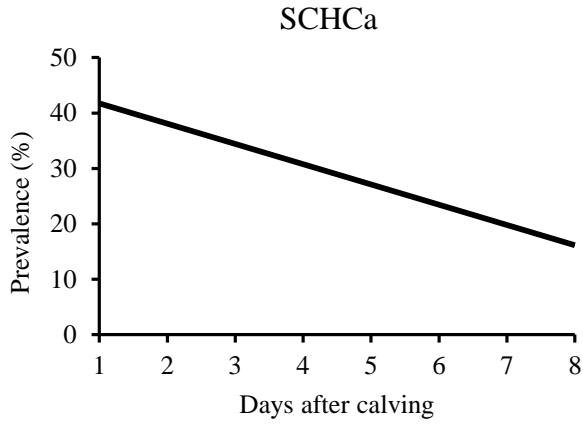
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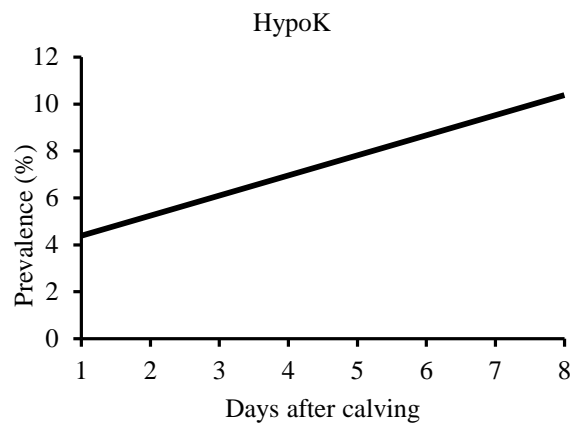
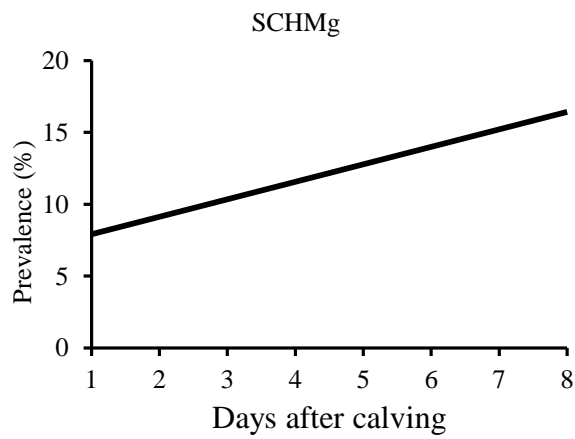
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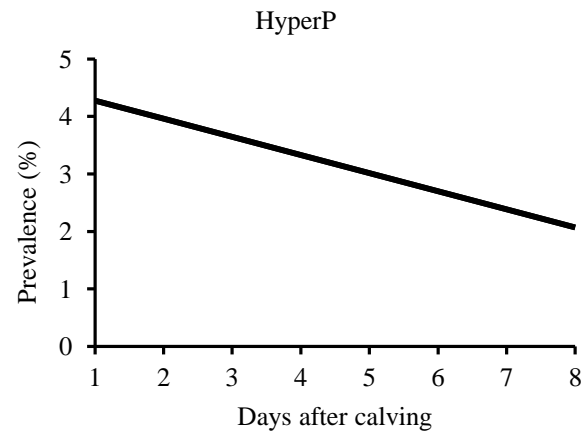
GENETIC PARAMETERS OF HEALTH DISORDERS



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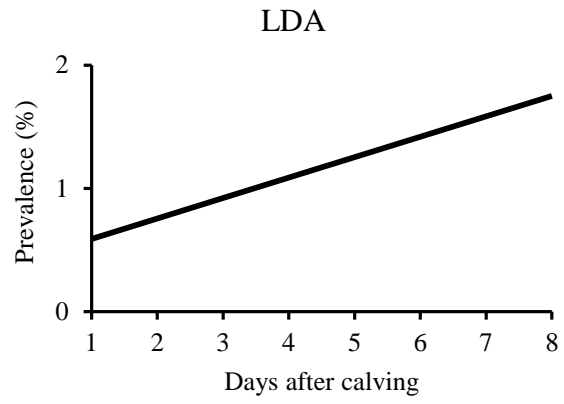
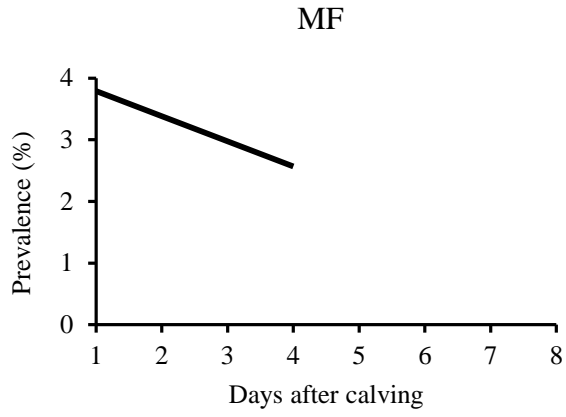
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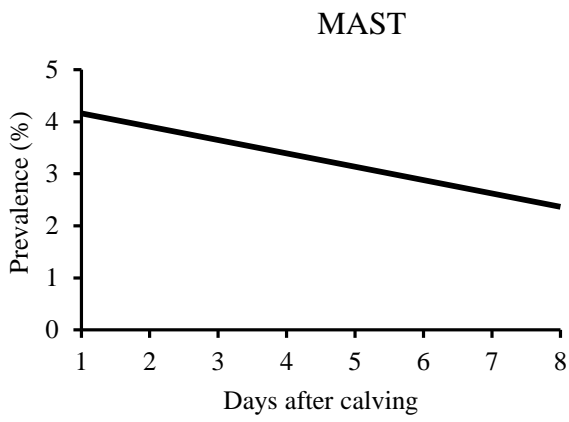
Tsiamadis Figure 1.



GENETIC PARAMETRS OF HEALTH DISORDERS



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679 **Tsiamadis Figure 2.**