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1 **Genetic Parameters of Calcium, Phosphorus, Magnesium and Potassium Serum**  
2 **Concentrations during the First Eight Days after Calving in Holstein Cows**

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14 ***Interpretive Summary***

15 Macromineral-related disorders immediately after calving are of great importance for the health  
16 and productivity of dairy cows. They predispose animals to other major diseases, increase culling  
17 rate and impair production. Our objective was to estimate the genetic parameters of  
18 macrominerals' concentrations during the first 8 days after calving in Holstein cows. Repeated  
19 measurements of blood serum macrominerals concentrations from 986 cows, in 9 commercial  
20 farms located in Northern Greece were analyzed with random regression models. Results  
21 revealed the presence of significant genetic variation. Achieving and maintaining normal

22 macromineral concentrations through genetic selection could contribute towards reduction of the  
23 related disorders.

24

## 25 **ABSTRACT**

26 Calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K) are of great importance for  
27 the health and productivity of dairy cows after calving. So far genetic studies have focused on  
28 clinical hypocalcemia, leaving the genetic parameters of these macroelements unstudied. Our  
29 objective was to estimate the genetic parameters of Ca, Mg, P and K serum concentrations and  
30 their changes during the first 8 days after calving. The study was conducted in 9 herds located in  
31 Northern Greece, with 1,021 Holstein cows enrolled from November 2010 until November 2012.  
32 No herd used any kind of preventive measures for hypocalcemia. Pedigree information for all  
33 cows was available. A total of 35 cows were diagnosed and treated for periparturient paresis and,  
34 therefore, excluded from the study. The remaining 986 cows were included in genetic analysis.  
35 The distribution of cows across parities was 459 (parity 1), 234 (parity 2), 158 (parity 3) and 135  
36 (parity 4 and above). A sample of blood was taken from each cow on day 1, 2, 4 and 8 after  
37 calving and serum concentrations of Ca, P, Mg and K were measured in each sample. A final  
38 data set of 15,390 biochemical records was created consisting of 3,903 Ca, 3,902 P, 3,903 Mg  
39 and 3,682 K measurements. Moreover, changes of these concentrations between day 1 and 4 as  
40 well as day 1 and 8 after calving were calculated and treated as different traits. Random  
41 regression models were used to analyze the data. Results showed that daily heritabilities of Ca, P  
42 and Mg concentrations traits were moderate to high (0.20 – 0.43;  $P < 0.05$ ), while those of K were  
43 low to moderate (0.12 – 0.23;  $P < 0.05$ ). Regarding concentration changes, only Mg change  
44 between day 1 and day 8 after calving had a significant heritability of 0.18. Genetic correlations

45 between Ca, P, Mg and K concentrations and their concentration changes from days 1-4 and 1-8  
46 after calving were not significantly different from zero. Most phenotypic correlations among Ca,  
47 P, Mg, and K concentrations were positive and low (0.09 – 0.16;  $P < 0.05$ ), while the correlation  
48 between P and Mg was negative and low (-0.16;  $P < 0.05$ ). Phenotypic correlations among  
49 macromineral concentrations on day 1 and their changes from day 1 to 4 and 1 to 8 after calving  
50 varied for each macromineral. This study revealed that genetic selection for normal Ca, P, Mg  
51 and K concentrations in the first week of lactation is possible and could facilitate the  
52 management of their deficiencies during the early stages of lactation.

53 **Key words:** macrominerals, genetic parameters

54

55

## INTRODUCTION

56 During the first critical days after calving, calcium (Ca), phosphorus (P), magnesium (Mg) and  
57 potassium (K) blood serum concentrations are of great importance for the health and productivity  
58 of the dairy cow. Possible deviations from normal levels of these macrominerals are interrelated  
59 (Goff and Horst, 1997; Goff, 2000; Lean et al., 2013).

60

61 Calcium plays a key role at the onset of lactation (DeGaris and Lean, 2008). Hypocalcaemia  
62 (serum  $\text{Ca} < 8.3 \text{ mg/dL}$ ) is the most important macromineral disorder of the transition dairy cow  
63 (Oetzel, 2011; Goff, 2014; Martinez et al., 2014) and is associated with health disorders  
64 including retained fetal membranes, mastitis, uterine infection, displaced abomasum and ketosis  
65 (Correa et al., 1990; Gröhn and Bruss, 1990; DeGaris and Lean, 2008), as well as reduced dry  
66 matter intake and milk production (Rajala-Schultz et al., 1999).

67

68 Phosphorus and Mg play important roles in the etiology of hypocalcemia, as well.  
69 Hypophosphatemia (serum P<4.0 mg/dL) is involved in the manifestation of the alert downer  
70 cow syndrome, while elevated phosphorus concentrations increase the risk of milk fever (Lean et  
71 al., 2013; Grünberg, 2014). Hypomagnesaemia (serum Mg<1.8 mg/dL) reduces parathormone  
72 (PTH) secretion, tissue sensitivity to PTH and synthesis of 1,25-dihydroxycholecalciferol  
73 (Littledike et al., 1983; Rude, 1998). Moreover, mild hypomagnesaemia (serum Mg between 1.3  
74 and 1.8 mg/dL) is common in anorectic fresh cows and in most cases is accompanied by mild  
75 hypophosphatemia (serum P between 2 and 4 mg/dL) and mild hypokalemia (serum K between  
76 2.6 and 3.9 mmol/L) (Peek and Divers, 2008).

77

78 Potassium homeostasis in transition dairy cows is affected by numerous factors. Off-feed fresh  
79 cows, increased milk production and concurrent diseases predispose to hypokalemia (serum K  
80 <3.9 mmol/L) (Pradhan and Hemken, 1968; Sattler et al., 1998; Sattler and Fecteau, 2014).

81

82 Blood Ca concentration is considered to reach its minimum 12 to 24 hours after calving and then  
83 it increases gradually (Goff, 2014). Relative estimates for the other three macrominerals are  
84 lacking from the literature.

85

86 Serum Ca, P, Mg and K concentrations are influenced by environmental factors, mainly nutrition  
87 (NRC, 2001; Kronqvist, 2011). Nutritional and management strategies for the prevention of  
88 these macromineral deficiencies have been developed (Bethard et al., 1998; Tauriainen et al.,

89 2003; Rérat et al., 2009). However, there is also a genetic component to these traits, as reported  
90 for serum Ca concentration by Tveit et al. (1991).

91  
92 Genetic studies so far have focused on heritability estimates of clinical hypocalcemia (milk  
93 fever) (Dyrendahl et al., 1972; Lin et al., 1989; Abdel-Azim et al., 2005) and genetic and  
94 phenotypic correlations between milk fever and various disease (Lin et al., 1989) and production  
95 traits (Lyons et al., 1991; Uribe et al., 1995; Heringstad et al., 2005). Tveit et al. (1991) reported  
96 heritability estimates for post-partum serum Ca concentrations in first lactation Norwegian cows.  
97 However, genetic studies of serum Ca, P, Mg and K concentrations in fresh Holstein dairy cows  
98 are lacking.

99  
100 Therefore, the objective of this study was to estimate the genetic parameters of Ca, Mg, P and K  
101 serum concentrations and their changes in Holstein cows during the first 8 days after calving.

102

## 103 **MATERIALS AND METHODS**

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

108

### 109 ***Animals and Management***

110 A total of 1,021 Holstein cows from 9 commercial free-stall dairy herds in Northern Greece were  
111 included in the study. The distribution across parities was 466, 242, 165 and 148 cows for

112 parities 1, 2, 3 and 4 and above, respectively. Farms were visited regularly between November  
113 2010 and November 2012 for data collection. No herd used any kind of preventive measures for  
114 hypocalcemia. Total mixed rations (TMR) were formulated to meet or exceed net energy and  
115 metabolizable protein requirements according to National Research Council recommendations  
116 (NRC, 2001).

117

### 118 *Clinical Examination, Blood Sampling and Analyses*

119 Each animal was clinically examined and blood sampled on day 1, 2, 4 and 8 after calving, by  
120 the first author. Blood samples, in all herds, were collected between 08:00 – 10:00 a.m., after the  
121 morning milking. Moreover, to standardize sampling and handling procedures, all samplings  
122 were performed in absence of unusual stressors and in proper containment systems that minimize  
123 stress and pain of the animal.

124

125 Blood sampling was performed by coccygeal venipuncture into 10-ml vacuum glass tubes  
126 without anticoagulant (BD Vacutainer<sup>®</sup>, Plymouth, United Kingdom) for serum macromineral  
127 measurements. Samples were placed in a cooler, transported to the Diagnostic Laboratory of the  
128 Faculty of Veterinary Medicine and centrifuged immediately upon arrival (3,000 x g for 15 min).  
129 Serum was transferred into polyethylene tubes and stored at -80°C until assay. All sera were  
130 analyzed for total Ca and Mg concentrations using flame atomic absorption spectrophotometry  
131 (Perkin ElmerAAAnalyst 100, Perkin Elmer Co, Norwalk, CT, USA), according to manufacturer's  
132 instructions. Serum inorganic phosphorus concentrations were determined photometrically using  
133 a Flexor E autoanalyzer (Vital Scientific, Netherlands), according to the procedure described by  
134 Daly and Ertingshausen (1972), with the use of standard commercial reagents (Thermo Fisher  
135 Scientific Inc. USA). Potassium serum concentrations were measured using an ion-selective

136 electrode according to manufacturer's instructions (Electrolyte Analyzer 9180, Roche Austria).  
137 The intra- and inter-assay coefficients of variation for all the above analyses were less than 3%.

138

### 139 *Data set*

140 Considering that pedigree information was available for all cows, the total population increased  
141 to 4,262 animals, spanning the last 5 generations. Calving date, parity number, calving ease and  
142 twinning was recorded.

143

144 A total of 35 cows were diagnosed with periparturient paresis, treated appropriately with  
145 intravenous Ca and excluded from the study. Therefore, the remaining 986 cows were finally  
146 included in the genetic analysis. The distribution across parities was 459, 234, 158 and 135 cows  
147 for parities 1, 2, 3 and 4 and above, respectively.

148

149 Following all analyses, a data set of 15,390 biochemical records was created (Table 1),  
150 consisting of 3,903 Ca, 3,902 P, 3,903 Mg and 3,682 K serum concentration measurements.  
151 Moreover, changes of these concentrations between day 1 and day 4 as well as day 1 and day 8  
152 were calculated and treated as different traits.

153

### 154 *Statistical Analysis*

155 Repeated cow records of Ca, Mg, P and K serum concentrations were analyzed with a random  
156 regression model which accounted for the covariance between successive records of the same  
157 animal; each trait was analyzed separately:



$$Y_{ijkm} = HYS_i + L_j + M_k + a_1 \cdot age + \sum_{n=0}^2 b_m P_m D_m + \sum_{n=0}^2 A_{km} P_m D_m + e_{ijkm}$$

158 (1)

159 where:

160  $Y_{ijkm}$  is the macromineral concentration of cow  $k$  on day from calving  $m$ ;

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

162  $L_j$  the fixed effect of number of lactation (4 levels);

163  $M_k$  the fixed effect of calendar month when the record was taken  $p$  (12 levels);

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

165  $D_m$  the number of days from calving;

166  $b_m$  the fixed regression coefficient on days from calving;

167  $A_{km}$  the random regression coefficient on day from calving associated with the additive  
168 genetic effect of cow  $k$  including all pedigree data (4,262 animals);

169  $P_m$  the  $m$ th orthogonal polynomial of day from calving ( $m$  the order of polynomial);

170  $e_{ijkm}$  the random residual term.

171

172 The fixed effects in the model were fitted after preliminary analyses had confirmed their  
173 statistically significant effect ( $P < 0.05$ ) on the traits. The final order of the random polynomial  
174 (third for either trait) was determined with the use of the log-likelihood test in sequential  
175 analyses of gradually increasing orders. The final order choice was also confirmed with the  
176 Akaike Information Criterion test. Four measurement error classes were defined using the time  
177 relative to calving as day 1, 2, 4 and 8. The definition of these classes, even at this small time

178 span, aimed to capture the day-to-day differences in health events at the beginning of lactation.  
179 Covariances between the error classes were assumed to be zero.

180

181 Estimates of variance components from model 1 were used to calculate heritabilities for each  
182 trait and day after calving.

183

184 Variance components and heritability estimated for Ca, K, P and Mg serum concentrations were  
185 also calculated across all days from calving using the following model:

186

$$187 \quad Y_{ijkm} = HYS_i + L_j + a_1 \cdot age + D_m + A_k + e_{ijkm} \quad (2)$$

188

189 where:  $Y_{ijkm}$  is the macromineral concentration change of cow k;  $A_k$  is the additive genetic effect  
190 of cow k and all effects are as in model 1.

191

192 Serum concentration changes between day 1 and day 4 (days 1-4), as well as day 1 and day 8  
193 (days 1-8) after calving were analyzed with the following model:

194

$$195 \quad Y_{ijk} = HYS_i + L_j + age + A_k + e_{ijk} \quad (3)$$

196

197 where:  $Y_{ijk}$  is the macromineral concentration change of cow k; All other effects are as in  
198 Model 2.

199

200 Genetic and phenotypic correlations among all traits analyzed with the above models were  
201 estimated with a series of bivariate analyses.

202

203 All analyses were conducted using the statistical software package ASREML (Gilmour and  
204 Gogel, 2006).

205

206

## RESULTS

### *Mean Macromineral Serum Concentrations and Prediction Lines for Concentrations*

208 Mean serum Ca concentration increased gradually from day 1 to day 8 after calving (P<0.001).  
209 In 1<sup>st</sup> and 2<sup>nd</sup> lactation cows, mean Ca concentration remained above the 8.3 mg/dL threshold  
210 throughout the sampling period, whereas in older cows it was below the threshold on days 1 and  
211 2 after calving. On the contrary, mean serum P, Mg and K concentrations decreased from day 1  
212 to day 8 after calving (P<0.001). Descriptive statistics and analysis of variance results by parity  
213 are presented in Table 1. Fixed curves of serum macromineral concentrations, across all  
214 lactations, during the first 8 days after calving from the random regression model analysis  
215 (Model 1) are shown in **Figure 1**. These curves are adjusted for all other effects included in  
216 Model 1.

217

### *Serum Macromineral Concentrations Variances and Heritabilities Estimates*

219 Estimates of day-to-day phenotypic, genetic and residual variances, and heritabilities for serum  
220 Ca, P, Mg and K concentrations are presented in Table 2. All estimates were statistically greater  
221 than zero (P<0.001). During the first 8 days after calving the estimated phenotypic ( $\sigma_p^2$ ) and  
222 residual variances ( $\sigma_r^2$ ) for Ca and P serum concentrations were high, while those of Mg and K

223 were low. During the same period, the estimated genetic variance ( $\sigma_a^2$ ) for Ca and P serum  
224 concentration was moderate and high, respectively, while for Mg and K was low. Day-to-day  
225 heritabilities of serum Ca, P and Mg concentrations were moderate ( $h^2 = 0.20 - 0.43$ ), while  
226 heritability estimates of K serum concentrations were low ( $h^2 = 0.12 - 0.15$ ) except on day 8 after  
227 calving ( $h^2 = 0.23$ ) (Figure 2).

228  
229 Heritability estimates of serum Ca, P, Mg, and K concentrations across all days using Model 2  
230 are in Table 3. Although smaller, they were comparable with the ones derived with the random  
231 regression model analysis. Regarding concentration changes, only Mg change between day 1 and  
232 day 8 after calving had a significant ( $P < 0.05$ ) heritability of 0.18.

233

#### 234 *Serum Macromineral Concentrations Correlations*

235 Significant genetic correlations between serum Ca, P, Mg and K concentrations and their  
236 concentration changes from days 1-4 and 1-8 after calving were not detected in the present study.

237

238 Statistically significant ( $P < 0.010 - 0.001$ ) phenotypic correlations among Ca, P, Mg, and K  
239 serum concentrations are shown in Table 3. Most correlations were positive and low ( $r_p = 0.09 -$   
240  $0.16$ ), while the P – Mg correlation was negative and low ( $r_p = -0.16 \pm 0.03$ ).

241

242 Significant phenotypic correlations among serum macromineral concentrations on day 1 and  
243 their changes from day 1 to 4 and 1 to 8 after calving are shown in Table 4. On day 1, there was  
244 a low positive correlation between Ca and P, Ca and K, as well as P and K; there was also a low  
245 negative correlation between P and Mg. Calcium and Mg serum concentrations on day 1 had

246 moderate negative correlations with both their changes from day 1 to 4 and 1 to 8. Phosphorus  
247 serum concentration on day 1 had moderate negative correlation with its change from day 1 to 8,  
248 while K serum concentration at day 1 had a moderate positive correlation with its change from  
249 day 1 to 8. Phosphorus serum concentration on day 1 had a low positive correlation with both  
250 Mg changes (days 1 – 4 and 1 – 8) and a low negative one with both K changes (days 1 – 4 and 1  
251 – 8). Phosphorus change from day 1 to 4 had a low negative correlation with both Mg changes.  
252 Both P changes (days 1 – 4 and 1 – 8) had a low positive correlation with both K changes (days 1  
253 – 4 and 1 – 8). For each macromineral, its serum concentration changes between day 1 to 4 and 1  
254 to 8 were positively and moderately correlated.

255

256

## DISCUSSION

257 The present study was designed to estimate the genetic parameters of serum Ca, P, Mg and K  
258 concentrations immediately after calving.

259

260 Normally, serum Ca concentration is maintained within a narrow range, between 8.3 and 10.4  
261 mg/dL (Goff, 2014). During the first 12 to 24 hours after calving, Ca concentration reaches the  
262 lower value and then gradually increases (Goff, 2014). In the present study, an increase across all  
263 lactations in serum Ca concentrations from day 1 to day 8 after calving was observed. Mean Ca  
264 serum concentrations from days 1 to 8 were different, depending on parity number and days after  
265 calving. Response of cows to the decreased serum Ca concentration was not similar across  
266 lactations. The homeorhetic mechanisms that determine the Ca balance (parathormone,  
267 cholecalciferol and calcitonin) restored Ca serum concentration in most 1<sup>st</sup> and 2<sup>nd</sup> parity cows.  
268 However, in older cows (3<sup>rd</sup> and 4<sup>th</sup>+ parities) the same homeorhetic mechanisms that affect the

269 Ca concentration did not react as efficiently, putting these animals in a profound hypocalcaemic  
270 status just after calving (day 1).

271  
272 The prediction curve generated with the random regression model denotes that there was a  
273 significant rise in Ca concentration from day 1 to day 8 across all lactations. This is in agreement  
274 with results from studies dealing with Ca physiology after calving (Littledike and Goff, 1987;  
275 Goff, 2000; DeGaris and Lean, 2008). Furthermore, mean serum P, Mg and K concentrations  
276 were within reference ranges (P: 4.2 – 7.7 mg/dL, Mg: 1.8 – 2.4 mg/dL, K: 3.9 – 5.8 mmol/L;  
277 Peek and Divers, 2008; Goff, 2008) during the 1<sup>st</sup> day after calving and then gradually decreased,  
278 but always remaining within those ranges. The prediction curves denote that there was a  
279 significant decline in P, Mg and K concentrations from day 1 to day 8 across all lactations.  
280 Serum Ca and P concentrations are regulated by the same hormones. The main regulatory  
281 hormone is PTH, which increases Ca and decreases P concentration, within normal ranges. The  
282 increase in PTH mobilization due to decreased Ca levels can explain the concurrent fall in P  
283 concentration observed in the present study. Regarding Mg and K, since there is no major  
284 hormonal control for these macrominerals (Kaneko et al., 2008), the observed decrease in their  
285 concentrations is difficult to explain but may be attributed to the demands of the increasing milk  
286 production.

287  
288 Large scale field studies on Ca, P, Mg and K serum concentrations during the first week after  
289 calving are lacking in literature. Recently, Reinhardt et al. (2011) conducted a field study for  
290 hypocalcaemia in 1,462 cows, with only one Ca measurement within 48 h postpartum. To our  
291 knowledge this is the first time that repeated measurements of Ca, P, Mg, and K concentrations

292 during the first 8 days after calving are reported. The observed variation allowed the  
293 development of Ca, P, Mg and K serum concentration prediction lines with the use of random  
294 regression model.

295

296 The estimated day-to-day heritabilities for serum Ca concentration were moderate (0.23 – 0.32).  
297 So far, genetic studies have focused on the estimation of clinical hypocalcemia (milk fever)  
298 heritability. Some studies reported moderate to high estimates (0.30 – 0.47) (Lin et al., 1989;  
299 Lyons et al., 1991, Abdel-Azim et al., 2005), while others (Dyrendahl et al., 1972; Pryce et al.,  
300 1997; Van Dorp et al., 1998; Heringstad et al., 2005) reported low ones (0.04 – 0.13), depending  
301 on lactation number, method of statistical analysis and method of data collection, with higher  
302 estimates being observed in later lactations. Heritability estimates for serum Ca concentration in  
303 Holsteins after calving are lacking. Only one study investigated the genetic variation of Ca  
304 concentration in Norwegian Reds cows and reported a low heritability (0.11±0.09) that was not  
305 statistically different from zero (Tveit et al., 1991).

306

307 Similarly, the estimated day-to-day heritabilities for serum P and Mg concentrations in the  
308 present study were moderate to high (0.30 – 0.43 and 0.20 – 0.39, respectively), while those for  
309 K were low to moderate (0.12 – 0.23). To our knowledge this is the first time that such estimates  
310 are reported. So far, only Kadarmideen et al. (2000) reported heritability estimates (0.004±0.004)  
311 for clinical hypomagnesaemia in dairy cattle, which was not statistically different than zero.  
312 Moreover, the information for hypomagnesaemia cases in that study was based on subjective  
313 clinical observations made by farmers and was not confirmed by serum Mg concentration  
314 measurements.

315

316 Genetic variance estimates of Ca and P were high (0.28 to 0.44 and 0.40 to 0.70, respectively),  
317 indicating high influence of additive genetic effects on these traits. Their serum concentrations  
318 are regulated mainly by PTH, 1,25-dihydroxyvitamin D and calcitonin (Kaneko et al., 2008). The  
319 existence of the above major hormonal mechanism that regulates Ca and P concentrations can  
320 help explain the moderate to high heritability estimates of these two elements. It was an early  
321 belief that milk fever resulted from the failure of parathyroid glands to respond to the reduced Ca  
322 concentration soon after calving. However, it has been shown that such cows have very high  
323 blood PTH concentrations. Therefore, this finding implies that PTH's target tissues cannot  
324 respond to its action (Goff, 2014). The main target of PTH is the skeleton. In humans the  
325 RANK/RANKL/OPG system is well known for its osteoclastic function. This axis has a genetic  
326 control and is hormonally stimulated by PTH and calcitonin, both of which control serum Ca and  
327 P concentrations (Asagiri and Takayanagi, 2007; Cappariello et al., 2014). Further investigation  
328 is needed in order to clarify **whether** this axis is also functional to dairy cows and **whether** is  
329 involved in the etiology of hypocalcemia at the genetic level.

330

331 Genetic variance estimates for Mg and K were low (0.03 to 0.07 and 0.03 to 0.05, respectively).  
332 In humans, PTH contributes towards a small increase of Mg concentration (Swaminathan, 2000).  
333 Moreover, aldosterone is the only known hormone that partly regulates K concentration. The  
334 absence of any major hormonal mechanism that regulates the serum concentration of Mg and K  
335 may help explain the low genetic variances. The high precision of the diagnostic methods for Mg  
336 and K measurements strongly contributed to our heritability estimates.

337



338 Our results indicate that genetic improvement is possible for these traits, probably to the same  
339 degree with traits such as milk yield ( $h^2 = 0.20 - 0.50$ ; Castillo-Juarez et al., 2000; Windig et al.,  
340 2006; Bastin et al., 2011) or BCS ( $h^2 = 0.34 - 0.79$ ; Berry et al., 2003; Banos et al., 2005;  
341 Oikonomou et al., 2008), which are already included in breeding programs worldwide. Both the  
342 amount of genetic variance and size of heritability for macromineral concentrations suggest that  
343 selection could be effective during the first critical days after calving. Especially for Ca, whose  
344 role in health status and disease development is of great importance (Goff and Horst, 1997), this  
345 genetic improvement could favor animal welfare and productivity. In the meantime, appropriate  
346 management and nutritional strategies during the close up part of the transition period are vital in  
347 order to establish normal macromineral concentrations at parturition.

348

349 In the present study, no genetic correlations among serum Ca, P, Mg and K concentrations and  
350 their changes from days 1-4 and 1-8 after calving were detected. If there are no genetic  
351 correlations, this probably denotes that there are no competitive mechanisms at genetic level that  
352 regulate the concentrations of macrominerals. Further research is needed in order to clarify this  
353 issue.

354

355 Although small, significant positive phenotypic correlations were found between Ca and P and  
356 Ca and K. These correlations are not easy to explain; e.g. one might expect that the action of  
357 PTH would result in a negative correlation between Ca and P. However, at the onset of lactation  
358 large amounts of macrominerals are excreted in the milk which are maintained almost constant,  
359 regardless of serum concentrations in the dam, so that adequate mineral supply can be offered to  
360 the newborn calf (Grünberg, 2014). This could explain the observed positive phenotypic

361 correlations. Moreover, the role of calcitonin in decreasing Ca and P blood concentration is well  
362 established (Allen and Sansom, 1985; Goff, 2000). Calcitonin actually counteracts PTH and,  
363 thus, it protects skeleton against major Ca losses during periods of intense Ca mobilization, such  
364 as pregnancy and, especially, lactation. It is likely that this might also explain the observed  
365 phenotypic correlation.

366  
367 An interesting finding was the negative phenotypic correlations of P with Mg. In humans, the  
368 presence of Mg ions in the binding regions of adenylate cyclase and phospholipase C –two  
369 intracellular molecules that are activated after the binding of PTH to its cell receptors– is  
370 essential for the full activation of these two secondary messengers and the manifestation of PTH  
371 action on target tissues (Rude, 1998; Potts and Gardella, 2007). Therefore, hypomagnesaemia  
372 reduces the secretion of PTH and decreases the sensitivity of tissues to PTH (Littledike et al.,  
373 1983; Goff, 2014). Consequently, this PTH reduction could contribute towards increasing serum  
374 P concentration. Moreover, in humans, PTH action in distal tubules reduces Mg renal excretion  
375 and contributes towards increased serum Mg levels, while at the same time decreases P  
376 concentration (Rude, 1998; Swaminathan, 2000). It remains uncertain whether these mechanisms  
377 apply to dairy cows, as well.

378  
379 Other interesting findings included the high negative correlations of Ca, P, Mg and K  
380 concentrations on day 1 with the respective changes between day 1 and 4 and day 1 and 8. This  
381 indicates that the higher the serum concentration on day 1 the smaller is the expected change  
382 during the following days (always within normal range). This seems to be particularly interesting  
383 especially for Ca. These observations imply that Ca homeostasis was effective, at a population

384 level and support the need for proper nutritional and management strategies during the transition  
385 period. Correlations between Ca serum concentration on day 1 and P serum changes corroborate  
386 the previous assumptions. Correlations between P serum concentration on day 1 and Ca serum  
387 changes follows the same pattern: high concentrations of P in plasma, at levels greater than 6.0  
388 mg/dL, inhibit the action of renal 1 $\alpha$ -hydroxylase 25-(OH)-D<sub>3</sub>, decreasing Ca reabsorption and  
389 thus limiting serum Ca concentration increase (Goff, 2014).

390

391 Phenotypic correlations between Mg serum concentration on day 1 and Ca changes from day 1 to  
392 8 and P changes from day 1 to 4 and 1 to 8, as well as K serum concentrations at day 1 and P  
393 changes from day 1 to 4 and 1 to 8 are difficult to interpret, as they usually remain within normal  
394 ranges. Cluster analysis may be the appropriate statistical method to analyze these phenomena.

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## CONCLUSIONS

397 In the present study, significant genetic variation was found in serum macromineral  
398 concentrations immediately after calving. During the first 8 days post-partum, day-to-day  
399 heritabilities of serum Ca, P and Mg concentrations traits were moderate to high, while those of  
400 K were low to moderate. Genetic evaluation of dairy cows for these traits seems possible and this  
401 would contribute to the selection of animals that are less prone to macromineral-related  
402 deficiencies during the early stages of lactation that can compromise health and productivity. As  
403 these results are the first of their kind, independent validation on different cattle populations  
404 would be desirable. Further studies should also focus on the identification of specific genomic  
405 regions affecting these traits.

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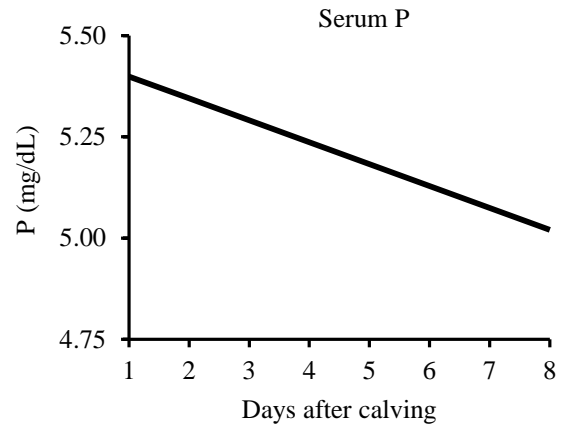
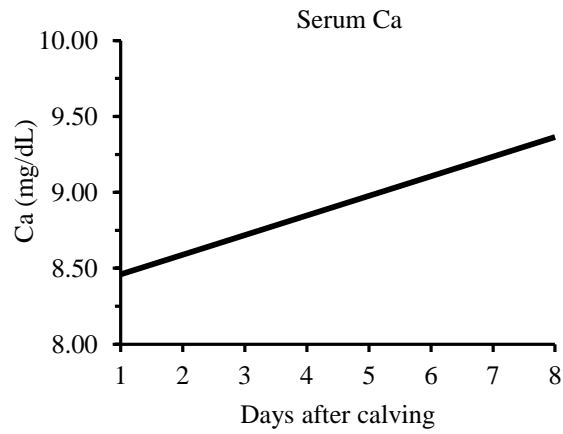
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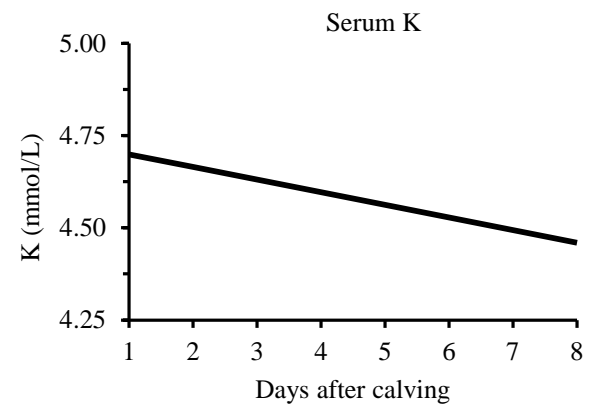
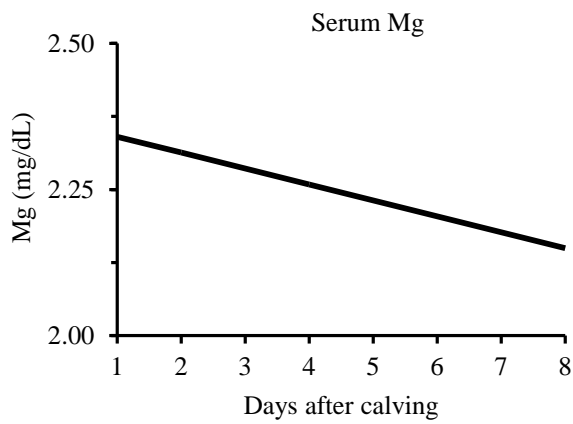
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GENETIC PARAMETERS OF Ca, P, Mg AND K



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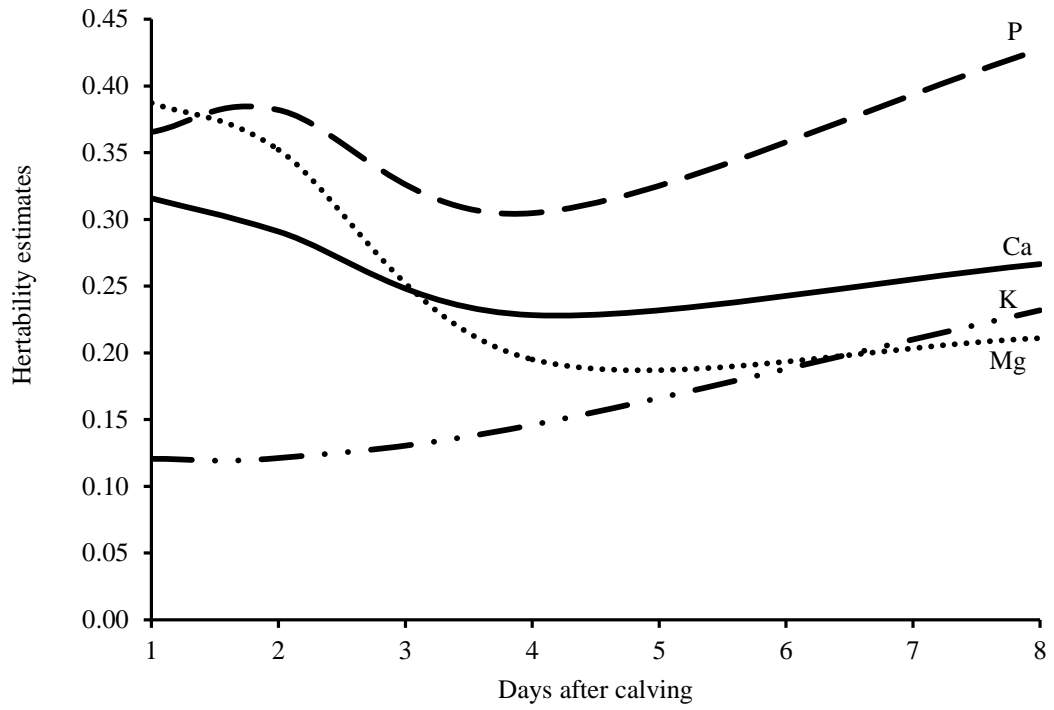


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535 **Tsiamadis Figure 1.**

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GENETIC PARAMETERS OF Ca, P, Mg AND K



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538 **Tsiamadis Figure 2.**