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

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Interpretive Summary

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

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

ABSTRACT

Calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K) are of great importance for the health and productivity of dairy cows after calving. So far genetic studies have focused on clinical hypocalcemia, leaving the genetic parameters of these macroelements unstudied. Our objective was to estimate the genetic parameters of Ca, Mg, P and K serum concentrations and their changes during the first 8 days after calving. The study was conducted in 9 herds located in Northern Greece, with 1,021 Holstein cows enrolled from November 2010 until November 2012. No herd used any kind of preventive measures for hypocalcemia. Pedigree information for all cows was available. A total of 35 cows were diagnosed and treated for periparturient paresis and, therefore, excluded from the study. The remaining 986 cows were included in genetic analysis. The distribution of cows across parities was 459 (parity 1), 234 (parity 2), 158 (parity 3) and 135 (parity 4 and above). A sample of blood was taken from each cow on day 1, 2, 4 and 8 after calving and serum concentrations of Ca, P, Mg and K were measured in each sample. A final data set of 15,390 biochemical records was created consisting of 3,903 Ca, 3,902 P, 3,903 Mg and 3,682 K measurements. Moreover, changes of these concentrations between day 1 and 4 as well as day 1 and 8 after calving were calculated and treated as different traits. Random regression models were used to analyze the data. Results showed that daily heritabilities of Ca, P and Mg concentrations traits were moderate to high (0.20 – 0.43; $P<0.05$), while those of K were low to moderate (0.12 – 0.23; $P<0.05$). Regarding concentration changes, only Mg change between day 1 and day 8 after calving had a significant heritability of 0.18. Genetic correlations between Ca, P, Mg and K concentrations and their concentration changes from days 1-4 and 1-8 after calving were not significantly different from zero. Most phenotypic correlations among Ca, P, Mg, and K concentrations were positive and low (0.09 – 0.16; $P<0.05$), while the correlation between P and Mg was negative and low (-0.16; $P<0.05$). Phenotypic correlations among macromineral concentrations on day 1 and their changes from day 1 to 4 and 1 to 8 after calving varied for each macromineral. This study revealed that genetic selection for normal Ca, P, Mg and K concentrations in the first week of lactation is possible and could facilitate the management of their deficiencies during the early stages of lactation.

Key words: macrominerals, genetic parameters

INTRODUCTION

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

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

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

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

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

Serum Ca, P, Mg and K concentrations are influenced by environmental factors, mainly nutrition (NRC, 2001; Kronqvist, 2011). Nutritional and management strategies for the prevention of these macromineral deficiencies have been developed (Bethard et al., 1998; Tauriainen et al., 2003; Rérat et al., 2009). However, there is also a genetic component to these traits, as reported for serum Ca concentration by Tveit et al. (1991).

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

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

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 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

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

Blood sampling was performed by coccygeal venipuncture into 10-ml vacuum glass tubes without anticoagulant (BD Vacutainer®, Plymouth, United Kingdom) for serum macromineral measurements. Samples were placed in a cooler, transported to the Diagnostic Laboratory of the Faculty of Veterinary Medicine and centrifuged immediately upon arrival (3,000 x g for 15 min). Serum was transferred into polyethylene tubes and stored at -80°C until assay. All sera were analyzed for total Ca and Mg concentrations using flame atomic absorption spectrophotometry (Perkin ElmerAAnalyst 100, Perkin Elmer Co, Norwalk, CT, USA), according to manufacturer's instructions. Serum inorganic phosphorus concentrations were determined photometrically using a Flexor E autoanalyzer (Vital Scientific, Netherlands), according to the procedure described by Daly and Ertingshausen (1972), with the use of standard commercial reagents (Thermo Fisher Scientific Inc. USA). Potassium serum concentrations were measured using an ion-selective electrode according to manufacturer's instructions (Electrolyte Analyzer 9180, Roche Austria). The intra- and inter-assay coefficients of variation for all the above analyses were less than 3%.

Data set

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

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

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

Statistical Analysis

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

$$Y_{ijkm} = HYS_i + L_j + M_k + a_1 \cdot age + \sum_{n=0}^{2} b_m P_m D_m + \sum_{n=0}^{2} A_{km} P_m D_m + e_{ijkm} \quad (1)$$

where:

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

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

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

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

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

D_m the number of days from calving;

b_m the fixed regression coefficient on days from calving;

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

P_m the *mth* orthogonal polynomial of day from calving (m the order of polynomial);

e_{ijkm} the random residual term.

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

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

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

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

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

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 with the following model:

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

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

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

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

RESULTS

Mean Macromineral Serum Concentrations and Prediction Lines for Concentrations

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

Serum Macromineral Concentrations Variances and Heritabilities Estimates

Estimates of day-to-day phenotypic, genetic and residual variances, and heritabilities for serum Ca, P, Mg and K concentrations are presented in Table 2. All estimates were statistically greater than zero (P<0.001). During the first 8 days after calving the estimated phenotypic (σ_p^2) and residual variances (σ_r^2) for Ca and P serum concentrations were high, while those of Mg and K were low. During the same period, the estimated genetic variance (σ_{α}^{2}) for Ca and P serum concentration was moderate and high, respectively, while for Mg and K was low. Day-to-day heritabilities of serum Ca, P and Mg concentrations were moderate (h^2 =0.20 – 0.43), while heritability estimates of K serum concentrations were low (h^2 =0.12 – 0.15) except on day 8 after calving (h^2 = 0.23) (Figure 2).

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

Serum Macromineral Concentrations Correlations

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

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

Significant phenotypic correlations among serum macromineral concentrations on day 1 and their changes from day 1 to 4 and 1 to 8 after calving are shown in Table 4. On day 1, there was a low positive correlation between Ca and P, Ca and K, as well as P and K; there was also a low negative correlation between P and Mg. Calcium and Mg serum concentrations on day 1 had moderate negative correlations with both their changes from day 1 to 4 and 1 to 8. Phosphorus serum concentration on day 1 had moderate negative correlation with its change from day 1 to 8, while K serum concentration at day 1 had a moderate positive correlation with its change from day 1 to 8. Phosphorus serum concentration on day 1 had a low positive correlation with both Mg changes (days 1 – 4 and 1 – 8) and a low negative one with both K changes (days 1 – 4 and 1 – 8). Phosphorus change from day 1 to 4 had a low negative correlation with both Mg changes. Both P changes (days 1 – 4 and 1 – 8) had a low positive correlation with both K changes (days 1 – 4 and 1 – 8). For each macromineral, its serum concentration changes between day 1 to 4 and 1 to 8 were positively and moderately correlated.

DISCUSSION

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

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

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

The prediction curve generated with the random regression model denotes that there was a significant rise in Ca concentration from day 1 to day 8 across all lactations. This is in agreement with results from studies dealing with Ca physiology after calving (Littledike and Goff, 1987; Goff, 2000; DeGaris and Lean, 2008). Furthermore, mean serum P, Mg and K concentrations 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; Peek and Divers, 2008; Goff, 2008) during the 1st day after calving and then gradually decreased, but always remaining within those ranges. The prediction curves denote that there was a significant decline in P, Mg and K concentrations from day 1 to day 8 across all lactations. Serum Ca and P concentrations are regulated by the same hormones. The main regulatory hormone is PTH, which increases Ca and decreases P concentration, within normal ranges. The increase in PTH mobilization due to decreased Ca levels can explain the concurrent fall in P concentration observed in the present study. Regarding Mg and K, since there is no major hormonal control for these macrominerals (Kaneko et al., 2008), the observed decrease in their concentrations is difficult to explain but may be attributed to the demands of the increasing milk production.

Large scale field studies on Ca, P, Mg and K serum concentrations during the first week after calving are lacking in literature. Recently, Reinhardt et al. (2011) conducted a field study for hypocalcaemia in 1,462 cows, with only one Ca measurement within 48 h postpartum. To our knowledge this is the first time that repeated measurements of Ca, P, Mg, and K concentrations during the first 8 days after calving are reported. The observed variation allowed the development of Ca, P, Mg and K serum concentration prediction lines with the use of random regression model.

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

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

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

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

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

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

Although small, significant positive phenotypic correlations were found between Ca and P and Ca and K. These correlations are not easy to explain; e.g. one might expect that the action of PTH would result in a negative correlation between Ca and P. However, at the onset of lactation large amounts of macrominerals are excreted in the milk which are maintained almost constant, regardless of serum concentrations in the dam, so that adequate mineral supply can be offered to the newborn calf (Grünberg, 2014). This could explain the observed positive phenotypic correlations. Moreover, the role of calcitonin in decreasing Ca and P blood concentration is well established (Allen and Sansom, 1985; Goff, 2000). Calcitonin actually counteracts PTH and, thus, it protects skeleton against major Ca losses during periods of intense Ca mobilization, such as pregnancy and, especially, lactation. It is likely that this might also explain the observed phenotypic correlation.

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

Other interesting findings included the high negative correlations of Ca, P, Mg and K concentrations on day 1 with the respective changes between day 1 and 4 and day 1 and 8. This indicates that the higher the serum concentration on day 1 the smaller is the expected change during the following days (always within normal rage). This seems to be particularly interesting especially for Ca. These observations imply that Ca homeostasis was effective, at a population level and support the need for proper nutritional and management strategies during the transition period. Correlations between Ca serum concentration on day 1 and P serum changes corroborate the previous assumptions. Correlations between P serum concentration on day 1 and Ca serum changes follows the same pattern: high concentrations of P in plasma, at levels greater than 6.0 mg/dL, inhibit the action of renal 1a-hydroxylase 25-(OH)-D_3, decreasing Ca reabsorption and thus limiting serum Ca concentration increase (Goff, 2014).

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

CONCLUSIONS

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

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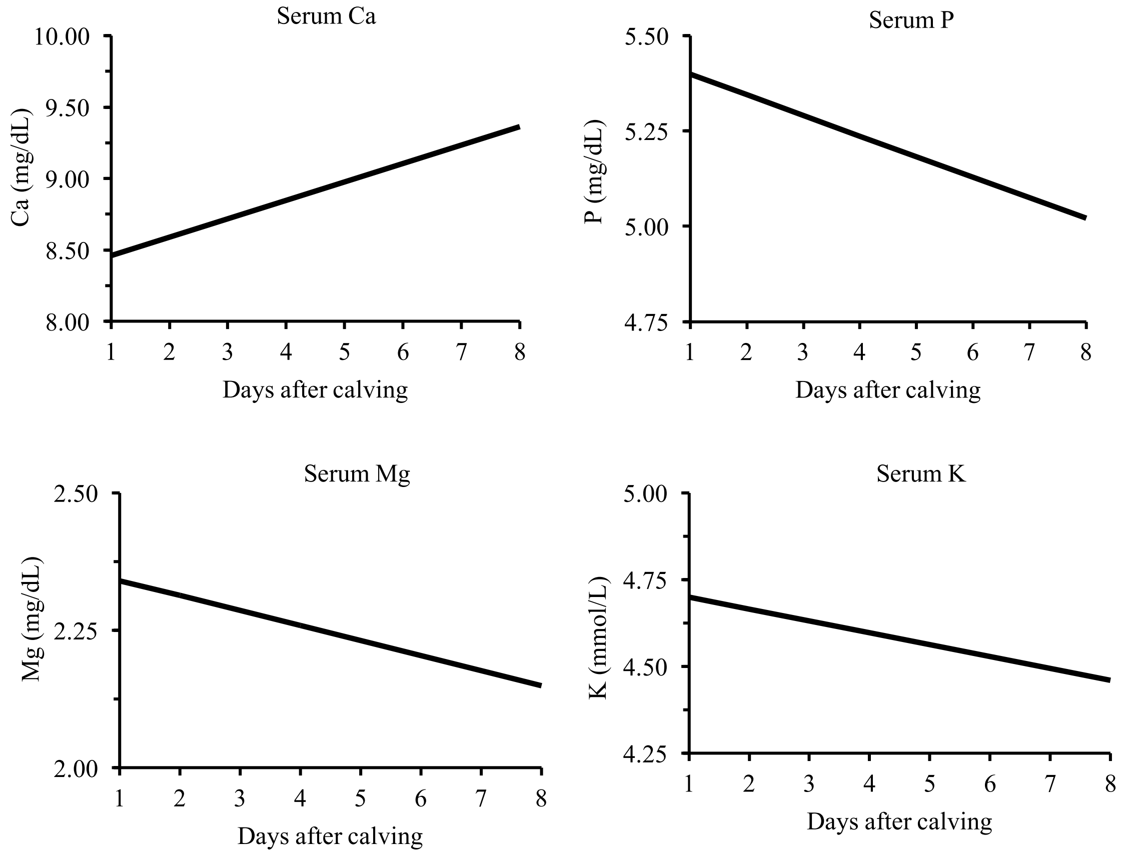
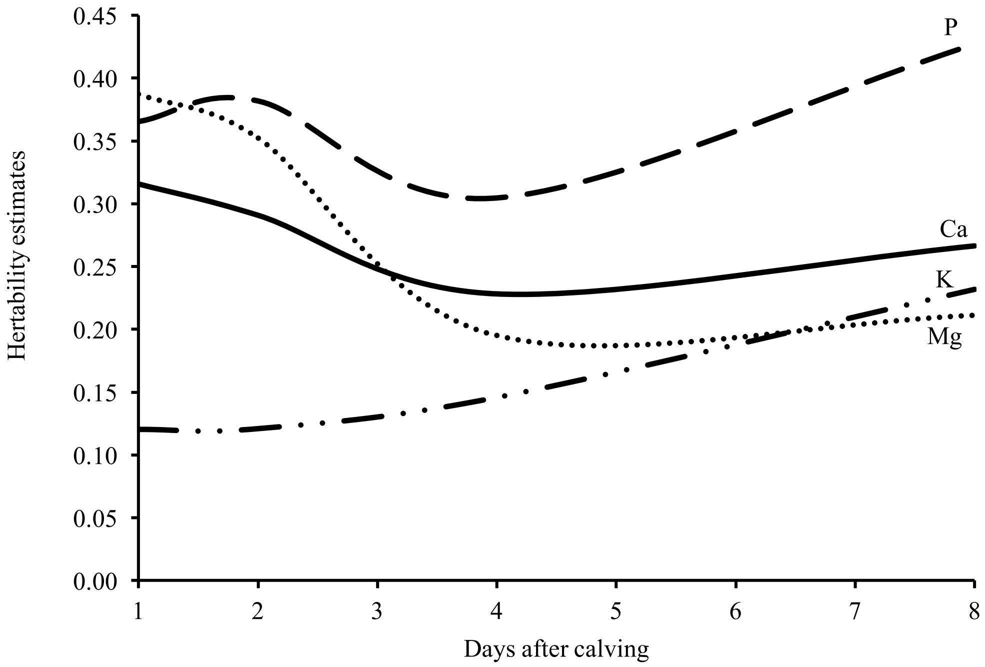


Tsiamadis Figure 1.



Tsiamadis Figure 2.

Figure captures

Figure 1. Fixed curves for serum Calcium (Ca), Phosphorus (P), Magnesium (Mg) and Potassium (K) concentrations across all lactations during the first 8 days after calving from random regression model analyses.

Figure 2. Heritability estimates of serum Calcium (Ca), Phosphorus (P), Magnesium (Mg) and Potassium (K) concentrations during the first 8 days after calving.

Table 1. Least square means for Calcium (Ca), Phosphorus (P), Magnesium (Mg) and Potassium (K) serum concentrations and analysis of variance by parity number and days after calving

		1st Day after calving				2nd Day after calving				4th Day after calving				8th Day after calving				
	Parity number	**Mean**	**SEM**	**St. dev**	**Min-Max**	**Mean**	**SEM**	**St. dev**	**Min-Max**	**Mean**	**SEM**	**St. dev**	**Min-Max**	**Mean**	**SEM**	**St. dev**	**Min-Max**	**P**
Ca (mg/dL)	1	9.14^{a}	0.05	1.02	3.90-12.90	8.84^{b}	0.04	0.95	4.45-11.65	9.06^{a}	0.05	1.06	4.10-13.90	9.45^{c}	0.05	1.09	5.75-12.85	**
	2	8.49^{a}	0.07	1.10	5.55-11.15	8.54^{a}	0.08	1.19	4.50-12.60	9.10^{b}	0.07	1.08	5.75-12.30	9.42^{c}	0.07	1.14	6.00-13.25	**
	3	8.30^{a}	0.10	1.29	5.25-11.85	8.20^{a}	0.10	1.31	4.05-11.85	8.98^{b}	0.09	1.13	5.15-11.30	9.21^{b}	0.10	1.18	4.45-11.50	***
	4	7.94^{a}	0.11	1.26	3.95-10.90	8.42^{b}	0.10	1.14	5.65-12.00	9.00^{c}	0.10	1.20	5.60-12.20	9.29^{c}	0.11	1.26	6.05-12.95	**
P (mg/dL)	1	5.54^{a}	0.06	1.18	2.20-9.30	5.26^{b}	0.06	1.19	2.10-10.20	5.14^{b}	0.05	1.10	2.70-10.00	4.98^{c}	0.05	1.05	2.10-8.70	*
	2	5.32^{a}	0.09	1.35	2.60-9.30	4.98^{b}	0.09	1.36	2.00-9.50	5.08^{ab}	0.08	1.25	2.00-9.40	5.13^{ab}	0.08	1.15	2.60-8.60	**
	3	5.18^{a}	0.11	1.44	2.30-9.80	5.14^{a}	0.11	1.35	2.20-10.30	5.18^{ab}	0.11	1.35	2.40-10.10	4.76^{ac}	0.09	1.10	2.20-7.70	**
	4	5.29^{a}	0.13	1.53	1.40-9.60	5.46^{ab}	0.12	1.41	3.00-10.20	5.65^{b}	0.11	1.30	3.00-10.50	5.34^{ab}	0.12	1.34	2.50-9.30	*
Mg (mg/dL)	1	2.37^{a}	0.02	0.35	1.50-3.90	2.35^{a}	0.02	0.35	0.95-3.55	2.21^{b}	0.02	0.46	0.38-7.20	2.18^{b}	0.01	0.31	1.10-3.35	***
	2	2.31^{a}	0.03	0.46	1.30-7.10	2.22^{b}	0.03	0.40	1.20-3.45	2.09^{c}	0.02	0.37	1.20-3.35	2.19^{b}	0.03	0.39	0.36-3.65	*
	3	2.41^{a}	0.03	0.44	1.30-4.15	2.31^{b}	0.03	0.42	1.40-3.90	2.07^{c}	0.03	0.36	1.40-3.55	2.13^{c}	0.03	0.35	1.10-3.20	***
	4	2.38^{a}	0.03	0.41	1.55-4.00	2.27^{a}	0.03	0.38	1.30-3.60	2.10^{b}	0.03	0.35	1.40-3.35	2.14^{b}	0.03	0.38	0.40-3.20	*
K (mmol/L)	1	4.7^{a}	0.03	0.59	2.8-6.3	4.6^{b}	0.01	0.55	2.5-6.3	4.5^{c}	0.01	0.47	2.9-6.3	4.4^{c}	0.01	0.47	2.8-6.3	*
	2	4.8^{a}	0.01	0.57	3.6-6.3	4.6^{b}	0.01	0.53	3.2-6.2	4.5^{cb}	0.01	0.50	3.5-6.2	4.5^{c}	0.01	0.54	3.0-6.2	**
	3	4.7^{a}	0.01	0.53	3.4-6.3	4.7^{a}	0.01	0.55	3.4-6.2	4.6^{a}	0.01	0.55	3.3-6.3	4.4^{b}	0.01	0.53	2.9-6.3	**
	4	4.7^{a}	0.10	0.63	3.5-6.3	4.7^{a}	0.10	0.61	3.3-6.3	4.6^{a}	0.01	0.54	3.5-6.3	4.6^{a}	0.10	0.65	3.2-6.3	NS

$^{a-c}$ Means in the same row having different superscripts differ significantly.

NS: Non-significant.

* P<0.05, ** P<0.001, *** P< 0.0001.

Table 2. Variances and heritability estimates of Calcium (Ca), Phosphorus (P), Magnesium (Mg) and Potassium (K) serum concentrations by days after calving from random regression model analyses. All estimates were statistically greater than zero at P<0.001 level

Trait	Day after calving	σ_p^2	σ_a^2	σ_r^2	h^2
Ca	1st	1.40 (0.06)	0.44 (0.05)	0.96 (0.06)	0.32 (0.03)
	2nd	1.26 (0.05)	0.37 (0.04)	0.89 (0.05)	0.29 (0.03)
	4th	1.22 (0.05)	0.28 (0.03)	0.94 (0.05)	0.23 (0.02)
	8th	1.30 (0.06)	0.35 (0.08)	0.95 (0.08)	0.27 (0.06)
P	1st	1.91 (0.08)	0.70 (0.07)	1.21 (0.07)	0.37 (0.03)
	2nd	1.48 (0.06)	0.57 (0.05)	0.92 (0.05)	0.38 (0.03)
	4th	1.31 (0.06)	0.40 (0.03)	0.91 (0.05)	0.30 (0.02)
	8th	1.05 (0.05)	0.45 (0.08)	0.60 (0.07)	0.43 (0.07)
Mg	1st	0.17 (0.01)	0.07 (0.01)	0.11 (0.01)	0.39 (0.03)
	2nd	0.16 (0.01)	0.06 (0.01)	0.10 (0.01)	0.35 (0.03)
	4th	0.19 (0.01)	0.04 (0.00)	0.15 (0.01)	0.20 (0.02)
	8th	0.12 (0.01)	0.03 (0.01)	0.10 (0.01)	0.21 (0.06)
K	1st	0.34 (0.02)	0.04 (0.01)	0.30 (0.02)	0.12 (0.03)
	2nd	0.29 (0.01)	0.04 (0.01)	0.26 (0.01)	0.12 (0.02)
	4th	0.21 (0.01)	0.03 (0.01)	0.18 (0.01)	0.15 (0.02)
	8th	0.22 (0.01)	0.05 (0.01)	0.17 (0.01)	0.23 (0.06)

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

Table 3. Heritability estimates of Calcium (Ca), Phosphorus (P), Magnesium (Mg) and Potassium (K) serum concentrations across days (diagonals) and statistically greater than zero phenotypic correlations (above diagonal); standard error in parentheses

Trait	Ca	P	Mg	K
Ca	0.20 (0.02)**	0.16 (0.03)**	NS	0.09 (0.03)*
P		0.25 (0.02)**	**-0.16 (0.03)**[a]**	0.20 (0.03)**
Mg			0.21 (0.02)**	NS
K				0.10 (0.02)**

[a] Bold letters indicate undesirable correlations.

NS: Non-significant.

* P<0.01, ** P<0.0001.

Table 4. Phenotypic correlations of Calcium, Phosphorus, Magnesium and Potassium serum concentrations on day 1 and corresponding change in days 1-4 and 1-8 after calving; standard error in parentheses

	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**
Ca_1	-0.56 (0.02)	-0.45 (0.03)	0.12 (0.03)	-0.12 (0.03)	NS	NS	NS	NS	0.08 (0.03)	NS	NS
Ca Change_1-4		0.53 (0.02)	-0.16 (0.03)	0.15 (0.03)	NS	NS	-0.06 (0.03)	NS	NS	0.09 (0.03)	NS
Ca Change_1-8			NS	NS	-0.11 (0.03)	-0.08 (0.03)	NS	NS	NS	0.07 (0.03)	NS
P_1				NS	-0.52 (0.02)	-0.14 (0.03)	0.18 (0.03)	0.11 (0.03)	0.19 (0.03)	-0.12 (0.03)	-0.08 (0.03)
P Change_1-4					0.66 (0.02)	0.09 (0.03)	-0.07 (0.03)	NS	-0.20 (0.03)	0.14 (0.03)	0.10 (0.03)
P Change_1-8						0.09 (0.03)	NS	NS	-0.16 (0.03)	0.09 (0.03)	0.09 (0.03)
Mg_1							-0.57 (0.02)	-0.55 (0.02)	NS	NS	NS
Mg Change_1-4								0.52 (0.02)	NS	NS	NS
K_1										NS	-0.57 (0.02)
K Change_1-4											0.54 (0.02)

NS: Non-significant.

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.