Genetic background of micellar and soluble calcium and phosphorus predicted from bovine milk mid-infrared spectra

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ABSTRACT:

Background

Mid-infrared spectroscopy (MIRS) is a valuable tool to determine milk composition and quality, and to collect data at population level. In milk, Ca and P are partitioned between micellar (MP) and soluble phase (SP), both with important effects on milk coagulation properties; in particular, greater mineral content in MP translates into better milk coagulation ability. Nevertheless, the high analytic costs of gold standard quantification methods hamper the possibility to deepen partition of minerals in MP and SP on a large scale. In this study, MP and SP of Ca and P were predicted from mid-infrared spectra of 111,653 individual milk samples from 9,519 Italian Holstein cows. Sources of non-genetic variation for MP and SP of Ca and P were investigated, and their genetic associations with milk yield, quality and coagulation properties were estimated.

Results

The MP of Ca and P decreased with parity but increased along the lactation, resembling the trend of protein content. Both MP and SP of Ca and P showed exploitable genetic variation and were heritable, and they were associated with traits of interest for the dairy industry, in particular milk yield and protein content. Furthermore, negative correlations between the two phases of the same mineral were estimated. The MP was negatively related to milk yield.

Conclusions

The MP and SP of the same mineral are negatively correlated, meaning that it is possible to improve mineral partition toward MP, in order to get milk with better technological properties. The current selection index of Italian Holstein breed gives positive emphasis on milk protein (content and yield) and thus it is indirectly improving the MP of Ca and P while reducing their SP content. Future research will focus on the genomic architecture of such traits to evaluate the role of potential specific genes in the determination of these mineral fractions in cow milk.

Keywords: milk mineral, coagulation, casein micelle, dairy industry, curd, genetic correlation
BACKGROUND

Milk and dairy products represent an important source of minerals in human nutrition. In particular, dairy products provide 59 and 27% of recommended daily intakes of Ca and P, respectively [1]. Both Ca and P are important for physiological processes such as bone growth, muscular and nervous functions, and cellular homeostasis. On the other hand, the amount of certain minerals in milk largely affects technological properties such as enzymatic coagulation and syneresis. In fact, Ca and P are present in casein micelles as amorphous nanoclusters of calcium phosphate, stabilizing interactions among casein molecules [2]. Moreover, soluble Ca acts as molecular bridge between micelles during the formation of paracasein reticulum [3]. If permitted, Ca salts are added before rennet coagulation during cheesemaking to improve milk technological traits; in fact, Ca salts accelerate the coagulation process and increase curd strength [4,5]. Nevertheless, the content of calcium phosphate nanoclusters in casein micelles is difficult to be tune and this hampers the possibility to study milk properties at different levels of colloidal minerals. For this reason, most studies have searched for differences in micellar content of Ca and P between naturally occurring 'good' and 'poor' coagulating samples [6].

Mid-infrared spectroscopy (MIRS) is a cost-effective non-destructive technique for the analysis of milk composition. Several studies have investigated the use of MIRS to analyse minor milk compounds and milk coagulation properties (MCP) in addition to the gross composition [7]. Some authors have demonstrated the feasibility of MIRS to predict minerals from milk spectra [8–10] but recently MIRS was shown to be inadequate to distinguish between micellar phase (MP) and soluble phase (SP) of minerals in bulk milk [11]. On the other hand, a recent study has demonstrated that MIRS, coupled with mathematical treatment of data and backward interval partial least squares analysis as prediction algorithm, may provide accurate prediction of SP and MP of Ca and P in individual bovine milk samples [12].

The total content of Ca and P in cow milk is affected by breed, month of sampling, lactation stage, parity and udder health [13]. However, to the best of our knowledge, no studies have characterized genetic and non-genetic sources of variation of SP and MP of Ca and P in cow milk. Therefore, in the present study sources of variation of Ca and P phases, and the ratio between MP and SP (MP/SP) predicted from test-day milk spectra of 9,519 Italian Holstein (HO) cows were
investigated. Moreover, heritability of and genetic correlations between these predicted new traits, and between them and other traits of interest for the dairy industry were estimated.

**METHODS**

**Data collection**

Milk samples of 23,450 HO cows were collected from January 2011 to February 2019 during monthly routine controls performed by the South Tyrolean Dairy Association (Bolzano, Italy). A total of 473,816 test-day records with information on milk yield (MY, kg/d), parity, days in milk (DIM), birth date, and calving date (Breeders Association of Bolzano Province, Bolzano, Italy) were originally available. According to the International Committee for Animal Recording (ICAR) guidelines for milk sampling and analysis, immediately after collection 50 mL of individual milk were added with 200 µl of preservative (Bronysolv; ANA.LI.TIK Austria, Vienna, Austria) and refrigerated at 4°C. All milk samples were then moved to the laboratory of the South Tyrolean Dairy Association (Bolzano, Italy) for MIRS analysis. Fat percentage (FP) and protein percentage (PP) were determined using a MilkoScan FT6000 until February 2017 and, from March of the same year, using a MilkoScan FT7 (FOSS Electric A/S, Hillerød, Denmark). To ensure the comparability of spectra between the two instruments, standard samples were routinely used for calibration, according to the manufacturer instructions [14,15]. Moreover, a principal component analysis on spectra was performed and no significant differences between the two instruments were observed. Somatic cell count (cells/µL) was determined using Fossomatic (FOSS Electric A/S, Hillerød, Denmark) and it was transformed to somatic cell score (SCS) to reach a normal distribution of data:

\[ SCS = 3 + \log_2 \left( \frac{\text{somatic cell count}}{100} \right) \]

**Calibration models**

Spectral data from 5,000 to 900 cm\(^{-1}\) were stored for all samples to allow *a posteriori* application of the available MIRS models to predict coagulation properties, total mineral composition [10], and mineral phases [12]. Briefly, prediction models for rennet coagulation time (RCT, min), curd-firming
time ($k_{20}$, min), and curd firmness 30 min after rennet addition to milk ($a_{30}$, mm) were developed using uninformative variable elimination procedure combined with partial least squares regression analysis on 923 individual milk samples of HO (n = 237), Brown Swiss (n = 223), Alpine Grey (n = 223), and Simmental cows (n = 240) collected in 2014 in the same province of the present study. The reference analysis for the coagulation traits was the lactodynamography and the 3 prediction models were validated through external validation with 80% of the samples as calibration set and 20% of the samples as validation set [10]. Coefficients of determination (root mean square error) in external validation were 0.54 (2.90 min), 0.56 (1.22 min), and 0.52 (9.00 mm) for RCT, $k_{20}$, and $a_{30}$, respectively. Moreover, prediction models for the total content (mg/kg) of Ca and P in milk were developed using a representative subset (251 samples) with the 4 breeds present in the same proportion and using inductively coupled plasma optical emission spectrometry as the reference analysis [10]. Coefficients of determination for total Ca and total P in external validation were 0.67 and 0.68, and the root mean square errors were 122.00 mg/kg and 88.12 mg/kg, respectively.

Finally, previously developed prediction models for MP, SP, and MP/SP of Ca and P were used [12]. Briefly, 93 HO samples were analysed for the content of Ca and P in both SP and MP [16] and calibration models were developed using backward interval partial least squares algorithm [12], ending up with coefficients of determination and root mean square error in cross-validation of:

i. 0.77 and 2.69 mg/100 mL for SP of Ca;

ii. 0.76 and 5.30 mg/100 mL for MP of Ca;

iii. 0.69 and 0.36 for the ratio MP/SP of Ca;

iv. 0.73 and 2.56 mg/100 mL for SP of P;

v. 0.73 and 4.76 mg/100 mL for MP of P;

vi. 0.68 and 0.22 for the ratio MP/SP of P.

Data editing

Spectral outliers were identified by means of the Mahalanobis distance between samples infrared data points (spectrum) and the centroid of spectra of the calibration dataset. Samples with a distance greater than 3 were excluded from the dataset. Moreover, observations with predicted values outside the range of the reference data used for calibrations were set to missing.
Values of MY, FP, PP, casein percentage (CP), and SCS deviating more than 3 standard deviations from the corresponding mean were set to missing. Parity ranged from 1 to 15 and DIM from 5 to 305 d; cows of parity ≥ 5 were grouped in the same class. Cows that changed herd during the investigated period and with unknown parents were removed from the data. Finally, lactations with less than 5 test-day records were discarded from the dataset, as well as contemporary groups (herd-test-date, HTD) with less than 5 cows sampled. The final dataset consisted of 111,653 test-day records from 9,519 HO cows in 338 herds. A summary (mean and variation) of the traits included in the study is reported in Table 1.

Statistical analysis
Sources of variation for SP, MP, and MP/SP of Ca and P were investigated using the HPMIXED procedure of SAS software v. 9.4 (SAS Institute Inc., Cary, NC), according to the following linear model:

\[ Y_{ijklmno} = \mu + M_i + Y_j + S_k + P_l + (S \times P)_{kl} + H_m + C_n + e_{ijklmno}, \]

where \( Y_{ijklmno} \) is the phenotypic record (SP, MP, or MP/SP of Ca or P); \( \mu \) is the overall intercept of the model; \( M_i \) is the fixed effect of the \( i \)th month of sampling (\( i = 1 \) to 12); \( Y_j \) is the fixed effect of the \( j \)th year of sampling (\( j = 2011 \) to 2019); \( S_k \) is the fixed effect of the \( k \)th DIM class of the cow (\( k = 1 \) to 30; 10 d classes); \( P_l \) is the fixed effect of the \( l \)th parity of the cow (\( l = 1 \) to 5); \( (S \times P)_{kl} \) is the fixed interaction effect between DIM class and parity; \( H_m \) is the random effect of the \( m \)th herd ~\( N(0, \sigma^2_H) \), where \( \sigma^2_H \) is the herd variance; \( C_n \) is the random effect of the \( n \)th cow ~\( N(0, \sigma^2_C) \), where \( \sigma^2_C \) is the cow variance; and \( e_{ijklmno} \) is the random residual ~\( N(0, \sigma^2_e) \), where \( \sigma^2_e \) is the error variance.

Six generations of ancestors were traced back, leading to 31,645 individuals in the pedigree. Variance and covariance components were estimated in ASReml software v. 4.1 [17] using single-trait and bivariate repeatability animal models, respectively. The general form of the model looked as:

\[ y = Xb + Z_ww + Z_awa + e, \]

where \( y \) is the vector of phenotypic records of the trait(s); \( b \) is the vector of fixed effects of contemporary group (10,504 HTD), parity (5 classes), and stage of lactation (30 DIM classes of 10 d each); \( w \) is the vector of solutions for random permanent environmental effect of the cow; \( a \) is the
vector of solutions for random additive genetic effect of the animal; and e is the vector of random residuals. The incidence matrices X, Z_w, and Z_a linked the corresponding effects to the dependent variable y. Random effects were assumed to be normally distributed with means zero and variance-covariance structures of additive genetic, permanent environmental, and residual effects in the bivariate analyses that were \( G \otimes A \), \( W \otimes I \), and \( R \otimes I \), respectively, where \( G \) is the 2 x 2 additive genetic (co)variance matrix, \( W \) is the 2 x 2 (co)variance matrix of permanent environmental effects, \( R \) is the residual (co)variance matrix, \( A \) is the additive genetic relationship matrix among individuals, \( I \) is an identity matrix of appropriate order, and \( \otimes \) is the Kronecker product. The phenotypic variance (\( \sigma^2_p \)), heritability (\( h^2 \)), repeatability (\( t \)), genetic correlation (\( r_a \)), and phenotypic correlation (\( r_p \)) were computed as [18]:

\[
\sigma^2_p = \sigma^2_w + \sigma^2_a + \sigma^2_e, \quad h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_w + \sigma^2_e}, \quad t = \frac{\sigma^2_a + \sigma^2_w}{\sigma^2_a + \sigma^2_w + \sigma^2_e}, \quad r_p = \frac{\sigma_{p12}}{\sqrt{\sigma_{p1}^2 \sigma_{p2}^2}}, \quad r_a = \frac{\sigma_{a12}}{\sqrt{\sigma_{a1}^2 \sigma_{a2}^2}},
\]

where \( \sigma^2_w \) is the permanent environmental variance; \( \sigma^2_a \) is the additive genetic variance; \( \sigma_{p12} \) and \( \sigma_{a12} \) are the phenotypic and additive genetic covariances between trait 1 and trait 2, \( \sigma^2_{p1} \) and \( \sigma^2_{p2} \) are the phenotypic variances of traits 1 and 2; and \( \sigma^2_{a1} \) and \( \sigma^2_{a2} \) are the additive genetic variances of traits 1 and 2.

RESULTS

Sources of variation of micellar and soluble calcium and phosphorous

Based on visual inspection of data, Ca and P in MP and SP followed a normal distribution. All the fixed effects included in the model were significant (\( p < 0.001 \)) in explaining the variation of Ca and P in MP and SP (Table 2). The MP, SP, and MP/SP of Ca were mostly influenced by the lactation stage similarly to MP and MP/SP of P. Instead, the fixed effect of parity was the most important to explain the variation of the SP of P (Table 2). Phosphorus and Ca showed a similar pattern across months of sampling (Figure 1). In particular, both the MP/SP of Ca and P exhibited the lowest values in late spring (May) and summer. The greatest MP/SP was observed in first-parity cows for Ca and in second-parity cows for P, with a decrease in subsequent lactations in both cases (Figure 2). The MP of Ca and P decreased by 3.4% and 6.4% from parity 1 to parity \( \geq 5 \), respectively,
whereas the SP of Ca had an opposite trend to that of SP of P. In fact, Ca in SP tended to increase with parity, while P in SP decreased with parity and was the greatest in first lactation (Figure 2).

The pattern of Ca and P in MP across DIM resembled the typical lactation curve of FP and PP, i.e., it showed a minimum in early lactation and progressively increased while approaching the end of lactation. At the same time, Ca and P in SP decreased from early to late lactation. As a result, the MP/SP of both minerals decreased during the first 30 DIM and increased thereafter.

**Genetic parameters**

Overall, the phases and the MP/SP of both Ca and P were moderately heritable (Table 3), with $h^2$ and $t$ that averaged 0.52 and 0.63, respectively. Moreover, $h^2$ of mineral phases mirrored that of PP ($0.469 \pm 0.014$) and was greater than that of FP ($0.356 \pm 0.011$). The trait with the lowest $h^2$ was the MP/SP of P ($0.472 \pm 0.013$).

The strongest and weakest $r_p$ were estimated between the two MP ($0.896 \pm 0.002$) and between the two SP ($-0.132 \pm 0.008$), respectively. As regards $r_a$, the MP and MP/SP of Ca were positively strongly correlated with MP and MP/SP of P, and the SP of both Ca and P were negatively genetically correlated with the other two fractions (Table 3).

The $r_p$ and $r_a$ of minerals fractions with MY, PP, FP, SCS, and MCP are reported in Table 4. The MP was negatively genetically associated with MY, with moderate magnitude for both Ca and P; on the other hand, the genetic correlation between MY and SP was $0.277 \pm 0.036$ in the case of Ca and close to zero in the case of P. As a result, the MP/SP ratios were negatively associated with MY (Table 4). The opposite was observed for correlations with PP; in fact, the MP of both minerals were positively strongly associated with PP. In general, correlations of Ca and P phases with FP were weaker than those with PP. On the other hand, correlations of Ca and P fractions with SCS were weak or close to zero. Except for SP of P, all fractions of Ca and P showed favourable $r_p$ and $r_a$ with RCT. The $r_p$ and $r_a$ with $k_{20}$ were favourable with MP and MP/SP of both minerals; in fact, despite weak, $r_p$ and $r_a$ between SP and $k_{20}$ were unfavourable in both minerals (Table 4). The same was observed for $a_{30}$, with $r_p$ and $r_a$ that were favourable with MP and MP/SP, and unfavourable with SP.
DISCUSSION

Data overview

The average MY and composition mirrored the official mean performance of Italian HO and was in accordance with the literature [9–11,13]. Considering MP/SP of Ca an P, previous research is not concordant about the average value in bovine milk, because both MP and SP are strongly affected by the method used for their quantification [19]. In the present study, the Ca MP/SP (3.44 w/w) was higher than the ratios (2.20 to 2.68 w/w) reported in the literature using filtration techniques to separate the micellar from the soluble phase [6,19,20]. Even if this is the most popular technique, it has been demonstrated to overestimate the soluble fraction, due to the solubilisation of colloidal minerals during the separation [19]. On the other hand, Ca MP/SP of 4.06 w/w based on rennet coagulation method was obtained from reconstructed milk [19]. For the calibration of this study, a modified version of rennet coagulation procedure was adopted; thus, results intermediate to those available in the literature were somehow expected [16].

On average, the MP/SP of P (1.55 w/w) was similar to the value of 1.45 w/w obtained after different trials on 12 milk samples [19]. However, it was higher than values (0.80 to 1.26 w/w) reported in other studies [6,19,20]; this was not surprising considering that only inorganic P was quantified in such studies and thus the contribution of phosphorylated protein was not taken into account.

The drop of MP/SP observed between May and June was due to a change in the partition of salts between the two phases (Figure 1), while the increasing trend of SP with the cow parity order was likely due to a reduction of casein and thus to a shift of the equilibrium to the SP. Both MP/SP and MP increased across DIM, being maximum at the end of lactation (Figure 3), whereas an opposite trend was observed for SP, likely due to a concentration effect of PP and CP.

Heritability, repeatability, and correlations

Calcium and P are involved in casein micelle structure and therefore a strong relationship between PP and these minerals was expected, also from a genetic point of view. Recently, genetic aspects of PP, CP, and minerals predicted by MIRS were investigated in the Italian HO [21]. Authors
reported $h^2$ of $0.423 \pm 0.027$, $0.430 \pm 0.027$, $0.446 \pm 0.024$, and $0.531 \pm 0.028$ for PP, CP, total Ca, and total P, respectively. Similarly, using random regression models, $h^2$ of MIRS-predicted Ca (average: $0.54 \pm 0.04$) and P (average: $0.42 \pm 0.04$) were estimated in the HO population [22].

Furthermore, the trend of $h^2$ across DIM was similar for Ca and P, with a minimum in early lactation and a subsequent increase until mid-lactation [21]. The moderate to high $h^2$ explained why the MP/SP of Ca and P were heritable as well. It was not possible to compare $h^2$ of MP/SP with other studies, since to the authors’ knowledge, no information on genetic parameters of mineral fractions in bovine milk is currently available. Apart from the MP/SP of P, the repeatability of other Ca and P fractions was greater than 0.60, suggesting that few records predicted from milk spectra are adequate to catch the variability of the phenotype and get a reliable estimate of cows’ mean for the phenotype itself.

According to the correlations, it can be stated that MP of Ca and P were closely related, which is likely attributable to their complementary and structural role in casein micelle. On the other hand, this was not valid for the soluble fractions. In fact, SP of Ca and P were negatively correlated each other (Table 3), suggesting that they have different genetic background. Moreover, within mineral the MP was negatively genetically correlated with the SP, meaning that it is possible to adopt selection strategies to shift mineral equilibrium according to the desired objective. From the cheesemakers point of view, a shift to MP is preferred.

Overall, all $r_p$ mirrored their respective $r_a$ (Table 4); the MP of Ca and P were favourably associated with MCP, confirming the role of these minerals in the structure of caseins [2]. In particular, greater values of milk Ca and P in the micellar fraction (i.e. greater MP and MP/SP) were associated with more favourable (shorter) RCT and $k_{20}$ and to a firmer curd. Considering that casein represents approximately 80% of milk PP [4], and that the trends of PP and MY are monitored in HO worldwide, $r_a$ and $r_p$ of mineral fractions with PP and MY were also estimated in the current study (Table 4). In general, the negative correlation between MP and MY confirmed that lower concentration of micellar Ca and P is observed in high-producing cows. This was in accordance with studies reporting both $r_a$ and $r_p$ of MY with PP and casein content [23]. The micellar Ca and P was correlated favourably with PP and FP, both phenotypically and genetically (Table 4). From the genetic point of view, it is possible to confirm that an indirect favourable selection for the MP of Ca
and P currently exists in Italian HO, since the selection index emphasises PP (3%), protein yield (36%), FP (2%) and fat yield (8%), and does not attribute any weight to MY [24]. Nevertheless, the $r_a$ and $r_p$ of Ca SP with MY were positive, while those of P SP with MY were close to zero (Table 4). In general, this further supports that MP and SP are characterized by a different genetic background in dairy cattle and perhaps this idea could be extended to other minerals involved in micellar structure, like Mg. Based on the correlations estimated between SP of the two minerals and milk PP (Table 4), the amount of Ca present in the SP is more associated than SP P with milk PP, both phenotypically and genetically. This also may suggest that there are different genetic and phenotypic factors affecting variation of the two phases of Ca and P. Finally, Ca and P phases did not result associated to SCS.

CONCLUSIONS

This study investigated the genetic and phenotypic aspects of Ca and P milk fractions on a large scale in dairy cattle. Micellar and soluble fractions of Ca and P showed exploitable genetic and phenotypic variation, and were associated with traits of interest for the Italian dairy industry. The content of Ca and P in casein micelle decreased with parity but increased along the lactation, following the trend of PP. Findings suggest that selection index currently giving a positive weight to protein are indirectly improving the micellar fraction of Ca and P in Italian HO, favourably affecting coagulation ability of milk and cheese-related performance. Future studies will focus on the genomic architecture of such traits in the HO population to evaluate the role of some specific genes in the determination of milk mineral fractions.
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ABBREVIATIONS

CP = casein percentage; DIM = days in milk; FP = fat percentage; $h^2$ = heritability; MCP = milk coagulation properties; MIRS = mid-infrared spectroscopy; MY = milk yield; PP = protein percentage; $r_g$ = genetic correlation; $r_p$ = phenotypic correlation; SCS = somatic cell score; t = repeatability, MP = micellar phase, SP = soluble phase, HO = Holstein.

AVAILABILITY OF DATA AND MATERIALS

Data are available on request by contacting the corresponding author.

ETHICS APPROVAL

For this study data routinely recorded on milk samples were used and the approval of the Ethical Committee for the Care and Use of Experimental Animals of the University of Padova was not needed.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS’ CONTRIBUTIONS

The work was conceived and designed by MF and MP. MF and AC performed the analyses and MF and AC interpreted the results. The manuscript was mainly written by MF and AC and revised by MP and MDM. All the authors contributed to read and approved the final manuscript.

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Table 1. Mean, range, and coefficient of variation (CV) of milk yield, quality traits, mineral fractions, and coagulation properties.

| Trait                  | Mean   | Range    | CV, %  |
|------------------------|--------|----------|--------|
| Milk yield, kg/d       | 29.71  | 47.00    | 25.35  |
| Protein, %             | 3.31   | 2.27     | 10.55  |
| Fat, %                 | 4.02   | 4.73     | 17.15  |
| SCS                    | 2.56   | 13.27    | 73.82  |
| **Minerals**           |        |          |        |
| Ca                     |        |          |        |
| Total, mg/kg           | 1,351.05 | 864.52    | 10.83  |
| MP, mg/kg              | 963.42 | 871.56   | 14.25  |
| SP, mg/kg              | 282.54 | 284.18   | 15.75  |
| MP/SP, w/w             | 3.44   | 4.98     | 23.01  |
| P                      |        |          |        |
| Total, mg/kg           | 1,032.35 | 774.78    | 13.37  |
| MP, mg/kg              | 602.94 | 579.97   | 15.31  |
| SP, mg/kg              | 365.19 | 313.66   | 13.57  |
| MP/SP, w/w             | 1.55   | 2.72     | 27.55  |
| **Milk coagulation properties** | | | |
| RCT, min               | 22.20  | 18.31    | 16.42  |
| $k_{20}$, min          | 6.22   | 7.53     | 19.32  |
| $a_{30}$, mm           | 14.00  | 42.13    | 63.24  |

1 SCS: somatic cell score; MP: micellar phase; SP: soluble phase; MP/SP: ratio of micellar to soluble phase; RCT: rennet coagulation time; $k_{20}$: curd-firming time; $a_{30}$: curd firmness 30 min after enzyme addition to milk.
Table 2. F-values of fixed effects. All the effects were highly significant (p < 0.001) for the traits.

| Mineral¹ | F-value | Parity | Lactation stage | Parity × Lactation stage | Year of sampling | Month of sampling |
|----------|---------|--------|-----------------|--------------------------|-----------------|------------------|
| Ca       |         |        |                 |                          |                 |                  |
| MP       | 267.89  | 1,356.54 | 28.83           | 255.23                   | 940.64          |
| SP       | 230.00  | 1,673.99 | 14.11           | 17.10                    | 281.18          |
| MP/SP    | 401.21  | 1,870.84 | 12.40           | 142.41                   | 537.22          |
| P        |         |        |                 |                          |                 |                  |
| MP       | 814.99  | 1,440.61 | 15.16           | 144.20                   | 289.17          |
| SP       | 3,410.38| 2,339.21 | 15.98           | 94.08                    | 1,088.99        |
| MP/SP    | 216.43  | 1,659.62 | 25.98           | 114.61                   | 844.46          |

¹ MP: micellar phase, mg/kg; SP: soluble phase, mg/kg; MP/SP: ratio of micellar to soluble phase, w/w.
Table 3. Additive genetic variance ($\sigma^2_a$), permanent environmental variance ($\sigma^2_w$), heritability ($h^2$), repeatability ($t$), phenotypic correlations (below diagonal), and genetic correlations (above diagonal) of Ca and P phases. Standard errors of heritability and repeatability ranged from 0.013 to 0.014 and from 0.004 to 0.005, respectively, and standard errors of correlations ranged from 0.002 to 0.025.

| Trait | $\sigma^2_a$ | $\sigma^2_w$ | $h^2$ | $t$ | Ca  |   |   | P  |   |   |
|-------|-------------|-------------|-------|-----|-----|---|---|---|---|---|
|       |             |             |       |     | MP  | SP| MP/SP | MP  | SP| MP/SP |
| Ca    |             |             |       |     |     |   |   |   |   |   |
| MP    | 55.637      | 10.316      | 0.520 | 0.616 | -0.299 | 0.735 |   |   |   |   |
| SP    | 7.149       | 1.272       | 0.518 | 0.610 | -0.256 | -0.834 |   |   |   |   |
| MP/SP | 0.238       | 0.044       | 0.548 | 0.650 | 0.720 | -0.809 |   |   |   |   |
| P     |             |             |       |     |     |   |   |   |   |   |
| MP    | 30.744      | 6.775       | 0.513 | 0.626 | 0.896 | -0.477 | 0.853 |   | -0.086 | 0.760 |
| SP    | 8.115       | 1.634       | 0.533 | 0.640 | -0.396 | -0.132 | -0.160 |   | -0.177 | -0.643 |
| MP/SP | 0.047       | 0.009       | 0.472 | 0.561 | 0.886 | -0.243 | 0.643 |   | 0.768 | -0.670 |

1MP: micellar phase, mg/kg; SP: soluble phase, mg/kg; MP/SP: ratio between micellar and soluble phase, w/w.
Table 4. Phenotypic ($r_p$) and genetic ($r_a$) correlations (standard errors within parentheses) of milk Ca and P mineral phases with milk yield, quality traits, and coagulation properties.

| Trait$^1$        |                 | Ca                                          |                 |
|------------------|-----------------|---------------------------------------------|-----------------|
|                  |                 | MP                                          | SP              |
| Milk yield, kg/d | $r_p$           | -0.256 (0.006)                              | 0.088 (0.007)   |
|                  | $r_a$           | -0.406 (0.035)                              | 0.277 (0.036)   |
| Fat, %           | $r_p$           | 0.399 (0.006)                               | -0.038 (0.007)  |
|                  | $r_a$           | 0.649 (0.016)                               | -0.358 (0.024)  |
| Protein, %       | $r_p$           | 0.647 (0.005)                               | -0.417 (0.006)  |
|                  | $r_a$           | 0.658 (0.015)                               | -0.449 (0.021)  |
| SCS              | $r_p$           | -0.018 (0.006)                              | -0.004 (0.006)  |
|                  | $r_a$           | -0.098 (0.043)                              | -0.042 (0.044)  |
| RCT, min         | $r_p$           | -0.134 (0.008)                              | -0.030 (0.008)  |
|                  | $r_a$           | -0.201 (0.026)                              | -0.020 (0.027)  |
| $k_{20}, \text{min}$ | $r_p$       | -0.509 (0.006)                              | 0.221 (0.007)   |
|                  | $r_a$           | -0.576 (0.017)                              | 0.295 (0.023)   |
| $a_{30}, \text{mm}$ | $r_p$       | 0.314 (0.007)                               | -0.108 (0.008)  |
|                  | $r_a$           | 0.376 (0.024)                               | -0.134 (0.027)  |

1 MP: micellar phase, mg/kg; SP: soluble phase, mg/kg; MP/SP: ratio of micellar to soluble phase, w/w; SCS: somatic cell score; RCT: rennet coagulation time; $k_{20}$: curd-firming time; $a_{30}$: curd firmness 30 min after enzyme addition to milk.
**Figure 1.** Effect of month of sampling on micellar and soluble phases of Ca and P, and their ratio.
**Figure 2.** Effect of parity on micellar and soluble phases of Ca and P, and their ratio.
Figure 3. Effect of lactation stage on micellar and soluble phases of Ca and P, and their ratio.