

Genetic parameters for milk mineral content and acidity predicted by mid-infrared spectroscopy in Holstein–Friesian cows

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The aim of the present study was to estimate genetic parameters for calcium (Ca), phosphorus (P) and titratable acidity (TA) in bovine milk predicted by mid-IR spectroscopy (MIRS). Data consisted of 2458 Italian Holstein – Friesian cows sampled once in 220 farms. Information per sample on protein and fat percentage, pH and somatic cell count, as well as test-day milk yield, was also available. (Co)variance components were estimated using univariate and bivariate animal linear mixed models. Fixed effects considered in the analyses were herd of sampling, parity, lactation stage and a two-way interaction between parity and lactation stage; an additive genetic and residual term were included in the models as random effects. Estimates of heritability for Ca, P and TA were 0.10, 0.12 and 0.26, respectively. Positive moderate to strong phenotypic correlations (0.33 to 0.82) existed between Ca, P and TA, whereas phenotypic weak to moderate correlations (0.00 to 0.45) existed between these traits with both milk quality and yield. Moderate to strong genetic correlations (0.28 to 0.92) existed between Ca, P and TA, and between these predicted traits with both fat and protein percentage (0.35 to 0.91). The existence of heritable genetic variation for Ca, P and TA, coupled with the potential to predict these components for routine cow milk testing, imply that genetic gain in these traits is indeed possible.

Keywords: milk mineral, milk acidity, mid-IR spectroscopy, Holstein–Friesian cow, genetic parameter

Implications

The present study quantified the phenotypic and genetic characteristics of cow milk calcium, phosphorus and acidity predicted by mid-IR spectroscopy. These milk features are very important for both human health and the technological properties of milk. The existence of heritable genetic variation coupled with the potential to predict these components for routine cow milk testing, imply that genetic gain in these traits is indeed possible.

Introduction

Global demand for dairy products is increasing, especially milk powder and butter, and further expansion of trade in dairy products is expected. Moreover, in recent years, there is a growing interest in milk components with potential benefits for human health. Milk mineral profile, in particular calcium (Ca) and phosphorus (P) content, play a key role in human health (Caroli *et al.*, 2011), especially for osteoporosis, which

is a progressive bone disease related to deficiencies in Ca and vitamin D. Both Ca and P are also important in cheese-making as they influence the coagulation process and the final consistency of the curd (Fossa *et al.*, 1994).

Another characteristic that affects the technological properties of milk is titratable acidity (TA), which has a key role in the aggregation rate of para-casein micelles, in the reactivity of rennet (De Marchi *et al.*, 2009) and also on milk coagulation properties (De Marchi *et al.*, 2007).

There is a paucity of studies that have estimated genetic variation for milk mineral content and acidity. Van Hulzen *et al.* (2009) reported heritability estimates of 0.57 and 0.62 for milk Ca and P content, respectively, in 1860 Dutch Friesian cows. Reported heritability estimates for milk TA range from 0.17 to 0.23 in Italian Holstein cows (Cassandro *et al.*, 2008; Cecchinato *et al.*, 2011). To our knowledge, only Soyeurt *et al.* (2008a) used 5502 multi-breed cows to estimate genetic parameters for mineral content of milk predicted by mid-IR spectroscopy (MIRS) and they reported heritabilities of 0.42 and 0.47 for Ca and P, respectively.

The limited number of studies on the genetics of milk mineral composition and acidity is likely related to the high

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analytical costs associated with measuring these traits, thereby hindering the generation of large data sets (De Marchi *et al.*, 2014). MIRS has already been proposed to predict milk Ca and P (Soyeurt *et al.*, 2008a; Toffanin *et al.*, 2015), thereby providing a low-cost approach to generate large datasets useful for genetic evaluations. The objective therefore of the present study was to estimate genetic parameters for milk Ca, P and TA predicted by MIRS as well as the genetic correlations between these traits with milk production and quality in Italian Holstein – Friesian cows.

Material and methods

Data and MIRS prediction models

A total of 2458 Holstein – Friesian cows representing the offspring of 210 AI sires were sampled once in 220 Italian dairy herds between May 2010 and March 2011. Cows were from parity 1 to 10, and between 5 and 600 days in milk (DIM). Information on parity, DIM and milk yield (MY, kg/day) at the time of milk sampling was provided by the Breeders Association of the Veneto region (Padova, Italy). Samples were preserved with Bronopol (Knoll Pharmaceuticals, Nottingham, UK), transported to the laboratory of the Breeders Association of the Veneto region (Padova, Italy), and analysed within 12 h of collection. The routine prediction of milk fat and protein percentage as well as pH were conducted with a Milko-Scan FT6000 (Foss Electric A/S, Hillerød, Denmark) and somatic cell count (SCC) was determined using a Cell Fossomatic 250 (Foss Electric A/S). SCC was transformed to somatic cell score (SCS) by taking the natural logarithm of SCC. Spectral information was stored for all milk samples.

Predicted Ca and P content, and TA were obtained using MIRS models developed by Toffanin *et al.* (2015). Briefly, calibration models were built using 208 cow milk samples collected from 13 herds chosen from those analysed in the present study. The gold standard method for the quantification of Ca and P was based on inductively coupled plasma optical emission spectrometry with previous milk mineralization; for TA the gold standard was milk titration. Prediction models were developed through partial least squares regression analysis using the PLS procedure of SAS (SAS Institute Inc., Cary, NC, USA). Coefficients of correlation of the models were 0.73, 0.84 and 0.86 for Ca, P and TA, respectively, and the ratio performance deviation was 1.47 for Ca, 1.84 for P and 2.01 for TA (Toffanin *et al.*, 2015).

Statistical analyses

Factors associated with the traits of interest were determined using the following linear model in PROC GLM (SAS Institute Inc., Cary, NC, USA):

$$Y_{ijkl} = \mu + herd_i + parity_j + stage_k + (parity \times stage)_{jk} + e_{ijkl},$$

where Y_{ijkl} is the dependent variable (Ca, P, TA, pH, fat percentage, protein percentage, SCS or MY); μ the overall

mean; $herd_i$ the fixed effect of the i^{th} herd of sampling ($i = 1$ to 220); $parity_j$ the fixed effect of the j^{th} parity of the cow ($j = 1, 2, 3, \geq 4$); $stage_k$ the fixed effect of the k^{th} class of DIM ($k = 5$ to 35, 36 to 65, 66 to 95, ..., 306 to 350, 351 to 450, >450 days); $(parity \times stage)_{jk}$ the fixed interaction effect between parity and stage of lactation; and e_{ijkl} the random residual $\sim N(0, \sigma^2_e)$. In the model, the herd and test-day effects were confounded because cows in each herd were sampled only once, all on the same test-day.

Variance and covariance components for the studied traits were estimated using univariate and bivariate animal models in ASREML (Gilmour *et al.*, 2009). Fixed effects considered in the analyses were the same as described previously, and the random effects were the additive genetic effect and the residual term. The number of animals in the additive relationship matrix was 33 197 and included individuals with phenotypic records and their ancestors up to 23 generations back. Pedigree information was provided by the Italian Holstein Friesian Cattle Breeders Association (Cremona, Italy).

Results

Descriptive statistics

Predicted Ca, P, TA and pH averaged 1171 mg/kg, 935 mg/kg, 3.41 °SH/50 ml and 6.63, respectively (Table 1). The coefficient of variation was 0.13 for both Ca and P, 0.08 for TA and 0.01 for pH.

The effect of lactation stage on Ca and P content was similar (Figure 1); both components decreased rapidly within the first 2 months of lactation and increased thereafter. The trend for TA and pH in early to mid-lactation was opposite to each other (Figure 2); milk TA decreased markedly in the 1st month after calving, reached a plateau during the 2nd month, and increased thereafter, whereas milk pH increased until the 5th month after calving and decreased slightly thereafter (Figure 2).

Table 1 Mean, coefficient of genetic variation (CV_g), estimated heritability (h^2 ; standard errors in parentheses) of milk mineral components, acidity, chemical composition and yield

| Trait ¹ | n | Mean | CV_g | h^2 (s.e.) ² |
|---------------------|------|-------|--------|---------------------------|
| Mineral component | | | | |
| Ca (mg/kg) | 2428 | 1171 | 0.03 | 0.10 (0.04) |
| P (mg/kg) | 2353 | 935 | 0.04 | 0.12 (0.05) |
| Acidity | | | | |
| TA (°SH/50 ml) | 2019 | 3.41 | 0.03 | 0.26 (0.07) |
| pH | 2440 | 6.63 | 0.004 | 0.16 (0.05) |
| Quality traits | | | | |
| Fat (%) | 2456 | 3.69 | 0.09 | 0.25 (0.06) |
| Protein (%) | 2458 | 3.37 | 0.04 | 0.20 (0.05) |
| SCS (units) | 2451 | 4.62 | 0.08 | 0.09 (0.04) |
| Milk yield (kg/day) | 2285 | 30.19 | 0.07 | 0.12 (0.05) |

Ca = calcium; P = phosphorus; TA = titratable acidity; SCS = somatic cell score; SCC = somatic cell count.

¹[SCS = ln(SCC)].

²All estimates of heritability are significantly different from zero ($P < 0.05$).

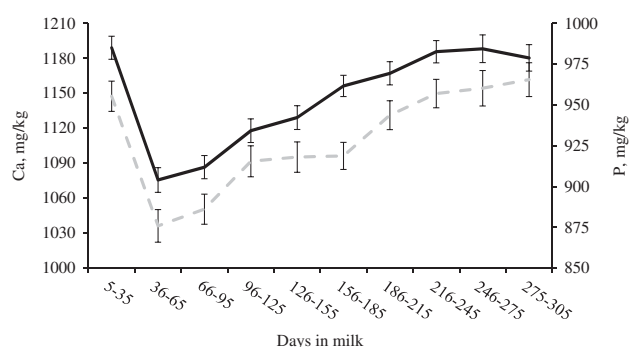


Figure 1 Least squares means (with standard errors) of predicted calcium (Ca – continuous line) and phosphorus (P – dashed line) across days in milk.

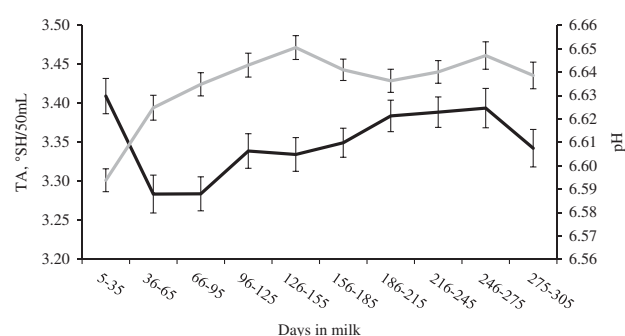


Figure 2 Least squares means (with standard errors) of predicted titratable acidity (TA – black line) and pH (grey line) across days in milk.

Table 2 Least squares means and pooled standard errors (s.e.) for milk mineral components, acidity, chemical composition and yield across parities

| Trait ¹ | Parity | | | | s.e. |
|---------------------|-------------------|-------------------|--------------------|--------------------|------|
| | 1 | 2 | 3 | 4 and later | |
| Mineral component | | | | | |
| Ca (mg/kg) | 1185 ^a | 1185 ^a | 1162 ^b | 1152 ^b | 6 |
| P (mg/kg) | 965 ^a | 942 ^b | 929 ^b | 931 ^b | 5 |
| Acidity | | | | | |
| TA (°SH/50 ml) | 3.49 ^a | 3.36 ^b | 3.30 ^c | 3.27 ^c | 0.01 |
| pH | 6.62 ^a | 6.64 ^b | 6.63 ^b | 6.64 ^b | 0.00 |
| Quality traits | | | | | |
| Fat (%) | 3.80 ^a | 3.80 ^a | 3.76 ^a | 3.77 ^a | 0.03 |
| Protein (%) | 3.44 ^a | 3.48 ^b | 3.43 ^a | 3.40 ^a | 0.01 |
| SCS (units) | 4.55 ^a | 4.82 ^b | 4.94 ^{bc} | 5.14 ^c | 0.06 |
| Milk yield (kg/day) | 26.8 ^a | 28.6 ^b | 30.5 ^c | 29.7 ^{bc} | 0.3 |

Ca = calcium; P = phosphorus; TA = titratable acidity; SCS = somatic cell score; SCC = somatic cell count.

^{a,b,c}Means with different superscripts within a row are significantly different ($P < 0.05$).

¹[SCS = $\ln(\text{SCC})$].

Least squares means for the milk characteristics in each parity are in Table 2. Predicted mineral content and TA were inversely related to parity ($P < 0.05$); pH did not change among parities with the exception of parity 1, which exhibited the lowest pH value ($P < 0.05$). Significant parity effects were

detected for MY, protein percentage and SCS, whereas no effects were detected for fat percentage.

Heritability

Estimates of heritability for predicted Ca, P and TA were 0.10, 0.12 and 0.26, respectively (Table 1), and they ranged from 0.09 (SCS) to 0.25 (fat percentage) for milk quality and yield. The coefficients of genetic variation for the minerals and TA were lower than those of fat percentage, SCS and MY, but comparable to that of protein percentage. Practically no genetic variation in milk pH existed with a detected coefficient of genetic variation of only 0.004.

Phenotypic and genetic correlations

Predicted Ca was moderately phenotypically correlated with both P (0.33) and TA (0.40), whereas P was strongly correlated (0.82) with TA (Table 3). A moderate negative phenotypic correlation (-0.38) existed between TA and pH. Phenotypic correlations of the milk minerals and TA with milk chemical composition varied from 0.00 (TA and SCS) to 0.45 (TA and protein percentage). MY was negatively phenotypically correlated with Ca, P and TA, albeit all correlations were weak (-0.23 to -0.11 ; Table 3).

Strong and significant ($P < 0.05$) genetic correlations existed between TA and P (0.92) and between Ca and P (0.67). Genetic correlations of Ca, P and TA with fat and protein percentages ranged from 0.35 (TA and fat percentage; $P < 0.05$) to 0.91 (P and protein percentage; $P < 0.05$), and with SCS and MY the correlations were weak to moderate and not significantly different from zero ($P > 0.05$; Table 3).

Discussion

Descriptive statistics

Mean milk Ca and P in the present study are within the ranges of values documented by van Hulzen *et al.* (2009), Cashman (2011) and Toffanin *et al.* (2015), who reported average values from 1120 to 1235 mg/kg and from 890 to 1027 mg/kg for measured Ca and P in bovine milk, respectively. Moreover, van Hulzen *et al.* (2009) and Toffanin *et al.* (2015) documented a phenotypic coefficient of variation between 9% and 12% for measured Ca and P in milk of Holstein – Friesian cows. Soyeurt *et al.* (2009) investigated the feasibility of MIRS to predict the content of milk minerals using the ICP-AES without previous mineralization as the reference method. Despite differences in pre-treatment for ICP-AES as well as the data originating from two separate populations of cows, similar means and coefficients of variation were observed between the present study and the aforementioned study of Soyeurt *et al.* (2009).

Parity least squares means for Ca and P (Table 2) followed a similar trend to that described by Kume *et al.* (1998) in Japanese Holstein cows, who reported a slight decrease in milk mineral concentration after first parity, even if not statistically significant. The decrease in milk Ca and P content within increasing parity is probably due to the reduced

Table 3 Phenotypic (above diagonal, with standard errors in parentheses) and genetic (below diagonal, with standard errors in parentheses) correlations between milk mineral components, acidity, chemical composition and yield

| Trait ¹ | Ca | P | TA | pH | Fat | Protein | SCS | MY |
|--------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Ca | | 0.33(0.02) | 0.40(0.02) | 0.01(0.02) | 0.32(0.02) | 0.44(0.02) | 0.06(0.02) | -0.23(0.02) |
| P | 0.67(0.24)* | | 0.82(0.01) | -0.10(0.02) | 0.25(0.02) | 0.39(0.02) | 0.03(0.02) | -0.14(0.02) |
| TA | 0.28(0.25) | 0.92(0.05)* | | -0.38(0.02) | 0.24(0.02) | 0.45(0.02) | 0.00(0.02) | -0.11(0.03) |
| pH | 0.19(0.29) | -0.06(0.25) | -0.30(0.19) | | -0.16(0.02) | -0.09(0.02) | 0.13(0.02) | 0.01(0.02) |
| Fat | 0.76(0.15)* | 0.83(0.14)* | 0.35(0.17)* | -0.29(0.19) | | 0.37(0.02) | 0.08(0.02) | -0.27(0.02) |
| Protein | 0.84(0.14)* | 0.91(0.10)* | 0.69(0.12)* | -0.02(0.22) | 0.75(0.11)* | | 0.16(0.02) | -0.35(0.02) |
| SCS | -0.02(0.33) | 0.20(0.30) | 0.38(0.25) | -0.14(0.29) | 0.38(0.24) | 0.40(0.24) | | -0.18(0.02) |
| MY | -0.26(0.30) | -0.31(0.28) | -0.46(0.24) | 0.28(0.25) | -0.19(0.23) | -0.44(0.21) | -0.08(0.31) | |

Ca = calcium; P = phosphorus; TA = titratable acidity; SCS = somatic cell score; MY = milk yield; SCC = somatic cell count.

¹[SCS = ln(SCC)].

*Genetic correlations are significantly different from zero at $P < 0.05$.

utilization of these minerals in the mammary gland (Kume and Tanabe, 1993). Indeed, serum Ca and P concentration is also inversely related to parity number (Shappell *et al.*, 1987; Romo *et al.*, 1991) justifying the hypothesis of a lower utilization in older cows. Van de Braak and Van't Klooster (1987) argued that osteoplastic activities are less intense in older than in younger cows, which could explain a decreased responsiveness for parathyroid hormone-mediated Ca resorption and intestinal absorption in older parity cows (Romo *et al.*, 1991). Moreover, the greater amount of milk produced by older cows during lactation could explain the lower mineral content because of the dilution.

The association between lactation stage and Ca in the present study (Figure 1) corroborates the observed increase in milk Ca content across lactation documented by van Hulzen *et al.* (2009) in 1860 Dutch Holstein – Friesian cows. Similar effects have been documented in other dairy species such as goat and sheep (Sahan *et al.*, 2005; Kondyli *et al.*, 2007). Trends of minerals across DIM might be due to changes in feeding composition across lactation (Cashman and Flynn, 2003) or to a dilution effect during lactation; negative phenotypic correlations between MY with both Ca (-0.23) and P (-0.14) were observed in the present study after adjustment for terms in the statistical model (Table 3).

Mean milk TA across all samples in the present study is similar to documented values of TA in the milk of Holstein – Friesian (Cassandro *et al.*, 2008) and Brown Swiss cows (De Marchi *et al.*, 2007 and 2008; Cecchinato *et al.*, 2011; Penasa *et al.*, 2014) and in herd bulk milk samples destined for Grana Padano cheese production (Pretto *et al.*, 2013). Moreover, mean TA in the present study is similar to reported values of MIRS-predicted TA by De Marchi *et al.* (2009), Colinet *et al.* (2010) and Toffanin *et al.* (2015). The lower pH in first compared with later parity cows corroborates results of Ikonen *et al.* (2004) and could be the consequence, at least partly, of the higher values of P and TA in first lactation cows (Table 2), and the inverse relationships between these minerals and pH (Table 3). The interrelationships between milk acidity and mineral content have been previously investigated (Mariani *et al.*, 1989). The similar patterns of TA and minerals across stages of lactation

(Figures 1 and 2) could be explained by the nature of these traits; TA is moderately phenotypically correlated with both protein percentage and Ca, as well as being strongly correlated with P (Table 3). As a consequence, it is expected that TA follows the trend of minerals across lactation, which decreases markedly in early lactation, increasing thereafter.

Descriptive statistics of milk chemical composition and MY are in agreement with those documented in several studies on Italian Holstein – Friesian cows (Cassandro *et al.*, 2008; Pretto *et al.*, 2011; Tiezzi *et al.*, 2013), with the exception of SCS, which exhibited a smaller phenotypic coefficient of variation in the present study. Parity mean milk composition and yield (Table 2) were generally in agreement with other studied international populations of dairy cows (Ikonen *et al.*, 2004; Hansen *et al.*, 2006; Bastin *et al.*, 2013).

Heritabilities

Heritability estimates of Ca and P were markedly lower than those reported by Soyeurt *et al.* (2008b) who predicted Ca and P using MIRS with similar accuracy to the equations used in the present study. Differences in number and variability of data could explain different estimates between the present study and that of Soyeurt *et al.* (2008b). Using data on measured Ca and P, Van Hulzen *et al.* (2009) reported similar heritability estimates for Ca and P to those documented by Soyeurt *et al.* (2008b). The lower heritability estimates obtained in the present study could be also related to the MIRS prediction models performance: the less than perfect predictions will likely increase the residual variance and thus reduce the heritability. Nevertheless, the presence of genetic variation in these traits, albeit with coefficients of genetic variation generally less than observed for other performance traits like milk production, suggests that genetic improvement is still nonetheless possible. This is especially true because milk MIRS information is routinely available on all milk samples taken during milk testing. Moreover, it has been reported that also in the case of quite low accuracy of prediction, the genetic correlations between predicted and true values are strong. This was demonstrated by Cecchinato *et al.* (2009) who compared genetic parameters from predicted (with accuracies similar to those obtained in the present

study) and measured values of milk coagulation properties: those authors concluded that the genetic response in milk coagulation properties from selection using MIRS predictions as indicator traits is expected to be equal to or slightly lower than the response achievable through direct measurement of the traits (Cecchinato *et al.*, 2009).

The accuracy of selection for both milk Ca and P based on information on 100 half-sib daughters (ignoring parental contribution) in a univariate genetic evaluation is 0.86. Assuming a genetic gain of 0.22 standard deviations annually, this equates to a potential genetic gain of 7.3 and 6.9 mg/kg for Ca and P, respectively. The actual gain achievable, however, will be dependent on the traits in the breeding goal, their covariances with Ca and P and their relative weighting within the breeding goal, as well as other factors like intensity of selection, accuracy of selection and generation interval (Rendel and Robertson, 1950).

To our knowledge, this is the first study to estimate genetic parameters for TA predicted by MIRS. Heritability estimates for actual TA, determined using Soxhlet – Henkel degrees, has been reported to range between 0.17 and 0.23 in Holstein – Friesian (Cassandro *et al.*, 2008) and Brown Swiss cows (Cecchinato *et al.*, 2011). The heritability of pH in the present study (0.16) is within the range of 0.06 (Cecchinato *et al.*, 2011) to 0.38 (Ikonen *et al.*, 2004) reported in other populations. The lack of genetic variation in pH observed in the present study suggests limited potential for change through breeding; the small phenotypic coefficient of variation for pH, also suggests limited potential for alteration of milk pH in the dairy cow through non-breeding means.

Phenotypic and genetic correlations

Both the phenotypic and genetic correlations between Ca and P observed in the present study were consistent with correlations reported by Soyeurt *et al.* (2008b) from a large multi-breed population of dairy cows. The phenotypic correlation between measures of P and TA has been reported to range from 0.54 to 0.81 (Mariani *et al.*, 1989; Toffanin *et al.*, 2015), depending on whether individual or bulk milk samples or whether single or multi-breed populations were considered. As expected, the phenotypic correlation between P and pH in the present study was negative, although weak, reflecting the link between P and TA (positive and strong) and between TA and pH (negative and moderate). The phenotypic correlation between TA and P was also confirmed by the effect of lactation stage on each trait being a mirror of the other (Figure 2). Similar correlations were reported by Mariani *et al.* (1989) who assessed a phenotypic correlation of -0.61 between pH and soluble P.

No study has previously estimated the genetic correlations between milk mineral content and other milk components. Predicted Ca and P were positively and moderately (phenotypic) to strongly (genetic) correlated with milk protein and fat percentage. After all, as reported by Malacarne *et al.* (2013), micellar P can be present as part of colloidal calcium phosphate or covalently bound to caseins as phosphate

groups, given that Ca and P are basic components of the milk micelles. MY was moderately and not significantly ($P > 0.05$) related to Ca, P and TA, suggesting independence of production from mineral content and acidity of milk. However, it is likely that the lack of significance in the present study was the consequence of the relatively small sample size, which led to quite high standard errors for some estimates of genetic correlations. The use of MIRS models to predict milk mineral content and TA at population level will reduce the sampling variance.

Conclusions

Improvement in milk mineral and acidity profiles could be of benefit for the human nutritional and technological characteristics of milk. The existence of heritable genetic variation coupled with the ability to predict these milk characteristics through routinely available MIRS suggests that genetic selection for these components in milk is indeed possible. Moreover, because Ca, P and TA are genetically correlated with milk protein and fat percentage, breeding goals that select for improved milk fat and protein concentration are likely to be indirectly selecting also for improved milk Ca, P and TA.

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