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Genetic and genomic evaluation of milk coagulation properties in Italian Holsteins using a reference population of bulls and cows

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ABSTRACT

In Italy, ~77% of milk is used for cheese production, with 55% allocated to certified Protected Designation of Origin/Protected Geographical Indication cheeses, making milk cheesemaking ability a crucial factor in the dairy industry. Milk coagulation properties (MCP)—rennet coagulation time (RCT), curd firmness after 30 min (a_{30}), and curd firming time (k_{20})—are pivotal for assessing milk's suitability for cheesemaking. The present study established a genetic and genomic evaluation system for MCP traits in Italian Holstein cattle. Over 4 million records from 546,944 animals were analyzed using a multiple-trait repeatability animal model. Variance components were estimated via Gibbs sampling, providing heritabilities of 0.33 for casein percentage, 0.11 for RCT, 0.16 for a_{30}, and 0.15 for k_{20}. Strong genetic correlations among MCP traits were observed, including −0.87 between RCT and a_{30}, highlighting their interdependence. Genomic evaluations, incorporating a mixed reference population of bulls and cows, significantly increased the reliability of genomic predictions by an average of 69% for MCP traits compared with using a bulls-only reference population. These findings demonstrate the potential for improving novel traits with moderate h^2, as MCP traits, through genomic selection and underscore the benefits of integrating females into reference populations to enhance the accuracy of genetic evaluations. This approach offers a robust pathway for enhancing milk quality and optimizing cheesemaking processes.

Key words: genomic selection, cheese production, mid-infrared spectrometry, animal model, genomic prediction

INTRODUCTION

In Italy, ~77% of the milk is intended for cheese production, with 55% of this directly involved in the manufacturing of the 56 cheeses certified as Protected Designation of Origin or Protected Geographical Indication officially acknowledged by the European Union (ISMEA, 2022). In this scenario, the importance of dairy production in the Italian agrifood sector is readily evident, and this implies the need to genetically identify elite animals in terms of production of suitable milk for cheesemaking. Milk coagulation properties (**MCP**), namely, rennet coagulation time (**RCT**, min), curd firming time (**k_{20}**, min), and curd firmness (**a_{30}**, mm), as well as casein percentage (**CAS**), are milk quality parameters informative on the capacity of milk to form a stable clot in a reasonable time, and therefore on its cheesemaking capacity. The RCT refers to the time taken to start milk coagulating from the addition of rennet. The k_{20} measures the time required for the curd to achieve a 20 mm thickness after the beginning of coagulation, and a_{30} is the curd thickness 30 min after rennet addition. Shorter RCT, stronger a_{30}, and higher CAS are associated with greater cheese yield (Aleandri et al., 1989; Ng-Kwai-Hang et al., 1989; Wedholm et al., 2006). The MCP have been proven to be heritable (Cassandro and Marusi, 2001; Cassandro et al., 2008; Visentin et al., 2017), as well as to have a significant economic value in the dairy market (Cassandro et al., 2016). The determination of MCP in individual milk samples, which is pivotal for the implementation of breeding programs to improve these characteristics in dairy cows' population, is hampered by costly and time-consuming reference laboratory techniques, such as lactodynamographic analysis. Mid-infrared spectrometry (**MIRS**), which is routinely employed for the analysis of individual milk samples, is a fast and low-cost method to measure multiple milk characteristics simultaneously by analyzing the interaction between molecules in milk and electromagnetic incident radiation between 5,000 and 900 cm^{-1} (Visentin et al., 2015). Within the frame of Livestock Environment Open Data (**LEO**) project (PSRN

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misura 16.2, AIA, 2023), MIRS prediction models have been implemented in the official national milk recording system, and individual predictions of RCT, k_{20}, and a_{30} are available for all analyzed milk samples from cows registered in the official Holstein herdbook operated by the National Breeders Association of Italian Holstein, Brown Swiss and Jersey (ANAFIBJ).

The availability of a large number of these phenotypes could allow the implementation of genomic selection (**GS**) strategies to identify genetically superior animals in terms of milk suitability for cheesemaking. The GS associates the performances of a reference population (**RP**) with their marker genotypes to predict the GEBV also of animals with no recorded performance (Meuwissen et al., 2001). Since GS has been implemented in dairy cattle breeding, genetic gain has been strongly accelerated due to the shortening of generation interval and the significant increase in reliability of EBV (Nejati-Javaremi et al., 1997; Meuwissen et al., 2001; Schaeffer, 2006; König et al., 2009; Meuwissen and Goddard, 2010; Gorjanc et al., 2015; García-Ruiz et al., 2016). In addition to that, GS had the effect of increasing the preselection intensity on young bulls, meaning that the number of bulls selected for breeding purposes per year decreased, resulting in an increased preselection bias (**PSB**) in breeding values estimation (Patry and Ducrocq, 2011a; Patry et al., 2013; Tyrisevä et al., 2018). The inclusion of females in the RP has the simultaneous effect of increasing prediction accuracy and decreasing PSB (Alkhoder et al., 2017; Liu et al., 2019; Togashi et al., 2019).

Given the economic importance of dairy production in Italy, the current availability of a large number of low-cost phenotypes related to it, and the potential benefits of including females in the RP, the purposes of the present study were (1) the implementation of a routine genetic and genomic evaluation of MCP for the Italian Holstein breed, and (2) exploring the feasibility of incorporating females into the RP for the estimation of the SNP effects, given the substantial availability of genotypes of Italian Holstein cows.

MATERIALS AND METHODS

Data Editing

The input data were composed of 6.7 million records from January 2017 until June 2023, originating from the LEO project (AIA, 2023) of the Italian National Breeders Association. Four phenotypes were considered: CAS and the 3 MCP traits (i.e., RCT, k_{20}, and a_{30}). Only records from regions that consistently provided data across calendar years were kept (10 regions out of 20). Records from parity 1 to parity 5 and from 5 to 405 DIM were considered. Regarding MCP traits, accepted range of values for RCT, k_{20}, and a_{30} were 5 to 60 min, 1 to 20 min, and 5 to 60 mm, respectively: all records out of these ranges were removed as obvious data errors (Cassandro et al., 2008). To detect laboratory measurement anomalies, the isolation forest algorithm implemented in the Python module Scikit-learn was employed (Pedregosa et al., 2011). The isolation forest algorithm identifies anomalies by recursively partitioning the data space using random feature and split-value selections. Due to their sparse nature, anomalous observations are more susceptible to isolation, requiring fewer partitions to be separated from the rest of the dataset. The algorithm constructs an ensemble of random trees and derives anomaly scores based on the average path length of data points. Observations with shorter path lengths are considered more likely to be outliers. This approach is computationally efficient and particularly effective in high-dimensional settings. To summarize, reference values from Visentin et al. (2015) were used for the phenotypic correlations between the 3 traits: −0.73 for RCT-a_{30}, 0.80 for RCT-k_{20}, and −0.79 for k_{20}-a_{30}. Phenotypic correlations within herd-year-test day (**HTD**) groups were calculated. All milk samples collected from the same herd on the same day (so, the HTD) are processed in the same laboratory; the anomaly indicates that the samples processed in that batch may not be fully reliable. All the HTD groups with an anomalous correlation coefficient between phenotypes compared with the reference have been excluded. All test day observations needed to have a record for casein percentage to be included in the analysis. As SCS was included in the statistical model, only values of SCC between 5,000 and 10,000,000 cells/mL were kept, in line with the thresholds applied by Dal Prà et al. (2022). The SCC was converted to SCS following Ali and Shook (1980):

$$\text{SCS} = \log_2\left(\frac{\text{SCC}}{100{,}000}\right) + 3. \qquad [1]$$

Regarding age at calving, records out of the range 18 to 41 mo for first parity, 30 to 59 mo for second parity, 42 to 77 mo for third parity, 55 to 91 mo for fourth parity, and 68 to 100 mo for fifth parity were excluded. Finally, only herd-year-season of recording groups with at least 20 contemporaries were kept. After all these edits, the final phenotypic dataset comprised 4,001,769 observations recorded between January 2017 and June 2023. The number of animals was 546,944, and the number of herds was 2,781. Pedigree was traced back to 4 generations (phenotyped animals plus 3 generations of ancestors).

Statistical Analysis

For the estimation of variance component and the subsequent development of the genetic evaluation, a multiple-trait repeatability linear animal model was employed, with CAS, RCT, a_{30}, and k_{20} as correlated dependent variables. The model for CAS was the following:

$$CAS_{ijklmnop} = hys_i + S_j \times Y_k + DIM_l \times PARC_m \times Y_k + AGEC_{PAR_n} \times Y_k + a_o + pe_o + e_{ijklmnop}, \quad [2]$$

with $CAS_{ijklmnop}$ as the pth phenotypic observation of casein percentage. Fixed effects were $S_j \times Y_k$ as the interaction effect of the jth season by the kth year of recording; $DIM_l \times PARC_m \times Y_k$ as the lth DIM class (10 classes of 40 d) by the mth parity class (3 classes: 1, 2, 3+) and the kth year; and $AGEC_{PAR_n} \times Y_k$ as the nth age at calving within parity class (9 classes: 3 ages at calving classes for every parity class) by the kth year. Random effects were hys_i as the ith contemporary group for herd-year-season of recording; a_o as the additive genetic effect of the oth animal; pe_o as the permanent environmental effect of the oth animal, fitted because of the presence of within- and across lactations repeated measures; and $e_{ijklmnop}$ as the residual of the pth observation.

The same model with the addition of the fixed linear regression of SCS was applied to MCP traits:

$$MCP_{ijklmnop} = hys_i + S_j \times Y_k + \mathrm{DIM}_l \times PARC_m \times Y_k + AGEC_{PAR_n} \times Y_k + \beta_{SCS} \times SCS_p + a_o + pe_o + e_{ijklmnop}. \quad [3]$$

Variance Components Estimation

Variance components were estimated with the Gibbs sampler THRGIBBS1F90 (Misztal et al., 2014), applying the models described previously. A pedigree-based relationship matrix was generated for the random additive genetic effect on a random sample of 150 herds (64,720 individuals) drawn from the final dataset after edits. Convergence was assessed with R (R Core Team, 2022) package BOA, Bayesian output analysis (Smith, 2007), using visual, Geweke, and Heidelberger and Welch criteria (Heidelberger and Welch, 1983; Geweke, 1992). A total of 500,000 samples were drawn, a burn-in of 10,000 samples, and a thinning rate of 10 were applied: the software POSTGIBBSF90 (Misztal et al., 2014) was employed to perform the post-Gibbs analysis on the retained 49,000 samples.

Heritabilities were calculated as follows:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}, \quad [4]$$

with σ_a^2 as the direct additive genetic variance, σ_{pe}^2 as the permanent environment effect variance, and σ_e^2 as the residual variance.

Genetic and Genomic Evaluation

Conventional EBV for CAS and MCP traits were estimated with MiX99 software (MiX99 Development Team, 2015), using the previously estimated variance components and the same aforementioned repeatability animal models described in Equations [1] and [2]. The solutions were standardized to the mean and SD of the genetic base, composed of cows born between 2015 and 2017. Finally, EBV were expressed on a scale with mean of 100 and SD of 5: proofs above 100 indicate animals with favorable genetic potential for each trait considered (i.e., high CAS and a_{30}, and low RCT and k_{20}). Genomic evaluation for direct genomic values (**DGV**) estimation was performed using GS3 software (Legarra et al., 2011) applying an SNPBLUP model:

$$\mathbf{y} = 1\mu + \mathbf{Mg} + \mathbf{e}, \quad [5]$$

with $\mathbf{y}$ as the pseudophenotypes vector, μ as overall intercept, $\mathbf{M}$ as the $n \times m$ matrix of genomic information (n is the number of individuals, and m is the number of SNP markers), $\mathbf{g}$ as the random SNP effects vector, and $\mathbf{e}$ as the vector of residuals. As pseudophenotypes, estimated deregressed proofs (**EDP**) calculated following Vicario et al. (2016) were considered. A conventional quality control was applied to SNP data, considering the following criteria: call-rate greater than or equal to 0.95, minimum allele frequency greater than or equal to 0.02, and Hardy-Weinberg equilibrium P-value greater than or equal to 0.005. Genotypes of 649,649 animals generated from 56 different DNA chips were imputed to a panel containing 70,012 SNP through the PedImpute software (Nicolazzi et al., 2013), with an allele error rate of 0.0053. The evaluation was performed with both an RP of only bulls (n = 3,276) and of bulls and cows (n = 43,754). For animals with EBV, GEBV were calculated as described in Galluzzo et al. (2024). To summarize,

$$\mathrm{GEBV} = \frac{\mathrm{EDC}_c \times \mathrm{EBV} + \mathrm{EDC}_g \times \mathrm{DGV}}{\mathrm{EDC}_c + \mathrm{EDC}_g}, \quad [6]$$

where EDC_c is the conventional effective daughter contribution (Fikse and Banos, 2001), and EDC_g is the genomic effective daughter contribution (Patry and Ducrocq, 2011b).

Validation of EBV and DGV

To implement a routine genetic evaluation for any trait, a validation process is needed. Regarding EBV, the LR method proposed by Legarra and Reverter (2018) was applied. Summarizing, EBV were obtained from both a full (with all data until June 2023) and a reduced (partial) dataset. In the reduced dataset, phenotypic observations of the last 2 yr were excluded: conventional validation is supposed to exclude the last 4 yr of records, but, as MCP trait records were available just from 2017, a 2-yr cutoff was applied. For all traits, the focal individuals were 84 bulls born between 2017 and 2019 with 10 to 20 daughters in the full dataset and no daughters in the partial one. Three statistics were computed on the focal groups:

(1) Bias ($\mu_{w,p}$) as $\dfrac{\hat{\mu}_p - \hat{\mu}_w}{\sigma_p}$, with $\hat{\mu}_p$ as the average of the solutions' vector in the partial dataset, $\hat{\mu}_w$ as the average of the solutions' vector in the full dataset, and σ_p as the solutions' SD in the partial dataset.

(2) Dispersion ($b_{w,p}$) as $\dfrac{Cov\left(\hat{\mu}_p, \hat{\mu}_w\right)}{Var\left(b_p\right)}$, with $\hat{\mu}_p$ and $\hat{\mu}_w$ as the solutions' vectors in the partial and full dataset, respectively, Cov denotes a covariance, and Var denotes a variance.

(3) Pearson correlation coefficient ($\rho_{w,p}$) between $\hat{\mu}_p$ and $\hat{\mu}_w$ as measure of accuracy.

To obtain a measure of uncertainty for the 4 statistics, a bootstrap method was applied on 1,000 samples with replacement of the focal group with the bootstrap module of the SciPy library of the Python programming language (Virtanen et al., 2020). The validation of DGV was performed as described in Finocchiaro et al. (2012) and Galluzzo et al. (2022). In summary, 2 datasets were considered: a complete dataset including all phenotypes, and a reduced dataset excluding the last 2 yr of phenotypes. The number of observations in the reduced dataset, which was subjected to the same editing criteria as the full dataset, as described in the "Data Editing" section, was 1,841,529, recorded between January 2017 and June 2021. The number of animals was 268,162, and the number of herds was 2,150. Estimated breeding values were calculated separately for each dataset, resulting in EBV from the complete and reduced runs. From these EBV, EDP were derived for both datasets. Genomic validation was carried out using the 1,371 validation animals that had EBV in the complete dataset but not in the reduced one; for these animals, only DGV were available from the reduced run. To validate the genomic predictions, the EDP obtained from the complete dataset (used as response variables, Y) were regressed on the DGV from the reduced dataset (used as predictors, X). The validation process was performed either using the only-bulls RP or the bulls and cows one. Parameters considered for the comparison were the dispersion coefficient and the reliability (Galluzzo et al., 2024) of the linear regression model. A graphical description of the whole process is depicted in Figure 1.

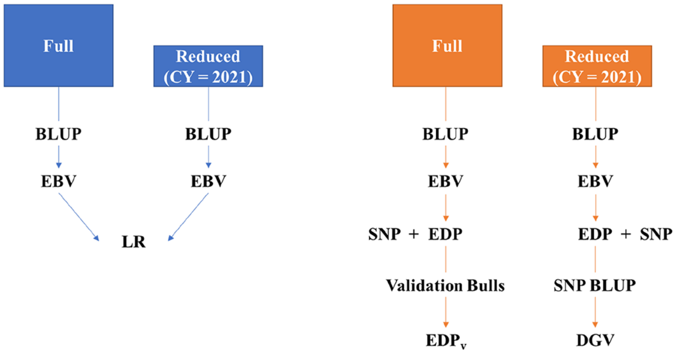

Figure 1. Steps for the validation of (blue) EBV and (orange) DGV using both a full dataset and a reduced dataset. Bulls selected for validation did not have daughters in the reduced dataset. Phenotypes recorded from June 2021 onwards were excluded. CY = cutting year; EDP$_v$ = validation estimated deregressed proof.

Effect of Females' Number on Validation Results

To quantify the effect of adding females to the training population, the genomic validation procedure previously described (1.0 scenario) was repeated by including 75% (0.75 scenario, 29,050), 50% (0.50 scenario, 18,930), and 25% (0.25 scenario, 8,811) of the females from the initial validation dataset (40,478), in addition to the 3,276 males present in all training population scenarios.

Relationship to Other Traits

As the indirect effect of the selection for a certain trait on other traits undergoing selection is crucial for the decision on whether to set up a routine genetic evaluation for that new trait or not, genetic relationships of CAS and MCP traits to other traits included in the official genetic and genomic evaluation were studied: milk, fat and protein yields, fat and protein percentages, SCS, longevity, cow conception rate at first service, udder health, and heat tolerance. For this purpose, approximate genetic correlations were calculated as Pearson correlation coefficients between GEBV of 87,569 heifers born after 2016.

RESULTS

Data and Data Editing

After edits, the dataset was composed of 4,001,769 observations on 546,944 animals (7.32 observations per animal on average) distributed in 2,781 herds (196.67 animals per herd on average). A total of 1,371,813 ob-

Table 1. Descriptive statistics by parity order (1, 2, and 3+)[1]

	CAS, %			RCT, min			a_{30}, mm			k_{20}, min		
Metric	1	2	3+	1	2	3+	1	2	3+	1	2	3+
Mean	2.75	2.77	2.70	25.53	26.27	26.47	21.72	21.51	21.22	7.39	7.37	7.51
SD	0.32	0.35	0.33	6.39	6.52	6.73	8.99	8.96	8.81	1.98	2.02	1.99
CV%	11.64	12.64	12.22	25.02	24.82	25.43	41.39	41.65	41.52	26.79	27.41	26.50
Min	1.20	1.06	1.22	5.00	5.00	5.00	5.00	5.00	5.00	1.00	1.00	1.00
Max	6.01	6.21	5.90	60.00	60.00	60.00	60.00	60.00	60.00	19.65	19.40	19.94

[1] a_{30} = curd firmness; CAS = casein %; k_{20} = curd firming time; RCT = rennet coagulation time; min = minimum; max = maximum.

servations were considered measurement anomalies, with 12,987 samples considered not coagulated because of RCT >60 min. General average ± SD for CAS and MCP traits were 2.74% ± 0.33%, 26.05 ± 6.56 min (RCT), 7.42 ± 2.00 min (k_{20}), and 21.50 ± 8.93 mm (a_{30}). Table 1 reports the descriptive statistics (mean, SD, min, and max) by parity order for these 4 evaluated traits. The CV was also computed as the ratio between SD and mean expressed as a percentage. For the purpose of having a balanced frequency of observations across lactations, cows above the second parity (third onwards) were grouped into a unique class (3+). Indeed, 38% of the dataset (n = 1,521,453) was composed of records belonging to first parity cows, 29% of second parity order cows records (n = 1,145,693), 19% of third parity (n = 750,008), 10% of fourth parity (n = 400,678), and the remaining 4% (n = 183,937) of fifth parity. The CV of all traits remained consistent across parities. For CAS, the CV ranged from 11.64% in first parity to 12.64% in second parity. The RCT and k_{20} showed higher variability, with CV ranging from 24.82% (RCT, second parity) to 27.41% (k_{20}, second parity). The most variable trait was a_{30}, with CV between 41.39% (first parity) and 41.65% (second parity). The mean CAS peaked in secondiparous cows (2.77%), whereas MCP tended to deteriorate across parity. Indeed, RCT was 54 s shorter in primiparous animals compared with 3+ parity class, whereas k_{20} was 12 s lower in primiparous cows compared with multiparous. Regarding a_{30}, the peak was reached in second parity. The phenotypic trends for all 4 evaluated traits across year of test are illustrated in Figure 2. Independently on the feature considered, the trends highlighted an increase across record years. This trend is considered favorable for CAS and a_{30}, but it is unfavorable for RCT and k_{20}.

Variance Component Estimation and Validation of Pedigree EBV and DGV

In Table 2 are depicted the results of variance components estimation. Variance component estimates revealed moderate h^2 for CAS and lower h^2 for the other MCP traits. Genetic correlations among MCP traits were generally strong, particularly between k_{20} and a_{30}, whereas CAS showed weaker genetic relationships with the others. Phenotypic correlations followed similar patterns but were consistently lower in magnitude. The results of the EBV validation through the LR method are reported in Table 3. Bias was −0.66 genetic SD for CAS, with a bootstrap CI (**BCI**) ranging from −0.67 to −0.65, whereas MCP bias was −0.06 (BCI = −0.07, −0.05), −0.43 (BCI = −0.43, −0.42), and 0.35 (BCI = 0.35, 0.36), for RCT, a_{30}, and k_{20}, respectively. Dispersion values were 1.06 (BCI = 1.05, 1.07; CAS), 1.10 (BCI = 1.09, 1.10; RCT), 1.26 (BCI = 1.25, 1.27; a_{30}), and 1.30 (BCI = 1.29, 1.31; k_{20}). Pearson correlation coefficient between pedigree EBV estimated in the full and reduced runs ranged from 0.61 (BCI = 0.60, 0.61; CAS) to 0.75 (BCI = 0.75, 0.76; a_{30} and k_{20}) for both a_{30} and k_{20}, whereas RCT was 0.65 (BCI = 0.65, 0.66). The results of the genomic validation are listed in Table 4. The genomic validation was undertaken using 2 different RP, one composed by daughter proven bulls-only, and the other mixed, thereby including both bulls and cows. These 2 different RP were tested to assess whether the accuracy of genomic prediction benefits from the increase of the RP size and representativeness. Regarding dispersion, values of 1.205, 1.359, 1.319, and 1.246 were calculated for CAS, RCT, a_{30}, and k_{20}, respectively, when the RP was composed of bulls only, whereas dispersion values of 0.898, 0.925, 0.911, and 0.895 were computed for the same traits when the RP was composed of both bulls and cows. With the mixed RP, the average distance from the theoretical optimal value of 1 for dispersion decreased by 67% (from 0.282 to 0.093). Finally, considering the RP composed of bulls only, validation reliabilities of 0.452, 0.421, 0.478, and 0.459 were calculated for CAS, RCT, a_{30}, and k_{20}, respectively. For the same traits, in the same order, the mixed RP resulted in reliability estimates of 0.790, 0.737, 0.767, and 0.763. Therefore, mean validation reliability for MCP increased by 69% using a mixed RP (from 0.453 to 0.764).

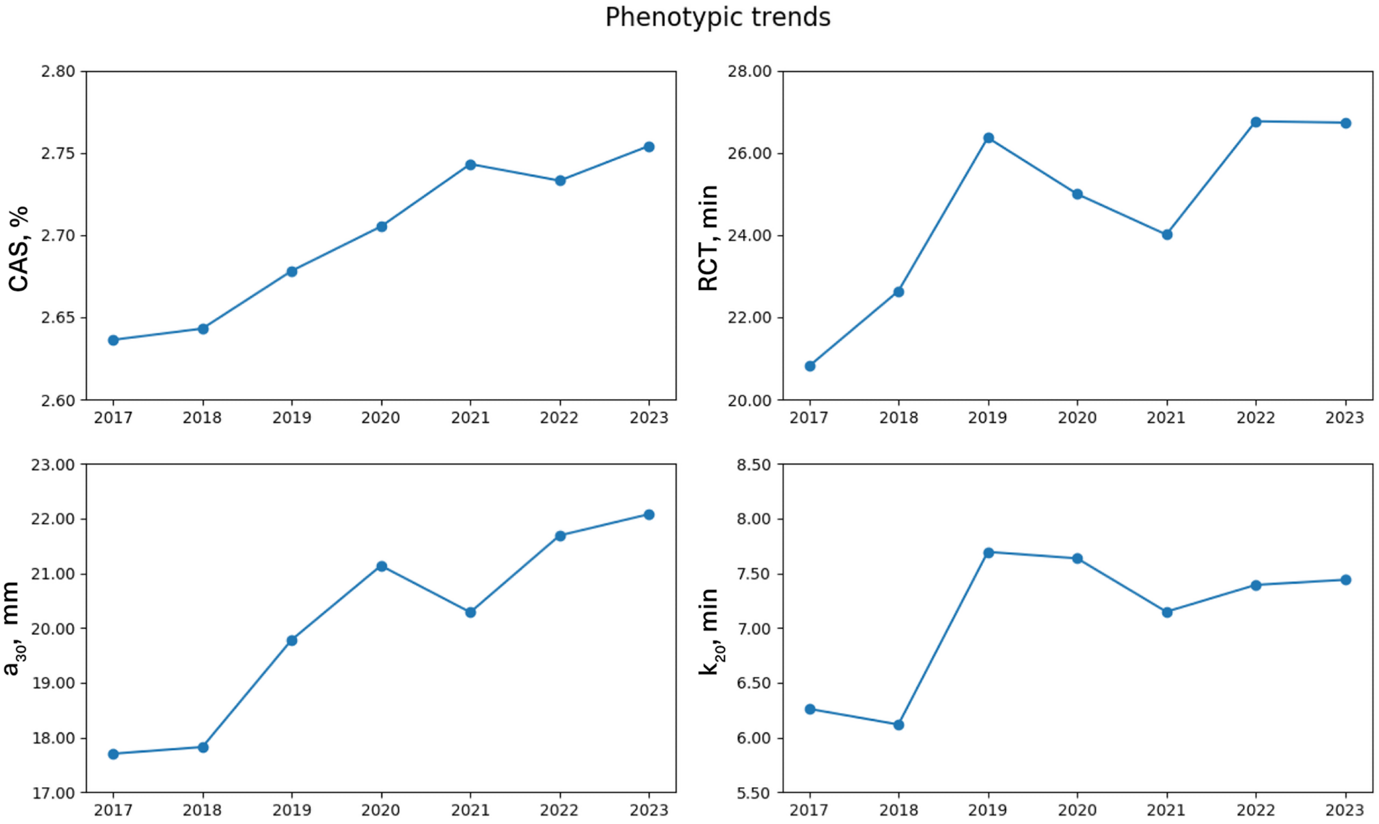

Figure 2. Phenotypic trends over recording years.

Effect of Females' Number on Validation Results

Table 5 provides a description of the 4 different training populations, including both males and females. The number of males remains constant across all scenarios, whereas the number of females is progressively reduced by 25% in each subsequent analysis. As a result, the female-to-male ratio decreases from 12.36 in the full training population to 2.69 when only 25% of females are retained. The effect of including different proportions of females in the training population on validation reliability is illustrated in Figure 3. For the CAS trait, reliability decreased from 0.790 when 100% of females are included to 0.774, 0.742, and 0.677 when only 75%, 50%, and 25% are retained, respectively. Following the same order, validation reliability for RCT: 0.737, 0.722, 0.694, and 0.621; for a_{30}: 0.767, 0.747, 0.722, and 0.658; and for k_{20}: 0.763, 0.734, 0.715, and 0.639. The average decrease in reliability was 2.23% from 100% to 75% of females, 3.83% from 75% to 50%, and 9.34% from 50% to 25%. The effect on the dispersion coefficient is illustrated in Figure 4. As the proportion of females in the training population decreased from 100% to 25%, the dispersion coefficients increased consistently across all traits. Specifically, CAS increased from 0.898 to 0.951; RCT from 0.925 to 0.955; a_{30} from 0.911 to 1.045; and

Table 2. Variance components estimation results[1]

Trait	CAS	RCT	a_{30}	k_{20}
CAS, %	0.33 (0.01)	−0.04	0.51	−0.67
RCT, min	0.07	0.11 (0.01)	−0.87	0.77
a_{30}, mm	0.33	−0.31	0.16 (0.01)	−0.98
k_{20}, min	−0.40	0.62	−0.43	0.15 (0.01)

[1] Posterior means (PM) and posterior SD (PSD, in parentheses) of heritabilities on diagonal, PM of genetic correlations above diagonal. Phenotypic correlations below diagonal. a_{30} = curd firmness; CAS = casein %; k_{20} = curd firming time; RCT = rennet coagulation time.

Table 3. The LR validation results of pedigree indexes; the 95% bootstrap CI is reported in parentheses[1]

Trait	Bias	Dispersion	Correlation
CAS, %	−0.66 (−0.67, −0.65)	1.06 (1.05, 1.07)	0.61 (0.60, 0.61)
RCT, min	−0.06 (−0.07, −0.05)	1.10 (1.09, 1.10)	0.75 (0.75, 0.76)
a_{30}, mm	−0.43 (−0.43, −0.42)	1.26 (1.25, 1.27)	0.75 (0.75, 0.76)
k_{20}, min	0.35 (0.35, 0.36)	1.29 (1.29, 1.31)	0.65 (0.65, 0.66)

[1] a_{30} = curd firmness; CAS = casein %; k_{20} = curd firming time; RCT = rennet coagulation time.

Table 4. The DGV validation results with mixed (M, bulls + cows) and bulls-only (B) reference population[1]

Trait	Training	Animals	b	Reliability
CAS, %	B	3,276	1.205	0.452
	M	43,754	0.898	0.790
RCT, min	B	3,276	1.359	0.421
	M	43,754	0.925	0.737
a_{30}, mm	B	3,276	1.319	0.478
	M	43,754	0.911	0.767
k_{20}, min	B	3,276	1.246	0.459
	M	43,754	0.895	0.763

[1] a_{30} = curd firmness; b = dispersion coefficient; CAS = casein %; k_{20} = curd firming time; RCT = rennet coagulation time.

Table 5. Description of the different training populations used to evaluate the effect of adding females to the training set

Female proportion	Number of animals	Number of male	Number of female	Female-to-male ratio
1.00	43,754	3,276	40,478	12.36
0.75	32,326	3,276	29,050	8.87
0.50	22,206	3,276	18,930	5.78
0.25	12,087	3,276	8,811	2.69

k_{20} from 0.895 to 0.994. When moving from 100% to 75% of females, the average increase in the dispersion coefficient was 0.17%, although the pattern was not uniform across traits (RCT slightly decreases), and the others indicated a mild increase. From 75% to 50% of females, the average increase was 2.62%, and from 50% to 25%, it was 4.97%. Notably, the a_{30} trait shifted from a slight overestimation (coefficient <1) to a mild underestimation (coefficient >1) when only 25% of females were retained in the training population.

Genetic Trend and Relationships to Other Traits

The genetic trend of bulls' GEBV, calculated by using the mixed RP, by birth year is represented in Figure 5 to assess the effect of selection on the traits under investigation, as bulls are the main drivers of genetic progress and the sex in which its effects become evident first. Although none of the traits considered in the present study are included in the official genetic and genomic evaluation of Italian Holstein Friesian, the genetic merit for CAS and MCP increased constantly across time, suggesting that a possible, although of low magnitude, indirect selection for these features is currently undergoing. This speculation is evident by considering results of Figure 6, which represents the approximate genetic correlations between CAS and MCP to a selected group of productive and functional traits included in the National Holstein breeding program. Indeed, for all traits, with the exception of milk yield, correlations were generally favorable (Figure 6). By interpreting these correlations, it should be underlined that EBV for CAS and MCP are standardized to a mean of 100 and SD of 5, with genetic merit above 100 indicating favorable values. Therefore, values above 100 for RCT and k_{20} should be interpreted as "shorter" (RCT and k_{20}), and values above 100 for the remaining 2 traits should be interpreted as "stronger" a_{30} and "greater" CAS. In the case of milk yield, a weak and negative correlation was observed for CAS, a_{30}, and k_{20} (−0.37, −0.24, and −0.31, respectively), with RCT being the only exception, showing no correlation. A plausible explanation is that RCT reflects coagulation time primarily influenced by enzyme activity, making it less dependent on milk solids concentration. In contrast, a_{30} and k_{20} are more affected by the concentration of

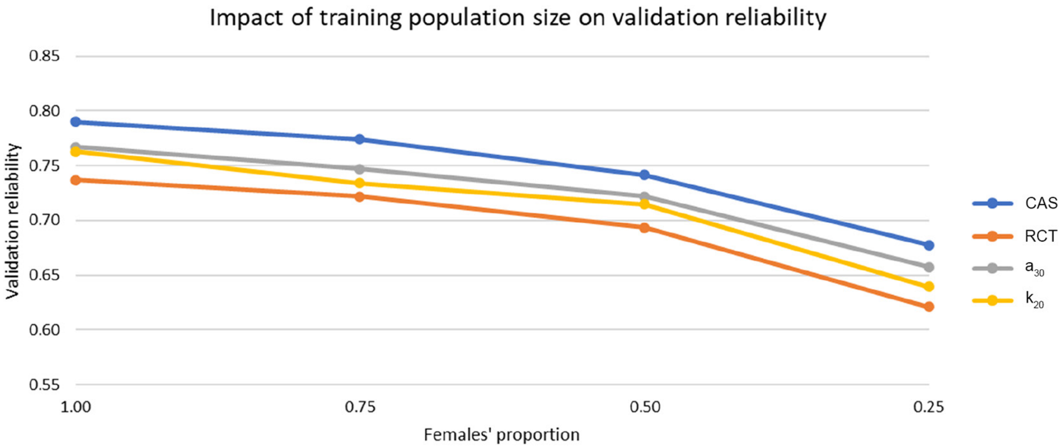

Figure 3. Impact on validation reliability of varying proportions of females included in the training population, relative to the complete males + females training (1.00).

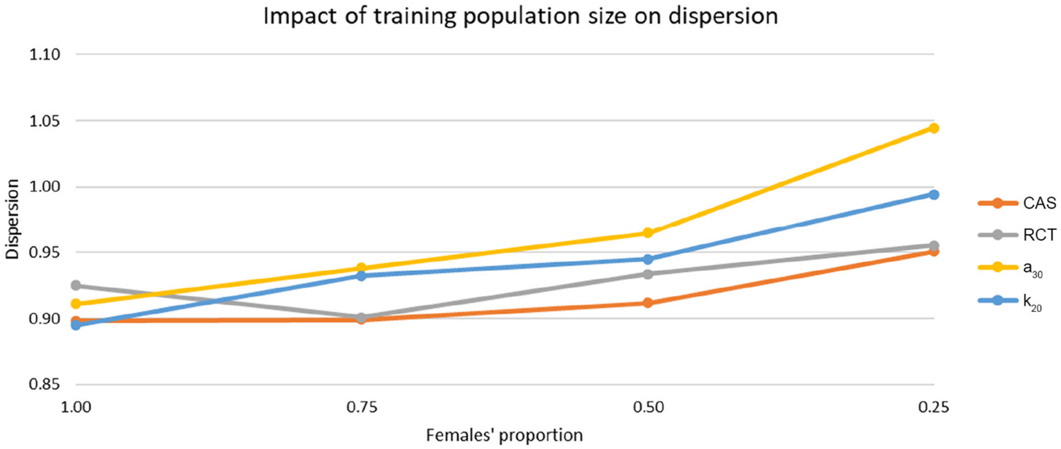

Figure 4. Impact on dispersion coefficient of varying proportions of females included in the training population, relative to the complete males + females training (1.00).

milk solids, which tends to be diluted in high-yielding cows. This distinction likely accounts for the lack of correlation between RCT and milk yield. These findings are consistent with previous studies (Cassandro et al., 2008), which also reported weak correlations between RCT and milk composition. The approximate genetic correlations between CAS and MCP to the other productive traits (yield and concentrations of fat and protein) were all positive and, in general, moderate to strong. For example, protein concentration was strongly correlated with both CAS (0.93) and k_{20} (0.67), moderately to a_{30} (0.53), although near zero to RCT. The latter was also weakly correlated with both fat concentration and yield (0.17 and 0.16, respectively). Fat concentration was also moderately genetically correlated with CAS (0.62), k_{20} (0.55), and a_{30} (0.48). Udder health traits (mastitis resistance and SCS) were all positively, although weakly, correlated with CAS and MCP (all approximate genetic correlations ≤0.20). The same applies also to fertility (defined as conception rate at first service), direct longevity, and heat tolerance, where the only approximate genetic correlations above 0.20 were the ones between CAS and both fertility (0.26) and heat tolerance (0.28), which was also weakly correlated with a_{30} (0.21) and k_{20} (0.26). All the other approximate genetic correlations (including those between CAS and MCP and direct longevity) were positive (i.e., favorable) but <0.20.

DISCUSSION

The present study aimed to assess the feasibility of genetically improving MCP in Italian Holstein population using MIR-predicted phenotypes, to develop a routine genetic evaluation for such traits, and to evaluate the possibility to include females in the RP for SNP effects estimation. The results of this study are consistent with previously reported h^2 estimates for MCP, which vary from 0.12 to 0.57 depending on the reference laboratory method used to quantify the traits, the statistical model applied, and the population studied (Ikonen et al., 1999; Cassandro et al., 2008; Vallas et al. 2010; Pretto et al., 2014). The h^2 range for CAS reported in the literature is narrower, being 0.23 to 0.46 (Ikonen et al., 1999; Cassandro et al., 2008; Vallas et al. 2010; Pretto et al., 2014).

The Use of Mid-Infrared Predictions for Breeding Purposes

The advantage of employing MIR-predicted traits for genetic evaluation is pivotal in case of difficult-to-measure traits such as MCP, where the reference laboratory technologies currently available for their quantification allow to assess RCT, k_{20}, and a_{30} only on a limited number of samples (10 samples/batch) in a relatively large amount of time (30 or 60 min; Visentin et al., 2015). Indeed, as indicated in Miglior et al. (2017), a potential trait to be considered for genetic selection must be (1) economically important, (2) exhibit genetic variation, and (3) clearly defined, measured at a low cost, and consistently recorded. Indicator traits, as in the case of MIR-predicted MCP instead of direct MCP measure (i.e., determined with the gold standard method), can be considered if strongly genetically correlated with the direct trait, or are measured cheaply and easily, or eventually earlier in life. Although the accuracy of MIR-prediction models for MCP allows their use only for screening purposes (De Marchi et al., 2013; Tiezzi et al., 2013), in general, genetic correlations between MIR-predicted and reference traits are stronger than their phenotypic counterpart. The coefficient of determination in cross-validation of MIRS

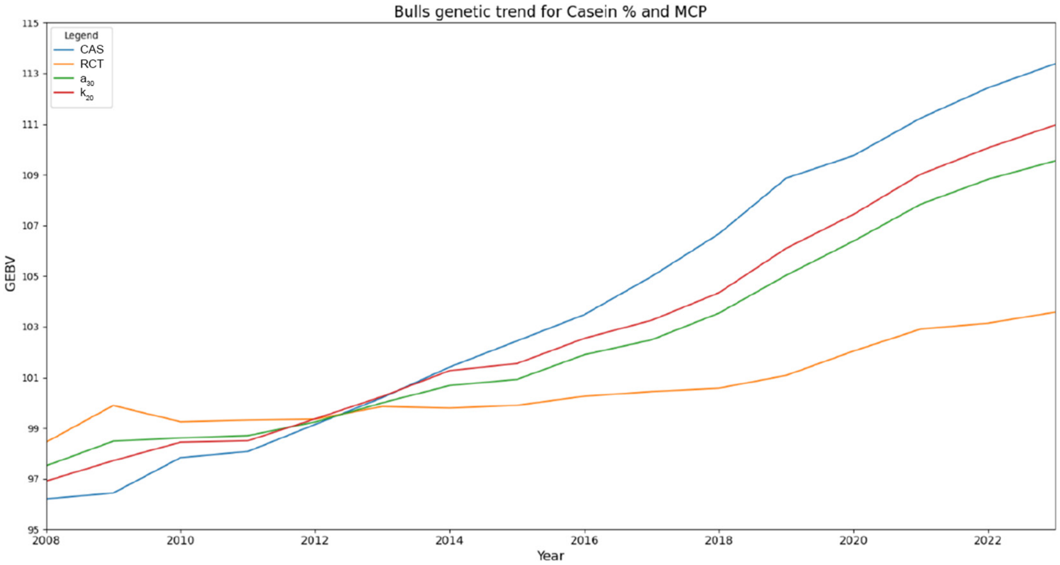

Figure 5. Genetic trends of genotyped bulls (average of the GEBV by birth year).

prediction models was 0.76, 0.72, and 0.70 for RCT, k_{20}, and a_{30} (De Marchi et al., 2013). As reviewed in Tiplady et al. (2020), this consideration has been proved for several milk quality characteristics, including MCP and SCC (Tiezzi et al., 2015), individual fatty acids (Rutten et al., 2010), and also milk protein fraction and free AA (McDermott et al., 2016; Visentin et al., 2022). One of the disadvantages of using MIR predictions as indicator traits, for breeding purposes, is related to the fact that the response to selection and the accuracy of selection when using MIR-predicted traits can be lower compared with the results achieved when using direct measures. This has been demonstrated in Costa et al. (2021), who developed near-infrared (**NIR**) spectrometry prediction models of IgG, IgA, and IgM in bovine colostrum. Indeed, in a simulated breeding base scenario where there is interest in improving colostrum IgG concentration, the use of NIR colostrum IgG prediction gave a response and accuracy of selection 25.69% and 26.03% lower, respectively, compared with the results achievable using direct measures (i.e., determined with the gold standard method) of IgG, at the same number of offspring with phenotypes/bull (set at 30; Costa et al., 2021). However, using NIR prediction of IgG and other correlated traits (such as NIR prediction of IgA and IgM) gave an equal response to selection and a slightly lower (−0.02 units) accuracy of selection, assuming again a constant number of offspring per bull. By increasing the number of daughters phenotyped/bull from 30 to 120, which is achievable using infrared technologies at no additional cost, the response of selection outperformed the base scenario by 17.27% (response to selection) and 17.81% (accuracy of selection). Therefore, these results highlight the potential of NIR/MIR spectrometry for large-scale phenotyping of traits that are otherwise difficult to measure. Although prediction accuracy may not be optimal, the method remains valuable due to the generally strong genetic correlations observed between predicted and reference values. However, careful consideration should be given to the accuracy and consistency of the predictions across different populations and environments. In the context of the present study, cheesemakers are particularly interested in the milk's ability to form a firm curd within an optimal timeframe, given the established association between a_{30} and cheese yield (Aleandri et al., 1989; Pretto et al., 2013). The strong genetic correlations observed among MCP traits and CAS, along with their moderate to high h^2 estimates, indicate that these 4 traits can be effectively included in a multitrait selection index to identify genetically superior individuals for enhancing the milk cheesemaking ability of Italian Holstein. Nevertheless, the results of the present study related to bulls' genetic trends indicated that an indirect selection for improved milk cheesemaking ability is currently in place, which can be explained by the genetic correlations calculated, particularly to protein and fat yields and concentrations.

Advantages of the Expansion of the RP Size

The current research proved the benefit of enlarging the RP size, with the inclusion of females, for the development of genomic prediction models. This has been demonstrated by comparing the results of dispersion and reliability of DGV calculated with a bulls-only or mixed

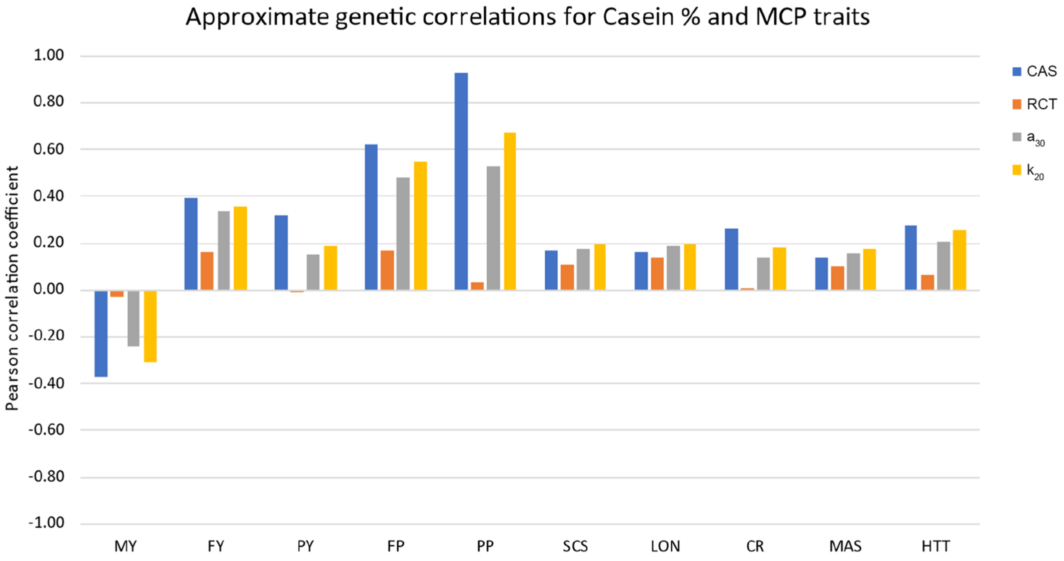

Figure 6. Approximate genetic correlations with other economically important traits under selection. CR = cow conception rate at first service; FP = fat percentage; FY = fat yield; HTT = heat tolerance; LON = direct longevity; MAS = mastitis resistance; MY = milk yield; PP = protein percentage; PY = protein yield.

RP. The National Holstein breeding program participates in the North American Intercontinental consortium for the exchange of bulls' genotypes and EBV between Italy, the United States, Canada, the United Kingdom, and Switzerland. The participation in this consortium is beneficial for the development of genomic prediction models for the national genomic evaluation, as it allows the expansion of the training population size by including closely related foreign bulls. However, this is possible just for the genomic evaluation of traits common to all the national breeding programs and part of Interbull multiple across-country genetic and genomic evaluation. The interest of genetically improving MCP is strictly related to the domestic dairy market and specialization, therefore, their large-scale recording internationally is limited. In this situation, which is applicable to all novel traits peculiar of a particular national breeding program, the training population can be limited in size, as it relies only on bulls with daughters phenotyped nationally. The expansion of the latter population, as indicated in Schöpke and Swalve (2016), can be achieved by combining data from different populations, such as countries or breeds (although dependent on the relationships between these populations), including bulls with imputed genotypes, or including female information. Nowadays the interest of farmers in herd genotyping is increasing due to the capacity to identify at a very early stage of life genetically elite heifers to be used as dams of cows through the use of sex-sorted semen. Therefore, the number of bulls genotyped, at the national level, is largely smaller than the number of nonselected females with genomic information. These data can therefore be used for setting up training populations for nationally recorded traits with ample benefits in terms of reliability, and simultaneously allowing the reduction of PSB, which is a common problem in multiple-step genomic evaluation based on bulls-only RP. Moreover, for traits where phenotypic recording has only recently begun—such as the MCP traits—including females in the training population is essential to achieve higher prediction accuracy (Buch et al., 2012). In the present study, the addition of the females increased the numerosity of the RP by 40,478 individuals, decreased the mean distance from one of the dispersion coefficients by 32%, and increased the mean reliability by 58%. The mixed training population performed better than the one composed of only bulls for both CAS and MCP. The advantages of females' inclusion have been proven also in 2 simulation studies (Plieschke et al., 2016; Togashi et al., 2019), as well as studies based on real data (Koivula et al., 2016), within the EuroGenomics consortium (Alkhoder et al., 2017), also for novel features such as health traits (Liu et al., 2019). When analyzing the effect of varying the number of females included in the training population, a marked improvement in accuracy was observed when moving from 25% to 50% female inclusion, corresponding to an increase in the female-to-male ratio from 2.69 to 5.78. At the same time, a progressive increase in overestimation was evident, particularly between these 2 levels of female representation in the training set.

CONCLUSIONS

In summary, the present study enhanced the understanding of the genetic parameters influencing MCP traits in the Italian Holstein population. The potential for genetic improvement of these traits within the breed was demonstrated and underscored the advantages of incorporating females into the RP for SNP effect estimation. Additionally, the results of this study allowed us to establish a routine genetic and genomic evaluation for CAS and MCP traits in Italian Holstein breed.

NOTES

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Nonstandard abbreviations used: a_{30} = curd firmness; BCI = bootstrap CI; CAS = casein percentage; CR = cow conception rate at first service; CY = cutting year; DGV = direct genomic values; EDP = estimated deregressed proofs; EDP_v = validation EDP; FP = fat percentage; FY = fat yield; GS = genomic selection; HTD = herd-year-test day; HTT = heat tolerance; k_{20}= curd firming time; LEO = Livestock Environment Open Data; LON = direct longevity; MAS = mastitis resistance; max = maximum; MCP = milk coagulation properties; min = minimum; MIRS = mid-infrared spectrometry; MY = milk yield; NIR = near-infrared; PM = posterior mean; PP = protein percentage; PSB = preselection bias; PSD = posterior SD; PY = protein yield; RCT = rennet coagulation time; RP = reference population.

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