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ORIGINAL ARTICLE

Genetic parameters for mid-infrared-spectroscopy-predicted mastitis phenotypes and related traits

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Abstract

Genetic improvement of udder health in dairy cows is of high relevance as mastitis is one of the most prevalent diseases. Since it is known that the heritability of mastitis is low and direct data on mastitis cases are often not available in large numbers, auxiliary traits, such as somatic cell count (SCC) are used for the genetic evaluation of udder health. In previous studies, models to predict clinical mastitis based on mid-infrared (MIR) spectral data and a somatic cell count-derived score (SCS) were developed. Those models can provide a probability of mastitis for each cow at every test-day, which is potentially useful as an additional auxiliary trait for the genetic evaluation of udder health. Furthermore, MIR spectral data were used to estimate contents of lactoferrin, a glycoprotein positively associated with immune response. The present study aimed to estimate heritabilities (h^2) and genetic correlations (r_a) for clinical mastitis diagnosis (CM), SCS, MIR-predicted mastitis probability (MIRprob), MIR + SCS-predicted mastitis probability (MIRSCSprob) and lactoferrin estimates (LF). Data for this study were collected within the routine milk recording and health monitoring system of Austria from 2014 to 2021 and included records of approximately 54,000 Fleckvieh cows. Analyses were performed in two datasets, including test-day records from 5 to 150 or 5 to 305 days in milk. Prediction models were applied to obtain MIR- and SCS-based phenotypes (MIRprob, MIRSCSprob, LF). To estimate heritabilities and genetic correlations bivariate linear animal models were applied for all traits. A lactation model was used for CM, defined as a binary trait, and a test-day model for all other continuous traits. In addition to the random animal genetic effect, the fixed effects year-season of calving and parity-age at calving and the random permanent environmental effect were considered in all models. For CM the random herd-year effect, for continuous traits the random herd-test day effect and the covariate days in milk (linear and quadratic) were additionally fitted. The obtained genetic parameters were similar in both datasets. The heritability found for CM was expectedly low ($h^2 = 0.02$). For SCS and MIRSCSprob, heritability estimates ranged from 0.23 to 0.25, and

for MIRprob and LF from 0.15 to 0.17. CM was highly correlated with SCS and MIRSCSprob ($r_a = 0.85$ to 0.88). Genetic correlations of CM were moderate with MIRprob ($r_a = 0.26$ and 0.37) during 150 and 305 days in milk, respectively and low with LF ($h^2 = 0.10$ and 0.11). However, basic selection index calculations indicate that the added value of the new MIR-predicted phenotypes is limited for genetic evaluation of udder health.

KEYWORDS

genetic correlation, heritability, lactoferrin, mastitis, mid-infrared spectroscopy, somatic cell count

1 | INTRODUCTION

The consideration of health traits for breeding purpose is of increasing importance in dairy cattle. It has started more than three decades ago in Scandinavian countries and is nowadays common in many countries around the globe (Philipsson & Lindhé, 2003; Zavadilová et al., 2021). In Austria, a genetic evaluation of direct health traits, based on diagnoses of veterinarians, was implemented for the breed Fleckvieh (dual purpose Simmental) in 2010 (Egger-Danner et al., 2012). Among health traits, mastitis is highly relevant as it is one of the most common diseases in dairy production. However, direct data on mastitis cases or diagnoses are often not available in large numbers or disease recording systems are not yet implemented. Therefore, a selection for udder health is in many countries based on genetically correlated or auxiliary traits, most commonly somatic cell count (SCC) or the corresponding logarithmic somatic cell score (SCS) (Interbull, 2022). SCC is the number of immune cells, mainly lymphocytes, polymorphonuclear neutrophils and macrophages, present in the milk. A higher SCC indicates an inflammatory response in the mammary gland (Schukken et al., 2003).

Besides data availability, the low heritability (0.001 to 0.06) of mastitis disease is another challenging aspect in the genetic evaluation of the trait (Fürst et al., 2021; Heringstad et al., 2000; Koeck et al., 2012; Koeck, Heringstad, Egger-Danner, Fuerst, & Fuerst-Waltl, 2010; Suntinger et al., 2022). Hence, the selection for auxiliary traits with a much higher heritability, such as SCC or the corresponding SCS (usually larger than 0.1), has a positive effect on selection response (Mrode & Swanson, 1998). The joint breeding value estimation program between Germany and Austria defined a 'udder health index' as follows: 30% clinical mastitis diagnoses +70% SCS + three auxiliary traits from linear udder scoring (Fürst et al., 2021).

An additional phenotype for genetic evaluation of udder health could potentially be provided by mid-infrared (MIR) spectral analyses of milk. MIR spectroscopy is the standard method in routine milk recording schemes to determine major milk components like fat, protein, urea, and lactose (Grelet et al., 2016), and has been applied to predict fine milk composition (Gengler et al., 2016). Since MIR spectroscopy is a fast, cheap, and high-throughput method, it has an involving role in the prediction of various traits relevant for herd management and animal breeding (Grelet et al., 2021; Tiplady et al., 2020).

For the purpose of udder health, diverse approaches using MIR spectral analyses have been applied (Dale & Werner, 2017; Rienesl, Khayatzdadeh, et al., 2022; Rienesl, Marginter, et al., 2022; Soyeurt et al., 2012). For instance, milk MIR spectra were used to estimate the content of the glycoprotein lactoferrin, which is known to be positively associated with immune response and hence a potential indicator for mastitis (Cheng et al., 2008; Li et al., 2004; Sordillo & Streicher, 2002). Reported heritability estimates of MIR-predicted lactoferrin (LF) were between 0.20 and 0.34 (Arnould et al., 2009; Nayeri et al., 2020; Soyeurt et al., 2007). Another approach was to use the MIR spectral information directly; models to predict clinical mastitis diagnoses were developed based on MIR spectra alone and in combination with SCS (Rienesl, Khayatzdadeh, et al., 2022), or combining MIR, SCS and differential somatic cell count (Rienesl, Marginter, et al., 2022). Those models classifying test-records of cows in "healthy" and "mastitis", reported balanced accuracies (mean of sensitivity and specificity) of 0.65 to 0.71. A probability of mastitis derived from the prediction models was available for each cow at every test-day.

To our knowledge, genetic parameters estimates have not been published yet for MIR-predicted mastitis probabilities. However, there are several studies on genetic parameter estimation of other phenotypes predicted from MIR spectra, such as blood β-hydroxybutyrate (Belay et al., 2017), energy intake and body energy status (McParland et al., 2015), and fertility (van den Berg et al., 2021), to name but a few.

The aim of the present study was to estimate heritabilities and genetic correlations for clinical mastitis diagnosis

(CM), SCS, MIR-predicted mastitis probability (MIRprob), MIR + SCS-predicted mastitis probability (MIRSCSprob) and lactoferrin estimates (LF). The results of the study will indicate whether MIR-predicted phenotypes are potentially useful for the genetic evaluation of udder health.

2 | MATERIALS AND METHODS

2.1 | Data and data editing

Data used for this study were routinely collected within the Austrian milk recording system and the Austrian health monitoring system (GMON) (Egger-Danner et al., 2012) between 2014 and 2021. Datasets, provided by ZuchtData GmbH, Vienna, Austria, consisted of comprehensive animal information, test-day milk records, MIR spectral data of milk, clinical mastitis diagnoses (acute and chronic) recorded by veterinarians, and pedigree information of approximately 54,000 dairy cows of the breed Fleckvieh (dual purpose Simmental). Only data of validated GMON farms with a minimum of 20 cows with records, where diagnoses were reported electronically by the herd veterinarian, were included (Egger-Danner et al., 2012; Suntinger et al., 2022). Data were further restricted to sires with at least 20 daughters to ensure an adequate data structure for variance component estimation.

Within the Austrian milk recording system, milk testing is done 9 to 11 times a year, which results in test-day performance information every 30–40 days. Each test-day record included the following information: encrypted animal and herd ID, date of test-day, parity, days in milk (DIM), milk yield, fat, protein, urea, lactose, SCC and MIR spectral data. To obtain MIR spectra of milk, samples are routinely analysed by laboratories of the Austrian milk recording system with spectrometers FT6000, FT+ and FT7 (FOSS® Instruments, Hillerød, Denmark). Resulting MIR spectral data express the absorbance of infrared light at 1060 wavenumbers from 926 to 5010 cm^{-1} and are routinely standardized to ensure comparability across time and laboratories (Grelet et al., 2015, 2017).

For analyses, two sets of data were created. Dataset 1 included test-day records from 5 to 150 days after calving, whereas mastitis diagnoses from −10 to 150 days from calving were considered, which is comparable with the data used for joint genetic evaluation of Austria and Germany (Fürst et al., 2021). Dataset 1 consisted of 399,961 test-day records. Dataset 2, which counted 772,069 records, included test-day records from 5 to 305 days after calving, in accordance with milk recording guidelines of ICAR (2022), were 305-days are defined as standard lactation. Records of cows with five or more lactations were grouped into one category, resulting into five parity classes (1, 2, 3, 4, 5+). The pedigree file included 190,864 animals and was generated by tracing the pedigrees of cows 5 generations back.

Data cleaning, merging and editing was done with the software SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Table 1 displays the characteristics of the datasets used for analyses.

2.2 | Trait definition

Investigations were carried out for five traits, clinical mastitis as reference trait and 4 additional continuous traits related to mastitis and udder health.

2.2.1 | Clinical mastitis (CM)

CM was defined as binary trait, 0 or 1 (healthy/diseased). Cows that had at least one clinical mastitis diagnosis (acute or chronic) by a veterinarian in the observation period were considered as 'diseased' in the respective lactation. The observation period was defined as −10 to 150 DIM for dataset 1, according to routine genetic evaluation in Austria and Germany (Fuerst et al., 2011), and −10 to 305 DIM for dataset 2. Cows without CM diagnosis were considered as 'healthy'. There was no distinction between acute and chronic diagnoses.

2.2.2 | Somatic cell score (SCS)

Measures of SCC were available continuously for every test-day. Records of SCC were logarithmically transformed into SCS by the formula

$$SCS = \log_2 (SCC / 100{,}000) + 3$$

according to Ali and Shook (1980), to achieve an approximately normal distribution of SCC.

TABLE 1 Characteristics of datasets (dataset 1: 5–150 DIM; and dataset 2: 5–305 DIM) used for analysis.

	Records (*n*)	
Unit	**Dataset 1**	**Dataset 2**
Animals (Pedigree)	189,800	190,864
Farms	1601	1610
Cows	53,639	53,910
Lactations total	117,952	118,754
With mastitis diagnosis	6384	12,933
Test-day records	399,961	772,069

Abbreviation: DIM, days in milk.

2.2.3 | MIR-predicted mastitis probability (MIRprob)

A calibration model to predict clinical mastitis diagnosis based on MIR spectral data, developed in a previous study (Rienesl, Khayatzdadeh, et al., 2022), was used to obtain a probability of mastitis, ranging from 0.0 to 1.0, for every cow's single test-day record. According to Rienesl, Khayatzdadeh, et al. (2022), prediction was done with partial least squares discriminant analysis (PLS-DA). Only selected parts of the MIR spectra, corrected for DIM, were used for modelling, and test-day records ±21 days from diagnosis were considered as 'mastitis cases'. Data, dataset 1 and dataset 2 respectively, were each randomly split into half by farm:

1. Model calibration was performed on the first half of data and validation on the second half of the data.
2. Datasets were exchanged; the second half of the data was used for model calibration and the first half of the data for validation.

The applied PLS-DA models classified records based on their prediction probability in 'healthy' ($p < 0.5$) or 'mastitis' ($p \geq 0.5$). Standard class statistics were used to describe accuracy of the models: sensitivity (proportion of records correctly classified as 'mastitis'), specificity (proportion records correctly classified as 'healthy'), and balanced accuracy (balanced mean of sensitivity and specificity).

Prediction probabilities, defined as MIR-predicted mastitis probabilities, of the validation datasets were finally used for further analyses and genetic parameter estimation.

2.2.4 | MIR + SCS-predicted mastitis probability (MIRSCSprob)

Again, a previously developed PLS-DA model based on MIR spectra and SCS was applied to derive probabilities of mastitis. SCS and selected parts of the MIR spectra, corrected for DIM, were used for modelling, and test-day records ±21 days from diagnosis considered as mastitis cases (Rienesl, Khayatzdadeh, et al., 2022). The procedure of prediction, including switching of calibration and validation datasets, was equal to the MIR model described above; MIR + SCS-predicted mastitis probabilities of the validation datasets were used for further analyses.

2.2.5 | Lactoferrin estimates (LF)

A prediction equation to estimate contents of lactoferrin (mg/L) was developed by Soyeurt et al. (2012). A second version (V2) of the lactoferrin prediction equation was developed in 2017 by the Walloon Agricultural Research Center (CRA-W) as part of an internal project of the European Milk recording EEIG organization (EMR) (C. Grelet, 2022, CRA-W, Gembloux, personal communication); the dataset ($n = 2189$) which was used for calibration was described by Soyeurt et al. (2020) as the second dataset in the document. The V2 lactoferrin equation is already implemented in routine and currently used by the European Milk recording EEIG organization (EMR EEIG, 2022).

2.3 | Descriptive statistics

The mean was calculated for CM (proportion in %) and means plus standard deviations (SD) for the four other continuous traits, separately for dataset 1 (5–150 DIM) and dataset 2 (5–350 DIM) (Table 2). Incidences of CM (in %) were plotted for parity classes (Figure 1a). For the continuous traits SCS, MIRprob, MIRSCSprob and LF, daily trend of means and 95% confidence intervals were visualized for each parity class in the lactation period from 5 to 305 days in milk (Figures 2–5).

TABLE 2 Descriptive statistics (mean and SD in parentheses) of analysed traits in dataset 1 (including records from 5 to 150 DIM) and dataset 2 (including records from 5 to 305 DIM).

Trait	Description of trait	Dataset 1 5–150 DIM	Dataset 2 5–305 DIM
CM, %	Cows with at least 1 CM diagnosis	5.73 (n. a.)	10.89 (n. a.)
SCS	Mean SCS in lactation	1.74 (1.79)	2.02 (1.73)
MIRprob	Probability of mastitis from MIR prediction model	0.47 (0.08)	0.47 (0.07)
MIRSCSprob	Probability of mastitis from MIR + SCS prediction model	0.44 (0.09)	0.45 (0.09)
LF, mg/L	Estimates of lactoferrin predicted from MIR spectra	88.64 (98.94)	126.46 (113.24)

Abbreviations: CM, clinical mastitis diagnosis; DIM, days in milk; MIR, mid-infrared spectra; n. a., not available; SCS, somatic cell score; SD, standard deviation.

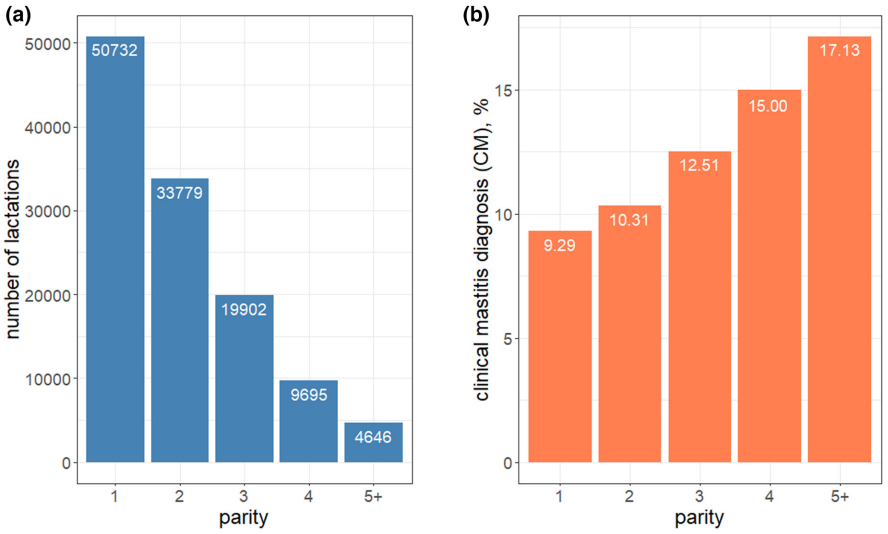

FIGURE 1 Total number of lactations per parity class (a) and percentage share of lactations with a clinical mastitis diagnosis (CM) per parity class (b), in dataset 2, considering test-day records from 5 to 305 days in milk and clinical mastitis diagnoses from −10 to 305 days in milk.

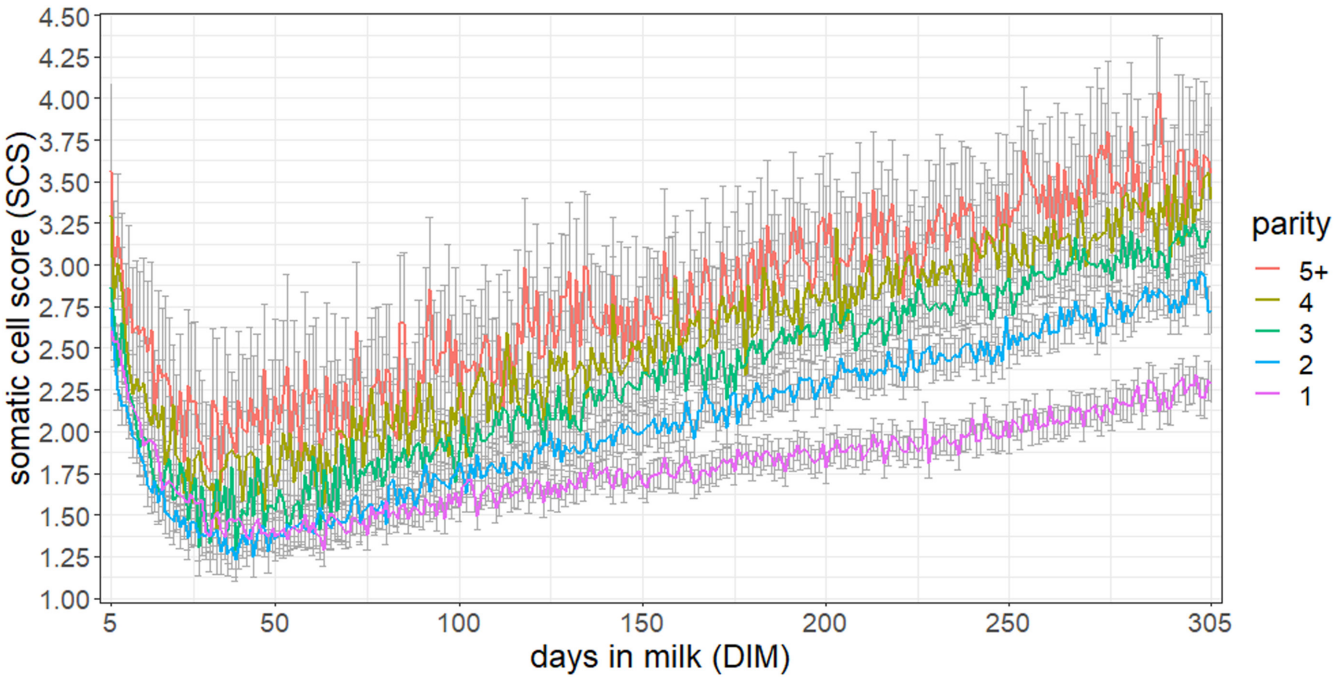

FIGURE 2 Trend of daily means and 95% confidence intervals of somatic cell score (SCS) in the lactation period from 5 to 305 days in milk (dataset 2), separately plotted for each parity class (1, 2, 3, 4, 5+).

2.4 | Statistical models for parameter estimation

Genetic parameters were estimated by applying the restricted maximum likelihood (REML) method as implemented in the software package VCE-6 (version 6.0.2; Groeneveld et al., 2010). Bivariate linear animal models were fitted for pairwise combinations of all traits. A lactation model was used for the binary trait CM:

$$Y = X\beta + Z_h h + Z_{pe} pe + Z_a a + e$$

where Y is the observation of interest; β is a vector of systematic effects, including fixed effects of year-season of calving, and parity-age at calving; h is a vector of random herd-year effects; pe is a vector of random permanent environmental effects; a is a vector of random animal effects; e is a vector of random residuals; and X, Z_h, Z_{pe}, and Z_a are the corresponding incidence matrices.

For the continuous traits SCS, MIRprob, MIRSCSprob, and LF, a test-day model was applied:

$$Y = X\beta + Z_h h + Z_{pe} pe + Z_a a + e$$

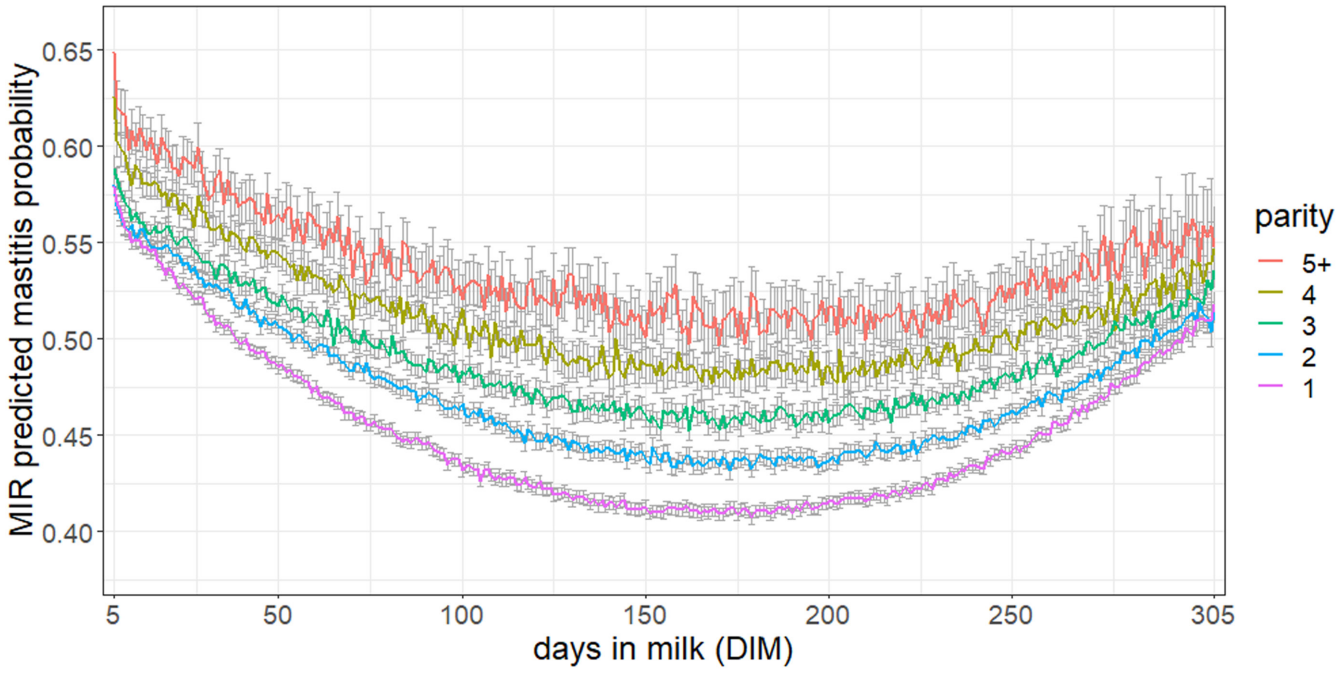

FIGURE 3 Trend of daily means and 95% confidence intervals of MIR-predicted mastitis probabilities (MIRprob) in the lactation period from 5 to 305 days in milk (dataset 2), separately plotted for each parity class (1, 2, 3, 4, 5+).

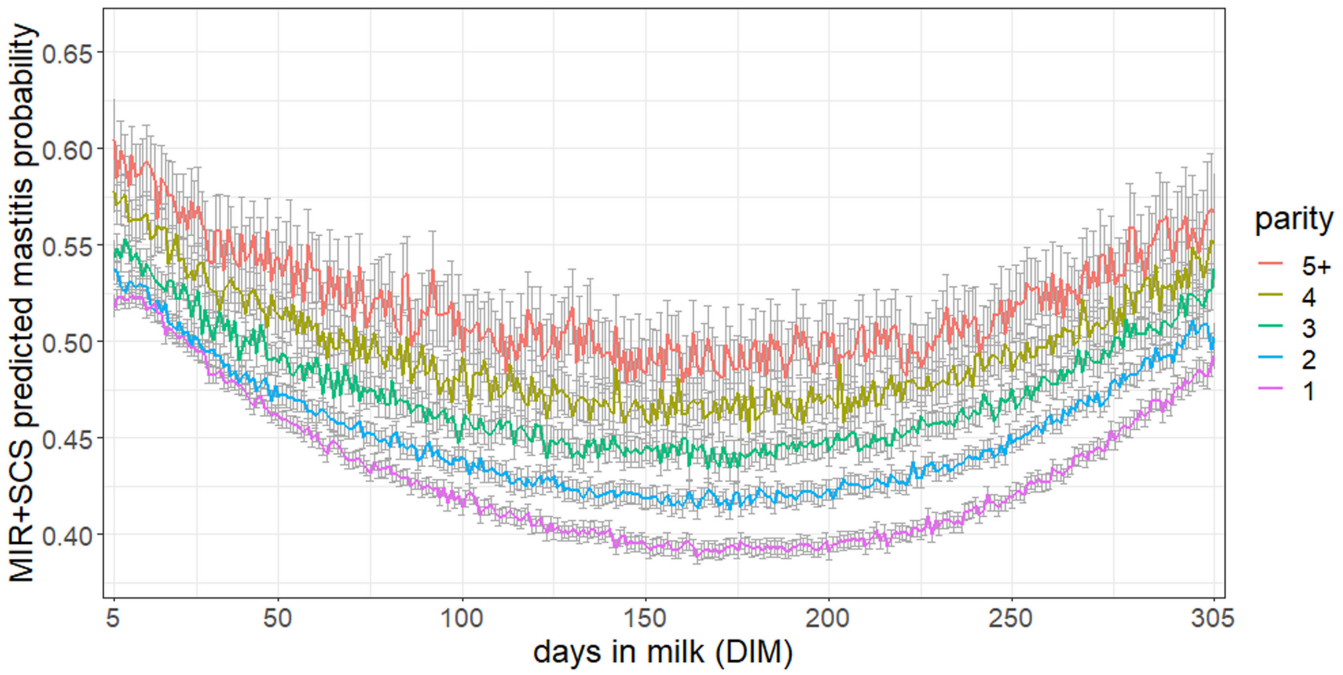

FIGURE 4 Trend of daily means and 95% confidence intervals of MIR + SCS-predicted mastitis probabilities (MIRSCSprob) in the lactation period from 5 to 305 days in milk (dataset 2), separately plotted for each parity class (1, 2, 3, 4, 5+).

where Y is the observation of interest; β is a vector of systematic effects, including fixed effects of year-season of calving, parity-age at calving, and DIM (linear and quadratic); h is a vector of random herd-test-day effects; pe is a vector of random permanent environmental effects; a is a vector of random animal effects; e is a vector of random residuals; and X, Z_h, Z_{pe}, and Z_a are the corresponding incidence matrices.

Statistical model for CM was similar with the model used for routine genetic evaluation in Austria (Fürst et al., 2021). For cows of first parity, eight calving-age classes were created. Parity-age of first lactating cows

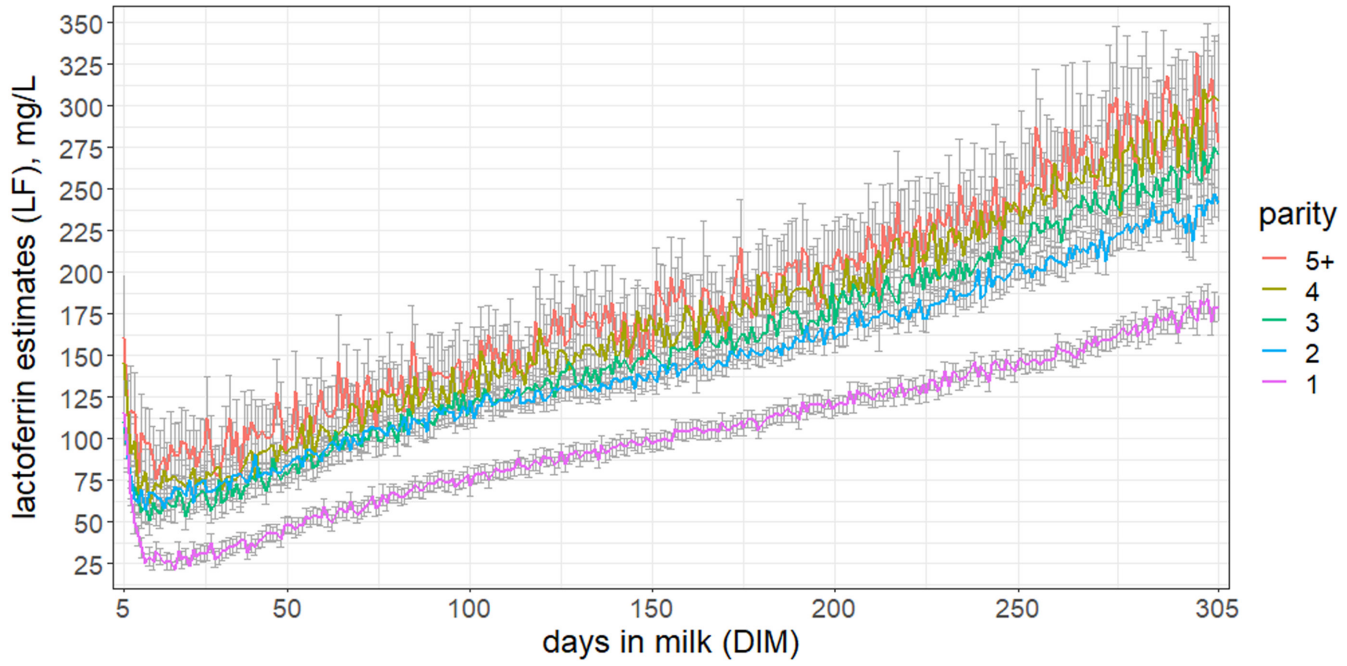

FIGURE 5 Trend of daily means and 95% confidence intervals of MIR-predicted lactoferrin estimates (LF) in the lactation period from 5 to 305 days in milk (dataset 2), separately plotted for each parity class (1, 2, 3, 4, 5+).

was grouped based on months: <23, 23–26, 27–29, 30–32, 33–35, 36–38, 39–41 and >41. For cows with more than one calving, parity-age classes were 2, 3, 4, and 5+. So, in total there were 12 classes of the effect parity-age at calving. The fixed effect year-season of calving expressed 32 levels in both datasets and the random herd-year effect expressed 65,458 levels in dataset 1 and 70,221 in dataset 2.

2.5 | Selection index calculations

For basic selection index calculations, a multi-trait selection index program (MTINDEX; Van der Werf, 2018) was used. Based on the estimated genetic parameters, accuracy of single trait EBV (estimated breeding value) was estimated for each trait (CM, SCS, MIRprob, MIRSCSprob and LF) and accuracy for multiple trait EBV was estimated for CM, considering different combinations of auxiliary traits. For the prediction of the accuracy of EBVs, only the presence of progeny information was considered, assuming individuals having 20 daughters with phenotype records.

3 | RESULTS

3.1 | MIR and MIR + SCS prediction models

The PLS-DA model to predict clinical mastitis diagnoses based on MIR spectra was evaluated with a sensitivity of 0.62, a specificity of 0.68, and a balanced accuracy of 0.65 in dataset 1 (5–150 DIM). The respective values for dataset 2 (5–305 DIM) were: 0.60, 0.68, and 0.64. The prediction model, combining MIR spectra and SCS, showed a sensitivity of 0.67, a specificity of 0.77; and a balanced accuracy of 0.72 in dataset 1 and corresponding values of 0.64, 0.75, and 0.69 in dataset 2.

3.2 | Descriptive statistics

Figure 1a shows the distribution of the total number of lactations among parity classes (1, 2, 3, 4, 5+); with the majority belonging to parity class 1 ($n = 50{,}732$) and the minority to parity class 5+ ($n = 4646$). The frequency of lactations with at least one CM diagnosis in dataset 1, where only diagnoses within −10 to 150 DIM were considered, was 5.73%. In dataset 2, considering diagnoses within −10 to 350 DIM, the frequency was 10.9% among all parity classes (Table 2); it was lowest for first parity cows with 9.29% and increased to 17.13% for cows with parities of 5 or more (Figure 1b).

Mean SCS (SCC) of all test-day records was 1.74 (123,527 cells/mL) in dataset 1 and 2.02 (131,111 cells/mL) in dataset 2 (Table 1). For the traits MIRprob and MIRSCSprob, the following mean probabilities were found in dataset 1 and dataset 2 respectively: 0.47/0.47 and 0.44/0.45. SDs for MIRprob and MIRSCSprob were low in both datasets, ranging from 0.07 and 0.09. Mean LF was 88.64 in dataset 1 and 126.46 mg/L in dataset 2.

The curve, from 5 to 305 days in milk, of SCS (SCC) was on average high shortly after calving with values above 2.8 (250,000 cells/mL), decreased to a minimum of 1.4 (>100,000 cells/mL) around DIM 40 and increased slowly up to 2.6 (>150,000 cells/mL) at the end of the observed lactation period (Figure S1). Figure 2 displays SCS curves separately for each parity class; similar patterns but different levels, namely mostly higher SCS with increased parity, could be observed. Only in the first 50 days of lactation, mean SCS was slightly higher for parity class 1 compared to parity class 2.

The trend across lactation of MIRprob are visualized in Figure 3, showing higher MIR-predicted mastitis probabilities for cows of higher parity. Among all parity classes, MIRprob was highest (0.58) at the beginning of the lactation period, slowly decreased to a minimum of 0.46 around DIM 170, and increased again towards DIM 305 to 0.52 (Figure S2).

Figure 4 displays the curves of probabilities derived from the MIR + SCS model (MIRSCSprob). Probabilities were higher for multiparous cows on each DIM, similar to MIRprob, whereas levels were generally lower for MIRSCSprob compared to MIRprob. Mean probability of all parity classes together was 0.54 at DIM 5, decreased to a minimum of 0.43 around DIM 170, and further increased until DIM 305 to 0.51 (Figure S3).

The trend of daily mean LF on DIM 5–305 is shown in Figure 5, separately for each parity class (1, 2, 3, 4, 5+). Estimates of lactoferrin were lowest for first parity cows on each DIM. Highest values were found for parity class 5+, whereas differences to parity classes 2–4 were small, especially until DIM 150. Mean LF among all parity classes was 120 mg/L on DIM 5, decreased very steeply to 50 mg/L around DIM 10, and increased almost linearly towards the end of the lactation period up to 215 mg/L (Figure S4).

3.3 | Genetic parameters

Heritabilities and genetic and phenotypic correlations for clinical mastitis (CM), MIR-predicted mastitis probability (MIRprob), MIR + SCS-predicted mastitis probability (MIRSCSprob), and lactoferrin estimates (LF) are given in Table 3 for dataset 1 (5–150 DIM) and in Table 4 for dataset 2 (5–305 DIM). Heritabilities displayed on the diagonal are means of the heritability estimates from all respective bivariate analyses.

In dataset 1 mean heritability estimates were 0.02, 0.25, 0.17, 0.26, and 0.17 for CM, SCS, MIRprob, MIRSCSprob, and LF. SE for heritability estimates showed a low variation among traits. CM was strongly genetically correlated

	Dataset 1: 5–150 DIM				
Trait	**CM**	**SCS**	**MIRprob**	**MIRSCSprob**	**LF**
CM	**0.02** (0.002)	0.85 (0.042)	0.26 (0.067)	0.85 (0.045)	0.10 (0.067)
SCS	0.16	**0.25** (0.009)	0.41 (0.020)	0.97 (0.001)	0.27 (0.022)
MIRprob	0.08	0.33	**0.17** (0.005)	0.50 (0.019)	0.60 (0.014)
MIRSCSprob	0.15	0.96	0.43	**0.26** (0.009)	0.31 (0.021)
LF	0.05	0.29	0.56	0.35	**0.17** (0.005)

Abbreviations: DIM, days in milk; MIR, mid-infrared spectra; SE, standard error.

TABLE 3 Mean heritabilities (in bold on diagonal, with mean SE in parentheses), genetic correlations (above diagonal, with SE in parentheses), and phenotypic correlations (below diagonal, SE not available) for clinical mastitis (CM), somatic cell score (SCS), MIR-predicted mastitis probability (MIRprob), MIR + SCS-predicted mastitis probability (MIRSCSprob), and lactoferrin estimates (LF) from bivariate linear animal models in dataset 1 (including records from 5 to 150 DIM).

	Dataset 2: 5–305 DIM				
Trait	**CM**	**SCS**	**MIRprob**	**MIRSCSprob**	**LF**
CM	**0.02** (0.002)	0.86 (0.038)	0.31 (0.067)	0.87 (0.034)	0.11 (0.067)
SCS	0.16	**0.23** (0.003)	0.38 (0.004)	0.97 (0.001)	0.25 (0.003)
MIRprob	0.07	0.31	**0.15** (0.002)	0.42 (0.004)	0.58 (0.003)
MIRSCSprob	0.15	0.90	0.49	**0.20** (0.003)	0.31 (0.004)
LF	0.06	0.30	0.52	0.32	**0.15** (0.002)

Abbreviations: DIM, days in milk; MIR, mid-infrared spectra; SE, standard error.

TABLE 4 Mean heritabilities (in bold on diagonal, with mean SE in parentheses), genetic correlations (above diagonal, with SE in parentheses), and phenotypic correlations (below diagonal, SE not available) for clinical mastitis (CM), somatic cell score (SCS), MIR-predicted mastitis probability (MIRprob), MIR + SCS-predicted mastitis probability (MIRSCSprob), and lactoferrin estimates (LF) from bivariate linear animal models in dataset 2 (including records from 5 to 305 DIM).

to SCS and MIRSCSprob (0.85). The genetic correlation between SCS and MIRSCSprob was also very high (0.97). The genetic correlation of CM was moderate with MIRprob (0.26) and low with LF (0.10). Moderate genetic correlations were also found between SCS and MIRprob (0.41), MIRprob and MIRSCSprob (0.50), SCS and LF (0.27), and MIRSCSprob and LF (0.31). A strong genetic correlation was also estimated between LF and MIRprob (0.60).

Estimates of heritabilities and genetic correlations were very similar in dataset 2 (5–305 DIM) compared to dataset 1 (5–150 DIM). Mean heritability was identical for CM (0.02) and slightly lower for SCS, MIRprob, MIRSCSprob, and LF (0.23, 0.15, 0.20, and 0.15). Genetic correlation of CM was again strongest with SCS (0.86) and MIRSCSprob (0.87). Compared to dataset 1, the genetic correlation between CM and MIRprob was somewhat higher with 0.31, and between SCS and MIRprob somewhat lower with 0.38. The biggest difference between the two datasets was found for the genetic correlation between MIRprob and MIRSCSprob, which was lower in dataset 2 (0.42 vs. 0.50). The genetic correlations between all other traits in dataset 2 differed only slightly from those in dataset 1.

Phenotypic correlations among all traits are displayed below the diagonal of Tables 3 and 4 respectively. The strongest phenotypic correlation was found between SCS and MIRSCSprob (>0.90). Rather high phenotypic correlations were also found between LF and MIRprob (>0.50) and between SCSMIRprob and MIRprob (>0.43). The phenotypic correlation of CM was moderate with SCS (0.16) and SCSMIRprob (0.15) in both datasets, and relatively low with MIRprob (0.08/0.07) and with LF (0.05/0.06). Moderate phenotypic correlations (approximately 0.30) were also found between SCS and MIRprob, SCS and LF, and SCSMIRprob and LF.

3.4 | Comparison of EBV accuracies for CM

The results of the estimation of EBV accuracies showed that the accuracy of single trait EBV for CM was 0.30 in both datasets (Tables 5 and 6). The multiple trait selection approach, combining CM + SCS resulted in an accuracy of multiple trait (MT) EBV of CM of approximately 0.65 in dataset 1 and 2. For the index CM + SCSMIRprob the accuracy of MT EBV of CM was 0.66 in dataset 1 and 0.64 in dataset 2. The corresponding values for CM + MIRprob were 0.34 and for CM + LF 0.31 in both datasets respectively. Indices of various trait combinations resulted in accuracies of MT EBV of CM between 0.66 and 0.68 in dataset 1 and between 0.65 and 0.66 in dataset 2.

4 | DISCUSSION

The aim of the present study was to estimate genetic parameters for MIR-predicted phenotypes associated with mastitis and udder health in a large dataset, collected during routine milk recording in Austria. The balanced

TABLE 5 Estimated accuracies of single trait estimated breeding values (EBV) for clinical mastitis (CM), somatic cell score (SCS), MIR-predicted mastitis probability (MIRprob), MIR + SCS-predicted mastitis probability (MIRSCSprob), and lactoferrin estimates (LF) and estimated accuracies of multiple trait EBV for CM using different auxiliary traits. Estimates are based on previously derived genetic parameters of dataset 1 (including records from 5 to 305 DIM), assuming information of 20 progeny.

Dataset 1: 5–150 DIM						
	Single trait EBV					Multi trait EBV
Trait (with 20 progeny records each)	CM	SCS	MIR prob	MIRSCS prob	LF	CM
CM + SCS	0.302	0.756	-	-	-	0.654
CM + MIRprob	0.302	-	0.686	-	-	0.336
CM + MIRSCSprob	0.302	-	-	0.763	-	0.660
CM + LF	0.302	-	-	-	0.686	0.307
CM + SCS + MIRprob	0.302	0.756	0.686	-	-	0.657
CM + SCS + MIRprob + LF	0.302	0.756	0.686	-	0.686	0.664
CM + SCSMIRprob + LF	0.302	-	-	0.763	0.686	0.676
CM + SCS + LF	0.302	0.756	-	-	-	0.676
CM + SCS + MIRprob + MIRSCSprob	0.302	0.756	0.686	0.763	-	0.674
CM + SCS + MIRprob + MIRSCSprob + LF	0.302	0.756	0.686	0.763	0.686	0.680

Abbreviations: DIM, days in milk; EBV, estimated breeding value; MIR, mid-infrared spectra.

TABLE 6 Estimated accuracies of single trait estimated breeding values (EBV) for clinical mastitis (CM), somatic cell score (SCS), MIR-predicted mastitis probability (MIRprob), MIR + SCS-predicted mastitis probability (MIRSCSprob), and lactoferrin estimates (LF) and estimated accuracies of multiple trait EBV for CM using different auxiliary traits. Estimates are based on previously derived genetic parameters of dataset 2 (including records from 5 to 150 DIM), assuming information of 20 progeny.

Dataset 2: 5–305 DIM						
	Single trait EBV					Multi trait EBV
Trait (with 20 progeny records each)	CM	SCS	MIR prob	MIRSCS prob	LF	CM
CM + SCS	0.302	0.741	-	-	-	0.649
CM + MIRprob	0.302	-	0.662	-	-	0.348
CM + MIRSCSprob	0.302	-	-	0.716	-	0.637
CM + LF	0.302	-	-	-	0.662	0.307
CM + SCS + MIRprob	0.302	0.741	0.662	-	-	0.650
CM + SCS + MIRprob + LF	0.302	0.741	0.662	-	0.662	0.659
CM + SCSMIRprob + LF	0.302	-	-	0.716	0.662	0.649
CM + SCS + LF	0.302	0.741	-	-	-	0.658
CM + SCS + MIRprob + MIRSCSprob	0.302	0.741	0.662	0.716	-	0.655
CM + SCS + MIRprob + MIRSCSprob + LF	0.302	0.741	0.662	0.716	0.662	0.664

Abbreviations: DIM, days in milk; EBV, estimated breeding value; MIR, mid-infrared spectra.

accuracy of the model to predict clinical mastitis based on MIR was 0.65 and for the model based on MIR + SCS it was 0.71. The MIR-based prediction equation applied to estimate contents of lactoferrin was evaluated with R^2cv of 0.66. Hence, such MIR-based prediction models and equations do not allow precise determination of lactoferrin content and accurate classification of cows with or without mastitis infection. However, they can be used for monitoring and screening purpose on individual cow and herd level (Rienesl, Khayatzdadeh, et al., 2022; Rienesl, Marginter, et al., 2022; Soyeurt et al., 2020). Further, several other MIR-predicted phenotypes were evaluated as being useful for genetic investigations at population level (Belay et al., 2017; Benedet et al., 2020).

The definition of the trait CM was applied according to routine genetic evaluation in Austria and Germany (Fuerst et al., 2011) to make results comparable and applicable. However, this definition implies that a cow that had one diagnosis of mastitis is equivalent to a cow that had multiple positive diagnoses in the defined period, whereas the traits SCS, MIRprob, MIRSCSprob, and LF are referred to test-days.

The frequency of lactations with at least one CM diagnosis in dataset 1 was lower compared to dataset 2 (5.7% vs. 10.9%), because in dataset 1 only diagnoses within −10 to 150 DIM were considered, whereas in dataset 2 diagnoses until DIM 305 were included. These frequencies are comparable to other studies on clinical mastitis in Austrian Fleckvieh cows, that reported frequencies of CM in the range of 7.7% to 9.6% (Koeck, Heringstad, Egger-Danner, Fuerst, Winter, & Fuerst-Waltl, 2010; Suntinger et al., 2022). An increase of mastitis incidence with parity was recently also reported by Lean et al. (2022), were mastitis incidence was 2.5 times greater in parity ≥5 than in parity 1. The patterns of the trends across lactation of SCS were consistent with findings reported by several other studies (De Haas et al., 2002; Græsbøll et al., 2016; Kirkeby et al., 2020; Suntinger et al., 2022; Wiggans & Shook, 1987).

Patterns of curves of MIR- and MIR + SCS-predicted mastitis probabilities (Figures 3 and 4) were very similar but deviated from the curves of SCS and LF (Figures 2 and 5). We did not find trends across lactation of similar MIR-predicted traits in literature.

Regarding MIR-predicted contents of lactoferrin, a large difference was found between the two datasets, 88.64 ± 98.94 in dataset 1 (5–150 DIM) and 126.46 ± 113.24 in dataset 2 (5–305 DIM). Soyeurt et al. (2007) and Arnould et al. (2009) used similar MIR-based equations to estimate contents of lactoferrin; the observed mean LF were 189.08 ± 155.88 and 137.80 ± 176.74, in datasets including different breeds and test-day records from 5 to 365 DIM. Hence, higher mean LF were found compared to the current study, and variation of LF contents was large in all three studies. According to Soyeurt et al. (2007), the large deviation in LF content could reflect the high variation during the lactation period or could be explained by the mix of breeds. The change of MIR-predicted LF contents during the lactation period, for each parity class, are displayed in Figure 5. Following a high concentration and a drop in the very first days of lactation, LF increased as lactation progressed. LF contents also increased with

parity number. Similar patterns were described by Soyeurt et al. (2007) and by Fleming et al. (2019).

The linear model estimates of heritability of CM (0.02) was in agreement with the heritability of clinical mastitis reported from the joint Austrian and German genetic evaluation (Fürst et al., 2021). Other studies on Austrian Fleckvieh cattle (Koeck, Heringstad, Egger-Danner, Fuerst, Winter, & Fuerst-Waltl, 2010; Suntinger et al., 2022) also found heritabilities in a similar range. Results were also in accordance with a literature review of Heringstad et al. (2000), that found heritability estimates in the range from 0.001 to 0.06 for mastitis, with most values ranging from 0.02 to 0.03. Heritability estimates of SCS, 0.25 in dataset 1 and 0.23 in dataset 2, were higher compared to other Austrian studies (Fürst et al., 2021; Koeck, Heringstad, Egger-Danner, Fuerst, Winter, & Fuerst-Waltl, 2010), where they were around 0.12. The Finnish Animal Breeding Association reported heritabilities of SCS in the range of 0.14 to 0.23, depending on the breed (Luttinen & Juga, 1997). Whereas a Swiss study (Dal Zotto et al., 2007) estimated a heritability of 0.06 for SCS. From these studies it is apparent that the heritability estimates of SCS and SCC show a large variability across countries and breeds. Heritability estimates for MIRSCSprob (0.26 and 0.20) were in the range of estimates for SCS, while estimated heritabilities of MIRprob were lower (0.17 and 0.15), indicating the large effect of SCS in the MIR + SCS prediction model. For MIR-predicted LF contents, heritability estimates (0.17 and 0.15) were equal to the trait MIRprob, similar and comparable with findings of Soyeurt et al. (2007), Arnould et al. (2009), and Lopez-Villalobos et al. (2009), who reported heritability estimates for MIR-predicted lactoferrin ranging from 0.16 to 0.22.

Estimated genetic correlations between CM and SCS were bit stronger (0.85 and 0.86) in the present study compared to values reported by Koeck, Heringstad, Egger-Danner, Fuerst, Winter, and Fuerst-Waltl (2010) and (Fürst et al., 2021), that were in the range of 0.64 to 0.71. Suntinger et al. (2022) found genetic correlation of 0.53 between SCS and acute CM, and genetic correlation of 0.61 between SCS and chronic CM. MIRSCSprob showed an equally strong genetic correlation to CM (0.85 and 0.87) as SCS did, underlining the large impact of SCS in the PLS-DA model based on MIR + SCS. Moreover, the genetic correlation between SCS and MIRSCSprob was 0.97, indicating that both traits provide largely the same information. The genetic correlations between CM and MIRprob were much lower compared to CM and SCS, but still at a moderate level. Further, the genetic correlations between SCS and MIRprob were also at a moderate level. These findings might indicate that MIR spectra of milk potentially provide additional information related to mastitis besides SCS. As stated before, the udder health index of the Austrian and German joint genetic evaluation, currently contains relative weights of 30% clinical mastitis diagnoses and 70% SCS and additionally considers auxiliary traits from linear udder scoring. Those auxiliary traits hold the following genetic parameters: $h^2 = 0.21$ and r_a to CM = 0.38 (fore udder attachment), $h^2 = 0.33$ and r_a to CM = 0.64 (udder depth), and $h^2 = 0.28$ and r_a to CM = 0.28 (teat placement front) (Fürst et al., 2021). Hence, the trait MIRprob showed a similar genetic correlation to CM as the traits fore udder attachment and teat placement front.

Regarding MIR-predicted contents of lactoferrin (LF), the genetic correlations with CM were very low (0.10 and 0.11). This result was not necessarily expected, since lactoferrin is known to contribute positively to resistance to mastitis infections (Cheng et al., 2008; Li et al., 2004; Sordillo & Streicher, 2002). However, infection of the mammary gland also leads to an increase in lactoferrin (Shimazaki & Kawai, 2017). Thus, in both cases, lactoferrin content in milk is high, but the udder health phenotype is opposite, which may explain the low genetic correlation of CM and LF. Another reason for the low correlation of CM and LF could be the fact that the applied prediction equation for LF was calibrated on an external dataset (Soyeurt et al., 2020), where it was evaluated with an R^2 cv of 0.66. Hence, in the current study R^2 between true and predicted contents of lactoferrin might be even lower.

The genetic correlations estimated between LF and SCS (0.27 and 0.25) were very similar to Arnould et al. (2009), who reported a value of 0.24. Soyeurt et al. (2007) and Nayeri et al. (2020) found low genetic correlations of 0.04 and 0.06 between SCS and MIR-predicted lactoferrin. Cheng et al. (2008) also found a stronger correlation of 0.38, but between ELISA-measured lactoferrin concentration and SCC. So, there is a large variation in estimates of genetic correlations between (MIR)-predicted lactoferrin and SCS/SCC. Further research is required to find explanations and influencing factors.

Results of the selection index calculation showed that adding MIRprob or LF could not increase accuracy of EBV for CM compared to the single trait EBV of CM. An index of CM + MIRSCSprob resulted in a similar accuracy of MT EBV for CM compared to CM + SCS, approximating the current udder health index. Indices combining various traits, e.g. CM + SCSMIRprob + LF, CM + SCS + LF, or CM + SCS + MIRprob + MIRSCSprob + LF, led to an increased accuracy of the MT EBV for CM in dataset 1, in dataset 2 there were hardly any differences. The index CM + SCS + MIRprob + MIRSCSprob + LF showed the highest accuracy (0.68), but also contains repeated information. Consequently, the performed basic selection index calculation indicate that MIR-predicted phenotypes evaluated in the present study may not considerably help to improve

accuracy of breeding values for clinical mastitis compared to the current index (70% SCS + 30% CM).

5 | CONCLUSIONS

Our study confirmed the good capability of SCS as auxiliary trait for genetic evaluation of udder health. Probabilities of mastitis derived from a MIR-spectra based prediction model showed a moderate heritability and a moderate positive genetic correlation to CM, which indicates a potential use as an additional auxiliary trait. Mastitis probabilities predicted by a model based on MIR + SCS resulted in similar genetic parameters as somatic cell score, suggesting that large part of the information in the joint model comes from SCS. However, basic selection index calculations indicate that the additional benefit of using the new MIR-predicted phenotypes is very limited for genetic improvement of udder health.

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CONFLICT OF INTEREST STATEMENT

Johann Sölkner is an Editorial Board member of Journal of Animal Breeding and Genetics and a co-author of this article. To minimize bias, Johann Sölkner was excluded from all editorial decision-making related to the acceptance of this article for publication. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions of the data provider and owner, the Austrian milk recording system (LKV Austria Gemeinnützige GmbH).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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