ABSTRACT

Disease-related milk losses directly affect dairy herds’ profitability and the production efficiency of the dairy industry. Therefore, this study aimed to quantify phenotypic variability in milk fluctuation periods related to diseases and to explore milk fluctuation traits as indicators of disease resilience. By combining high-frequency daily milk yield data with disease records of cows that were treated and recovered from the disease, we estimated milk variability trends within a fixed period around the treatment day of each record for 5 diseases: udder health, reproductive disorders, metabolic disorders, digestive disorders, and hoof health. The average milk yield decreased rapidly from 6 to 8 d before the treatment day for all diseases, with the largest milk reduction observed on the treatment day. Additionally, we assessed the significance of milk fluctuation periods highly related to diseases by defining milk fluctuations as a period of at least 10 consecutive days in which milk yield fell below 90% of the expected milk production values at least once. We defined the development and recovery phases of milk fluctuations using 3,847 milk fluctuation periods related to disease incidences, and estimated genetic parameters of milk fluctuation traits, including milk losses, duration of the fluctuation, variation rate in daily milk yield, and standard deviation of milk deviations for each phase and their genetic correlation with several important traits. In general, the disease-related milk fluctuation periods lasted 21.19 ± 10.36 d with a milk loss of 115.54 ± 92.49 kg per lactation. Compared with the development phase, the recovery phase lasted an average of 3.3 d longer, in which cows produced 11.04 kg less milk and exhibited a slower variation rate in daily milk yield of 0.35 kg/d. There were notable differences in milk fluctuation traits depending on the disease, and greater milk losses were observed when multiple diseases occurred simultaneously. All milk fluctuation traits evaluated were heritable with heritability estimates ranging from 0.01 to 0.10, and moderate to high genetic correlations with milk yield (0.34 to 0.64), milk loss throughout the lactation (0.22 to 0.97), and resilience indicator (0.39 to 0.95). These results indicate that cows with lower milk losses and higher resilience tend to have more stable milk fluctuations, which supports the potential for breeding for more disease-resilient cows based on milk fluctuation traits. All milk fluctuation traits evaluated were heritable with heritability estimates ranging from 0.01 to 0.10, and moderate to high genetic correlations with milk yield (0.34 to 0.64), milk loss throughout the lactation (0.22 to 0.97), and resilience indicator (0.39 to 0.95). These results indicate that cows with lower milk losses and higher resilience tend to have more stable milk fluctuations, which supports the potential for breeding for more disease-resilient cows based on milk fluctuation traits. Overall, this study confirms the high effect of diseases on milk yield variability and provides insightful information about their relationship with relevant traits in Holstein cattle. Furthermore, this study shows the potential of using high-frequency automatic monitoring of milk yield to assist on breeding practices and health management in dairy cows. Key words: daily milk yield, dairy cattle, disease resilience, milk variability

INTRODUCTION

Health issues represent serious limitations to farm productivity and animal welfare in the dairy industry and have a substantial effect on dairy farm profitability and sustainability (Vukasinovic et al., 2017; Sumner et al., 2018). The long-term disruption of the physiology of dairy cows caused by diseases is usually not fully recovered even after the disease is cured, which affects subsequent performance, fertility, and livability (Ribeiro et al., 2016). Diagnostics, veterinary treatment, labor, and infrastructure associated with diseased cows are also part of the direct costs of dairy farms (Carvalho et al., 2019). Quantifying milk losses caused by diseases might be a feasible approach for enhancing awareness of the effect of diseases on productivity and providing tools for breeding cows for greater health,
welfare, and productive efficiency. The low-frequency of test-day records makes it more difficult to explore the relationship between diseases and milk production. In recent years, the availability of high-frequency daily milk yield recording equipment and the substantial improvement of farm management and data recording have facilitated analyses of milk losses and fluctuations. Adriaens et al. (2021) quantified fluctuations in daily milk yield around treatment day for clinical mastitis and evaluated milk losses at both the lactation and udder quarter levels. However, it is not clear whether different diseases have consistent effects on daily milk yield. The precise quantification of milk fluctuation periods may provide a good opportunity for management and breeding for improved health and disease resilience. For the purposes of this study, disease resilience can be defined as the capacity of the animal to be minimally affected by diseases or to rapidly return to the state it was in before exposure to a disease (Knap and Doeschl-Wilson, 2020).

The efficiency and sustainability of the dairy industry can be improved by reducing the incidence of disease in dairy cows. Balanced diets and improved farm management can be effective, and breeding for greater disease resilience is also an important strategy, including indirect selection for related traits (Egger-Danner et al., 2015) or direct selection for health traits (Vukasinovic et al., 2017). SCS has been widely used for indirect selection for clinical mastitis incidence and are included in worldwide dairy cattle selection indexes (Miglior et al., 2005; Martin et al., 2018). Additional indicators of clinical mastitis include electrical conductivity (Norberg et al., 2006; Pedrosa et al., 2023) and acceleration measured by sensors (Halachmi et al., 2019). However, there are limited indicator traits and records for most diseases and breeders usually rely on direct selection based on diagnostic records done by farmers or veterinarians in commercial dairy farms. Incomplete or absent disease recording systems in dairy farms constrain the effectiveness of direct selection for disease resilience. As a result, recent studies have investigated approaches to reduce the effect of various diseases on production performance and herd productivity (Elgersma et al., 2018). Poppe et al. (2020) and Wang et al. (2022a) both reported a notable association between the variability in daily milk yield and resilience and udder health disease incidence. However, to our best knowledge, there are no breeding companies directly selecting for disease resilience based on variability in longitudinal variables in dairy cattle. Therefore, there is an urgent demand for reliable indicators of disease resilience with practical breeding applications. In this context, the main objectives of this study were (1) to quantify and describe milk yield fluctuations related to various diseases in Holstein cattle, and (2) to investigate the systematic factors, genetic background, and breeding potential of milk fluctuation traits as indicators of disease resilience. The findings of this study will provide insights on the relationship among functional traits in dairy cattle, contribute to improving disease monitoring and animal welfare in commercial farms, and serve as a reference for further research on variation in daily milk yield and resilience in dairy cattle populations. This study also highlights the limitations and challenges of using disease data recorded in commercial farms.

MATERIALS AND METHODS

Ethics Committee Approval

The animal care and data collection process were carried out in strict accordance with the protocol approved by the Animal Welfare Committee of the China Agricultural University (Protocol Number: DK996).

Phenotypic Data

Daily Milk Yield Phenotypes. The initial daily milk yield data set contained 11,536,488 milk yield records of 22,666 Holstein cows measured from January 2017 to January 2021 in 3 herds (owned by a single entity) located in Hebei, China. The daily milk yield information of each cow was extracted from the farm management software. Cows were housed in freestall barn systems, fed TMR, and milked 3 times per day on a rotary milking system.

From the initial data set, only records from DIM 1 to 305 d with milk yield ranging from 2.5 to 100 kg/d were kept for further calculations. Only lactations with more than 220 records throughout the lactation and interval between the first and last milk recording greater than 260 d were retained for further analyses. A linear regression model was used to impute missing records within each lactation. For each missing value, a first-order regression model was fitted with 5 actual records around the missing value, and the regression value was used as the filled value on that day until all missing values were filled in for a lactation. After imputation, 305-d records of daily milk yield for 22,366 lactations were obtained. A theoretically undisturbed lactation curve (expected lactation curve, ELC) was predicted to enable the characterization of the lactation potential of each cow in the absence of environment perturbations (Ben Abdellkrim et al., 2021). The ELC was fitted by combining 4 lactation curves (Wood [Wood, 1967], Nelder [Nelder, 1966], Wilmink [Wilmink, 1987], and Ali-Schaeffer [Ali and Schaeffer, 1987] models) with an
iterative procedure which was based on the deviations between imputed daily milk yield and the ELC fitted values. In each iteration, the model with the highest R² among the 4 lactation curves was selected and the outliers with the deviation below the lower threshold (first quartile − 1.5 × interquartile range) were removed to eliminate the values where the actual milk yield was extremely lower than the fitted value, and the ELC would gradually increase. The iterations stopped when no more outliers were detected and the final ELC was obtained for each lactation. Finally, 305 records of actual daily milk yield and the ELC for each lactation were obtained for 8,935 lactations of 6,816 cows. The initial data set, data processing, and the fitting of ELC are described in more details in Wang et al. (2022a). Supplemental Table S1 (https://doi.org/10.6084/m9.figshare.22820741.v1; Wang, 2023a) reports the number of records kept after each data editing step.

The deviations between actual milk yield and the fitted values from the ELC for each lactation were calculated, and a milk fluctuation period was defined as a period of at least 10 consecutive days of negative deviations in which the milk yield dropped at least once below 90% of the ELC fitted values. A typical milk fluctuation period would show a gradual decrease of deviations from the beginning of the milk fluctuation stage until the maximum milk loss, and then a gradual increase in milk yield until it returned to the ELC level. An example illustrating the milk fluctuation period is presented in Figure 1. The zero value on the vertical axis refers to the expected milk yield based on the ELC values. The scatters are the deviations between the actual milk yield and the ELC fitted values in a fluctuation phase and the points A and B represent the beginning and end of the milk fluctuation period. To simplify the calculation process, the day with the most negative deviation (the DIM at point C in Figure 1) was taken as the tipping point of this fluctuation, regardless of other lesser variations of deviation within this fluctuation phase. The days before and after the day with the most negative deviation were defined as the fluctuation development phase and the fluctuation recovery phase, respectively.

**Phenotypes for Health Traits.** A total of 120,821 diseases records from 34,322 Holstein females recorded from January 2017 to January 2021 were available for this study. The phenotypic records were extracted from the herd management software backup files. The disease records were from daily diagnosis and recording by farm veterinarians. The data set included diagnosis date, symptoms, treatment date, medication used for treatment, and treatment outcome (cure, worsening, culling, and death; these outcomes were defined based on the veterinarian evaluations and did not include laboratorial analyses for confirming the cure of the disease). The traits evaluated included udder health, reproductive disorders, metabolic disorders, digestive disorders, and hoof health. Udder health included clinical mastitis (scores 1, 2, and 3; the 3 scores were defined by veterinarians and higher values indicated more severe cases), subclinical mastitis, and bloody milk; reproductive disorders included metritis; metabolic disorders included ketosis, displacement of abomasum, and rumen acidosis; digestive disorders included diarr-
rhea, dyspepsia, and atony of forestomach; and, hoof health included hoof disease, footrot, sole ulcer, and deformed hoof. Additional details about the definition of the diseases are provided in Wang et al. (2022b). As the original disease records included over 125 descriptions of diseases, the raw records were organized and standardized during the data editing process and categorized into the 5 groups described above.

**Phenotypes for Milk Production, Loss, and Variability Traits.** Milk production and 4 milk loss traits were evaluated in the genetic correlation analyses, including 305-d milk yield (MY305), milk loss (in kg), number of milk loss events, duration of milk loss events (in days), and percentage of milk loss (in %). The MY305 was calculated by summing up all daily milk yield within a lactation (recorded and imputed values). Milk loss referred to the sum of the deviations in all milk fluctuation periods within a lactation based on the definition of fluctuation. The number of milk loss events referred to the number of fluctuations within a lactation. The duration of milk loss events referred to the total duration of all fluctuations with a lactation. The percentage of milk loss referred to the proportion of milk loss to MY305 per lactation. The log-transformed standard deviation of milk deviations from ELC based on the lactation when removing first and last 10 DIM (Lnsd2) was also considered. The Lnsd2 was the most promising indicator for breeding for improved resilience in Chinese Holstein cattle through genetic analyses and phenotypic validation (Wang et al., 2022a). Detailed descriptions about the milk production, milk losses, and milk variability traits can be found in Wang et al. (2022a). The descriptive statistics of the traits used for estimation of genetic correlations are presented in Supplemental Table S2 (https://doi.org/10.6084/m9.figshare.22787519.v6; Wang, 2023b).

**Pedigree.** The pedigree used in the genetic analyses was traced back up to 12 generations, and 72.88% of the cows with phenotypic records had sire information. There were no animals with both parents unknown. The final pedigree used for the genetic analyses included 21,838 females and 2,447 males.

**Defining Milk Fluctuation Traits**

Several milk fluctuation traits were calculated to reflect the characteristics of milk fluctuation periods, including the duration of the milk fluctuation period (DOP; in days), duration of the development phase (DOD; in days), and duration of the recovery phase (DOR; in days); milk loss in each milk fluctuation period (MLF; in kg), in the development phase (MLD; in kg), and in the recovery phase (MLR; in kg); the absolute value of variation rate of daily milk yield in the development phase (ROD; in kg/d) and in the recovery phase (ROR; in kg/d), the log-transformed SD of milk fluctuation period in the development phase (SDD; in kg) and in the recovery phase (SDR; in kg). The DOF, DOD, and DOR referred to the duration of the AB, AC, and CB phases of each milk fluctuation in Figure 1. The MLF referred to the sum of the deviations of the fluctuation phase. The MLD and MLR referred to the sum of the milk deviations of the development phase and the recovery phase, respectively. To ensure that the sum of milk losses in both phases was the same as MLF, half of the milk loss on the day with the most negative deviation was added to each of the 2 traits (MLD and MLR) as this day was set as the threshold between the 2 phases and had not been included in their calculations. The deviations within the development phase and the recovery phase were used to fit a simple linear regression model for each phase. The ROD and ROR represented the slope of the model of the corresponding phase which were expected to be negative and positive, respectively, and were subsequently transformed to absolute values. The SDD and SDR represented the SD of the milk deviations during the development and recovery phases, respectively. We applied logarithmic transformation to the SD to make these traits normally distributed.

**Editing of Disease Records and Milk Fluctuation Records**

The number of records in the raw data set with a noncured treatment outcome (worsening, culling, or death) was only 1.3%. Therefore, only diseases with a treatment and cure were included in this study. Whether the disease was cured or not depended on the evaluation and recording of the herd veterinarians. Only disease records of cows were retained, while the records of heifers and calves were removed. The gap between 2 diagnoses of the same disease was required to be more than 10 d, so that they were considered as separate disease cases. When the interval was less than 10 d, only the case with the first diagnosis was retained. Furthermore, only diseases diagnosed between DIM 1 to 305 and with a gap between the treatment and diagnosis between 1 to 10 d were included in the analyses. The specific disease data set editing steps, with the number of cows, lactations, and records after each editing step are presented in Supplemental Table S3 (https://doi.org/10.6084/m9.figshare.22787639.v6; Wang, 2023c).

Based on the definition of milk fluctuation periods, 31,606 milk fluctuation events were identified in 8,935 lactations. Only the fluctuations with negative ROD and positive ROR before being transformed to absolute
value were included in further analyses (>96.4%). The fluctuations with DOF, ROD, and ROR deviating by 3 or more SD from the mean were sequentially removed. The editing steps of the fluctuation events are presented in Supplemental Table S4 (https://doi.org/10.6084/m9.figshare.22787726.v4; Wang, 2023d). Finally, the lactations present in both the edited disease data set and in the milk fluctuation data set were retained for further analyses, which included 8,865 disease cases, and 16,173 fluctuation events for 4,735 lactations (parity 1 = 1,836, parity 2 = 1,592, parity 3+ = 1,307) of 4,098 cows.

To evaluate the milk variability trends during the disease period, we calculated the average relative deviations of each day within a window of 51 d surrounding the treatment day (d −25 to 25) for the 5 disease groups based on the edited disease data set (8,865 disease cases). The relative deviation referred to the ratio between the deviation and the ELC fitted values for each DIM within a lactation. In addition, we calculated milk variability trends for the 3 scores of clinical mastitis in the same way based on the clinical mastitis data set (5,260 clinical mastitis cases).

Detection of Milk Fluctuation Periods Highly Related to Diseases

Disease cases were categorized into 3 disease-fluctuation relationship groups (DFR). The groups are as follows: DFR1, diseases highly related to milk fluctuation periods; DFR2, diseases associated with milk fluctuation periods; and DFR3, diseases unrelated to milk fluctuation periods. An illustration of the categorization is presented in Figure 2. From the perspective of milk fluctuation, the section AB refers to a milk fluctuation period, point C refers to the day of the maximum milk loss within this fluctuation period, and point M refers to the 5 d before the DIM of the first record within this fluctuation period. From the perspective of disease, points a and b refer to the diagnosis and the treatment day of the disease, respectively. We considered cases in which the points a and b were both within the date range of points M and C (including points M and C) as DFR1; cases with at least one date of points a and b within the date range of points C and B as DFR2, while the other cases were considered as DFR3. Based on this definition, 4,185, 445, and 4,235 disease cases were categorized into DFR1, DFR2, and DFR3, respectively. However, there was an overlap in the fluctuations corresponding to different diseases. A total of 3,551 milk fluctuation periods were highly related to a single disease, whereas 296 milk fluctuation periods were co-induced by more than one disease. We expected to determine milk fluctuation periods highly related to disease only (DFR1) based on the common dataset of disease and milk fluctuation, with the recovery of milk yield due to treatment as its characteristic. Milk fluctuation periods in DFR2 may not be strictly caused by the disease event, but by other factors with a greater probability, and therefore may show characteristics that were not diagnosed or treated until the recovery phase. In DFR3, the critical time points of diseases and milk fluctuation periods were completely separated and it could be assumed that no notable milk fluctuations were caused by the disease. However, because it did not correspond to specific milk fluctuation periods, it was not possible to detect the critical time point of deviation around the disease, and the milk fluctuation traits were not available. Therefore, DFR2 and DFR3 were not included in the calculation of the milk fluctua-

Figure 2. An example illustrating the categorization of the disease cases into 3 disease-fluctuation relationship (DFR) groups. Section AB is a milk fluctuation period, points A and B are the beginning and ending of the milk fluctuation period, respectively, point C is the day of the maximum milk loss within this fluctuation, and point M refers to the 5 d before the DIM of the first record within this fluctuation. Points a and b refer to the diagnosis and the treatment day of a disease case, respectively. When both points a and b were within the date range of M and C (including points M and C), diseases were considered highly related to milk fluctuation periods and abbreviated as DFR1. Cases with at least one DIM of points a and b within the date range of points C and B were considered diseases associated with milk fluctuation periods and abbreviated as DFR2.
tion traits. Finally, 3,847 milk fluctuation periods for 2,905 lactations from 2,685 cows were retained for the calculation of milk fluctuation traits hypothesized to be highly related to diseases, whereas the other 12,326 milk fluctuation periods were likely caused by other environmental factors. Supplemental Figure S1 (https://doi.org/10.6084/m9.figshare.22787762.v1; Wang, 2023h) shows the relationship among the diseases, milk fluctuation periods, and DFR groups.

**Statistical Modeling**

The GLM procedure of the SAS software (version 9.4, SAS Institute Inc.) was used to test the significance of nongenetic effects on milk fluctuation traits. Multiple testing correction was done based on the Bonferroni adjustment (α = 0.05). The linear model for each trait had the following general form (model 1):

\[ y_{ijkmn} = \mu + HYS_i + P_j + S_k + D_m + \varepsilon_{ijkmn}, \]  

where \( y_{ijkmn} \) is the phenotypic record for each milk fluctuation trait; \( \mu \) is the overall mean; \( HYS_i \) is the effect of the herd-disease year-disease season \( i \) (48 levels; including 3 herds, 4 years [2017 to 2020], and 4 seasons [March to May, June to August, September to November, and December to February]); \( P_j \) is the effect of the parity \( j \) (4 levels, including 1, 2, 3, and 4+); \( S_k \) is the effect of the lactation stage \( k \) at the diagnosis day (4 levels, including DIM 1 to 44, 45 to 99, 100 to 199, and 200 to 305); \( D_m \) is the effect of the disease group \( m \) (6 levels, including udder health, reproductive disorders, metabolic disorders, digestive disorders, and hoof health, and more than one disease); and \( \varepsilon_{ijkmn} \) is the random residual term. We assumed that \( \varepsilon_{ijkmn} \sim N(0, \sigma^2) \), where \( \sigma^2 \) is the residual variance. The interaction between parity and lactation stage was tested but not included in the final model as it was not statistically significant (P-value = 0.113). We also tested the significance of the effect of the mastitis scores on milk fluctuation traits based on the dataset of milk fluctuation periods highly related to clinical mastitis. The fixed effects were the same as defined in the above statistical model and the scores of mastitis (3 levels: scores 1, 2, and 3) were included for all milk fluctuation traits.

**Genetic Analyses**

**Estimation of Genetic Parameters.** Variance and covariance components were estimated based on a repeatability animal model, using the Average Information Restricted Maximum Likelihood algorithm implemented in the DMU software (Madsen et al., 2006). The model used can be described in matrix form as

\[ y = X\beta + Z_a a + Z_{pe} p + e, \]  

where \( y \) is the vector of phenotypic records for the milk fluctuation traits; \( \beta \) is the vector of systematic effects, including the same fixed effects described for model 1; \( a \) is the vector of random animal additive genetic effects; \( p \) is the vector of random residual effects; \( X, Z_a, \) and \( Z_{pe} \) are the incidence matrices linking phenotypic records to \( \beta, a, \) and \( p, \) respectively. It was assumed that \( a \sim N(0, A\sigma^2_a), p \sim N(0, I\sigma^2_p), \) and \( e \sim N(0, I\sigma^2_e), \) where \( A \) is the matrix of additive genetic relationships constructed based on pedigree information, \( \sigma^2_a \) is the additive genetic variance, \( I \) is an identity matrix, \( \sigma^2_p \) is the permanent environmental variance, and \( \sigma^2_e \) is the residual variance.

**Calculation of Genetic Correlation Among Milk Fluctuation Traits.** The genetic correlations between all pairs of milk fluctuation traits were calculated based on bivariate repeatability animal models. The bivariate-trait repeatability animal model included the same effects as described for model 2. The model assumptions are as follows:

\[
\begin{bmatrix}
    a_1 \\
    a_2
\end{bmatrix}
\sim
N\left(0, A \otimes \begin{bmatrix}
    \sigma^2_a & \sigma_{a_1a_2} \\
    \sigma_{a_1a_2} & \sigma^2_a
\end{bmatrix}\right),
\]

\[
\begin{bmatrix}
    p_{e_1} \\
    p_{e_2}
\end{bmatrix}
\sim
N\left(0, I \otimes \begin{bmatrix}
    \sigma^2_p & \sigma_{p_1p_2} \\
    \sigma_{p_1p_2} & \sigma^2_p
\end{bmatrix}\right),
\]

\[
\begin{bmatrix}
    e_1 \\
    e_2
\end{bmatrix}
\sim
N\left(0, I \otimes \begin{bmatrix}
    \sigma^2_e & \sigma_{e_1e_2} \\
    \sigma_{e_1e_2} & \sigma^2_e
\end{bmatrix}\right),
\]

where \( a_i \) is the vector of additive genetic effects for the trait \( i, \sigma^2_{a_i} \) is the additive genetic variance of trait \( i, \sigma_{a_ia_j} \) is the additive genetic covariance between traits \( i \) and \( j, p_e_i \) is the vector of permanent environmental effect for the trait \( i, \sigma^2_{p_e_i} \) is the permanent environmental variance of trait \( i, \sigma_{p_e_ip_e_j} \) is the permanent environmental covariance between traits \( i \) and \( j, e_i \) is the vector of residual effect for trait \( i, \sigma^2_e \) is the residual variance of trait \( i, \sigma_{e_1e_2} \) is the residual covariance between traits \( i \) and \( j. \)
and $j$. The heritability, repeatability, genetic correlations, and standard errors were calculated as described in Su et al. (2007).

**Calculation of Genetic Correlation Between Milk Fluctuation Traits with Milk Production, Loss, and Variability Traits.** Genetic correlations between milk fluctuation traits with several important traits were calculated based on bivariate repeatability animal models. For the milk fluctuation traits, the animal models used were the same as model 2. For the milk production, milk losses, and milk variability traits, the models included the fixed effects of herd-calving year, cluster group, lactation curve (4 lactation curve models used in ELC fitting), and the age at first calving, and the random effects of animal additive genetic effect, permanent environmental effect, and residual effect, which were detailed in Wang et al. (2022a). These analyses were implemented using the DMU software (Madsen et al., 2006).

**RESULTS**

**Variability of the Milk Deviations**

The milk variability trends around the treatment day are presented in Figure 3. The largest decreases were observed on the treatment day (d 0), with 21.02% (udder health), 16.40% (reproductive disorders), 19.55% (metabolic disorders), 20.98% (digestive disorders), and 16.42% (hoof health), respectively. For udder health [Figure 3(a)], the deviation gradually decreased to the maximum from d −7. The deviations were more variable for other disease groups as shown in Figure 3(b–e), whose decreases were observed from d −20 to −15 on average, although the magnitude was small. The deviation for all diseases did not recover to zero within 25 d after treatment day, with udder health showing the fastest recovery as it had stabilized in about 10 d after treatment day. The relative deviations for the 3 mastitis scores are presented in Figure 3(f). The deviations gradually increased for scores 1 to 3 of mastitis on the treatment day, with 19.75%, 24.15%, and 27.44%, respectively. However, the maximum decrease was reached on d 1 and d 2 at 24.78% and 31.20% for the scores 2 and 3, respectively. After the treatment day, the deviation recovered faster for score 1 and slower for score 3.

**Descriptive Statistics for Milk Fluctuation Traits**

A total of 4,185 disease cases (47.21% of the total number of cases) were in the DFRI group. The largest disease group (udder health) included 2,779 cases (31.35%), while the smallest disease group (hoof health) included 54 cases (0.61%). Table 1 shows the descriptive statistics for milk fluctuation traits. In general, the DOF was highly related to diseases ranging from 10 to 53 d and the average DOD and DOR was 8.45 ± 5.90 d and 11.75 ± 8.79 d, respectively. The average MLF was 115.54 ± 92.49 kg and the average MLD was 11.04 kg lower than MLR. The average ROD were 1.53 ± 1.11 kg/d, which was 0.35 kg/d faster than ROR. Furthermore, the coefficients of variation for milk fluctuation traits in the development phase were smaller than those in the recovery phase, except for SDD (58.04%) and SDR (53.92%). The numbers of cases included in each DFR groups are shown in Supplemental Table S5 (https://doi.org/10.6084/m9.figshare.22787735.v5; Wang, 2023e) and the numbers of specific disease cases are shown in Supplemental Table S6 (https://doi.org/10.6084/m9.figshare.22787744.v4; Wang, 2023f).

**Influence of Nongenetic Effects on Milk Fluctuation Traits**

The least squares means estimates of various levels and multiple comparisons are presented in Supplemental Table S7 (https://doi.org/10.6084/m9.figshare.22787750.v4; Wang, 2023g) and Table 2. The effects of parity, lactation stage, and disease on milk fluctuation traits were significant ($P < 0.05$). Milk fluctuation periods highly related to more than one disease had extreme values of milk fluctuation traits. For milk fluctuation periods highly related to one disease, DOF and DOD for hoof health were the highest. The ROD for milk fluctuation periods highly related to digestive disorders was the fastest at 1.57 ± 0.10 kg/d, followed by the udder health at 1.54 ± 0.07 kg/d. The milk fluctuation periods highly related to digestive disorders and hoof health had the highest SDD and SDR, respectively. The least squares means of the mastitis scores are presented in Table 3. The cows with more severe mastitis showed significantly ($P < 0.05$) longer DOF, higher MLF, faster ROD, slower ROR, and higher SDD and SDR. The DOF and MLF for milk fluctuation periods highly related to the score 3 were 9.99 d and 110.62 kg higher than those of score 1.

**Genetic Parameters for Milk Fluctuation Traits**

The estimates of variance components and heritability for milk fluctuation traits are presented in Table 4, while the genetic correlations among milk fluctuation traits are shown in Table 5. The heritability estimates for milk fluctuation traits ranged from 0.01 ± 0.01 (DOF and DOR) to 0.10 ± 0.03 (SDD and SDR). High positive genetic correlations were observed between DOF and other milk fluctuation traits, ranging from
There was a positive but negligible genetic correlation of 0.13 ± 0.04 between DOD and DOR. The genetic correlations between MLF and other milk fluctuation traits were all positive. The genetic correlation between ROD and ROR was −0.55 ± 0.14. Furthermore, the genetic correlations between SDD and SDR were 0.99 ± 0.03, and both had significant ($P < 0.05$) positive genetic correlations with the other milk fluctuation traits, ranging from 0.41 ± 0.12 (SDR and DOD) to 0.98 ± 0.07 (SDR and MLF).

**Figure 3.** The average, 25th, and 75th percentiles of average relative deviations of each day within a window of 51 d around the treatment day (d −25 to 25) for the 5 disease groups and the 3 scores of mastitis. (a) Udder health, (b) reproductive disorders, (c) metabolic disorders, (d) digestive disorders, (e) hoof health, (f) 3 scores of mastitis.

**Genetic Correlation Between Milk Fluctuation Traits with Milk Production, Loss, and Variability Traits**

The genetic correlations of milk fluctuation traits with milk production, milk losses, and variability traits are presented in Table 6. Several milk fluctuation traits (MLF, ROR, SDD, and SDR) were positively genetically correlated with MY305, ranging from 0.34 ± 0.12 (MLF) to 0.64 ± 0.14 (SDR). Moderate to high positive genetic correlations were observed between milk losses and all other milk fluctuation traits, ranging from 0.22
± 0.09 (ROD) to 0.97 ± 0.05 (MLF). The genetic correlations between milk losses and milk fluctuation traits during the recovery phase were higher than the corresponding traits from the development phase. For Lnsd2, the genetic correlation estimates were positive and significant ($P < 0.05$) and ranged from 0.39 ± 0.17 (DOD) to 0.95 ± 0.05 (SDR). In addition, the genetic correlations between Lnsd2 with SDD (0.92 ± 0.06) and SDR (0.95 ± 0.05) were high.

**DISCUSSION**

**Evaluation of Milk Losses and Variability**

Assessing and quantifying milk losses related to diseases is still challenging (Fourichon et al., 1999). Due to limitations on the frequency of milk yield recording, many studies considered yield differences between healthy and diseased cows throughout lactation as the long-term effect of diseases (Dubuc et al., 2011; Nikkhah et al., 2021). In this study, cows suffering from all 5 disease groups did not recover to the pre-disease levels within 25 d after the treatment day, indicating a long-term reduction. In contrast, this study focused on the short-term effects of diseases on milk yield and stopped calculating milk fluctuation periods once it recovered to the proposed normal level. This is the main reason why milk losses were generally lower than in previous studies. The diagnostic records of naturally occurring diseases from large commercial herds were used in our study. Cows had the highest proportion of diseases in the early lactation and udder health was the most prevalent of the 5 disease groups. This is consistent with the pattern of disease incidence in dairy herds (Zwald et al., 2004; Gernand et al., 2012). However, several factors could have biased the results due to the

### Table 1. Descriptive statistics of milk fluctuation traits related to diseases in Holstein cattle

<table>
<thead>
<tr>
<th>Trait</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOF (d)</td>
<td>3,847</td>
<td>21.19</td>
<td>10.36</td>
<td>10</td>
<td>53</td>
<td>48.89</td>
</tr>
<tr>
<td>DOD (d)</td>
<td>3,847</td>
<td>8.45</td>
<td>5.90</td>
<td>1</td>
<td>46</td>
<td>69.82</td>
</tr>
<tr>
<td>DOR (d)</td>
<td>3,847</td>
<td>11.75</td>
<td>8.79</td>
<td>1</td>
<td>47</td>
<td>74.81</td>
</tr>
<tr>
<td>MLF (kg)</td>
<td>3,847</td>
<td>115.54</td>
<td>92.49</td>
<td>4.35</td>
<td>798.46</td>
<td>80.05</td>
</tr>
<tr>
<td>MLD (kg)</td>
<td>3,847</td>
<td>52.25</td>
<td>46.66</td>
<td>1.22</td>
<td>499.27</td>
<td>89.30</td>
</tr>
<tr>
<td>MLR (kg)</td>
<td>3,847</td>
<td>63.29</td>
<td>60.91</td>
<td>2.08</td>
<td>634.89</td>
<td>96.24</td>
</tr>
<tr>
<td>ROD (kg/d)</td>
<td>3,847</td>
<td>1.53</td>
<td>1.11</td>
<td>0.22E-02</td>
<td>4.64</td>
<td>72.55</td>
</tr>
<tr>
<td>ROR (kg/d)</td>
<td>3,847</td>
<td>1.18</td>
<td>0.94</td>
<td>0.23E-02</td>
<td>4.00</td>
<td>79.66</td>
</tr>
<tr>
<td>SDD (kg)</td>
<td>3,847</td>
<td>1.04</td>
<td>0.60</td>
<td>-4.35</td>
<td>2.52</td>
<td>58.04</td>
</tr>
<tr>
<td>SDR (kg)</td>
<td>3,847</td>
<td>1.02</td>
<td>0.55</td>
<td>-2.64</td>
<td>2.52</td>
<td>53.92</td>
</tr>
</tbody>
</table>

1$N =$ number of records; DOF = duration of milk fluctuation period; DOD = duration of the development phase; DOR = duration of the recovery phase; MLF = milk loss in milk fluctuation period; MLD = milk loss in the development phase; MLR = milk loss in the development phase; ROD = variation rate of daily milk yield in the development phase; ROR = variation rate of daily milk yield in the recovery phase; SDD = log-transformed SD of milk deviations in the development phase; SDR = log-transformed SD of milk deviations in the recovery phase.

### Table 2. Estimates of disease group fixed effect on milk fluctuation traits in Holstein cattle

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Udder health</th>
<th>Reproductive disorder</th>
<th>Metabolic disorder</th>
<th>Digestive disorder</th>
<th>Hoof health</th>
<th>More than one disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOF</td>
<td>20.61 ± 0.61</td>
<td>21.00 ± 0.81</td>
<td>24.31 ± 0.89</td>
<td>24.15 ± 0.96</td>
<td>25.54 ± 1.54</td>
<td>28.56 ± 0.86</td>
</tr>
<tr>
<td>DOD</td>
<td>8.60 ± 0.35</td>
<td>9.90 ± 0.46</td>
<td>10.81 ± 0.51</td>
<td>9.39 ± 0.55</td>
<td>13.02 ± 0.89</td>
<td>13.46 ± 0.49</td>
</tr>
<tr>
<td>DOR</td>
<td>11.01 ± 0.53</td>
<td>10.11 ± 0.70</td>
<td>12.50 ± 0.77</td>
<td>13.77 ± 0.83</td>
<td>11.53 ± 1.34</td>
<td>14.10 ± 0.74</td>
</tr>
<tr>
<td>MLF</td>
<td>115.23 ± 5.36</td>
<td>110.03 ± 7.10</td>
<td>142.69 ± 7.84</td>
<td>136.30 ± 8.45</td>
<td>174.44 ± 13.52</td>
<td>183.05 ± 7.53</td>
</tr>
<tr>
<td>MLD</td>
<td>54.81 ± 2.70</td>
<td>59.42 ± 3.59</td>
<td>71.16 ± 3.96</td>
<td>64.52 ± 4.27</td>
<td>93.09 ± 6.83</td>
<td>97.61 ± 3.80</td>
</tr>
<tr>
<td>MLR</td>
<td>60.34 ± 3.61</td>
<td>50.48 ± 4.78</td>
<td>71.58 ± 5.28</td>
<td>71.77 ± 5.69</td>
<td>81.33 ± 9.10</td>
<td>85.40 ± 5.07</td>
</tr>
<tr>
<td>ROD</td>
<td>1.54 ± 0.07</td>
<td>1.13 ± 0.09</td>
<td>1.20 ± 0.10</td>
<td>1.57 ± 0.10</td>
<td>1.19 ± 0.17</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>ROR</td>
<td>1.25 ± 0.06</td>
<td>1.19 ± 0.08</td>
<td>1.13 ± 0.08</td>
<td>1.05 ± 0.09</td>
<td>1.06 ± 0.14</td>
<td>1.16 ± 0.08</td>
</tr>
<tr>
<td>SDD</td>
<td>0.98 ± 0.03</td>
<td>0.81 ± 0.05</td>
<td>0.96 ± 0.05</td>
<td>1.01 ± 0.06</td>
<td>0.98 ± 0.09</td>
<td>1.07 ± 0.05</td>
</tr>
<tr>
<td>SDR</td>
<td>0.99 ± 0.03</td>
<td>0.87 ± 0.04</td>
<td>1.02 ± 0.05</td>
<td>1.00 ± 0.05</td>
<td>1.06 ± 0.08</td>
<td>1.14 ± 0.04</td>
</tr>
</tbody>
</table>

$^{a,b}$Different letter superscripts among different levels means significant difference ($P < 0.05$).

$^1N =$ number of records; DOF = duration of milk fluctuation period; DOD = duration of the development phase; DOR = duration of the recovery phase; MLF = milk loss in milk fluctuation period; MLD = milk loss in the development phase; MLR = milk loss in the development phase; ROD = variation rate of daily milk yield in the development phase; ROR = variation rate of daily milk yield in the recovery phase; SDD = log-transformed SD of milk deviations in the development phase; SDR = log-transformed SD of milk deviations in the recovery phase.
quality of the disease records. First, not all diseases might have been recorded. Hoof health was the least recorded of all diseases. Malchiodi et al. (2017) indicated that hoof health was the third most prevalent diseases on herds after mastitis and reproductive disorders, indicating a lack of comprehensiveness of hoof health events derived from herd records. Omissions of other diseases were also presumed, which may explain the weak matching between milk yield and disease records. Second, for some herds, the effects of disease on milk fluctuation periods may be further magnified by the fact that milk may be discarded due to quality issues such as mastitis. Finally, cows with nonsignificant milk fluctuation periods, cows that recovered spontaneously without treatment, and cows that did not recover from the diseases were not considered in this study. These biases were inevitable due to the inability to calculate milk fluctuation traits for these cows. To simplify the calculations, this study defined a threshold for substantial milk fluctuation periods. Therefore, it is difficult to define specific key time points for those nonsubstantial milk fluctuation periods. The cows that did not have fluctuations highly related to diseases (e.g., DFR3 group) may be more suitable for further investigation of disease resilience compared with DFR1 group. However, this requires a more specialized research approach and modeling. Furthermore, the latter 2 biases were caused by the unavailability of the corresponding treatment and milk yield records, respectively. Nevertheless, we infer that the present joint analyses provide a valuable insight into the effect of diseases on milk losses based on incomplete data from commercial herds.

Relationship Between Diseases and Milk Fluctuation Periods

Udder health is the main disease affecting commercial farms, with the most common one being clinical mastitis (Capuco et al., 2003). van Soest et al. (2016) estimated milk losses of 336 kg caused by clinical mastitis, which were much higher than MLF highly related to udder health in this study. In addition to differences in the duration of effect in different studies and estimation procedures, the treatment protocol (Heikkilä et al., 2018) and pathogen difference (Hertl et al., 2014) can also cause high variability of estimated milk loss. Adriaens et al. (2021) measured milk deviations around

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM ± SE for the mastitis score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOF</td>
<td>20.33 ± 0.80a</td>
<td>22.49 ± 0.85b</td>
<td>30.32 ± 1.34a</td>
</tr>
<tr>
<td>DOD</td>
<td>9.20 ± 0.46</td>
<td>8.73 ± 0.49</td>
<td>8.88 ± 0.77</td>
</tr>
<tr>
<td>DOR</td>
<td>10.13 ± 0.69a</td>
<td>12.77 ± 0.73b</td>
<td>20.44 ± 1.15a</td>
</tr>
<tr>
<td>MLF</td>
<td>105.96 ± 6.54a</td>
<td>133.84 ± 6.96b</td>
<td>216.58 ± 10.89a</td>
</tr>
<tr>
<td>MLD</td>
<td>55.14 ± 3.31a</td>
<td>62.63 ± 3.50b</td>
<td>85.27 ± 5.54a</td>
</tr>
<tr>
<td>MLR</td>
<td>50.84 ± 4.42a</td>
<td>71.32 ± 4.67b</td>
<td>131.17 ± 7.36a</td>
</tr>
<tr>
<td>ROD</td>
<td>1.42 ± 0.09a</td>
<td>1.58 ± 0.09b</td>
<td>1.71 ± 0.14ab</td>
</tr>
<tr>
<td>ROR</td>
<td>1.33 ± 0.07a</td>
<td>1.23 ± 0.07a</td>
<td>0.80 ± 0.12b</td>
</tr>
<tr>
<td>SDD</td>
<td>0.98 ± 0.04a</td>
<td>1.07 ± 0.05a</td>
<td>1.29 ± 0.07a</td>
</tr>
<tr>
<td>SDR</td>
<td>0.96 ± 0.04a</td>
<td>1.07 ± 0.04b</td>
<td>1.21 ± 0.06a</td>
</tr>
</tbody>
</table>

**Note:** Different letter superscripts among different levels mean significant difference (P < 0.05).

1N = number of records; DOF = duration of milk fluctuation period; DOD = duration of the development phase; DOR = duration of the recovery phase; MLF = milk loss in milk fluctuation period; MLD = milk loss in the development phase; MLR = milk loss in the recovery phase; ROR = variation rate of daily milk yield in the recovery phase; SDR = log-transformed SD of milk deviations in the development phase; SDD = log-transformed SD of milk deviations in the development phase; ROD = variation rate of daily milk yield in the development phase; MLF = milk loss in milk fluctuation period; MLD = milk loss in the development phase; MLR = milk loss in the recovery phase; ROR = variation rate of daily milk yield in the recovery phase; SDD = log-transformed SD of milk deviations in the development phase; SDR = log-transformed SD of milk deviations in the recovery phase.

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the treatment day (d −5 to 30), showing similar trends in milk fluctuations as this study. We identified the characteristics of milk fluctuation periods highly related to more severe mastitis cases, including a longer DOR, higher milk losses, and slower recovery rate in daily milk yield. The inability of current veterinary treatments in this population to inhibit milk losses in a timely manner may cause a continued decrease for the more severe mastitis within 1 to 2 d after treatment day, which contributes to the diversified understanding of mastitis risk and farm assessment of mastitis recovery.

Postpartum clinical diseases develop in about 40% to 70% of cows during the first month of lactation (Ribeiro et al., 2016). Masia et al. (2022) showed that the milk yield of cows with postpartum clinical diseases was about 150 kg lower than that of healthy cows, which was similar to the results of this study. The greater variation in yield trends before the treatment day for reproductive disorders, metabolic disorders, and digestive disorders compared with udder health may be related to the concentration in early lactation, where the number of days before the treatment day would be lower than 25 d, resulting in a greater distribution of DIM with larger variations (e.g., DIM 1 to 5 d) included in the calculations. Therefore, the trend in the recovery phase of these 3 diseases is more obvious and homogeneous compared with the development phase. Hoof health is a serious welfare concern and results into compromised health, early culling, and reduced milk production (Fourichon et al., 1999; Huxley, 2013). Many studies showed that hoof health causes milk losses of 270 to 540 kg throughout the lactation (Huxley, 2013; van den Borne et al., 2022). In this study, hoof health showed the poorest recovery after treatment day and therefore should be the most concerning disease on farms in addition to udder health from the milk losses perspective. Milk losses highly related to multiple diseases had longer DOF, and higher MLF compared with milk losses highly related to one disease. These findings highlight the effect of multiple diseases on milk loss due to severe decrease in cow immunity caused by multiple diseases.

Milk Fluctuation Traits as Disease Resilience Indicators

Considering the large effect of disease on production and the high selection pressure on milk yield that aggravates disease incidence, disease resilience has become a highly regarded novel breeding goal in Holstein cattle breeding programs (Brito et al., 2021). A potential direction is to genetically select for reducing the effect of disease on milk production. Cows with less
milk fluctuation, faster recovery rate, shorter duration of fluctuations, and lower milk losses are considered to be more disease resilient. For breeding purposes, all milk fluctuation traits can be considered collectively as they may capture complementary biological mechanisms. As anticipated, milk fluctuations that are more prominent in terms of longer DOF, higher MLF, and more abrupt fluctuations (SDD and SDR), tend to occur simultaneously. The genetic correlations of milk fluctuation duration and rate between development and recovery phase are low or negative, which indicate different mechanisms involved in the 2 phases. During the development phase, milk losses depend mainly on the relative relationship between the intensity of the disturbance and individual disease resilience. Each cow has a unique threshold in which milk yield would decrease rapidly until the intensity of the disease challenge is reduced or an intervention (e.g., veterinary treatment) is applied. During the recovery phase, milk loss is largely dependent on the ability to recover, and therefore the worse the disease resilience, the more difficult for the cows to recover productive performance, which makes ROR slower and DOR longer. A more in-depth study of these 2 milk fluctuation phases is needed using data from a larger population and ideally, with a better disease recording system.

Cows are expected to be selected for increased disease resilience while maintaining high production performance. However, it is often quite difficult to achieve balanced breeding within the constraints of resource allocation theory (Rendel, 1963). The positive genetic correlations between MY305 and some milk fluctuation traits suggest that high-yielding cows tend to be less resilient. There is a positive genetic correlation between ROR and MY305, indicating that high-yielding cows may also be more capable of recovering from disturbances, which is a good signal for resilience breeding. We speculate that the recovery phase is more critical in determining milk losses than development phase, which may explain why the genetic correlations between milk fluctuation traits and milk loss traits are mostly higher in the recovery phase than in the development phase. Therefore, monitoring the recovery phase after treatment in cows is recommended. In addition, we observed a significant negative genetic correlation of the number of milk loss with duration (DOF) and milk loss (MLF, MLD, and MLR), likely due to the natural inverse relationship under a certain total milk loss per lactation. The Lnsd2 was the optimal resilience indicator in Chinese Holstein cattle obtained by Wang et al. (2022a). Lower Lnsd2 refers to less fluctuation in milk yield and closer to expected milk yield, and hence, greater resilience. The cows with lower Lnsd2 had shorter DOF, less MLF, and smoother and slower milk fluctuation rates. Poppe et al. (2021) reported the stability of resilience indicators across parities and lactation stages. In this study, the high genetic correlation between Lnsd2 and SDD and SDR shows the high stability of Lnsd2 as an indicator of resilience between global and local lactations which also shows that fluctuations following disturbances are the major component of overall milk fluctuations. The positive correlation between Lnsd2 and ROR is of interest. It is desirable to select cows with rapid recovery rate after disease. However, an increase of ROR would be accompanied by increased intensity of milk fluctuations, leading to greater Lnsd2. This is one of the limitations of Lnsd2 in reflecting resilience as it focuses mainly on reducing fluctuations in daily milk yield and neglects the effect of partial rate increase on recovery of milk production performance.

Despite the genetic correlations of milk fluctuation traits showing attributes reflecting disease resilience,
there are challenges in the selection of the optimal indicator for achieving notable genetic gains in disease resilience. Knap and Doeschl-Wilson (2020) noted that it is unclear whether the efficacy of such deviation-based indicators is the same across environments and multiple pathogen loads. Consequently, it is a potential direction to integrate multiple protocols for the assessment and selection for disease resilience. In summary, when selecting disease-resilient cows, it is essential to require balanced breeding on different milk fluctuation traits to reduce fluctuations in production performance and increase the rate of recovery of daily milk yield, while also considering the stability of milk production. With the advent of rapidly developing data tools, integrating different approaches can enhance the modeling of milk fluctuation periods, thereby providing a promising direction for future research for disease resilience.

CONCLUSIONS

This study evaluated phenotypic variation of daily milk yield around treatment day for various diseases and derived milk fluctuation traits to quantify the effect of disease on milk production. The average daily milk yield related to all disease cases showed a maximum decrease on treatment day, and the recovery of production performance was more challenging and time-consuming than during the development phase. Although the heritability estimates for milk fluctuation traits were low, the variability and genetic correlations with production, milk loss, and resilience traits revealed the potential to develop new traits based on daily milk yield to help farms in managing and monitoring disease conditions of dairy cows, as well as opportunities for breeding for improved disease resilience. This study also showed longitudinal data and methods to facilitate the construction of informative disease resilience indicators to optimize the understanding of dairy cow responses to specific environmental challenges.

ACKNOWLEDGMENTS

This study was supported by the National Key R&D Program of China (2021YFD1200093; Beijing, China); National Agriculture Key Science & Technology Project (NK20221201; Beijing); Ningxia Agricultural Breeding Program (Dairy 2019NYY205; Yinchuan, China); the Key Research Project of Ningxia Hui Autonomous Region (2022BBF02017; Yinchuan), the earmarked fund for CARS-36; and, the Program for Changjiang Scholar and Innovation Research Team in University (IRT_15R62; Beijing). Author Dengke Liu is employed by Hebei Sunlon Modern Agricultural Technology Co. Ltd., and Gang Guo is employed by Beijing Sunlon Livestock Development Co. Ltd. The authors have not stated any other conflicts of interest.

REFERENCES


