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Mapping genomic regions affecting resilience traits in a large dairy farm of Holstein cows

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Introduction: This study evaluated the genetic architecture of resilience indicators in Holstein cows managed in a herd equipped with automatic milking systems (AMS) from 2017 to 2024.

Methods: Four resilience indicators were calculated based on deviations in daily milk yield: log-transformed variance (LnVar), autocorrelation of residuals (rauto), weighted frequency of perturbations (wfPert), and accumulated milk losses due to perturbations (dPert). Polynomial quantile regression models were applied to 594,481 daily records from 966 cows, with data filtered for completeness and lactation duration. Genome-wide association studies (GWAS) were performed using selective genotyping coupled with DNA pooling statistics.

Results: Descriptive statistics revealed that LnVar increased with parity, indicating greater production variability in older cows, while rauto remained stable, suggesting a consistent ability of cows to recover from production perturbations. Both dPert and wfPert increased across lactations, reflecting greater cumulative losses and perturbation frequencies. Genes related to immune response, energy metabolism, and tissue integrity were identified.

Discussion: These findings suggest a multifactorial complex genetic nature of resilience and disclose the involvement of several genes that can explain both the physiology related to production and response to stressors.

KEYWORDS

resilience, Holstein, milk yield, GWAS, QTL

1 Introduction

Over the past few years, the introduction of advanced monitoring systems in dairy farms such as automatic milking systems (AMS), have significantly enhanced data collection capabilities. The adoption of AMS provides continuous and high-resolution data. This system enables an unprecedented ability to monitor fluctuations in milk yield and to detect health-related disturbances. Such systems offer valuable insight into resilience, particularly by facilitating the longitudinal monitoring required to capture production responses to environmental stressors and management interventions (Niloofar et al., 2021).

Resilience in dairy cows is a critical aspect, especially in the face of environmental disruptions such as calving, infection by various pathogens, extreme weather (e.g. heat waves), or fluctuations in feed availability and management practice (Hansen et al., 2012; Aradotlu Parameshwarappa et al., 2019). Maintaining high milk yield is essential for the profitability of dairy farms, but health issues can lead to substantial losses in milk production and its quality (Liang et al., 2017). These losses often appear as disruptions in the lactation curve (Hertl et al., 2014; Ben Abdelkrim et al., 2021), due to reduced feed intake, immune system activation (Daniel et al., 2016) and intramammary infections affecting the udder's functionality (Heikkilä et al., 2018). Resilient cows are minimally affected by these disturbances and are able to recover quickly, resulting in reduced labor requirements, lower treatment costs, and decreased milk yield losses compared to their less resilient counterparts (Colditz and Hine, 2016; Berghof et al., 2019b). In order to quantify general resilience, two primary indicators have been recently proposed: the natural log-transformed variance of deviations from an expected lactation curve (LnVar) and the lag-1 autocorrelation of yield deviations (rauto). These indicators are based on the hypothesis that cows with stable production (low LnVar) and quick recovery from disturbances (low rauto) exhibit greater resilience (Scheffer et al., 2018; Poppe et al., 2020). Lower LnVar values indicate more stable production and higher resilience to disturbances (Scheffer et al., 2018). Differently, the parameter rauto assesses recovery times from disturbances by measuring the autocorrelation of yield deviations over time. A lower rauto value signifies a faster return to baseline performance following disturbances, indicating quicker recovery (Poppe et al., 2020; Wang et al., 2022a). In addition to these established indicators, more recent published research has introduced novel metrics for resilience assessment, daily perturbations (dPert) and weighted frequency of perturbations (wfPert) (Chen et al., 2023). These metrics propose a more dynamic and real-time evaluation of resilience by capturing the frequency and impact of deviations in daily milk yield.

Similar approaches have been applied to dairy cattle using daily step counts and to other livestock species, including pigs, chickens, and lambs, using longitudinal records of feed intake and body weight (BW) (Berghof et al., 2019a; Nguyen-Ba et al., 2020; Ben Abdelkrim et al., 2021; Garcia-Baccino et al., 2021; Poppe et al., 2022).

Despite these advances, the integration of genomic information to uncover the genetic basis of resilience is still in its infancy and remains an open topic that requires further investigation. Studies have shown that LnVar has the highest heritability among resilience indicators, ranging from 0.13 to 0.21 depending on the lactation stage (Poppe et al., 2021a; Chen et al., 2023). It is also significantly genetically correlated with health, longevity, fertility, and metabolic traits, highlighting its potential value for breeding more resilient cows. In contrast, rauto exhibits low heritability, ranging from 0.02 to 0.08, suggesting that while it provides some insight into recovery times, it may be less effective for selection purposes. Similarly, the heritability values of wfPert and dPert are low, spanning from 0.01 to 0.06 across different parities (Chen et al., 2023).

Genome-wide association studies (GWAS) have revealed differences in the genetic architecture of resilience indicators derived from milk yield variability in North American Holstein cattle (Chen et al., 2024). Relevant genomic regions and biological pathways, particularly those related to intestinal homeostasis, were identified and Mendelian Randomization (MR) analyses indicated an unfavorable causal association between daily milk yield (DMY) and LnVar, suggesting caution in its use for breeding resilient cattle (Chen et al., 2024). Additionally, herd management significantly affects resilience indicators, as variations across herd-years highlight the impact of practices like feed management on environmental disturbances (Chen et al., 2024).

Based on this concept, the aims of the present study were: i) to calculate the resilience indicators described above for cows farmed in a single dairy herd with a large amount of longitudinal data available from an AMS; and ii) to perform a GWAS based on a selective genotyping (Darvasi and Soller, 1992) using the selective DNA pooling statistics methodology to identify linkage between QTLs and SNP markers (Darvasi and Soller, 1994).

2 Materials and methods

2.1 Ethics statement

No animal care committee approval was necessary for the purposes of this study, as all genotypes and data were available from the pre-existing database of the GENORIP project, funded by the Lombardy Region

2.2 Data editing

In this study, we used data collected from nine AMS units between January 2017 and August 2024 on a farm in the Lombardy region (Italy). The dataset consisted of 594,481 daily observations from 966 individual cows, 113 of which had three completed lactations. To ensure the use of high-quality data, we conducted rigorous preprocessing. The first step involved thorough data editing, focusing on filtering out records with missing values or instances where daily milk production was recorded as zero. Next, we removed from the analysis the production of the first 10 Days in Milk (DIM). Then the data analysis was stratified by parity while only those from 1 to 3 were retained. Lactations were filtered by selecting only those that started before the 30th DIM in our database and had at least 250 daily recordings (i.e. DIM); we considered for the study the conventional lactation at 305 DIM. Table 1 provides a detailed summary of the number of observations at the start of the analysis and the remaining counts after each data filtering step, alongside the corresponding relative distribution of cows analyzed. After the application of all filtering criteria, the final

TABLE 1 Workflow adopted before computing the resilience indicators.

Editing steps	N. of observations	N. of cows
Initial data set	594,481	966
Removed daily records with missing information	520,509	965
Removed first 10 days	452,891	946
Lactation 1	193,113	681
Max DIM < 305 & Min DIM < 10	178,069	627
Lactation 2	139,690	582
Max DIM < 305 & Min DIM < 10	92,213	320
Lactation 3	62819	277
Max DIM < 305 & Min DIM < 10	38,535	136

dataset included 627 cows in first parity, 320 in second parity, and 136 in third parity. This progression ensures a robust dataset with representative samples across the different parity orders for subsequent analyses.

2.3 Expected lactation curve

For each cow and each parity order, we applied a fourth-degree polynomial quantile regression using the 'quantreg' package in R and the poly function to generate orthogonal polynomial terms (Koenker et al., 2018; Poppe et al., 2020). This approach allowed us to capture nuanced variations in milk production. For each cow and parity, a polynomial quantile regression curve with the 70th percentile (τ = 0.7) was fitted using daily milk production data and days in milk (DIM), capturing the expected milk yield under normal conditions

$$y_i = \beta_0 + \beta_1 \cdot DIM_1 + \beta_2 \cdot DIM_i^2 + \beta_3 \cdot DIM_i^3 + \beta_4 \cdot DIM_i^4 + e_i$$

where: *yi* is the predicted daily milk production for day DIMi; β_0, β_1, β_2, β_3, β_4 are the intercept and the first to fourth order coefficients of the polynomial regression describing the relationship between *DIMn* and y_i; e_i is the error term.

The choice of the 70th percentile in the quantile regression was guided by its ability to provide a balanced estimate of the expected milk yield under normal physiological conditions. This quantile reflects production levels that are less influenced by short-term fluctuations or extreme negative deviations, making it particularly suitable for evaluating how well cows sustain higher yields in the face of potential disturbances. This approach has been supported in previous studies (Poppe et al., 2020) which employed the 70th percentile for similar purposes in resilience assessment. Then we calculated the daily yield deviations (residuals) as follows:

$$Res_i = Observed \ Daily \ Milk \ Production - y_i$$

where Res_i represents the deviation between the observed and expected milk yield on day DIMi, as derived from model (1)

2.4 Resilience indicators calculation

Four resilience indicators were then calculated starting from the daily yield deviations residuals, Resi, all of which are based on the cited studies (Poppe et al., 2020; Adriaens et al., 2021; Chen et al., 2023):

i. Natural log-transformed variance (LnVar): obtained as the natural logarithm of the variance of all Resi:

$$LnVar = log(\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2),$$

where n represents the total number of observations, x denotes the ith observation, spanning from the first to the last available DIM, and $\bar{x}$ represents the average of the *i* observations.

LnVar provides a comprehensive measure of milk production stability within each lactation.

ii. Autocorrelation of Residuals (rauto): is calculated as the autocorrelation of residuals over consecutive days:

$$rauto = \frac{n}{s^2(n-1)}\sum_{i=1}^{n_i}(x_i - \bar{x})(x_{i+1} - \bar{x})$$

where n represents the total number of observations, *x* denotes the i^{th} observation, spanning from the first to the last available DIM, $\bar{x}$ represents the average of the *i* observations, and *s* represents the standard deviation of the *I* observations.

This indicator helps identify patterns of persistence or volatility in milk production dynamics over time.

iii. Weighted occurrence frequency of yield perturbations (wfPert): this indicator quantifies the occurrence of perturbations. Only sequences of at least four consecutive days with negative deviations from expected values in milk production were considered. Each sequence was required to include at least one day in which total daily milk production fell below 80% of the estimated unperturbed lactation curve. The duration of each sequence was weighted (1/4 per day) to assess its overall impact on milk production efficiency (Adriaens et al., 2021; Chen et al., 2023):

$$wfPert = \sum_{p=1}^{n} \frac{l_p}{4}$$

where *lp* is the length of the perturbation and n is the total number of perturbation events. At the end the indicator was normalized to a ratio per 100 DIM, dividing it by the number of DIM and multiplied by 100.

iv. Accumulated milk losses of yield perturbations (dPert): it stands for the total milk losses associated with perturbation and was calculated as the differences between expected productions and actual ones, in percentage (Adriaens et al., 2021; Chen et al., 2023).

$$dPert = \sum_{p=1}^{n}\sum_{d=1}^{lp} \frac{yexp_d - yobs_d}{yexp_d} * 100$$

where n is the total number of perturbation events, *lp* is the length of each perturbation (DIM); *yexp* and *yobs* are the estimated and observed daily milk yield, respectively. Finally, the indicator was normalized to a ratio per 100 DIM, dividing it by the number of DIM and multiplied by 100.

The Pearson correlations between pairs of indicators were assessed both within and across lactations. Within each lactation, correlations were calculated between the four resilience indicators (LnVar, rauto, dPert, wfPert), grouped by parity order. Additionally, to evaluate consistency across lactations, correlations were computed between the same indicators for cows with complete records across all three lactations.

2.5 Heritability of resilience indicators

Genetic parameters were estimated using the following animal model:

$$y = X\beta + Zu + e$$

where y is the vector of phenotypic observations for each resilience indicator, β is the vector of fixed effects including age at calving (in months), month of calving (January to December), and year of calving (2017–2024), u is the vector of random additive genetic effects assumed to follow a distribution $u \sim N(0, A\sigma^{2u})$ where A is the pedigree-based relationship matrix, and e is the vector of residual errors $e \sim N(0, I\sigma^{2e})$.

Heritability was estimated using AIREMLF90 software (Misztal et al., 2018) based on the animal model described above. Estimates were obtained separately for parity order 1 and 2, not for parity order 3 because of the small sample size. The fixed effects include: age at calving expressed in months (from month 22 to 36 for parity 1 and 33 to 49 for parity 2), the month of calving (January to December), the year of calving (from 2017 to 2024). The relationship matrix was calculated using the available genealogical information. All cows included in the study were genotyped using the GGP 100K SNP chip, and the pedigrees were all previously verified by ANAFIBJ (Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana; National Association of Breeders of the Friesian, Brown and Jersey Italian Breed). This validation step based on genomic information ensured the accuracy and consistency of the genealogical data provided for all the animals included in the study, reducing possible bias in estimating variance components due to incorrect genealogical recording. The pedigree information for each individual, included all available generations of ancestors and was employed to build the relationship matrix. The overall pedigree file included a total of 3158 animals, including animals with phenotypes and individuals non-phenotyped. The pedigree file was used to build the additive relationship matrix A implemented in the animal model.

2.6 Genome-wide association studies

To investigate the genetic basis of resilience in dairy cows, we conducted a genome-wide association study (GWAS) using a selective genotyping approach (Darvasi and Soller, 1992), an efficient strategy that involves genotyping only individuals with extreme phenotypes to enhance the detection of SNPs associated with the trait (Darvasi and Soller, 1992). This methodology is particularly efficient in detecting QTL, as a considerable amount of genetic information resides in individuals with extreme phenotypes (Xing and Xing, 2009; Lipkin et al., 2016).

Cows were selected based on the 10% most extreme values (highest and lowest) for each indicator: the most resilient (RE) and least resilient (NRE) cows. Since each indicator captures different aspects of lactation curve perturbations, animals ranked in the top or bottom 10% for one indicator are not necessarily the same as those in another (Medugorac and Soller, 2001). All genotypes (mapped according to the ARS-UCD1.2 bovine genome assembly) were already available for both selected RE and NRE cows. To simulate the selective DNA pooling strategy (Darvasi and Soller, 1994) using individual genotype data, each RE and NRE group was randomly divided into two biological replicates (RE1/RE2 and NRE1/NRE2) with comparable sample sizes. For each replicate, allele frequencies at each SNP marker were calculated using the ‘genotype statistics by marker’ function of Golden Helix’s SNP & Variation Suite (SVS v8.9, Golden Helix Inc., Bozeman, MT, USA). The GWAS was conducted only for resilience indicators with heritability greater than 0.05, both in our dataset and consistently reported in the literature (Poppe et al., 2020; Chen et al., 2023). This dual criterion ensured a focus on traits with a strong biological and genetic basis, making them more reliable for downstream genomic analysis. The analysis was further restricted to the first and second lactations, which included the largest number of animals (627 and 320 cows, respectively), to ensure robust estimation of both phenotypic indicators and genetic parameters.

The GWAS analyses were conducted by comparing the allele frequency of each SNP (for one of the possible alleles) between resilient (RE1 and RE2) and non-resilient (NRE1 and NRE2) cows using an in-house R script (R version 4.0.5). Monomorphic markers, which do not provide useful information, were excluded from the analysis. Additionally, SNPs showing a high degree of variability between replicates (i.e., the top 5% of the absolute value of allele differences between RE1 vs RE2 and NRE1 vs NRE2) were also removed to reduce potential false positives calls.

After the filtering process, the following numbers of SNPs were retained, out of the original 89,764 autosomal markers, for the analysis: i) LnVar1: 63,862 SNPs; ii) LnVar2: 60,513 SNPs; iii) rauto1: 69,347 SNPs; iv) rauto2: 65,066 SNPs; v) dPert2: 67,124 SNPs.

This filtered dataset ensures high-quality genetic markers are available for robust downstream analyses.

According to (Darvasi and Soller, 1994) a single-marker association test was applied, calculating a Z-test for each marker to assess the association between the A allele frequency difference and resilience status. The Z-test was defined as follows:

$$Ztest = \frac{Dtest}{SD(Dnull)}$$

where *Dtest* is the difference in the A allele frequencies between the resilient and non-resilient groups, and *Dnull* is the difference within groups (i.e., between the two biological replicates within RE or NRE groups).

The association results were visualized using a Manhattan plot, generated with the qqman R package (Turner, 2018). After the analysis, the False Discovery Rate (FDR) and Bonferroni correction thresholds were used for determining the statistically significant SNPs.

2.7 Gene annotation

All SNPs over the 5% FDR threshold were annotated, and the SNP's rsID code (Reference SNP cluster ID) of each of the Illumina SNP markers has been obtained. The Variant Effect Predictor (VEP) tool of the Ensembl database allowed to annotate the significant SNPs through the rsID codes according to the Bos taurus genome assembly ARS-UCD1.2 (Annotation Release: 106). Candidate genes were identified as: i) if a significant SNP was annotated within a gene, this latter was considered as a candidate gene; ii) for intergenic SNPs, the candidate gene was the one mapping closest, either upstream or downstream, within a maximum distance of 500 kb.

QTL associated with each indicator were identified using the QTLdb's 'Search by associated gene' option available within the Cattle Quantitative Trait Locus (QTL) Database of Animal QTLdb (https://www.animalgenome.org/cgi-bin/QTLdb/BT/index).

3 Results and discussion

3.1 Modeling lactation curves

The modeling of lactation curves using polynomial quantile regression allowed for the estimation of the expected daily milk production. This approach, as detailed in Poppe et al (Poppe et al., 2020), captures the variability in milk production across different days in milk (DIM) and provides a robust basis for calculating deviations from expected milk yield and consequently the resilience indicators. The graphs in Figure 1 illustrate the lactation performance of two cows: one classified as more resilient (Figure 1a) and the other as less resilient (Figure 1b).

The first visible difference between the curves is the fluctuations of the daily milk yield. In Figure 1a, with LnVar value 1.06, the cow shows a relatively stable milk production over time: the milk yield remains consistent, with minor day-to-day variability, assuming the cow's strong ability to cope with stressors. In contrast, the cow in Figure 1b has a value of LnVar equal to 2.98 and reveals more significant fluctuations in daily milk yield and a sharper decline in production. The pattern of milk yield by DIM respect to the expected lactation curve suggests that the resilient cow is more capable to maintain high levels of productivity along the lactation. The less resilient cow, on the other hand, exhibits greater variability in daily milk production, which is hypothesized to reflect a reduced capacity to recover from stressors. Periods of stress are marked by sharp declines in production, followed by slower recoveries, which can be interpreted as a diminished ability to maintain production efficiency over time.

3.2 Descriptive statistics of the derived resilience indicators

Table 2 shows the descriptive statistics for the calculated resilience indicators across different lactations. When compared to recent studies, LnVar across parity orders are similar to the results of (Chen et al., 2023) (average from 1.39 to 1.83 and SD from 0.57 and 0.62), while they are different when compared to (Poppe et al., 2021a) (average from 4.40 to 4.99 and SD from 0.66 and 0.79). The observed increase in LnVar through the parity orders is consistent with findings from the mentioned studies, showing that milk yield variability tends to rise proportionally with aging. Possibly, this is due to physiological changes that occur with aging, such as changes in mammary gland function or in metabolic regulation. Additionally, it is possible that prolonged exposure to environmental stressors, including variation in management or in environmental conditions over time, may contribute to the increase of the variability (Friggens and Badsberg, 2007; Wathes et al., 2007).

The rauto values indicate a consistent pattern of autocorrelation through parities. In our study, the mean rauto values were 0.62, 0.63, and 0.62 for the first, second, and third parity, respectively. In comparison, Chen et al (Chen et al., 2023). reported mean rauto values of 0.37, 0.43, and 0.44 for these parities, while Poppe et al (Poppe et al., 2021a). observed values of 0.55, 0.56, and 0.55. This stability suggests that the persistence in milk production patterns stays relatively constant over different parity orders. This somehow contrasts with the increasing variability observed in LnVar, highlighting that while overall variability in milk yield increases with parity, the persistence of production patterns (as measured by autocorrelation) may remain stable. This difference underscores the complexity of lactation dynamics, where variability and persistence can be influenced by different factors and may not always align.

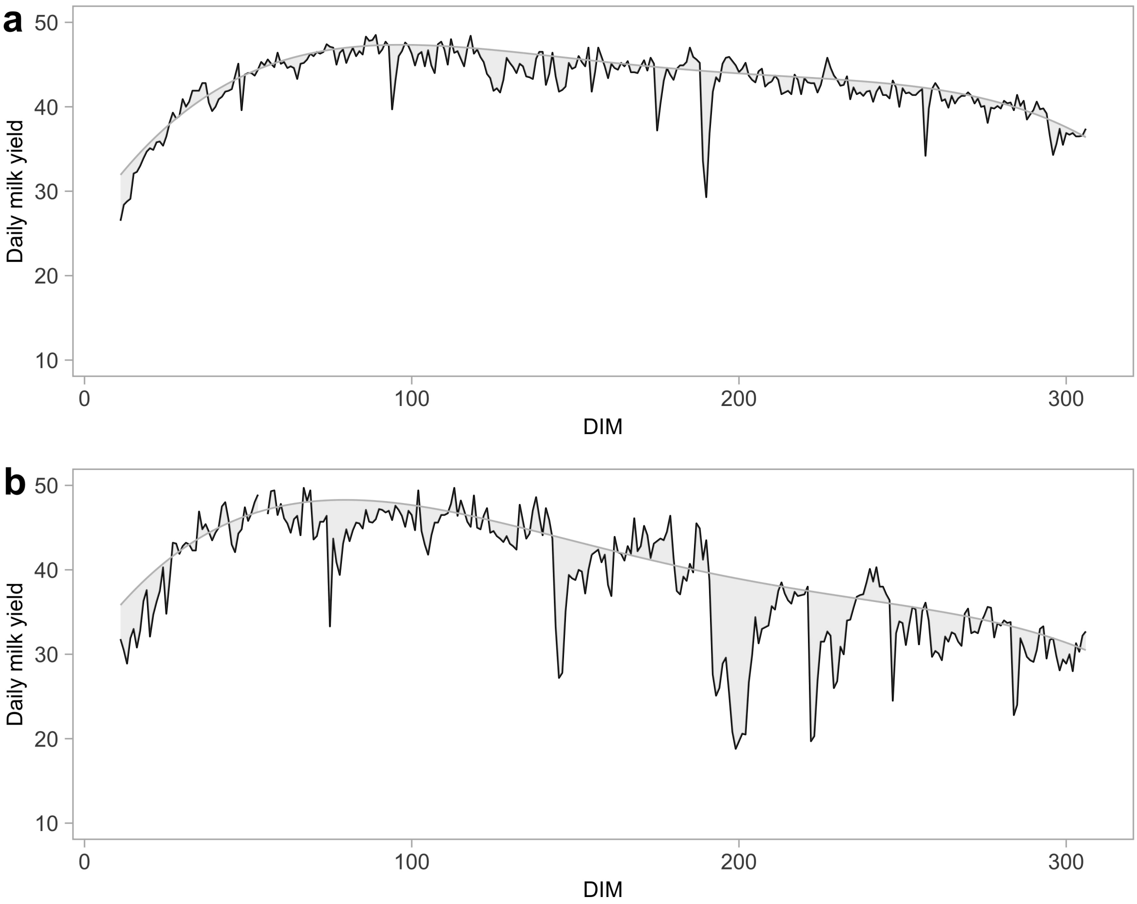

FIGURE 1
In this figure two lactation curves are reported as examples of how resilience is captured and how two different cows are considered resilient **(a)** and non-resilient **(b)**. The represented lactations belong to cows in their first order of parity.

The upward trend in dPert across lactations suggests that milk production losses due to perturbations accumulate more significantly as cows' parity increases. In our study, the average dPert ranged from 19.1 to 20.2, with a standard deviation (SD) ranging from 2.44 to 2.74 across lactations. These values are higher than those reported by Chen et al (Chen et al., 2023), who found averages between 15.62 and 17.43 for the same parities. This discrepancy may be explained by the generally higher milk production in multiparous cows, where any deviation from expected yield results in proportionally greater losses. Consequently, higher yields in older cows are likely to contribute to more substantial accumulated milk losses during perturbation periods. The wfPert values show a relatively consistent frequency of perturbations across parties, with only slight variations seen in the third one. In our study, there were 24 cows in the first lactation without any perturbations (3.8%), 10 in the second lactation (2.8%), and 4 in the third lactation (2.9%).

The wfPert showed an average of 4.68 ± 2.67 s.d. in the first lactation, compared to 5.03 ± 2.81 in the second lactation and 5.57 ± 2.45 in the third lactation. Although the average for the third lactation is a bit higher compared to the first and second lactations, the differences are not large. In Chen et al (Chen et al., 2023). the results are slightly bigger: from the first to the third parity order, averages and standard deviations are 5.43 ± 1.75, 5.85 ± 1.91 and 6.11 ± 1.94, respectively.

The correlation analysis of resilience indicators within lactation and across lactations provides valuable insights into the relationships between the various measures of milk production stability and perturbations. The results of the correlation matrix are visualized in Figure 2.

In the first lactation (Figure 2a), the pairwise correlations between the indicators are all positive and range from moderate to high, varying from 0.22 (between rauto and wfPert) to 0.78 (between wfPert and dPert). This pattern is expected, as all indicators are derived from the same residuals obtained through the quantile regression model and are specifically designed to capture different aspects of resilience. The strongest correlation, observed between dPert and wfPert, reflects the similarity in their definitions—both quantify production losses due to perturbations, albeit from different perspectives.

Moreover, it makes biological sense that a cow with more frequent production losses exhibits a greater total loss. Strong correlations are also observed between LnVar – dPert and LnVar – wfPert. This suggests that cows with higher variability in milk

TABLE 2 Descriptive statistics of the resilience indicators.

Indicator	Average			SD			Min			Max		
Lactation	1	2	3	1	2	3	1	2	3	1	2	3
LnVar	1.86	2.31	2.49	0.48	0.57	0.53	0.44	0.64	1.38	3.40	4.25	4.07
rauto	0.62	0.63	0.62	0.11	0.12	0.23	0.23	0.15	0.29	0.90	0.93	0.87
dPert	19.07	19.39	20.02	2.44	2.74	2.65	13.87	13.17	15.20	28.47	31.00	27.39
wfPert	4.68	5.03	5.57	2.67	2.81	2.45	0.00	0.00	0.00	13.01	12.46	13.18

LnVar, log-transformed variance of daily milk yield residuals; rauto, lag-1 autocorrelation of daily milk yield residuals; dPert, accumulated milk losses of yield perturbations; wfPert, weighted occurrence frequency of yield perturbations.

production (LnVar) also experience more frequent and severe production losses. In contrast, the lowest correlations involved rauto, which measures the persistence of deviations. Interestingly, in lactations 2 and 3 (Figures 2b, c), the correlations between rauto and the other indicators became stronger, while the remaining pairwise correlations remained relatively stable. As shown in Figures 2a–c, correlations within lactations tended to strengthen across parities. However, this pattern is not evident in Figure 2d, where correlations between indicators across lactations are generally low and tend to weaken. As cows age, they do not exhibit consistent resilience patterns across parities, suggesting that their responses to perturbations evolve over time. Furthermore, Figure 2d confirms that strong correlations between indicators are primarily observed within the same lactation. Calculating these resilience indicators within a single farm has the potential to help farmers better understand the productive life of cows at the end of their lactation. The indicators can also be used to measure the ongoing production and behavior of cows, so that attention can be paid to those animals that show different behavioral patterns. Productive animals are known to be less resilient, depending on how we measure the resilience indicators. Since some resilience indicators are constructed such that higher values reflect greater instability or poorer recovery, they are often positively genetically correlated with milk yield. In this context, higher values of these indicators correspond to lower resilience and higher milk production (Poppe et al., 2021b). This is an explicit trade-off between resilience and production. However, accurate measures of cow life events that may be available to help calculate fertility and reproductive traits, together with treatment records kept by farmers, could help to assess a better definition of resilience. In addition, AMS can provide more than just daily milk yield, but longitudinal traits such as feeding behavior and milk contents, which can be used to improve understanding of the physiological responses of lactating cows throughout their lactation. This was not the aim of this study.

3.3 Variance components and heritability

The results of the heritability calculations for the traits are shown in Table 3 and briefly discussed below. However, an important premise must be made before the discussion. As the heritability estimates shown here were obtained in only one herd, they may not fully capture the existing population additive genetic variability, even if the sires used in this farm are selected by the owner himself from all available bulls on the market, i.e. from different AI companies, countries and genetic programs, and as such they are a representative sample of the population. Furthermore, since our goal was not to estimate heritability per se or to introduce it into breeding programs, heritability values were retained for comments and to select the resilience indicator to be the subject of the GWAS, also based on the fact that they confirmed already published values, as hereinbelow commented.

The heritabilities of LnVar in the first and second lactation are both 0.086, which is considerably lower than previous results where 0.13 and 0.15 were estimated for the first lactation and 0.18 and 0.20 for the second lactation (Poppe et al., 2021a; Chen et al., 2023). As shown here and in a previous study, heritability exhibits small variations across parities but does not follow a specific trend, suggesting that the heritability of LnVar may be considered stable throughout a cow's life (Poppe et al., 2021b). For rauto, the estimated values are 0.14 and 0.09 for lactations 1 and 2. These results are slightly higher than those reported previously, ranging from 0.08 for the first lactation to 0.07 for the second one (Poppe et al., 2021a). Our results are also higher than those in another study, where estimates range from 0.04 for the first lactation to 0.02 for the second lactation (Chen et al., 2023). Only one study has estimated the heritability of the resilience indicators dPert and wfPert (Chen et al., 2023). The results we obtained are compared by placing in curly brackets the corresponding values from that study. The heritability of dPert ranges from 0.01 (0.03) in the first lactation to 0.13 (0.06) in the second lactation. Regarding wfPert, the estimated heritabilities obtained here were zero, while another study reported values of 0.04 and 0.02 for the first and second lactation, respectively (Chen et al., 2023).

3.4 Genome wide association study

As hereinbefore described, GWAS were performed using the selective genotyping approach coupled to the statistics of DNA pooling to investigate the genetic basis of resilience in the

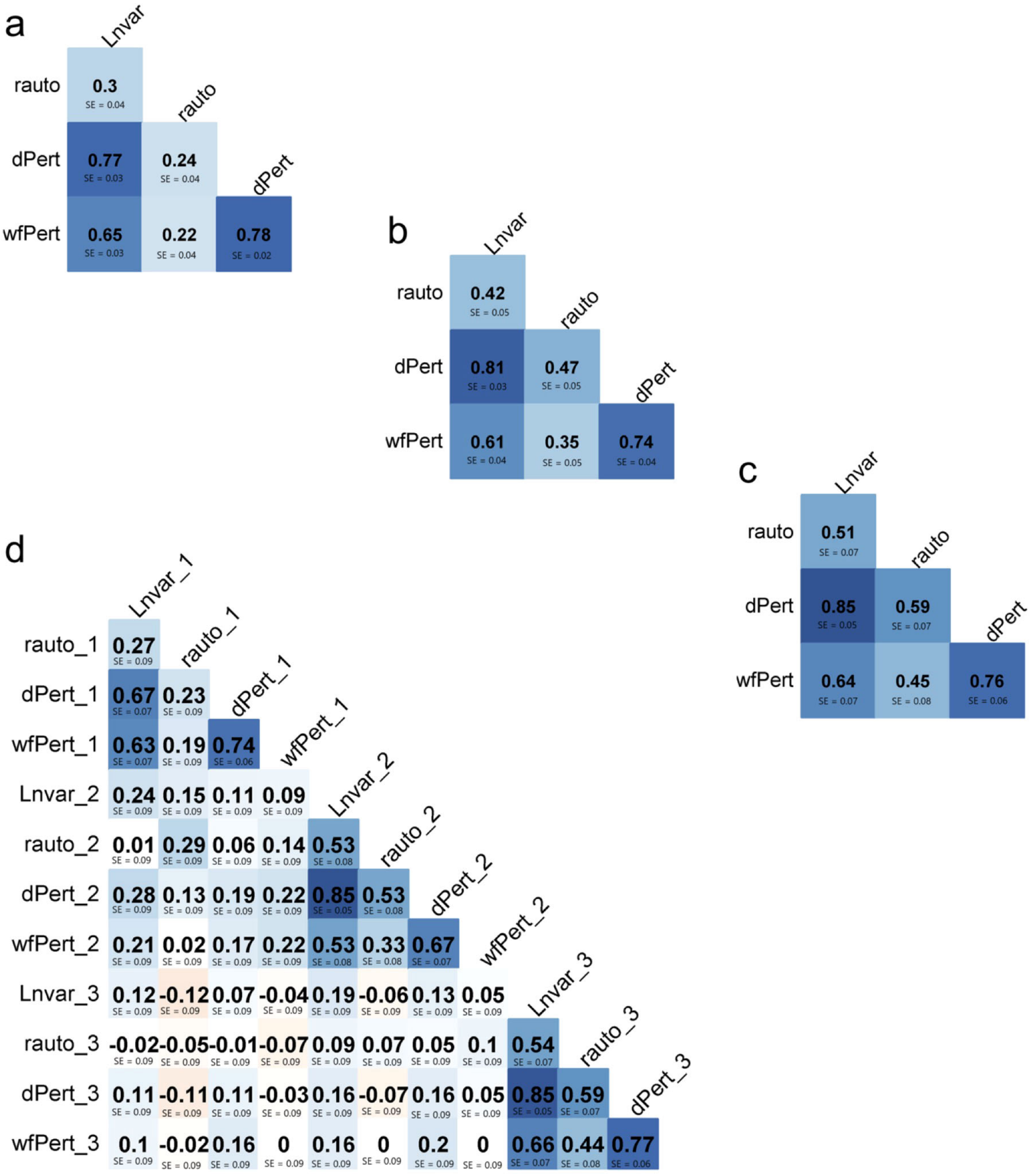

FIGURE 2
Correlation between resilience indicators within lactation one, two and three [respectively, **(a–c)**] and across lactation **(d)**.

population only for those indicators that have shown heritability. The descriptive statistics shown in Supplementary Table S1 refer to the full RE and NRE groups prior to replicate creation.

For the animals selected for the GWAS (top 10% most resilient - RES and bottom 10% least resilient - NRES) the average gEBVs for milk yield and SCC of the cows were plotted in Supplementary Figure S1. The figure includes only the resilience indicators selected for GWAS analysis (i.e., LnVar, rauto, and dPert). Interestingly, the analysis revealed that RES animals consistently exhibited lower gEBV values for milk yield and higher value for SCC across the three resilience indicators (Supplementary Figure S1). This trend was particularly evident for LnVar-based indicators. The results here obtained are in agreement with the findings of (Chen et al., 2024) that showed an unfavorable causal association between daily milk yield (DMY) and LnVar. Results of each GWAS are graphically shown in the Manhattan plots of Figures 3A–E and reported in Table 4.

A total of 66, 22, 60, 33, and 8 significant SNPs were identified above the 5% FDR threshold (blue line) for LnVar1, LnVar2, rauto1, rauto2, and dPert2, respectively (Supplementary Table S2). These SNPs were located in intronic (n = 79), intergenic (n = 99), 5'UTR (n = 2), 3'UTR (n = 4), and intragenic regions (n = 2; one missense and one synonymous). Based on the location of these SNPs, a total of 124 candidate genes were identified for functional interpretation. Out of these, the Animal Genome Cattle Database linked 40 genes to six main "QTL Terms – Trait_class" categories, comprising 77 unique "QTL Trait_Name" entries (Supplementary Table S2). As shown in Supplementary Figure S2, milk-related traits were more frequently

TABLE 3 Genetic (Gen.) and residual (Res.) variance components and heritability (h2) for each indicator within lactations 1 and 2.

Indicator	Lactation	Gen. variance (SE)	Res. variance (SE)	Heritability (SE)
LnVar	1	0.019 (0.017)	0.204 (0.019)	0.086 (0.076)
rauto	1	0.0016 (0.0011)	0.0098 (0.0011)	0.143 (0.092)
dPert	1	0.033 (0.261)	5.85 (0.42)	0.01 (0.044)
wfPert	1	0 (0)	7.08 (0.42)	0 (0)
LnVar	2	0.026 (0.038)	0.275 (0.042)	0.086 (0.128)
rauto	2	0.0014 (0.002)	0.014 (0.0022)	0.09 (0.14)
dPert	2	0.895 (1.00)	6.14 (1.05)	0.127 (0.14)
wfPert	2	0 (0)	7.34 (0.63)	0 (0)

Estimates were found with the use of the Average Information REML algorithm. The values minor than 0.01 (1%) for the heritability are written as 0, while the values for variance components (Gen. and Res.) and their standard errors are written as 0 when minor than 0.001.

associated with LnVar1 than with LnVar2. QTL terms associated with LnVar2 were predominantly linked to milk-related traits (87.0%), whereas for LnVar1, only 30.8% were related to milk production. The remaining QTLs associated with LnVar1 were distributed among other categories, including exterior traits (26.2%), reproduction (16.9%), production traits (16.9%), meat and carcass traits (6.2%), and others. This discrepancy may reflect physiological and genetic differences in milk production dynamics between first and second parity. The genetic correlations between milk yield in the first and second lactations have been estimated at approximately rg = 0.82, indicating a strong but not perfect relationship between the two (Dong and Van Vleck, 1989). This suggests that while a common genetic component influences both lactations, specific factors unique to each parity also contributes to milk production dynamics. The potential roles of some of candidate genes in resilience are then herein below discussed by resilience indicator.

3.4.1 LnVar

For LnVar1, on BTA 1 a total of 9 significant SNPs were above the FDR threshold (Figure 3a). Among them, rs110792885 is located in intron position of the *EPHB1* gene (EPH Receptor B1), one of the Eph (erythropoietin-producing hepatocellular carcinoma) receptors representing one of the largest known family of receptor tyrosine kinases in mammals: there is evidence the Eph receptors and ephrin ligands may mediate immune cell activation and the immune cell trafficking required for optimal functioning of immune system (Darling and Lamb, 2019). Given that Eph receptors are involved in various normal cellular processes during development and play a crucial role in maintaining adult tissue homeostasis, their role in both non-infectious and infectious diseases is well established. As a result, the pathways in which they operate may indirectly impact stress responses and resilience traits. The *EPHB1* gene was also associated with udder traits (udder suspension and teat score) in Angus cattle (Devani et al., 2020). On BTA 2, three above FDR threshold SNP are in intron sequences of *SCN2A* and *SCN3A* genes, but none of their functions that may be related to resilience has been reported to date. The SNP rs110164494, is located in the intron of the *PDSS1* gene on BTA 13. *PDSS1* encodes the enzyme decaprenyl diphosphate synthase subunit 1, which is involved in the synthesis of coenzyme Q (ubiquinone), a key component of mitochondrial electron transport and cellular energy production. The association of *PDSS1* with female fertility traits has been highlighted in previous studies, such as Mohammadi et al (Mohammadi et al., 2020), who identified it in a GWAS focused on Iranian Holstein cattle. This study found that *PDSS1* could play a role in female fertility through pathways linked to cellular energy metabolism, which is crucial for reproductive performance and resilience during energy-intensive periods like lactation.

On BTA14 at about 6 Mbp, two intergenic markers (rs110769987 and rs42211697) were found to be significantly associated with LnVar1 and were located closely to *KHDRBS3*. This gene has previously been associated with milk production traits in both Chinese Holstein and crossbred populations (Jiang et al., 2010; da Cruz et al., 2020).

The *ST3GAL1* gene was also found to be associated with milk traits, including milk yield, milk fat yield, and milk fat percentage (Wickramasinghe et al., 2011). Thyroglobulin gene (*TG*), identified as candidate gene on BTA14 (rs29021775), is a key glycoprotein involved in the synthesis of thyroid hormones. *TG* plays an important role in different physiological processes including regulating the metabolism, adipocyte growth, differentiation and homeostasis of fat depots (Dubey et al., 2014). Given its function TG can be easily linked to resilience. In fact, studies in rat and livestock have demonstrated that thyroid function, influenced by *TG*, is critical for coping with temperature extremes, supporting its role in resilience to environmental stressors (Sejian et al., 2018; Rial-Pensado et al., 2022).

At about 82 Kbp from rs41629530 on BTA 15 is located the *GRAMD1B* gene, part of the GRAM (glucosyltransferases, Rab-like GTPase activators and myotubularin) domain- containing gene group, involved in maintaining cholesterol homeostasis, apoptosis and cancer (Yang et al., 2011). In livestock, *GRAMD1B* was already associated with feed efficiency, production and reproduction traits in cattle (Kunej et al., 2024). *SERGEF* at positions about 34 Mbp (identified as candidate gene by rs110010916 and rs109595542) has been linked to pig adaptation to high-altitude conditions, suggesting

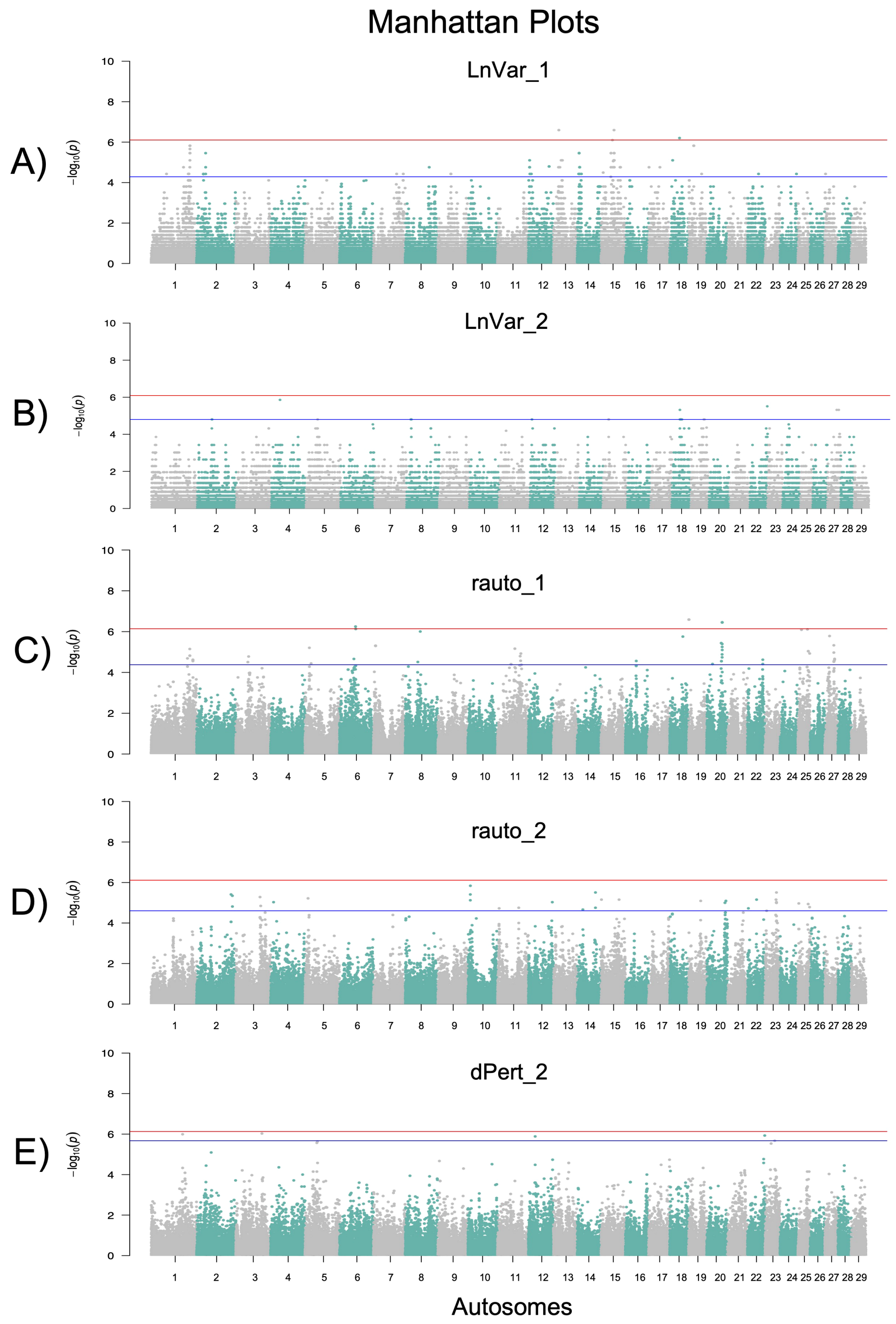

FIGURE 3
Manhattan plots of the GWAS results for the resilience indicators showing hereditability: LnVar – first **(A)** and second Lactation **(B)**; rauto – first **(C)** and second Lactation **(D)**; dPert – second Lactation. The lines represent the False Discovery Rate (blue) and Bonferroni (red) thresholds, respectively, set at 5% genome-wide.

potential resilience to environmental stressors that may indirectly influence lactation stability (Dong et al., 2014).

The significant rs110249272 maps in the 3'UTR region of the *RNF135* gene (Ring Finger Protein 135) involved in the activation of the NF-κB signaling pathway, which is essential for immune responses, inflammation regulation, and cell survival (Kiser et al., 2018). In cattle, activation of this pathway helps increase resilience to infections like *Mycobacterium avium paratuberculosis* (Map), the pathogen causing Johne's disease. The activation of NF-κB by these proteins boosts the cattle's resilience by ensuring the survival of immune cells, especially monocytes, which are key to fighting infection. This ability to withstand and adapt to immune challenges contributes to overall health and disease resistance (Calderón-Chagoya et al., 2023).

For LnVar2, the major part of candidate genes seems to be involved both in functional and productive traits (as reported in

TABLE 4 Results of the GWAS analysis.

SNP*	CHR	bp_Start	bp_End	P-value (*)	Gene	Variant
Lnvar1						
Hapmap60237-rs29019540	1	112581930	112581930	1.74E-05	MME	intron
BovineHD0100038618	1	134531830	134531830	1.74E-05	EPHB1	intron
ARS-BFGL-BAC-29669, BovineHD0200009212, Hapmap27145-BTA-116154	2	30980589	31096836	1.94E-05	SCN2A	intron
BovineHD0900012694, BovineHD0900012701	9	45190557	45228937	3.73E-05	HACE1	intron
BovineHD1300005194	13	17948346	17948346	1.74E-05	PDSS1	intron
ARS-BFGL-NGS-17819	13	31353706	31353706	7.91E-06	CUBN	intron
ARS-BFGL-BAC-24839	14	4586387	4586387	3.73E-05	FAM135B	intron
DB-838-seq-rs109494080	14	7881268	7881268	3.73E-05	ST3GAL1	intron
Hapmap53910-rs29021775	14	8266712	8266712	1.74E-05	TG	intron
BovineHD1500001927	15	7384011	7384011	3.21E-05	TRPC6	intron
BovineHD1500009549, ARS-BFGL-NGS-118826, BovineHD1500009559	15	34468497	34497030	1.74E-05	TPH1, SERGEF	intron
BovineHD1500011481	15	41240619	41240619	1.74E-05	GALNT18	intron
BovineHD1500013117	15	45782481	45782481	7.91E-06	ZNF215	intron
BovineHD1500019157	15	65873261	65873261	1.74E-05	SLC1A2	intron
Hapmap53277-rs29025667	18	10305903	10305903	7.91E-06	NECAB2	synonyomus
chr19_18330921	19	17991086	17991086	1.50E-06	RNF135	3' UTR
BovineHD2200010872	22	38049578	38049578	3.73E-05	SYNPR	intron
BovineHD2700000363	27	2286136	2286136	3.73E-05	CSMD1	intron
Lnvar2						
BovineHD0400009324, BovineHD0400009326, BovineHD0400009327, BovineHD0400009328, BovineHD0400009334	4	32682347	32692288	1.65E-05	RUNDC3B	intron
BovineHD0500011732, BovineHD0500011741	5	40817911	40874992	9.33E-06	SLC2A13	intron
BovineHD0500012967	5	44906731	44906731	6.09E-06	CPM	intron
BovineHD0500013030	5	45080266	45080266	2.15E-06	NUP107	intron
BovineHD0500013061	5	45169905	45169905	7.27E-07	RAP1B	intron
ARS-BFGL-NGS-19337	19	41942159	41942159	6.09E-06	HAP1	intron
BovineHD1900012186	19	42107233	42107233	1.65E-05	ODAD4	5'UTR
BovineHD2400006407	24	23149184	23149184	1.65E-05	NOL4	intron
rauto_1						
BTB-01935567	1	126208399	126208399	2.05E-05	PCOLCE2	intron
ARS-BFGL-NGS-106296, ARS-BFGL-NGS-101030, BovineHD0100038576	1	134365317	134439762	1.24E-05	EPHB1	intron
BovineHD0300013636	3	44311093	44311093	1.66E-05	PLPPR5	intron
BovineHD0500006098	5	21026129	21026129	3.64E-05	DCN	intron
Hapmap25928-BTA-18390	6	55450495	55450495	5.64E-07	ARAP2	intron
BovineHD0800015665	8	51978193	51978193	9.89E-07	PCSK5	intron
Hapmap43807-BTA-21732	11	59822134	59822134	6.86E-06	C11H2orf74	3' UTR
BovineHD1100022461	11	78167522	78167522	1.60E-05	LDAH	intron

(Continued)

TABLE 4 Continued

SNP*	CHR	bp_Start	bp_End	P-value (*)	Gene	Variant
rauto_1						
Hapmap50260-BTA-121056	16	37673618	37673618	2.75E-05	KIFAP3	intron
ARS-BFGL-NGS-18897	18	45789853	45789853	1.76E-06	HPN	intron
BovineHD1900000212, ARS-BFGL-NGS-92544, BovineHD1900002018	19	1120842	1131844	2.58E-07	CA10	intron
BovineHD2000005959	20	19858203	19858203	3.87E-05	PDE4D	intronic
BovineHD2000014687, BTB-00788976, BovineHD2000014688, BovineHD2000014691, BovineHD2000014724	20	53442839	53557191	1.87E-06	CDH18	intron
ARS-BFGL-NGS-117960	22	52783681	52783681	3.87E-05	TMIE	intron
BovineHD2500003400	25	12057939	12057939	8.16E-07	SHISA9	intron
ARS-BFGL-NGS-59828	25	32764383	32764383	7.77E-07	NCF1	intron
BovineHD2500011569	25	40434604	40434604	1.18E-05	CARD11	intron
BovineHD2700004421	27	16219404	16219404	1.65E-06	FAM149A	5' UTR
BovineHD2700008510, BovineHD2700008511, BovineHD2700008539	27	31017430	31056395	1.51E-05	UNC5D	intron
rauto_2						
BTB-01466122	3	83872828	83872828	5.22E-06	PATJ	intron
BTA-68603-no-rs	3	86312172	86312172	1.43E-05	FGGY	intron
BTA-00702-no-rs	4	10147900	10147900	9.33E-06	CDK6	intron
ARS-BFGL-NGS-69509	5	10190011	10190011	6.04E-06	PTPRQ	intron
ARS-BFGL-NGS-108825, chr10_7895525	10	7960731	7961933	5.70E-06	F2R	synonymous, 3' UTR
BovineHD1100020894	11	73073273	73073273	1.76E-05	OTOF	missense
BovineHD1200025294	12	82887622	82887622	9.33E-06	FAM155A	intron
BovineHD1400018052, BovineHD1400018067	14	62668956	62692420	1.04E-05	NCALD	intron
BovineHD1500018110	15	62392291	62392291	6.99E-06	ELP4	intron
BovineHD2200000724	22	2715544	2715544	1.89E-05	CMC1	intron
Hapmap51784-BTA-97575	23	5530326	5530326	2.49E-05	FAM83B	intron
BovineHD2300011375, BovineHD2300011378, BovineHD2300011379, BovineHD2300011381, BovineHD2300011380	23	39585474	39610535	8.49E-06	KIF13A	intron
ARS-BFGL-NGS-74596	25	35220858	35220858	1.16E-05	COL26A1	3' UTR
Hapmap51005-BTA-60474	25	40111236	40111236	1.64E-05	SDK1	intron
dPert_2						
BovineHD0100031225	1	109410007	109410007	1.02E-06	RSRC1	intron
BovineHD0500011732	5	40817911	40817911	2.70E-06	SLC2A13	intron
BovineHD0500012310	5	42830228	42830228	2.30E-06	PTPRR	intron
BovineHD2200017497	22	59585131	59585131	1.18E-06	KBTBD12	intron

The Table lists only SNPs located within genes, grouped by chromosome. When multiple SNPs map to the same gene, the reported P-value (*) corresponds to the average of the individual p-values. Full results, including all significant SNPs (both genic and intergenic), the corresponding flanking genes, and the associated QTLs for each gene, are provided in Supplementary Table S2.

Supplementary Table S3, QTL). In addition, on BTA 4, all the significant SNPs mapped in the intronic position of *RUNDC3B* gene, that is considered a backfat gene (Yang et al., 2011). As of now, the *RUNDC3B* gene has not been directly associated with backfat thickness in Holstein cattle. However, it is well known, that backfat genes have role in body composition, energy reserves, and overall productivity (Schmidtmann et al., 2024). We may speculate that genes influencing fat deposition (backfat genes) might relate to resilience indirectly, as fat reserves can buffer energy deficits during stress, thereby supporting resilience. The gene *Rap1b*, identified as a candidate by the SNP rs133340933, belongs to the Ras superfamily, a group of proteins involved in regulating B-cell development, homing, and T-cell-dependent humoral immunity (Chu et al., 2008). It is well known, that adaptive immune response, characterized by its specificity and memory, plays a critical role in maintaining long-term resilience against pathogens (Alotiby, 2024). Two significant SNPs were annotated in intronic positions of *SLC2A13*, one of the glucoses transporters, resulted associated with milk, protein and fat yields in Buffaloes species (Du et al., 2019). Even the gene *NOL4* seem to be involved in milk traits according with was reported in different cattle breeds (Bekele et al., 2023).

3.4.2 rauto

Regarding rauto1, the QTL region at about 134 Mbp and defined by three significant SNPs (in intronic position of the *EPHB1* gene) overlaps the one identified for LnVa1. Based on its functions (as described in the LnVar1 GWAS results), we hypothesize that for rauto (reflecting stability and recovery after perturbation) *EPHB1* could regulate pathways that maintain homeostasis under stress by guaranteeing robust intercellular signaling. Instead, for LnVar1 (which measures variability, where lower variability suggests more robust responses to environmental or physiological stress) *EPHB1* might reduce variability by ensuring consistent signaling pathways, supporting immune responses across diverse environmental challenges, and thereby contributing to more consistent physiological outcomes.

On BTA 1, a second significant region is defined by three SNPs, all mapping close to the *POFUT2* gene (max distance 59 Kbp). This gene has been associated both with female fertility in Nordic dairy cattle (Mesbah-Uddin et al., 2022) and with body conformation traits in Holstein (Wang et al., 2022b).

On BTA 3, the genetic variant rs133042560 is located in the intronic position of *DCN* gene, that encodes for decorin, a small leucine-rich proteoglycan involved in connective tissue structure. Decorin binds collagen fibrils and regulates collagen assembly, influencing fibril uniformity (Khatib, 2005). This function is essential for tissue integrity and may impact resilience, particularly in response to metabolic and physical changes during early lactation. *DCN* is maternally expressed in placental tissue in mice, highlighting its potential role in tissue development and adaptation, which could be relevant for bovine resilience during the first lactation (Mizuno et al., 2002).

On BTA 11, two significant SNPs are located near the *IL1B* gene. The *IL1B* gene, as a member of the interleukin-1 (IL-1) family, plays a crucial role in inflammation and immune responses (Moghaddam et al., 2019). Its expression is upregulated in milk somatic cells as part of the immune response during udder infections (Lee et al., 2006). Additionally, increased expression of *IL1B* has been reported in response to infectious agents, such as *Mycoplasma bovis* and *Klebsiella pneumoniae* (Bannerman et al., 2004; Kauf et al., 2007). The *IL1B* gene is also associated with bovine respiratory disease susceptibility (Tizioto et al., 2015), a key trait impacting animal health and productivity (Neupane et al., 2018).

The rs135712530 is an intronic variant of the *LDAH* gene. *LDAH* has been associated with hoof and leg disorders (Wu et al., 2016), suggesting a potential role in health traits. Hoof and leg issues are critical in livestock, affecting both productivity and welfare, indicating the relevance of this locus for improving resilience and health in cattle. It may influence rauto1 by modulating the stability of physiological responses under stress.

On BTA 20, the SNP rs109908751, located in the intergenic region near the *PDE4D* may be associated with resilience. The expression of *PDE4D* has been detected in mammary glands, indicating its potential involvement in milk production. In fact, previous studies have suggested a possible role of *PDE4* in the regulation of mammary gland function and lactation (Dostaler-Touchette et al., 2009). This function may be related with rauto1, as *PDE4D* could influence the consistency and stability of physiological responses during early lactation. In fact, the *PDE4D* gene is part of the *PDE4* family, involved in regulating cAMP signaling pathways, which are critical for cell desensitization, signal compartmentalization, and cross-talk between cellular signals. *By* maintaining cAMP homeostasis, *PDE4D* plays a key role in regulating various physiological processes (Dostaler-Touchette et al., 2009). The regulation of cAMP signaling by *PDE4D* could also contribute to the stability of immune responses and metabolic adaptations, both critical for resilience in dairy cattle during the early lactation period.

Another SNP, rs42070678, is located in an intronic region of *NCF1* on BTA 25. This gene encodes a cytosolic subunit of neutrophil NADPH oxidase, which plays a critical role in the production of reactive oxygen species (ROS). ROS are key mediators in host defense and the regulation of inflammation (Kennedy and DeLeo, 2009). Although this gene has not been directly associated with a known QTL, the role of *NCF1* in modulating ROS production suggests it may be involved in the immune responses and inflammatory control, both of which are crucial for maintaining resilience during the early lactation period. Also, the *CARD11* gene in which rs109938921 maps, is involved in immune signaling, being crucial in the activation of T-cells and the differentiation of peripheral B-cells (Stepensky et al., 2013). In dairy cattle, *CARD11* has been linked to feed efficiency (FE), with studies showing its involvement in residual feed intake (RFI) in Danish Holstein cattle. *CARD11* was downregulated in animals with high RFI compared to those with low RFI, suggesting its role in regulating energy balance and metabolic efficiency (Salleh et al., 2017). *CARD11* may then influence resilience to metabolic and immune stress during early lactation.

On BTA 20, a genomic region at approximately 53.4 Kbp is defined by 9 significant SNPs, including 5 intronic variants and 4 intergenic ones (Table 3). This region harbors the *CDH18* gene, which belongs to the canonical cadherin (*CDH*) gene family. The cadherin family is composed of a series of cell adhesion molecules that play a dominant role in tissue morphogenesis and regulate adhesion interactions. Some studies have shown that the *CDH18* gene locus resulted strongly associated with milk and fat yields in dairy cattle (Laodim et al., 2017).

Finally, three significant SNPs (rs133164649, rs42130478, and rs109905892) are all located in the intron position of the *UNC5D* (BTA 27). *UNC5D* is implicated in the development and maintenance of udder structure and conformation (Cole et al., 2011), which are crucial for efficient milk production and the animal's ability to cope with physiological stress during lactation. Rauto1 may reflect how well an animal's udder structure and other related traits maintain stability during early lactation.

The genes associated with rauto2 are involved in various traits, as described by the examples reported below. On BTA 3, two significant SNPs were located in the intronic regions of the *PATJ* and *FGGY* genes. These genes have been reported to be associated with fertility and reproduction traits, as well as body structure and finishing precocity, respectively. *CDK6* gene can be considered a candidate gene involved in body traits (Liu et al., 2011; Silva et al., 2019). *PTPRQ* on BTA 5, as identified by (Robakowska-Hyżorek et al., 2016), may influence meat production traits in beef cattle, possibly through the regulation of MRF (myogenic regulatory factors) gene expression. Two closed SNPs on BTA 10 lie in the coding sequence (synonymous and 3'UTR) of the *F2R* gene, proposed as novel and promising candidates for regulation of hypoxic adaptation in the heart by Wang et al (Wang et al., 2021), a study that compared the hypoxic adaptation of the yak (*Bos grunniens*) against different cattle species.

On BTA 14, rs110970186 and rs43430961 are annotated in intronic position of the *NCALD* (Neurocalcin Delta) gene that was associated with the Bovine Respiratory Disease (Kiser et al., 2017). On BTA 25, rs42073064, located in the 3'UTR region of *COL26A1*, which encodes collagen type XXVI, was identified as one of the differentially expressed genes potentially involved in host resistance against ticks (Mantilla Valdivieso et al., 2022). *KRT14*, located 193 bp from the rs43727762 SNP, plays a role in mammary epithelial cell lineage changes, which are essential for the proper development of the mammary epithelium during the cow's life and, consequently, for milk production (Finot et al., 2019). The same authors described also the role of *KRT14* (together with other cell line) in the development of the bovine mammary gland at puberty (Finot et al., 2018).

Finally, we found five significant SNPs mapping in the intronic position of *KIF13A*. This gene belongs to the kinesin superfamily, a large group of motor proteins involved in intracellular transport and recycling endosome dynamics. These functions are crucial for maintaining cellular homeostasis and responding to environmental stressors, particularly when *KIF13A* interacts with other proteins such as Rab GTPases (Thankachan and Setty, 2022). The interaction between these two classes of proteins could be a key mechanism in stress adaptation and recovery. Therefore, we may speculate that this represents an indirect link with rauto, which measures autoregulation—the ability of a system to autonomously regulate itself without external intervention.

3.4.3 dPert

Eight above FDR threshold SNPs sparse along the chromosomes were associate with dPert2. One of these SNPs was already associated with LnVar2 (rs133894374 annotated in intronic position of *SLC2A13*). As reported here and by Chen et al (Chen et al., 2023), LnVar2 and dPert2 were highly correlated (0.85, Figure 2d). This strong association is expected, as both metrics reflect an animal's ability to maintain stable performance despite challenges.

The *PTPRR* gene harbors the intronic rs43440584. The protein encoded by *PTPRR* is a member of the protein tyrosine phosphatase (PTP) family and appears to be involved in mammary gland involution, possibly contributing to the remodeling of udder tissue for subsequent parturitions. Researchers reported that weaning (in mice) increased PTP activity in the mammary gland (Tolleson et al., 2017). *PTPRR* gene has also been associated with various mammary traits in different cattle breeds (Tolleson et al., 2017; Sinha et al., 2023).

The rs43219764 and rs132905517 are intronic SNPs of *RSRC1* and *KBTBD12* genes, that were associated with reproduction traits and with milking temperament in Holstein cattle, respectively (Chen et al., 2020; Grigoletto et al., 2020). Opposite genetic correlation between immune response traits (the most ones related to resilience) and fertility traits are reported by Konig and May (König and May, 2019). In their review, the gestation length resulted positively and negatively correlated with antibody- and cell-mediated immune response, respectively (+0.17; -0.17). The same opposite correlation values were found also for other fertility traits including calving ease, maternal calving ease, and daughter fertility (König and May, 2019). The potential link between milk temperament and resilience in milk production suggests that also behavioral traits might indirectly influence the physiological stability of lactating cows. However, some evidences are contrasting: i) Stepancheva et al., 2024, investigating how milking temperament affects milk productivity, found that Buffalos with higher milking behavior scores (4 or 5, more reactive cows) had the quite higher LS means for TDMY; ii) Marçal-Pedroza et al., 2023 reported that calm and intermediate cows produced more milk and a shorter milking time and a greater average milk flow; iii) Antanaitis et al., 2021, observed a negative genetic correlation between the temperament of cows and milk yield; instead, temperament was positively correlated with SCS.

4 Conclusions

This study offers a high-resolution analysis of resilience indicators in Holstein cows, leveraging daily milk yield data from automatic milking systems collected under standardized management and environmental conditions. The four indicators assessed (LnVar, rauto, dPert, and wfPert) captured different resilience dimensions. LnVar showed the most robust biological and genetic signals, particularly in the second parity. Rauto showed moderate heritability and improved biological coherence with age, while dPert and wfPert, despite lower heritabilities, provided valuable insights into the dynamics of short-term production perturbations.

Genome-wide association studies identified a complex genetic basis for resilience, involving immune function, metabolic regulation, and tissue integrity. Candidate genes such as *EPHB1, IL1B, PDSS1*, *GRAMD1B*, and *DCN* were associated with processes including inflammation, energy homeostasis, and extracellular matrix remodeling. The *EPHB1* and *SLC2A13* genes were linked to multiple indicators or parities, suggesting shared regulatory mechanisms, while others appeared only in later parities, pointing to age-related physiological adaptations. Several genes have also been previously associated with production traits, supporting potential pleiotropy and the importance of considering resilience in breeding decisions.

Working within a single, large and well-monitored herd minimized environmental variability, allowing clearer detection of individual differences and genetic signals. This approach delivers practical value to farmers by supporting herd management decisions, especially for low-resilience cows, and informing breeding strategies that prioritize resilient phenotypes. Future studies across diverse herds and environments, enriched with health records and external stressor data (e.g., disease, heat), may improve and validate these findings and further disentangle intrinsic resilience from environmental effects.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

All the procedures were approved by the Animal Welfare Body of the Università degli Studi di Milano (OPBA) and by the Italian Minister of Health (protocol number OPBA_68_2023). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CP: Formal analysis, Writing – original draft, Writing – review & editing. AD: Formal analysis, Writing – original draft, Writing – review & editing. CF: Data curation, Writing – original draft, Writing – review & editing. AB: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. MS: Formal analysis, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that Generative AI was used in the creation of this manuscript. Generative AI was used solely to improve the English language and grammar of the text. No content was generated or interpreted by AI.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2025.1627086/full#supplementary-material

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