



Mastitis has a cumulative and lasting effect on milk yield and lactose content in dairy cows

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ABSTRACT

Milk lactose content (LC) physiologically decreases with parity order in dairy cows, but also after udder health inflammation(s) and in presence of elevated milk SCC in subclinical cases. Therefore, the progressive decrease in milk LC observed along cows' productive life can be attributed to a combination of factors that altogether impair the epithelial integrity, resulting in weaker tight junctions, e.g., physiological aging of epithelium, mechanical epithelial stress due to milking, and experienced clinical or subclinical mastitis. Mastitis is also known to affect the udder synthesis ability, so our intention through this study was to evaluate if there is a cumulative and lasting effect of mammary gland inflammation(s) on milk yield (MY) and LC. For this purpose, we used diagnoses of clinical mastitis and milk data of Austrian Fleckvieh cows to evaluate the effect of cumulative mastitis events on LC and MY. Only mastitis diagnoses recorded by trained veterinarians were used. Finally, we investigated if cumulative mastitis is a heritable trait and whether it is genetically correlated with either LC or MY. Estimates were obtained using univariate and bivariate linear animal models. A significant reduction in LC and MY was observed in cows that suffered from mastitis compared with those that did not experience udder inflammation. The h^2 of cumulative mastitis is promising and much greater (0.09) than the h^2 of the binary event itself (≤ 0.03). The genetic correlations between cumulative mastitis with LC and MY were negative, suggesting that cows with a great genetic merit for MY and LC are expected to be more resistant

to repeated inflammations and less recidivist. When we used number of lifetime SCC peaks ($\geq 200,000$ or $400,000$ cells/mL) to calculate cumulative inflammation events, h^2 was even higher (up to 0.38), implying that subclinical mastitis also has a relevant negative impact on both LC and MY. Finally, the present study demonstrated how repeated mastitis events can permanently affect the mammary gland epithelial integrity and synthesis ability, and that the number of cumulative mastitis is a promising phenotype to be used in selection index in combination with other indicator traits toward more resistant and resilient mammary glands.

Key words: SCC, udder health, milk synthesis, alveolar permeability, mammary gland inflammation

INTRODUCTION

In dairy cattle, the negative correlation between milk lactose content (LC) and SCC has been reported in various breeds and contexts, and several authors have discussed the causal relationship and mechanisms responsible for the LC decrease in correspondence of mastitis or high SCC (Costa et al., 2019a,b). A reduction in LC also occurs in buffaloes and small ruminants when the SCC increases, even in animals with no clinical signs (Stelwagen et al., 1999; Costa et al., 2020b; Carta et al., 2023). The effect of a single mastitis event on milk yield (MY) and its major solids is well known (Guinard-Flament et al., 2011; Costa et al., 2019b; Herve et al., 2019). Ebrahimie et al. (2018), for instance, concluded that LC is the most promising predictor of subclinical mastitis in cows, followed by electrical conductivity. Genetic and phenotypic correlations between LC and SCC, and between LC and clinical mastitis available in the literature confirmed the antagonistic relationship of these features. The same applies to milk minerals such as Na and K,

Received July 19, 2024.

Accepted August 28, 2024.

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

whose concentration is usually high in milk with elevated SCC and inversely correlated with LC (Visentin et al., 2018; Costa et al., 2019c; Singh et al., 2024). Moreover, cows with greater LC in milk seem to have better udder health, fertility, and longevity, and are less likely to be early culled than those with lower LC concentration (Miglior et al., 2006; Haile-Mariam and Pryce, 2017). A genome-wide investigation carried out in the Austrian Fleckvieh population revealed some interesting overlaps in this sense (Costa et al., 2019d). In fact, part of the genomic regions significant for LC contained signals for mastitis and udder health-related traits (Meredith et al., 2013; Tiezzi et al., 2015).

In cows, LC decreases with parity and age (Costa et al., 2019b). The progressive drop in milk LC observed along the cow's productive life might be attributed to a combination of factors, namely physiological aging, mechanical epithelial stress due to repeated milkings, and the cumulative effect of the experienced inflammation events (Herve et al., 2018; Costa et al., 2020a). It can be therefore hypothesized that, to a different extent, the alveolar structure undergoes damage during the productive life of a specialized dairy cow, which in turn impairs epithelial functionality and integrity due to leaky tight junctions (Zhao and Lacasse, 2008). When the mammary gland epithelial cells produce lactose, they release it in the alveolar lumen. This disaccharide draws the water in the lumen up to reach physiological osmotic equilibrium, thus determining the alveolar LC or, in other words, the milk LC (Wellnitz and Bruckmaier, 2021). According to Herve et al. (2019), weak tight junctions are associated with a reduction in LC and MY and, consequently, with lactosemia. In the presence of damaged epithelial tissue, leakage of lactose occurs from the alveolar lumen (i.e., milk) to the blood and finally to the urine. Considering that lactose is the major milk osmole, any reduction in LC within the alveolar lumen alters the blood-milk barrier equilibrium, causing a change in the concentration of other osmotic components, such as Na and K. This explains why it is possible to consider LC as an indicator of the mammary gland history and memory (Costa et al., 2020a).

In the present study, we tested the hypothesis that there is a cumulative and lasting effect of clinical and subclinical mastitis on LC, the major milk osmole, as well as MY, possibly due to effects on alveolar permeability and mammary gland secretion ability. For this purpose, milk test-day records (TD) with SCC and gross composition traits were used together with validated clinical mastitis data (Egger-Danner et al., 2012) recorded in Austrian Fleckvieh cattle. In addition, heritability of cumulative mastitis and its genetic correlation with LC and MY were estimated.

MATERIALS AND METHODS

Phenotypes

Data used in the present study were retrieved from official Austrian national databases upon agreement with ZuchtData EDV-Dienstleistungen GmbH (Vienna, Austria). Milk TD and health records of Austrian cows of different breeds born between 1997 and 2017 were initially available for all farms under the validated health data recording system. The system was created within the framework of the Austrian project "Health Monitoring in Cattle" started in 2006 and fully described in Egger-Danner et al. (2012). Farms located in the federal provinces of Styria and Lower Austria were considered. The focus was exclusively on Fleckvieh cows and with TD available from parity 1 onwards. The maximum parity was set at 10 and TD recorded from 5 to 500 DIM were considered to capture the entire possible variability. The inclusion criteria were the presence of at least 3 cows/farm and of at least 3 TD/lactation. Furthermore, date of first calving was restricted from January 2011 onward to guarantee the presence of reliable health records, as the recording system (after a preliminary validation period) started to be routinely used for genetic evaluations in December 2010 (Egger-Danner et al., 2012). A restriction was made so that all cows (especially those born in 2017) were able to potentially close the lactation, as the TD used in this study were collected from 2011 to 2020.

The final dataset consisted of 432,839 TD from 53,099 lactations and 18,404 Fleckvieh cows in 566 farms. To retain as much variability as possible for LC, a wide range was considered. In particular, values from 3.00% to 7.00% were kept, leading to a negligible loss of 217 (LC <3%) and 2 (LC >7.00%) observations. Other traits available for each TD included the MY (kg/d), fat content (%), and protein content (%), whose distribution was preliminarily visually inspected with histograms to evaluate normality of distribution (Supplemental Figure S1; see Notes). Milk SCC was also present for all TD, ranging from 1,000 to 9,999,000 cells/mL, and was converted to SCS as follows: $SCS = 3 + \log_2(SCC/100,000)$.

Cumulative Mastitis Events

For all the 53,099 lactations, whenever present, the cumulative number of diagnoses of acute (AM) and chronic (CM) clinical mastitis were retrieved. In the Austrian validated health data recording system, the 2 forms of clinical mastitis are coded differently, but regardless whether the conditions are acute or chronic, they are always associated with a specific drug treatment whose registration is regulated by law and is managed by the

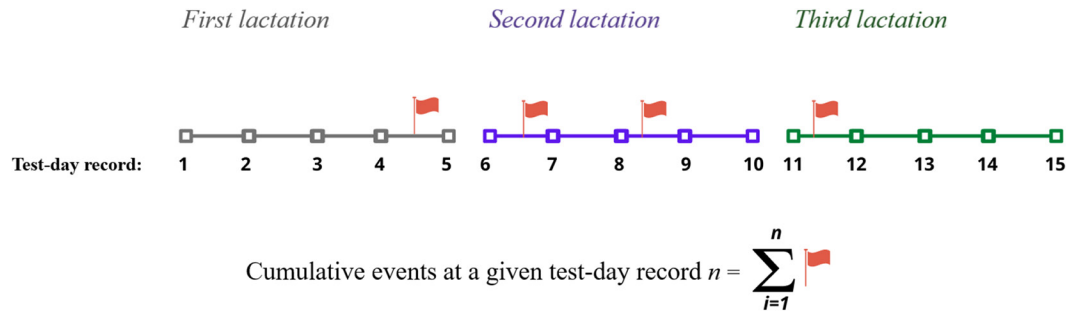


Figure 1. Visual example of cumulative number of mastitis events calculation at a given test-day record for a cow whose productive life consisted of 3 lactations.

veterinarian (Süntinger et al., 2022). Given the inclusion criteria of the present study, only farms where the diseases/treatments were recorded by a trained veterinarian were selected ($n = 566$). The choice between AM and CM relies on the veterinarian’s diagnosis which also takes into account the cow’s historical SCC trend within the lactation and clear signs of clinical mastitis (Süntinger et al., 2022).

A cumulative number of past mastitis events was assigned to each TD of a cow starting from the first available TD in parity 1, regardless of the time between the date of diagnosis and milk TD (Figure 1). In this study, mastitis events were defined in different ways, i.e., based on

- Diagnosis of AM,
- Diagnosis of CM,
- Sum of the diagnosis of AM+CM,
- Through the presence of elevated milk SCC.

For the latter, both the TD $\text{SCC} \geq 200,000$ cells/mL (SCC_{200}) and TD $\text{SCC} \geq 400,000$ cells/mL (SCC_{400}) were

used as a threshold to identify a potential subclinical mastitis. In fact, subclinical cases are often latent and not detected by the veterinarian. The limit at 200,000 cells/mL was selected because it is conventionally considered as the threshold for subclinical mastitis, whereas 400,000 cells/mL was a more arbitrary threshold empirically identified based on experience and the existing literature on mastitis (Malek dos Reis et al., 2013). Briefly, in the present study, each TD with SCC above the threshold (either 200,000 or 400,000 cells/mL) was considered as a mastitis event.

The TD were assigned a value representing the number of past cumulative events (Table 1). For binary data (AM and CM), TD were categorized in 3 classes based on the presence of 0, 1, or ≥ 2 mastitis events experienced. For cumulative AM+CM, 4 classes were defined, from 0 (no diagnosed mastitis) to 3 (≥ 3 diagnosed mastitis). Finally, 5 classes of SCC_{200} and 5 classes of SCC_{400} were created based on the number of previous TD presenting above-threshold SCC. Specifically, the classes 0, 1, 2, 3, and 4 included TD records linked to 0, 1, 2, 3, and ≥ 4 mastitis events based on SCC_{200} or SCC_{400} , respectively. For each

Table 1. Descriptive statistics of the traits available for each test-day (TD) record ($n = 432,839$); data refer to 53,099 lactations of 18,404 Austrian Fleckvieh cows in 566 farms

Trait	Description	Mean	Median	CV, %	Minimum	Maximum
Lactose content	Concentration (%) of lactose in milk	4.79	4.80	4	3.00	5.63
Milk yield	Daily milk yield (kg/d)	27.60	27.00	32	2.00	82.00
SCS	Score of SCC, calculated as $\text{SCS} = 3 + \log_2(\text{SCC}/100,000)$	2.26	2.16	76	-3.64	9.64
Health ¹						
AM _{BIN}	Acute mastitis (binary) recorded by a trained veterinarian ²	0.01	0.00		0	1
AM	Cumulative number of lifetime ² events of AM _{BIN}	0.17	0.00		0	8
CM _{BIN}	Chronic mastitis (binary) recorded by a trained veterinarian ²	0.00	0.00		0	1
CM	Cumulative number of lifetime ² events of CM _{BIN}	0.07	0.00		0	7
AM+CM	Cumulative number of lifetime ² events of both AM _{BIN} and CM _{BIN}	0.24	0.00		0	12
SCC ₂₀₀	Cumulative number of lifetime TD records with $\text{SCC} \geq 200,000$ cells/mL	2.06	0.00		0	52
SCC ₄₀₀	Cumulative number of lifetime TD records with $\text{SCC} \geq 400,000$ cells/mL	0.82	0.00		0	34

¹More details regarding the definition of AM_{BIN} and CM_{BIN} are available in Süntinger et al. (2022).
²All diagnoses present in the Austrian health data recording system until the given TD record date were used.

definition of mastitis, the number of classes was empirically chosen based on the frequency of TD in each class (Table 2).

The different mastitis definitions are justified by the fact that high SCC, which is often an indicator of the subclinical form, does not necessarily mean that there is a clinical record of mastitis (Heringstad et al., 2006; Pinzón-Sánchez and Ruegg, 2011; Jamali et al., 2018).

Analysis of TD Milk Yield and Lactose Content

The following repeatability linear animal model was used to estimate changes in the milk secretion ability (proxy: MY) and alveolar epithelial integrity (proxy: LC) with increasing cumulative number of inflammations, i.e., by including the fixed effect of AM, CM, AM+CM, SCC₂₀₀, or SCC₄₀₀ in 5 separate analyses:

$$y_{ijklmnop} = u + P_i + D_j + M_k + S_l + Y_m + a_n + c_o + f_p + e_{ilklnop}, \quad [1]$$

where $y_{ijklmnop}$ is MY or LC at the given TD; u is the overall intercept of the model; P_i is the fixed effect of the i th parity ($i = 1$ to 6, with the last class including parities from 6 to 10); D_j is the fixed effect of the j th lactation stage ($j = 1$ to 7, with each class covering 50 d, except for the last class, which included TD from 306 to 500 DIM); M_k is the fixed effect of the k th cumulative past mastitis events ($k =$ number based on AM, CM, AM+CM, SCC₂₀₀, or SCC₄₀₀); S_l is the fixed effect of the l th calving season (December to February, March to May, June to August, and September to November); Y_m is the fixed effect of the

m th calving year (from 2011 to 2020); and a_n , c_o , f_p , and $e_{ilklnop}$ are the random effects of additive genetic animal, permanent environment (cow), farm, and residual, respectively. Random effects were assumed to be independent and normally distributed, with the following variance structure: $\text{var}(a) = \mathbf{A}\sigma_a^2$, $\text{var}(c) = \mathbf{I}\sigma_{pe}^2$, $\text{var}(f) = \mathbf{I}\sigma_f^2$, and $\text{var}(e) = \mathbf{I}\sigma_e^2$, where σ_a^2 is the additive genetic variance, σ_{pe}^2 is the permanent environmental variance, σ_f^2 is the farm variance, σ_e^2 is the residual variance, $\mathbf{A}$ is the pedigree-based relationship matrix, and $\mathbf{I}$ is an identity matrix of appropriate order. The pedigree size was 185,948 animals and included all cows with phenotypic data and their ancestors up to 6 generations back. The LSM of the fixed effects were compared considering a significance level of $P \leq 0.05$.

For a comprehensive overview, in addition to LC and MY, fat and protein content were also analyzed using the model in Equation 1.

For the genetic correlations, the additive genetic covariance between LC or MY and cumulative mastitis events was estimated using 5 bivariate analyses, whose starting values were preliminarily obtained from univariate runs. The model looked as in Equation 1, but without the inclusion of the fixed effect M because cumulative past mastitis events was one of the 2 dependent variables:

$$y_{ijklmno} = u + P_i + D_j + S_k + Y_l + a_m + c_n + f_o + e_{ilklnno}, \quad [2]$$

where $y_{ijklmno}$ is the phenotypic observation of either LC or MY and cumulative mastitis at a given TD record.

The h^2 of each trait was calculated after convergence by using the estimated σ_a^2 , σ_{pe}^2 , σ_f^2 , and σ_e^2 , whose sum corresponded to the total phenotypic variance (σ_p^2). Genetic ($\mathbf{r}_a$), permanent environmental ($\mathbf{r}_{pe}$), and phenotypic ($\mathbf{r}_p$) correlations were obtained through conventional formulas specified in the parameter file by using the covariance components (Falconer and MacKay, 1996).

The SAS software v. 9.4 (SAS Institute Inc., Cary, NC) was used for data inspection, manipulation, and editing, as well as for the calculation of the Pearson, Spearman, and Kendall correlations. The ASReml software v. 4.1 (Gilmour et al., 2015) was adopted for the ANOVA, LSM estimation and comparison, and genetic parameter estimation.

Analysis of Lifetime Lactose

Three lifetime lactose traits were proposed to disentangle the effects of parity and aging and cumulative mastitis. This analysis was not carried out for MY because it does not progressively reduce with parity in dairy cows.

Table 2. Distribution of cows' test-day records ($n = 432,839$) across classes of cumulative mastitis¹

Mastitis	Class frequency, %				
	0	1	2	3	4
AM	88.01 ($n = 380,956$)	8.01	3.98	—	—
CM	94.94 ($n = 410,940$)	3.66	1.40	—	—
AM+CM	84.68 ($n = 366,521$)	9.41	4.07	1.84	—
SCC ₂₀₀	51.39 ($n = 222,421$)	13.74	10.2	5.79	18.88
SCC ₄₀₀	69.42 ($n = 300,494$)	12.86	7.51	3.48	6.73

¹AM = cumulative number of acute mastitis diagnoses; CM = cumulative number of chronic mastitis diagnoses; AM+CM = cumulative number of acute plus chronic mastitis diagnoses; SCC₂₀₀ = cumulative number of lifetime test-day records with SCC $\geq 200,000$ cells/mL; SCC₄₀₀ = cumulative number of test-day records with SCC $\geq 400,000$ cells/mL. Classes were created based on the presence of 0, 1, or ≥ 2 events for both AM and CM; 0, 1, 2 or ≥ 3 events for AM+CM; 0, 1, 2, 3, and ≥ 4 events for SCC₂₀₀ and SCC₄₀₀.

Starting from TD, the following lactose phenotypes, whose distribution is given in the Supplemental Figure S2 (see Notes), were obtained for each cow:

- Endpoint LC, measured at the last available TD in the lifetime ($n = 18,404$);
- Coefficient of variation of LC (CV_{LC} , %), as lifetime variability calculated using all TD available ($n = 18,404$);
- Δ of LC, which is the absolute difference between the content measured in early (parity 1 and <100 DIM) and late productive life (last available TD; $n = 18,249$).

The model adopted in this case was as follows:

$$y_{ijklmno} = u + P_i + M_j + S_k + Y_l + a_m + f_n + e_{ijklmno}, \quad [3]$$

where $y_{ijklmno}$ is the lifetime trait of lactose (endpoint, CV_{LC} , or Δ); u is the overall intercept of the model; P , S , and Y are the fixed effects of the parity, calving season, and calving year, respectively, at the last TD as described for Equation 1; M is the fixed effect of the cumulative past mastitis events as described for Equation 1 across productive life (i.e., at the last TD); and a , f , and e are the random effects of additive genetic animal, farm, and residual, respectively, with the same characteristics as in Equation 1.

RESULTS AND DISCUSSION

Lactose Peculiarities

Milk LC is expected to have a low day-to-day variability for physiological constraints, i.e., osmotic pressure, especially in healthy cows. However, small variations exist and can be partly attributed to differences among cows in terms of breed, blood-milk barrier, epithelial integrity and permeability, and/or udder health status (Costa et al., 2020b). Therefore, when compared with other milk components, the variation of LC is generally low (Costa et al., 2019b), making it the least variable bovine milk solid. The LC mirrors the metabolic activity of the mammary gland for the synthesis of milk constituents, and the limiting factor for lactose synthesis is the concentration of hematic glucose, which is taken up by the udder and partitioned between intracellular metabolic pathways. It has been observed that milk LC in cows milked once a day is lower compared with cows milked twice a day (Boutinaud et al., 2013). Boutinaud et al. (2013) observed that the reduction in LC with once-a-day milking is more pronounced than that of total daily MY, suggesting that lactose yield/synthesis is negatively affected by a reduction in the number of milkings and

therefore additional milking events stimulates the synthesis of lactose.

Nevertheless, the LC official data routinely available for the different dairy species have some peculiarities that deserve discussion. In fact, LC in bovine milk is predicted via mid-infrared spectroscopy (MIRS) worldwide, and the models' prediction accuracy or instruments performance may be sometimes below the optimum. In some cases, in fact, the prediction accuracy for the gross composition traits of milk (fat, protein, and lactose) is rather far from being considered acceptable for punctual prediction of the solid concentration. A moderate accuracy is acceptable in instruments installed in the automatic milking systems or in the milking parlors and in portable devices that usually operate in the near- or visible infrared region. Few manufacturers of the most popular MIRS benchtop instruments publish the prediction error of their models. For instance, FOSS (Hillerød, Denmark) declares the prediction error for the milk solids, including LC (Application Note 5373, Rev. 3, MilkoScan 7RM/FT+/6000). Uncertainty, up to a certain extent, is always expected when dealing with MIRS prediction equations. The International Organization for Standardization (ISO) and the International Dairy Federation (IDF) with ISO 9622 | IDF 141 ("Milk and liquid milk products—Guidelines for the application of mid-infrared spectrometry") recognize MIRS as comparable to gold standards for the major milk solids. At the same time, it is obvious that MIRS instruments that are not subjected to regular inspection and standardization may provide biased predictions (ISO, 2013; Tiplady et al., 2019). Surveillance in Europe is generally made by the International Committee for Animal Recording (ICAR), which periodically performs proficiency and ring tests involving the vast majority of the milk laboratories producing data for official and legal use, including those used in the framework of the DHI. The same applies to MY; in this case, the local official technicians are responsible for the MY recording. In the case of the milk TD data used in this study, samples were analyzed in a laboratory that participates in ICAR proficiency tests (ICAR, 2024) following ISO 9622 | IDF 141.

The frequent absence of manufacturer-declared MIRS prediction error, coupled with the moderate/low intra- and intercow variability of LC, makes data interpretation and identification of perturbations even more challenging. For fat, protein, and lactose, the models approved by IDF allow for a maximum CV up to 1% and 1.5% in MIRS predictions of bulk and individual milk, respectively (Italian Breeders Association, 2024). However, for LC, even minimal errors should be considered as important given its small variability. In general, assuming that the instrument is standardized on a regular basis, LC repeatability is excellent, and changes in LC from

one TD to the subsequent one in a cow are informative. Although Luinge et al. (1993) and Pinto et al. (2021) affirmed that MIRS can accurately predict LC, there is no clear evidence about the limit of detection of the current benchtop devices.

Apart from the intercow variability of LC, for the purpose of this study, it is important to consider that the focus should be placed on the intracow LC pattern. In fact, dramatic drops in LC at cow level should be considered as anomalous and as a potentially informative alerts of the udder health status.

Phenotype Overview

After edits and application of inclusion criteria, the cows left belonged to different parity orders (1 to 10). In particular, parity averaged 2.47, with median of 2.00. Milk LC averaged 4.79% and ranged from 3.00% to 5.63% (Table 1). The minimum SCS was -3.64 , which corresponds to 1,003 cells/mL, and the maximum was 9.64, which corresponds to 9,973,307 cells/mL. The observed median of the health binary events was zero, whereas nonzero means were observed for the cumulative events. The maximum number of cumulative events associated with a given TD was 8 for AM, 7 for CM, 12 for AM+CM, 52 for SCC₂₀₀, and 34 for SCC₄₀₀ (Table 1).

Regardless if the form was acute or chronic, approximately one-fourth of mastitis cases belonged to primiparous cows and nearly half belonged to parity 1 and 2 (Figure 2). Although single diagnoses of acute (AM_{BIN}) and chronic mastitis (CM_{BIN}) were mainly recorded in early and late lactation, respectively, flags of SCC₂₀₀ and SCC₄₀₀ were generally observed throughout the whole lactation, but linearly increased together toward the end (Figure 3). This confirms the different nature of the mastitis definitions adopted in this study and corroborates the idea that the presence of very high SCC at a given TD does not necessarily translate into a diagnosed (binary) clinical mastitis event. In the edited dataset, the first and third quartiles of DIM were at 86 and 246 d, and the median and the mean were at 166 and 170.08 d, respectively. Most (95%) TD were recorded between DIM 5 and 337, but a non-negligible number of cows ($n = 3,191$) underwent prolonged lactations, with one or more TD recorded between 400 and 500 DIM. Each TD was assigned to the number of experienced mastitis events to account for the cumulative effect.

The distribution of the TD across classes of cumulative events is presented in Table 2. When the SCC₂₀₀ or SCC₄₀₀ rather than veterinarians' AM or CM diagnoses were used to flag mastitis, more events were counted. Moreover, the percentage of TD classified as healthy (class 0) was greater ($\geq 84.68\%$) for mastitis defined using binary events compared with mastitis defined using

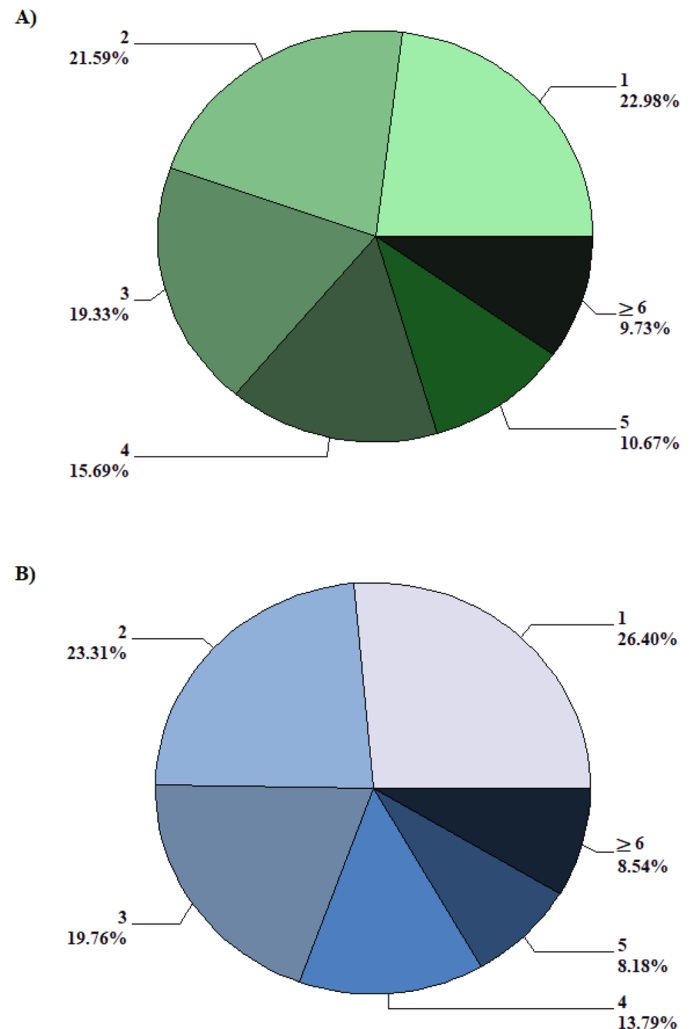


Figure 2. Distribution of (A) acute and (B) chronic mastitis diagnoses across the cows' parities. Parity ≥ 6 included cows from the sixth up to the 10th lactation.

milk SCC₂₀₀ ($\leq 51.39\%$). This suggests that in Fleckvieh dual-purpose cows, a subclinical form of mastitis is present, as in highly specialized dairy breeds.

Table 3 summarizes the amount of TD in common between the different classes of cumulative events and demonstrates that subclinical mastitis is latent and not detected by the veterinarians. For example, 212,903 TD were present at the same time in class 0 of AM and in class 0 of SCC₂₀₀, but only 8,786 TD were simultaneously present in the highest class of AM (class 2) and SCC₂₀₀ (class 4). In other words, there were cases in which cows with one or more peak in SCC never had clinical mastitis: e.g., the 58,526 TD in class 0 of AM and in the fourth class of SCC₂₀₀. Half of the 17,228 TD with ≥ 2 assigned cumulative AM events belonged to the highest SCC₂₀₀ class (class 4) and only 17.75% of the same were located in SCC₂₀₀ class 0. The same distribution and percentages

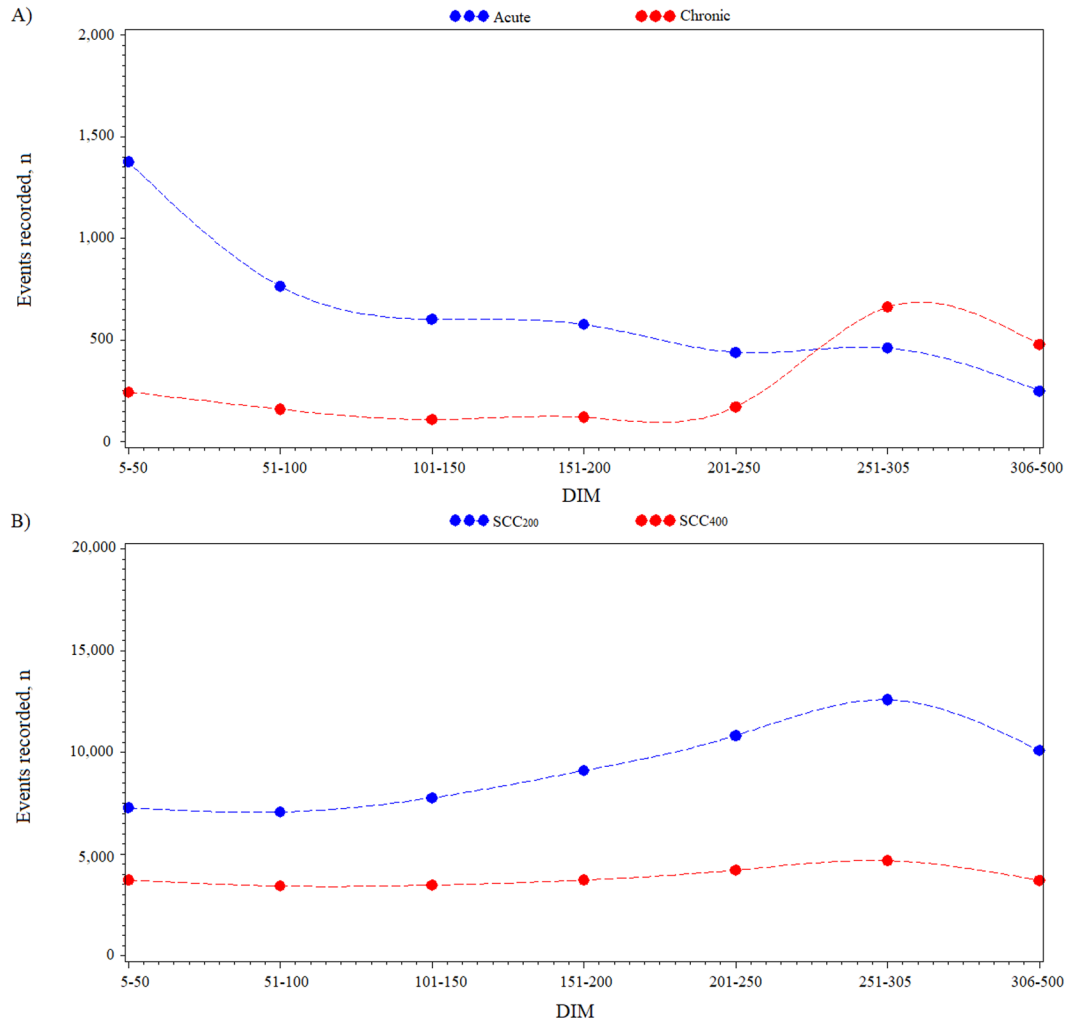


Figure 3. Distribution of mastitis events across DIM identified using (A) acute or chronic clinical diagnoses or (B) the number of milk test-day records with SCC $\geq 200,000$ cells/mL (SCC₂₀₀) or SCC $\geq 400,000$ cells/mL (SCC₄₀₀).

were observed when CM was considered instead of AM. Overall, this table suggests that rather frequently, a diagnosis of clinical mastitis is not associated with a peak in milk SCC or vice versa.

Direct comparison of these results with the literature is rather difficult, as this is the first study dealing with classes of cumulative mastitis and using various definitions of mastitis for the analysis of LC and MY.

Effect of Cumulative Mastitis

Subclinical mastitis is expected to be mostly undiagnosed because milk does not present visual anomalies and there are no evident signs at either the cow or udder level. Thus, indicator traits that routinely and easily available, such as milk SCC, LC, electrical conductivity, and minerals (e.g., Na), are important to identify cows

with latent cases even if only 1 quarter is inflamed (Fox et al., 2015; Ebrahimie et al., 2018; Costa et al., 2019c). Unfortunately, in the present study, data of milk electrical conductivity and minerals were not available.

Pearson correlations were preliminarily calculated among the traits using all the TD records available to disclose the direction of the relationships (Table 4). Milk LC was positively correlated only to MY (0.228), which, however, was in a weak association with AM, CM, AM+CM, SCC₂₀₀, and SCC₄₀₀ (Table 4). Given the dichotomic nature of the number of cumulative events, the Spearman and Kendall correlations were also calculated (Supplemental Table S1; see Notes). In all cases, the coefficients and the direction of the correlations were in line with the Pearson values in Table 4.

All fixed effects in Equation 1, including the number of cumulative mastitis events, were highly significant

Table 3. Confusion matrix with number of test-day records for the classes considered¹

		Class				
Item	SCC	0	1	2	3	4
AM						
0	SCC ₂₀₀	212,903	51,748	37,467	20,312	58,526
1		6,460	5,986	4,454	3,329	14,426
2		3,058	1,724	2,245	1,415	8,786
0	SCC ₄₀₀	282,276	44,699	25,048	10,630	18,303
1		12,877	8,295	4,730	2,884	5,869
2		5,341	2,667	2,747	1,543	4,930
CM						
0	SCC ₂₀₀	217,989	56,470	41,495	23,244	71,742
1		3,310	2,313	1,980	1,383	6,858
2		1,122	675	691	429	3,138
0	SCC ₄₀₀	292,369	51,465	29,591	13,342	24,173
1		6,182	3,224	2,011	1,209	3,218
2		1,943	972	923	506	1,711
AM+CM						
0	SCC ₂₀₀	209,018	49,461	35,623	19,181	53,238
1		8,940	7,257	5,270	3,738	15,532
2		3,944	2,127	2,560	1,472	7,527
3	SCC ₄₀₀	519	613	713	665	5,441
0		275,432	41,886	23,229	9,747	16,227
1		17,068	9,507	5,241	2,960	5,961
2		6,822	2,958	2,925	1,536	3,389
3		1,172	1,310	1,130	814	3,525
SCC ₄₀₀						
0	SCC ₂₀₀	222,421	38,897	21,445	8,595	9,136
1			20,561	11,951	8,176	14,973
2				10,770	6,050	15,705
3					2,235	12,822
4						29,102

¹AM = cumulative number of acute mastitis diagnoses (3 classes); CM = cumulative number of chronic mastitis diagnoses (3 classes); AM+CM = cumulative number of acute plus chronic mastitis diagnoses (4 classes); SCC₂₀₀ = cumulative number of lifetime test-day records with SCC $\geq 200,000$ cells/mL (5 classes); SCC₄₀₀ = cumulative number of test-day records with SCC $\geq 400,000$ cells/mL (5 classes). Classes were created based on the presence of: 0, 1, or ≥ 2 events for both AM and CM; 0, 1, 2 or ≥ 3 events for AM+CM; 0, 1, 2, 3, and ≥ 4 events for SCC₂₀₀ and SCC₄₀₀.

($P < 0.001$), being able to explain variation of LC and MY (Table 5). The only exception was the effect of CM on milk LC ($P > 0.05$). Regarding LC, the cumulative mastitis effect with the greatest importance was SCC₂₀₀ ($F = 1,307.97$; Table 5). Moreover, when SCC₂₀₀ or SCC₄₀₀ was the fixed effect, their importance (F -value) was greater than the effects of calving year and calving season. When considering the LSM (Table 6), the great-

est LC was always observed in class 0, i.e., when the cow did not experience mastitis events. In all the mastitis types, the LSM of LC in class 0 significantly differed from the others (Table 6). This is particularly evident in the models with either SCC₂₀₀ or SCC₄₀₀. Supporting this, the density plots in Figure 4 show how evident the LC change is when grouping TD according to the number of SCC₂₀₀ and SCC₄₀₀ flags. The LSM of LC have been

Table 4. Pearson correlations ($P < 0.001$) calculated among milk lactose content (%), milk yield (kg/d), and cumulative mastitis¹ on the raw data

Trait	Milk yield	AM	CM	AM+CM	SCC ₂₀₀	SCC ₄₀₀
Lactose	0.228	-0.095	-0.064	-0.107	-0.288	-0.233
Milk yield		0.037	0.012	0.035	-0.006	-0.008
AM			0.171	0.879	0.284	0.299
CM				0.621	0.180	0.186
AM+CM					0.313	0.328
SCC ₂₀₀						0.861

¹AM = cumulative number of acute mastitis diagnoses; CM = cumulative number of chronic mastitis diagnoses; AM+CM = cumulative number of acute plus chronic mastitis diagnoses; SCC₂₀₀ = cumulative number of lifetime test-day records with SCC $\geq 200,000$ cells/mL; SCC₄₀₀ = cumulative n. test-day records with SCC $\geq 400,000$ cells/mL.

Table 5. Summary of the analyses of variance carried out for test-day lactose content and milk yield¹

Mastitis ³	F-value ²				
	Cumulative mastitis	Parity	Lactation stage	Calving season	Calving year
Lactose content					
AM	33.95	10,235.04	10,869.09	179.27	24.95
CM	0.39 ^{NS}	10,233.43	10,867.89	179.21	22.81
AM+CM	15.36	10,234.50	10,868.46	179.27	24.52
SCC ₂₀₀	1,307.97	10,360.06	10,991.91	181.99	68.11
SCC ₄₀₀	987.99	10,328.56	10,964.57	181.09	72.16
Milk yield					
AM	183.19	11,070.58	68,207.00	465.51	29.95
CM	49.35	11,065.19	68,145.42	465.60	26.99
AM+CM	145.42	11,072.33	68,217.66	465.59	30.40
SCC ₂₀₀	584.09	11,117.50	68,549.88	466.99	54.73
SCC ₄₀₀	550.91	11,113.90	68,536.23	466.73	62.27

¹Unless otherwise stated, the effects are highly significant ($P < 0.001$). NS = not significant.

²Incremental F -value provided by ASReml software v.4.1 (Gilmour et al., 2015).

³AM = cumulative number of acute mastitis diagnoses; CM = cumulative number of chronic mastitis diagnoses; AM+CM = cumulative number of acute plus chronic mastitis diagnoses; SCC₂₀₀ = cumulative number of lifetime test-day records with SCC $\geq 200,000$ cells/mL; SCC₄₀₀ = cumulative number of test-day records with SCC $\geq 400,000$ cells/mL.

adjusted for the cow's parity in Equation 1, meaning that the effect of cumulative mastitis events is significant regardless of the parity. This, coupled with the LSM estimated for the fixed effect of parity (Supplemental Table S2; see Notes), corroborates the hypothesis that the lifetime decline in LC is partly due to parity (aging) and partly to cumulative events, i.e., to the mammary gland history and memory (Costa et al., 2020a). Within lactation, some variation in LC is expected, with the greatest concentration usually recorded in early lactation, followed by a linear reduction up to late stages (Costa et al., 2019c).

As for LC, in the case of MY the difference between LSM of class 0 and class 1 was wider with SCC₂₀₀ and

SCC₄₀₀ compared with AM, CM, and AM+CM (Table 6). In addition, cows in the class 0 of SCC₂₀₀ yielded almost 1.8 kg/d more milk than those with 4 or more flags (class 4). A relevant difference is present when the effect of AM is considered: 26.08 versus 24.97 kg/d. Overall, Table 6 suggests that daily MY declines considerably even in the presence of nonclinical (subclinical) mastitis, in agreement with Gonçalves et al. (2018). Notably, the loss in MY (i.e., direct farmer's income) calculated in the present study may be underestimated because cows more susceptible to mastitis are those with high productivity potential but that are culled early in life. The LSM of MY estimated for the fixed effect of parity (Supplemental Table S2) confirm the opposite trend of MY across

Table 6. Least squares means and SE of test-day lactose content and milk yield for the fixed effect of cumulative mastitis

Mastitis ¹	Class				
	0	1	2	3	4
Lactose content, %					
AM	4.738 $\pm$ 0.008 ^a	4.731 $\pm$ 0.008 ^b	4.725 $\pm$ 0.008 ^b	—	—
CM	4.737 $\pm$ 0.008 ^a	4.735 $\pm$ 0.008 ^a	4.736 $\pm$ 0.008 ^a	—	—
AM+CM	4.738 $\pm$ 0.008 ^a	4.733 $\pm$ 0.008 ^b	4.730 $\pm$ 0.008 ^c	4.722 $\pm$ 0.009 ^c	—
SCC ₂₀₀	4.775 $\pm$ 0.008 ^a	4.745 $\pm$ 0.008 ^b	4.730 $\pm$ 0.008 ^c	4.720 $\pm$ 0.008 ^d	4.699 $\pm$ 0.008 ^c
SCC ₄₀₀	4.761 $\pm$ 0.008 ^a	4.726 $\pm$ 0.008 ^b	4.716 $\pm$ 0.008 ^c	4.704 $\pm$ 0.008 ^d	4.687 $\pm$ 0.008 ^c
Milk yield, kg/d					
AM	26.08 $\pm$ 0.28 ^a	25.52 $\pm$ 0.28 ^b	24.97 $\pm$ 0.29 ^c	—	—
CM	26.00 $\pm$ 0.28 ^a	25.65 $\pm$ 0.28 ^b	25.10 $\pm$ 0.29 ^c	—	—
AM+CM	26.10 $\pm$ 0.28 ^a	25.66 $\pm$ 0.28 ^b	25.15 $\pm$ 0.28 ^c	23.64 $\pm$ 0.34 ^d	—
SCC ₂₀₀	26.80 $\pm$ 0.28 ^a	26.50 $\pm$ 0.28 ^b	25.90 $\pm$ 0.28 ^c	25.70 $\pm$ 0.28 ^d	24.97 $\pm$ 0.28 ^c
SCC ₄₀₀	26.60 $\pm$ 0.28 ^a	25.95 $\pm$ 0.28 ^b	25.48 $\pm$ 0.28 ^c	25.02 $\pm$ 0.28 ^d	24.41 $\pm$ 0.28 ^c

^{a-c}Estimates with different lowercase letters within a row differ significantly ($P < 0.05$).

¹AM = cumulative number of acute mastitis diagnoses; CM = cumulative number of chronic mastitis diagnoses; AM+CM = cumulative number of acute plus chronic mastitis diagnoses; SCC₂₀₀ = cumulative number of lifetime test-day records with SCC $\geq 200,000$ cells/mL; SCC₄₀₀ = cumulative number of test-day records with SCC $\geq 400,000$ cells/mL.

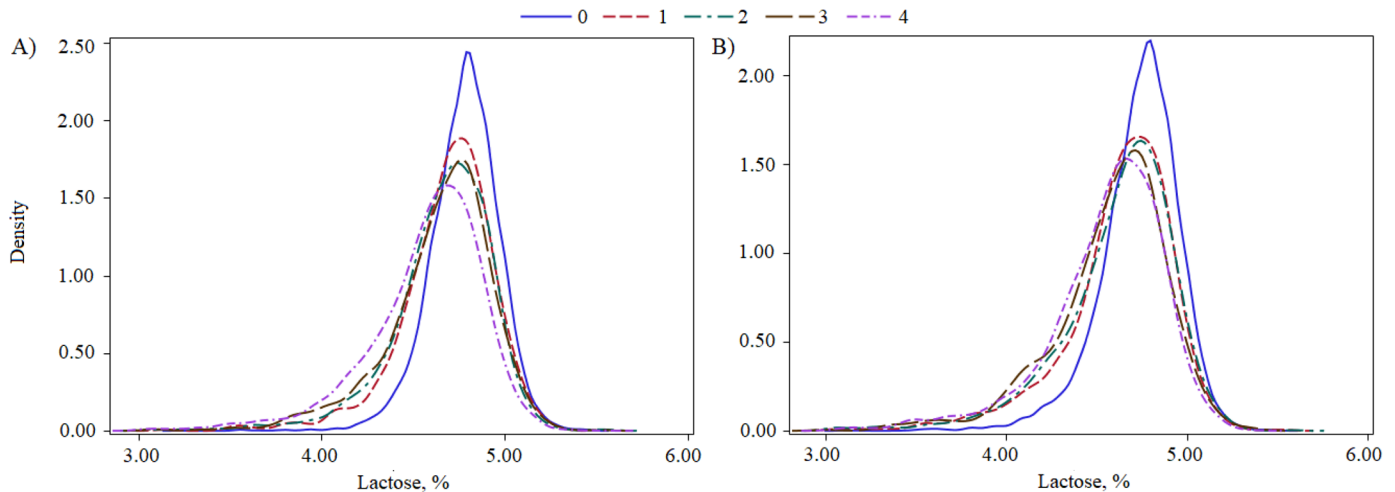


Figure 4. Kernel density plot of endpoint milk lactose content (last test-day available in life) according to the (A) SCC_{200} and (B) SCC_{400} class. Class 0, 1, 2, 3, and 4 includes test-day records associated with 0, 1, 2, 3, and ≥ 4 previous peaks of SCC. The SCC_{200} is the cumulative number of lifetime test-day records with $SCC \geq 200,000$ cells/mL and SCC_{400} is the cumulative number of lifetime test-day records with $SCC \geq 400,000$ cells/mL.

parities compared with LC. The greatest daily production has been observed in older cows in parity 5 (27.664 kg/d) and the lowest in primiparous (21.707 kg/d).

In this study, the use of AM and AM+CM might plausibly have led to less glaring findings compared with SCC_{200} and SCC_{400} because after a diagnosis of CM_{BIN} in late lactation (Figure 3) the cows could have been culled, with no possibility to track events in the final stages of lactation and in subsequent lactations.

The ANOVA results support the idea that there is a carry-over permanent effect of mastitis on the alveolar epithelium integrity (i.e., LC) and secretion ability of the udder (i.e., MY), but only LC reduces with parity, i.e., with the progressive physiological epithelial aging, as per Supplemental Table S2. Overall, the alveolar epithelium of young cows (primiparous) is expected to be intact and endowed with strong tight junctions; older cows, on the other hand, are characterized by physiologically weaker junctions and greater permeability due to the mechanical stress alveoli are exposed to at milking during lactating periods. The concentration of Na and Cl in milk increases with parity, i.e., with aging of the epithelium (Visentin et al., 2018; Wellnitz and Bruckmaier, 2021).

Another potential explanation for the LC lifetime reduction in cows with multiple mastitis could be modulation of the microbiota. It has been demonstrated that the microorganisms present in the digestive tract of cattle are able to modify the epithelium barrier at the gut level and thereby alter the osmotic equilibrium. It can be hypothesized that the same happens in the mammary gland, so that microbiota can play a role in the milk osmotic equilibrium in quarters or udders that experienced a clinical or subclinical mastitis, even for a long time. Microbiome

alteration may result from the infection itself or can be associated with the antibiotic treatment (Falentini et al., 2016).

Accurate identification of mastitis (especially the subclinical form) is challenging in modern dairy cattle farming, and it frequently happens that a clinical case could be missed or recorded late by the farmer or veterinarian for various reasons. In addition, both worldwide and in Austria, binary events can be recorded by trained personnel instead of veterinarians, and therefore the reliability and consistency of diagnoses may differ if the recording system does not undergo validation. These data should be therefore used and interpreted with caution. At the same time, the milk TD (with SCC and LC) are available generally every 4 wk at cow level, which is quite a low frequency to allow for a constant monitoring. Automatic milking systems or milking parlors equipped with infrared spectroscopy-based instruments may be a concrete solution, allowing the farmers to detect fluctuations in important indicator traits such as LC. However, as previously discussed, the prediction error of such devices is generally higher compared with the benchtop instruments of laboratories regulated by the ICAR guidelines.

The use of additional indicators other than SCC is advisable to recognize udder inflammations, as the baseline SCC of healthy cows could differ according to several, including intrinsic, factors. For this reason, SCC-based lactation traits have been explored by some authors in recent years (Bobbo et al., 2019). The way and the extent to which SCC increases in presence of mastitis also depends on the pathogen(s) involved (de Haas, 2003; Malek dos Reis et al., 2013). The same can be valid for the LC decrease pattern, but at present, there is no information

in the literature that can support this idea. As reviewed by Cobirka et al. (2020), the species and the type (e.g., contagious, environmental) of the microorganism(s) responsible for the infection and inflammation affect the quickness and the modality of the immune response activation, as well as the epithelium damage level and the extent to which milk composition is altered.

To better interpret the findings of the present study, it is important to consider that in the Austrian system, in the presence of a binary diagnosis of mastitis, there is always an antimicrobial treatment administered to the cow, regardless of AM_{BIN} or CM_{BIN} . Of course, this is not the case when milk SCC exceeds the thresholds of 200,000 or 400,000 cells/mL. Such a treatment in sick quarter(s) could speed up the recovery after mastitis and can likely reduce the damage due to the inflammation for which bactericidal and bacteriostatic factors are responsible. Using fluctuations in LC and SCC to identify inflammation events seems a less restrictive approach, allowing for detection of problematic animals in the herd apparently healthy, with no clinical mastitis. The literature is rich in studies proposing novel indicators of udder infection or (sub)clinical inflammation and methods to identify the pathogen causing mastitis. To give some examples, serum amyloid A, bacteria identification and quantification via target culture, and cytokines (especially interleukins) may potentially be very accurate (Otsuka et al., 2021), but at the same time, they are costly and not easy to implement in the field, making them scarcely interesting and available compared with routinely available TD data (Kour et al., 2023; Tommasoni et al., 2023).

To evaluate if there is a cumulative positive or negative effect of cumulative mastitis also on other milk major solids, the ANOVA was carried out for fat and protein content. Both were significantly ($P < 0.001$) affected by presence of mastitis at least once, but the LSM suggest that there is not a progressively decreasing trend as was observed for LC and MY. In fact, the LSM ($SE = 0.01$) were 4.35%, 4.33%, and 4.30% for fat content in AM classes 0, 1, and 2, respectively, and 3.65%, 3.64%, and 3.62% in these classes for protein content. Therefore, although significant, the biological impact of mastitis in these traits was negligible in the current study. The trend was opposite for the LSM across the CM classes, with the following estimates ($SE = 0.01$) for classes 0, 1, and 2, respectively: 4.32%, 4.33%, and 4.36% for fat, and 3.63%, 3.64%, and 3.65% for protein content. When the 2 mastitis types were considered together as a single fixed effect (AM+CM), the estimates of the 2 milk solids had a different direction. In particular, fat content had an erratic trend, with LSM ($SE = 0.01$) of 4.33%, 4.32%, 4.34%, and 4.32% for cows with 0, 1, 2, or ≥ 3 cumulative

events, respectively. Instead, the LSM of protein content for these groups had an increasing trend, i.e., 3.62%, 3.63%, 3.64%, and 3.65% ($SE = 0.01$). Overall, results indicate that greater protein content can be observed in TD associated with multiple inflammation events. It has been demonstrated that even in presence of a stable total protein content, the casein index is negatively affected by high SCC and mastitis (Magro et al., 2023). Unfortunately, data on casein and serum proteins were not available to verify if there is a fraction for one that compensates for a reduction in the other. Also, the fixed effects of SCC_{200} and SCC_{400} were investigated for fat and protein content. The erratic patterns suggest that cumulative events did not affect fat and protein content linearly, which is opposite to what has been observed for LC and MY. The following LSM ($SE = 0.01$) were obtained for fat content: 4.31%, 4.34%, 4.34%, 4.33%, and 4.44% for the SCC_{200} class 0, 1, 2, 3, and 4, respectively, and 4.32%, 4.35%, 4.34%, 4.33%, and 4.33% for the SCC_{400} class 0, 1, 2, 3, and 4, respectively. Finally, the LSM of protein ($SE = 0.01$) were: 3.62%, 3.62%, 3.63%, 3.63%, and 3.64% for the 5 SCC_{200} classes, and 3.62%, 3.63%, 3.63%, 3.64%, and 3.65% for the 5 SCC_{400} classes. Findings confirm that milk fat and protein content are not impaired by the number of mastitis episodes and, more generally, by the cow's mammary gland history.

Cumulative Mastitis and Lifetime Lactose Traits

Although MY increased with parity and decreased with the number of cumulative mastitis events, the LC declined in both cases. Therefore, to better understand the lifetime evolution of LC and its association with the mammary gland history, 3 lifetime traits based on TD data of LC were studied using Equation 3, and the results are depicted in Figure 5. The Δ was expressed as a percentage and ranged from -2.10 to 1.12 with average, first quartile, median, and third quartile being -0.24% , -0.38% , -0.20% , and -0.06% , respectively. The 5% of the cows with the largest drop ($\Delta < -0.76$, quantile 0.05), experienced on average 6.93 events of SCC_{200} and 3.02 events of SCC_{400} within the whole productive life. In these cows, on average cumulative AM and CM were 0.36 and 0.14, respectively. As regards the CV_{LC} , the average, first quartile, median, and third quartile were 2.81%, 1.98%, 2.54%, and 3.30%. The top 5% of cows were those with $CV_{LC} > 5.29\%$ and experienced on average 7.03 events of SCC_{200} and 3.19 events of SCC_{400} within the whole productive life. The same had on average 0.31 and 0.14 cumulative mastitis of AM and CM, respectively. Finally, the endpoint LC averaged 4.68% and had first and third quartile at 4.55% and 4.85%, respectively. The bottom

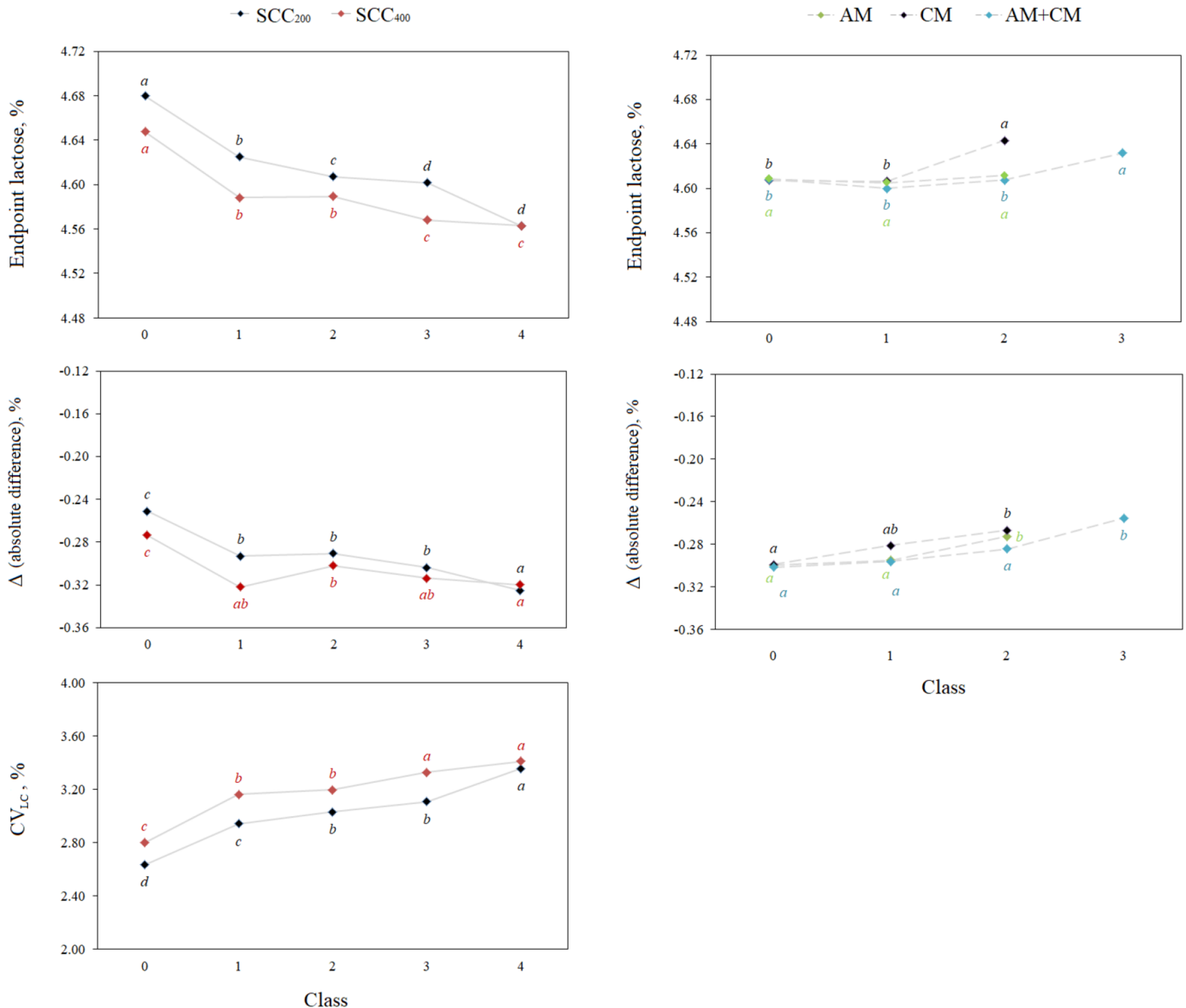


Figure 5. Least squares means of lifetime lactose traits estimated for the fixed effect of cumulative mastitis. Least squares means with different letters (a–d) within fixed effect differ significantly ($P < 0.05$). Endpoint = lactose content measured at the last available TD in the lifetime; CV_{LC} = within-cow coefficient of variation (%) of lactose content calculated using all TD available; Δ = absolute difference between the lactose content measured in early (parity 1 and <100 DIM) and late productive life (last available cow's TD; $n = 18,249$); AM = cumulative number of acute mastitis diagnoses; CM = cumulative number of chronic mastitis diagnoses; AM+CM = cumulative number of acute plus chronic mastitis diagnoses; SCC_{200} = cumulative number of lifetime test-day records with $SCC \geq 200,000$ cells/mL; SCC_{400} = cumulative number of test-day records with $SCC \geq 400,000$ cells/mL. Classes were created based on the presence of 0, 1, or ≥ 2 events for both AM and CM; 0, 1, 2 or ≥ 3 events for AM+CM; 0, 1, 2, 3, and ≥ 4 events for SCC_{200} and SCC_{400} . Primiparous accounted for the 26%, 27%, 26%, 18%, and 22% of cows in the class 0 of AM, CM, AM+CM, SCC_{200} , and SCC_{400} , respectively.

5% consisted of 910 cows with LC endpoint <4.17% that experienced on average 7.21 events of SCC_{200} , 3.08 events of SCC_{400} , 0.34 AM, and 0.15 CM in their life.

To the best of the authors' knowledge, this study reports for the first time the effect of multiple inflammations on phenotypes derived from LC. The existing literature can support the findings, but a direct comparison with similar studies is not possible.

Genetic Aspects of Cumulative Mastitis

The h^2 of clinical mastitis estimated via linear animal model has been reported as equal to 0.02 in Austrian Fleckvieh cattle (Koeck et al., 2010; Costa et al., 2019a). This value is overall comparable to the h^2 reported for the same binary trait in other populations, both dairy-specialized and dual-purpose breeds (Govignon-Gion et

Table 7. Heritability of lactose content and milk yield (h^2), and heritability of the cumulative mastitis trait (h^2_{MAST}) at each bivariate analysis, along with the genetic (r_a), permanent environmental (r_{pe}), and phenotypic correlation (r_p); values presented $\pm$ SE

Mastitis ¹	h^2	h^2_{MAST}	r_a	r_{pe}	r_p
Lactose					
AM	0.243 $\pm$ 0.007	0.090 $\pm$ 0.009	-0.180 $\pm$ 0.045	-0.013 $\pm$ 0.024	-0.033 $\pm$ 0.005
CM	0.243 $\pm$ 0.007	0.013 $\pm$ 0.004	-0.105 $\pm$ 0.088	-0.031 $\pm$ 0.022	-0.012 $\pm$ 0.006
AM+CM	0.244 $\pm$ 0.007	0.082 $\pm$ 0.009	-0.178 $\pm$ 0.045	-0.023 $\pm$ 0.024	-0.033 $\pm$ 0.006
SCC ₂₀₀	0.250 $\pm$ 0.008	0.376 $\pm$ 0.008	-0.260 $\pm$ 0.023	-0.185 $\pm$ 0.034	-0.161 $\pm$ 0.006
SCC ₄₀₀	0.247 $\pm$ 0.008	0.293 $\pm$ 0.010	-0.217 $\pm$ 0.025	-0.128 $\pm$ 0.031	-0.125 $\pm$ 0.006
Milk yield					
AM	0.127 $\pm$ 0.006	0.087 $\pm$ 0.009	-0.098 $\pm$ 0.054	0.054 $\pm$ 0.019	0.004 $\pm$ 0.007
CM	0.129 $\pm$ 0.006	0.014 $\pm$ 0.004	-0.339 $\pm$ 0.096	0.043 $\pm$ 0.017	0.001 $\pm$ 0.008
AM+CM	0.127 $\pm$ 0.006	0.078 $\pm$ 0.008	-0.147 $\pm$ 0.054	0.063 $\pm$ 0.019	0.004 $\pm$ 0.008
SCC ₂₀₀	0.128 $\pm$ 0.006	0.372 $\pm$ 0.011	-0.270 $\pm$ 0.028	0.098 $\pm$ 0.027	-0.097 $\pm$ 0.009
SCC ₄₀₀	0.127 $\pm$ 0.006	0.289 $\pm$ 0.010	-0.222 $\pm$ 0.031	0.083 $\pm$ 0.025	-0.071 $\pm$ 0.009

¹AM = cumulative number of acute mastitis diagnoses; CM = cumulative number of chronic mastitis diagnoses; AM+CM = cumulative number of acute plus chronic mastitis diagnoses; SCC₂₀₀ = cumulative number of lifetime test-day records with SCC $\geq$ 200,000 cells/mL; SCC₄₀₀ = cumulative n. test-day record with SCC $\geq$ 400,000 cells/mL.

al., 2016). Low h^2 estimates are also generally expected for health and other functional traits in Austrian Fleckvieh, such as longevity, fertility, and twinning in cattle (Koeck et al., 2010; Zuchtwert Austria, 2023; Caccin et al., 2025).

In this study, however, the h^2 was estimated for cumulative mastitis events and was characterized by greater h^2 (Table 7) compared with their binary counterparts (Koeck et al., 2010; Costa et al., 2019a). Cumulative AM, for example, has exploitable h^2 (0.090), and the h^2 of SCC₂₀₀ (0.376) was greater than that of major milk traits under selection. In general, when the cumulative mastitis was given by SCC₂₀₀ and SCC₄₀₀, the h^2 was considerably higher (overall $h^2 > 0.280$; Table 7) compared with AM, CM, and AM+CM (overall $h^2 < 0.100$; Table 7). This is the first time that the h^2 of cumulative mastitis, (i.e., recidivist mastitis) has been presented. Findings suggest that it would be possible to select for cows more resistant to recursive inflammations and more resilient at the udder level. In the practice, the genomic evaluation for cumulative mastitis should be properly designed to avoid selection bias and to take into account that cows may be culled earlier than the diagnosis of one or more event. A potential strategy to overcome this issue is to use exclusively first lactation data to carry out the evaluations.

The h^2 of LC obtained from different analyses were quite stable, ranging from 0.243 to 0.250 (Table 7). In the case of MY, the minimum (0.127) and maximum h^2 (0.129) were also close. Overall, these estimates are in line with the literature based on the same or different populations (e.g., Miglior et al., 2007; Haile-Mariam and Pryce, 2017; Costa et al., 2019a).

For LC, animals' solutions were estimated with univariate analysis, and when extracting the bottom 5%

(EBV < -0.092) and the top 5% sires (EBV > 0.064), 3,313 and 2,832 offspring cows were isolated from the total database, i.e., 12,122 cows remained in the intermediate class. Retrospectively, the daughters' endpoint LC was on average 4.617%, 4.675%, and 4.741% in the bottom, intermediate, and top group, respectively (data not shown). The same had different productive life length, i.e., average parity of 2.61, 2.89, and 3.31, and average age at last TD of 1,777, 1,914, and 2,100 d for bottom, intermediate, and top groups corresponding to approximately 4.9, 5.2, and 5.8 yr of age, respectively (data not shown). These findings suggest that estimating the genetic merit for LC in addition to other milk traits could be meaningful because cows staying longer in the herd are expected to have greater LC compared with the rest of the population.

Regardless of the mastitis type considered, r_a , r_{pe} , and r_p with LC were always negative (Table 7). In particular, the strongest associations were obtained between LC and SCC₂₀₀, with r_a , r_{pe} , and r_p values of -0.260, -0.185, and -0.161, respectively. In the case of MY, the r_a (but not the r_{pe}) always had negative direction (Table 7). Estimates suggest an existing negative genetic covariance between cows' productivity and mammary gland memory, i.e., animals with medium to low genetic merit for MY are those carrying favorable alleles for udder health. Nevertheless, the weak r_{pe} and r_p with traits such as AM and CM suggest that there is no or very limited covariance due to nongenetic reasons (Table 7). This supports the Pearson correlations in Table 4 on the raw data, i.e., without accounting for the additive genetic variance and for presence of repeated measurements on the same cow.

The decrease in MY is not expected to be as pronounced as for LC in presence of mastitis or high SCC because of the milk osmolality equilibrium: the amount

of water pulled into the alveoli determines the milk volume and is regulated by osmoles that play an important role in the milk-blood barrier, e.g., lactose, Na, Cl, and K. When needed, minerals re-establish the osmotic equilibrium and compensate for the LC reduction (loss) due to the impaired epithelial integrity. Therefore, although the LC reduction can be clearly observed in the milk of sick quarters and cows, the decrease in MY should be milder, especially if the udder yield (as a whole) is considered rather than the quarter yield. Mastitis reduces the mammary epithelial cells number in the inflamed alveoli (Boutinaud et al., 2019). Consequently, scar tissue plus cells apoptosis impair the synthesis and the secretion ability of the tissue at the quarter level (Boutinaud et al., 2019). Further studies should focus on the quarter-level variability of MY and LC rather than on the pooled milk to identify the variability related to mastitis in the quarter affected. In fact, clinical mastitis usually occurs in 1 quarter, which is thereby responsible for the cow's LC drop in the pooled milk.

CONCLUSIONS

In dairy cows, the lifetime progressive depression in LC and MY is due to an impaired epithelial integrity caused by both mastitis and aging. The findings confirm that there is a lasting and cumulative effect (i.e., cows that experienced several mastitis events produce less and have a significantly lower LC in their milk compared with cows that experienced only 1 or no events). In addition, LC is negatively phenotypically and genetically correlated with the number of mastitis events experienced. In the case of MY, the genetic but not the phenotypic correlation was negative. These results make the cumulative number of cumulative mastitis events a promising phenotype, as more heritable than the binary diagnoses themselves. Quarter-level investigations are advisable to better disclose the evolution of milk osmolality mechanisms in presence of mastitis or its permanent alteration after that. Pursuing selection toward cows that are more resistant to recursive inflammations is therefore possible and meaningful, especially if phenotypes available early in life are used.

NOTES

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (Piano Nazionale di Ripresa e Resilienza (PNRR) – Missione 4 Componente 2, Investimento 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them. The research

also received support from the Project D4Dairy (Digitalization, Data Integration, Detection, and Decision support in Dairying, grant 872039) funded by the Federal Ministry of Climate Action, Environment, Energy, Mobility, Innovation and Technology (BMK; Vienna, Austria) and the Federal Ministry for Digital and Economic Affairs (BMDW; Vienna, Austria), and the provinces of Lower Austria (St. Pölten, Austria) and Vienna (Vienna, Austria) in the framework of COMET-Competence Centers for Excellent Technologies program handled by the Austrian Research Promotion Agency (FFG; Vienna, Austria). Supplemental material for this article is available at <https://doi.org/10.7910/DVN/K1ENXJ>. No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: AM = cumulative number of acute mastitis diagnoses; AM_{BIN} = single diagnosis of acute clinical mastitis; CM = cumulative number of chronic mastitis diagnoses; CM_{BIN} = single diagnosis of chronic clinical mastitis; CV_{LC} = CV of lactose content; h²_{MAST} = heritability of the cumulative mastitis trait; ICAR = International Committee for Animal Recording; IDF = International Dairy Federation; ISO = International Organization for Standardization; LC = lactose content; MIRS = mid-infrared spectroscopy; MY = milk yield; NS = not significant; r_a = genetic correlation; r_p = phenotypic correlation; r_{pe} = permanent environmental correlation; SCC₂₀₀ = test day SCC ≥200,000 cells/mL; SCC₄₀₀ = test day SCC ≥400,000 cells/mL; TD = test day record.

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