

## The lasting effect of udder inflammation on milk lactose content in dairy cows

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### Abstract

Milk lactose content (LC) reduces with parity in dairy cattle and shows an inverse relation with somatic cell count. The aim of this study was to investigate if the lifetime decrease in LC is due to cumulative number of experienced udder inflammations. For this purpose, milk test-day data of Austrian Fleckvieh cows were available together with records of acute (AM) and chronic mastitis (CM) cases. In addition, inflammations were also defined by using high test-day somatic cell count:  $\geq 200,000$  (SCC<sub>200</sub>) or  $\geq 400,000$  cells/mL (SCC<sub>400</sub>). Inflammation events were calculated for each test-day using data collected across the cows' lifespan, i.e. across all available lactations. The effect of cumulative inflammations on LC was highly significant, with LC being the highest when 0 mastitis events were experienced and significantly different from LC estimated for test-day records linked to  $\geq 1$  event. Findings demonstrate that the lifetime LC decrease can be seen as a mammary memory indicator and represents the combined result of physiological aging and past inflammations.

### Introduction

A negative correlation between milk lactose content (LC) and somatic cell count (SCC) has been observed in several studies, but only in recent years some attempts have been made to explore why and how LC changes when mastitis occurs. Both the genetic and the phenotypic correlations between LC and SCC and between LC and mastitis confirm the opposite trend of these features. A progressive drop in average milk LC is observed during productive life of cows, i.e., across parities. Costa et al. (2020) suggested that LC decreases with parity for two main reasons: physiological aging and cumulative effect of inflammation events. In both cases, the alveolar permeability is subjected to damage and impairment of its functionality, which lead to a loss of lactose from the alveolar lumen (milk) to blood and finally to urine. The reduction in alveolar LC causes an alteration of the osmotic equilibrium, which is compensated by sodium and potassium. In this study, we explored the role of cumulative mastitis events on milk LC using cows' test-day (TD) records and validated health data.

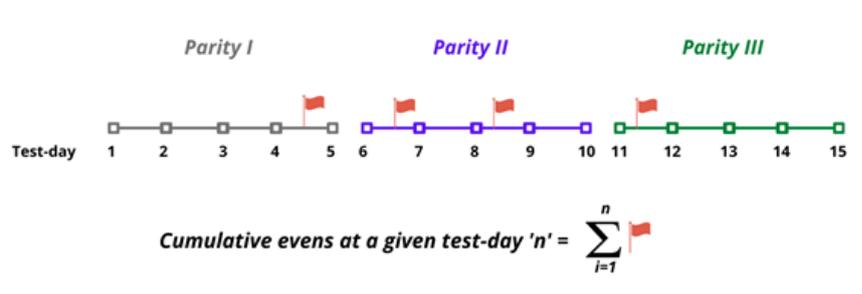
### Materials & Methods

**Data.** Data used in the present study included milk and health records of cows born between 1997 and 2017 reared in Austrian farms under validated health data recording system (Egger-Danner et al. 2012) located in Styria and Lower Austria. Only Fleckvieh cows with information available from parity 1 were retained and the maximum parity was set at 10. In addition, only TD records from 5 to 500 days in milk were considered and  $\geq 3$  cows/farm and  $\geq 3$  TD/lactation were required. After editing, date of first calving was within a window from 1 January 2011 to 29 February 2020. This guaranteed the presence of reliable health records and allowed the completion of those lactations started in spring 2020. Finally, 432,839 TD from

53,099 lactations and 18,404 cows located on 566 farms were left. Both acute (AM) and chronic mastitis (CM) events were available. The cumulative number of mastitis events were assigned to each TD of a cow starting from the first available TD in parity 1 onwards, i.e. across lifespan, regardless of the distance between the diagnosis date and the date of TD. Mastitis events were defined using diagnosed AM, AM+CM or based on high SCC. In particular, TD SCC  $\geq 200,000$  (SCC<sub>200</sub>) or TD SCC  $\geq 400,000$  cells/mL (SCC<sub>400</sub>) were used as threshold. The TD records were classified as reported in Table 1 according to cumulative AM events, i.e., 0, 1, and 2, with the latter including TD records with 2 or more AM. For the sum of cumulative AM+CM, 4 classes were defined, i.e., from 0 (no diagnoses) to 3 ( $\geq 3$  diagnoses). Furthermore, 5 classes of SCC<sub>200</sub> and 5 classes of SCC<sub>400</sub> were created based on the number of TD with ‘high’ SCC. Specifically, the classes 0, 1, 2, 3, and 4 included 0, 1, 2, 3, and  $\geq 4$  ‘high’ TD SCC, respectively. Counting of mastitis events did not stop at each lactation, so that for each TD all events recorded previously in life were considered (Figure 1). Seasons of calving were defined as: Mar-May, Jun-Aug, Sep-Nov, and Dec-Feb.

**Table 1. Distribution of test-day records across classes of fixed effects.**

Fixed effect	Cumulated traits	Frequency (%)				
		0	1	2	3	4
AM	n. past acute mastitis	88.01	8.01	3.98	-	-
AM+CM	n. past acute plus chronic mastitis	84.68	9.41	4.07	1.84	-
SCC <sub>200</sub>	n. past test-day somatic cell count $\geq 200,000$	51.39	13.74	10.2	5.79	18.88
SCC <sub>400</sub>	n. past test-day somatic cell count $\geq 400,000$	69.42	12.86	7.51	3.49	6.72



**Figure 1. Example of mastitis events calculation for a given test-day record along a cow's lifespan.**

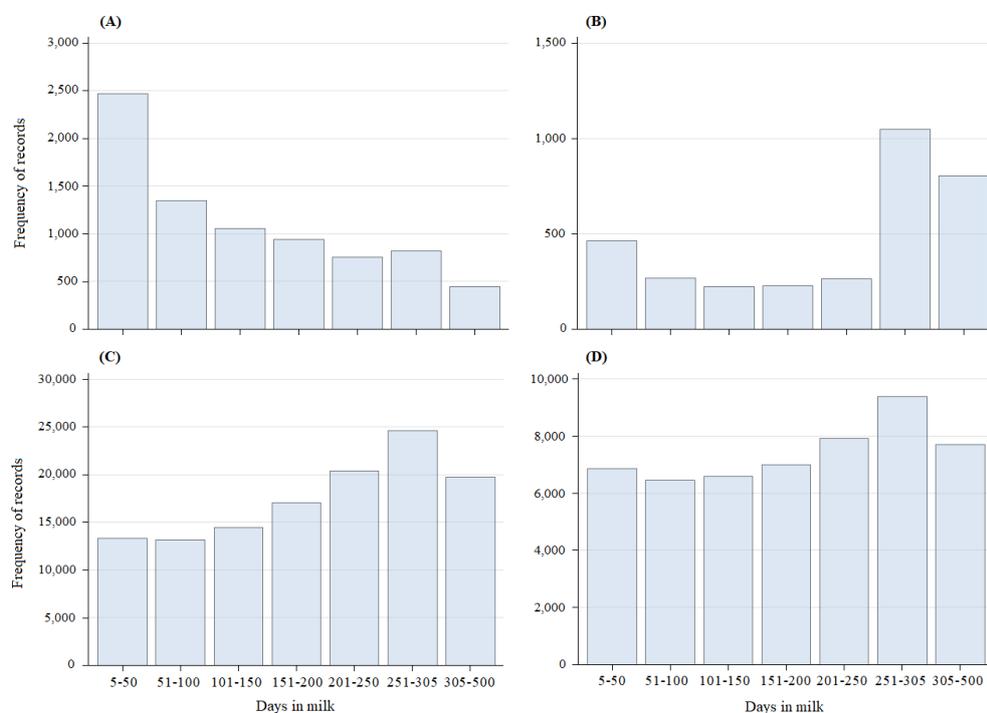
**Effect of mastitis.** The following mixed model was used in SAS software v. 9.4 (PROC HP MIXED) to estimate LC with increasing number of experienced inflammations, i.e. including the fixed effect of AM, AM+CM, SCC<sub>200</sub>, or SCC<sub>400</sub> separately in four analyses:

$$y_{ijklmn} = u + PO_i + LS_j + ME_k + (PO*LS)_{ij} + (LS*ME)_{jk} + YS_l + FC_m + DF_n + e_{ijklmn} \quad (1)$$

where  $y_{ijklmn}$  is the milk LC at the given TD,  $u$  is the overall intercept of the model,  $PO_i$  is the fixed effect of the  $i$ th parity ( $i = 1$  to 6, with parities from 6 to 10 grouped together),  $LS_j$  is the fixed effect of the  $j$ th lactation stage ( $l = 1$  to 7, each class being 50 days wide, except for the last class which included TD from 305 to 500 days in milk),  $ME_k$  is the fixed effect of the  $k$ th cumulative mastitis event (Table 1),  $YS_l$  is the fixed temporal effect of the  $l$ th year-season of calving ( $l = 1$  to 43), and  $FC_m$ ,  $DF_n$ , and  $e_{ijklmn}$  are the random effects of the  $m$ th cow, the  $n$ th herd, and the residual, respectively. Random effects were assumed to be independent and normally distributed.

## Results

Milk LC averaged 4.79% and varied from 3.00 to 5.63%. Each TD was assigned to the cumulative number of mastitis events defined as previously described. The binary events AM and CM were mainly recorded in early and late lactation, respectively, while high SCC values were observed throughout the whole lactation (Figure 2).



**Figure 2. Distribution of mastitis events identified using acute (A) and chronic (B) diagnoses and milk TD SCC  $\geq 200,000$  (C) and  $\geq 400,000$  cells/mL (D) across days in milk.**

All fixed effects in model (1), including the number of mastitis events, were highly significant in all analyses ( $p < 0.001$ ). The highest level of LC was observed in class 0 and this estimate was significantly different than those of other classes (Table 2). In addition, the gap between class 0 and class 1 was wider when fixed effect of mastitis was based on  $SCC_{200}$  and  $SCC_{400}$ . The lifetime decline in LC is thus due to both parity and past inflammations, and this is particularly evident when looking at the estimates of  $SCC_{200}$  or  $SCC_{400}$ . In fact, the LC decrease was less noticeable if the effect was either AM or AM+CM. The F-value of fixed effect of parity ranged from 3,104.09 to 5,438.63, while the F-value of mastitis event was 29.87, 18.46, 1392.03, and 998.99 for AM, AM+CM,  $SCC_{200}$  and  $SCC_{400}$ , respectively.

**Table 2. Least squares means<sup>1</sup> of lactose (%) for cumulative number of mastitis events.**

Fixed effect <sup>2</sup>	Class 0	Class 1	Class 2	Class 3	Class 4
AM	4.725 <sup>a</sup>	4.717 <sup>b</sup>	4.714 <sup>b</sup>	-	-
AM+CM	4.724 <sup>a</sup>	4.719 <sup>b</sup>	4.719 <sup>b</sup>	4.712 <sup>c</sup>	-
$SCC_{200}$	4.763 <sup>a</sup>	4.730 <sup>b</sup>	4.715 <sup>c</sup>	4.705 <sup>d</sup>	4.686 <sup>e</sup>
$SCC_{400}$	4.746 <sup>a</sup>	4.710 <sup>b</sup>	4.699 <sup>c</sup>	4.687 <sup>d</sup>	4.678 <sup>e</sup>

<sup>1</sup> Superscript letters within row indicate significantly different estimates ( $p < 0.05$ ).

<sup>2</sup> Cumulative number of past acute mastitis (AM), acute plus chronic mastitis (AM+CM), and test-day somatic cell count  $\geq 200,000$  ( $SCC_{200}$ ) and  $\geq 400,000$  cells/mL ( $SCC_{400}$ ).

## Discussion

Costa et al. (2020) opened the debate on the LC reduction across parities in dairy cows and suggested that the drop in LC may be used as a marker of mammary memory. A larger drop in LC within the productive life indicates that the cow faced up more infections, i.e. inflammations. Findings suggest that there is a lasting effect of udder inflammation on the alveolar structure and epithelium, that is detectable through changes in LC. The effect of parity (e.g. tissue aging plus milking mechanical stress) was separated from the effect of cumulative inflammations and, particularly when considering the results of SCC<sub>200</sub> or SCC<sub>400</sub>, the hypothesis that the lifetime LC reduction is due to both aging and cumulative udder inflammations was confirmed. Accurate identification of mastitis is challenging, particularly in presence of binary records. On the other hand, identification of mastitis based on milk SCC may led to false positive cases. Thus, in the present study the use of AM and AM+CM may plausibly have led to less glaring findings compared to continuous SCC data. It frequently happens that cows may not be sampled for official TD milk analyses if one or more diagnosis of mastitis is recorded during the lactation. For instance, farmers could opt for culling or for an earlier drying off. In the Austrian system specifically, when AM or CM is recorded it means that a treatment was administered to the lactating cow, speeding up the recovery and likely reducing the potential damage due to inflammation process. Conversely, using milk SCC for identification of inflammations seems a less restrictive approach, allowing detection of LC change after the event(s) in those cows with i) considerable number of cumulated high SCC TD and ii) likely no or few official recorded mastitis diagnoses. The distribution of the different mastitis events within lactation (Figure 2) supports the idea that binary health traits have a different connotation compared to SCC quantitative data. Understanding if there is interaction between parity and cumulative event on LC sounds interesting, even if a small number of TD is expected to be associated with multiple inflammation events in parity 1, inevitably leading to distorted results. Vice versa, it is plausible that very old cows, e.g. in parity >5, have a limited number of TD linked to 0 past inflammations. In conclusion, this study demonstrates that the lifetime change in LC can be considered a mammary memory indicator, representing the combined result of aging and mastitis events in Fleckvieh cows.

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