

# Increased somatic cell counts at first milk control are associated with decreased milk yield in the first lactation in dairy heifers

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## Abstract

Intramammary infections before calving and in early lactation have a negative impact on the development of the mammary gland, udder health, the risk of clinical mastitis in early lactation, the risk of premature culling and future milk production in dairy heifers. The aim of this study was to reveal whether and how the somatic cell count of the first dairy herd improvement (DHI) affects the 305 day milk yield (energy corrected milk) of the first lactation in German dairy heifers. It should also be examined whether the threshold of 100,000 cells/ml used for herd analysis in Germany is suitable for estimating possible economic losses as the threshold of 200,000 cells/ml is often used in other countries. In addition, it should be examined whether a milk loss, which is associated with an increased initial SCC, differs between different German dairy cattle breeds. For this study, the DHI data of 49,467 heifers reared on 2413 German dairy farms located in Schleswig-Holstein between October 2015 and June 2016 were analyzed. Heifers with a SCC in the first DHI test of > 100,000 cells/ml showed a significantly ( $p < 0.001$ ) lower milk yield (on average -98.3 kg; 95% confidence interval (CI): -116.4 - -80.2) during the first lactation compared to heifers with an SCC  $\leq$  100,000 cells/ml. Heifers with a SCC in the first DHI test of > 200,000 cells/ml also showed a significantly ( $p < 0.001$ ) lower milk yield (on average -96.3 kg; 95% CI: -118.4 - -74.2) in the first lactation compared to heifers with a SCC  $\leq$  200,000 cells/ml. The effect of milk loss due to an increased initial SCC did not differ significantly between the breeds analysed. These results show that it is possible to use both thresholds of the SCC for assessing udder health in individual heifers and at herd level. However, the present study only shows the relationship between increased SCC and the loss of milk yield measured in different German breeds of dairy heifers. Future studies must validate further risk factors that influence the udder health of dairy heifers.

**Keywords:** mastitis, intramammary infection, udder health, dairy heifers, milk production

## Introduction

Bovine mastitis is one of the most important economic diseases in dairy cows and still a serious problem in dairy farms. Mastitis causes additional costs due to a lower milk production, higher workload and

more veterinary treatments [1, 2]. Intramammary infections (IMI) before calving and in early lactation can negatively affect the development of the mammary gland, udder health, the risk of clinical mastitis in early lactation, the risk of culling and future milk production in dairy heifers [3-7].

In addition, subclinical mastitis (SCM) in early lactation increases the risk of chronic mastitis and leaving the herd prematurely as well as it decreases the reproductive performance during early lactation [8,9].

In the past, heifers received little consideration in mastitis control programs [8,10]. As early as 1942, Schalm (1942) [11] described the occurrence of mastitis in dairy heifers. Several studies followed in the 1980s which showed that the incidence of IMI in the udder quarters of heifers is high at calving or in early lactation [6,12,13]. Nitz et al. (2020) [14] showed a prevalence of intramammary infections (IMI) in udder quarters of dairy heifers of 19.8% on day 3 postpartum and 14.3% on day 17 postpartum, respectively. Results from other studies show that prevalence of IMI in heifers at the time of calving varies between 18% and 80% [10, 15, 16].

The specific risk factors for IMI in heifers before and after calving are not fully understood. However, some risk factors are described in the literature. Infections with non-aureus staphylococci (NAS) are the most common cause of mastitis in heifers before and after calving. However, *Staphylococcus (S.) aureus*, environmental streptococci and coliforms also play a role in mastitis in heifers [14, 17]. IMIs often occur before calving and are facilitated, for example, by the early loss of the keratin plug and the associated opening of the teat canal [15]. Poor hygiene at the calving area, dirty udders, juvenile intersucking, age at calving and an inadequate supply of vitamin E and selenium also play a role in heifers' mastitis. However, new infections also occur after calving. The risk of IMI in early lactation is increased by udder edema and problems with milking such as transmission of infectious mastitis pathogens like *S. aureus* during milking or teat cup fall-offs [4, 10, 14, 18, 19, 20]. A high incidence of clinical mastitis at herd level or a high bulk milk SCC are also a risk factor for poor udder health in dairy heifers [21].

To evaluate the udder health of heifers at herd level, the measurement of the SCC in the first dairy herd improvement test (DHI) and a threshold of 100,000 cells/ml is used in Germany. The proportion of heifers with an SCC in the first DHI after calving with > 100,000 cells/ml in all heifers

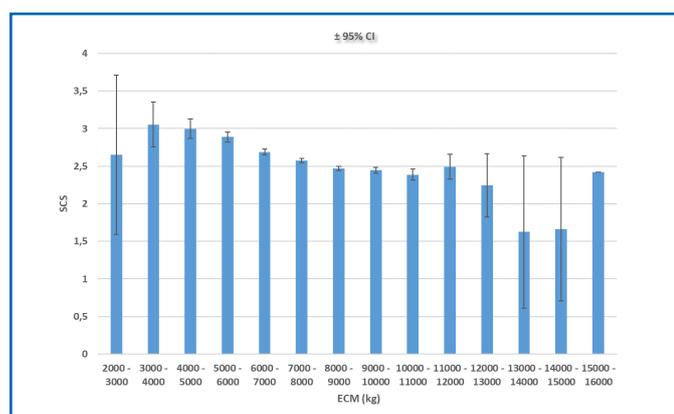
tested provides the heifer mastitis rate. The heifer mastitis rate can be used to compare farms with one another, but it is criticized because an increased SCC in the first DHI often normalizes by the next DHI. Nonetheless, increased cell counts at the start of lactation can lead to a reduced milk yield in the first lactation and are associated with economic losses [7, 22]. The annual costs caused by subclinical heifer mastitis are estimated at an average of 626 € on farm level (range: 85-1657 €) and 31 € (range: 4.29-82.86 €) per heifer reared on the farm [22]. It becomes clear that also heifer mastitis is a serious problem in dairy farms and can lead to financial losses as well as animal welfare issues. So far there are no studies on possible associations of increased SCC in the first DHI after calving and reduced milk yield in the first lactation for German dairy heifers. The aim of this study was to reveal whether and how the SCC of the first DHI affects the milk yield of the entire first lactation in German dairy heifers. It should also be examined whether the limit value of 100,000 cells/ml used for herd analysis in Germany is suitable for estimating possible economic losses as the threshold of 200,000 cells/ml is often used in other countries. In addition, it should be examined whether a possible milk loss, which is associated with an increased initial SCC, differs between different breeds.

## Material and Methods

The data was collected between October 2015 and June 2016. For this study, the DHI data of 49,467 heifers reared on 2413 German dairy farms located in Schleswig-Holstein were analysed. These are all heifers in this federal state which have calved during this time period originating from farms taking part in the DHI system and which reached a 305 days milk yield. Of these, 43,052 were of the breed German Holstein, 1,540 Angeln cattle, 2,393 German red or black pied cattle and 2,482 belonged to other cattle breeds and crossbreeds, respectively. The dataset was made available by the LKV Schleswig-Holstein and contains the following data: Animal date of birth, date of calving, test date, SCC 1. DHI, days in milk, milk kg 305 days, fat kg 305 days, protein kg 305 days, breed. Energy corrected milk (ECM; 4.0% fat, 3.4% protein) was calculated for each heifer [23]:

$$ECM (kg) = milk (kg) \times (0.38 \times fat\% + 0.21 \times protein\% + 1.05) / 3.28.$$

**Statistical Analysis:** the collection and processing of data was carried out with Microsoft excel (Microsoft Corp., 2010). For analysing the dataset, the program SPSS 26.0, Chicago IL, USA was used with heifers considered as statistical unit. Associations between the 305 days milk yield (ECM) and predictors (independent variables) were examined with linear mixed models. The normal distribution of the outcome vari-



**Figure 1: SCC at first DHI by milk yield category (ECM: Energy corrected milk; CI: Confidence Interval; SCS =  $\log_2 (SCC/100,000) + 3$ )**

able milk yield (ECM) was tested and confirmed using the Kolmogorov Smirnow test. The independent variables breed [categorical variable with four expressions], first calving age (covariate), SCC 100, SCC 200 were subjected to univariable analyses. Variables with  $P \leq 0.3$  (Z-test) were retained for inclusion in the multivariable models. Based on this preselection, no variable was excluded. To avoid multicollinearity, correlation with one another were checked based on  $r > 0.70$ . For this reason, no variables were excluded. Two different models were calculated. In both models, the categorical variable breed and the covariate first calving age in months were examined as fixed effects and herd as a random effect.

The models used were described as :

$$305 \text{ days milk yield (ECM)} = \text{Breed (fixed)} + \text{SCC 100/200 (fixed)} + \text{1st calving age (fixed, covariate)} + \text{herd (random)} + e.$$

We used the Welch-Satterthwaite equation to calculate the pooled degrees of freedom. In the first model (model 1), the categorical variable SCC100 (somatic cell count in first milk control was above a SCC threshold of 100 (1) or not (0)) and in the second model (model 2), the categorical variable SCC200 (somatic cell count in first milk control was above a SCC threshold of 200,000 cells/ml (1) or not (0)) were used additionally. The multivariable analysis was performed using a backward stepwise selection and elimination procedure until each independent variable had a p-value of  $\leq 0.05$ . Confounding effects were monitored by observing regression coefficient changes. Variables that modified regression coefficients by  $> 20\%$  were considered confounding factors. No confounding was observed. The models were evaluated using the Akaike information criterion (AIC) [24], where an AIC closest to zero was used as final model. In the final model, all biologic credible two-way interactions were tested but eliminated again due to lack of significance. Model fit was evaluated by checking normality of the residuals. The random farm effect was significant in the models. Estimated marginal means from the models were calculated. To adjust for multiple comparisons we used Bonferroni correction. The significance level for the linear mixed model was 0.05. To investigate SCC at the time of the first DHI after calving SCC was transformed to the logarithmic scale according to the formula:

$$SCS = \log_2 (SCC/100,000) + 3 \text{ [25,26].}$$

## Results

As part of this study, the milk yield and the composition of the milk in the first lactation (305 days) of 49,467 heifers in relation to the SCC at first dairy herd improvement test after calving were analysed. Least square means were used.

Heifers with an SCC of  $\leq 100,000$  cells/ml in the first DHI test showed an average milk yield of 7,412.6 kg (ECM) within 305 days. Heifers with a SCC  $> 100,000$  cells/ml had an average milk yield of 7,314.3 kg (ECM). This means that heifers with a SCC in the first DHI test of  $> 100,000$  cells/ml showed a significantly ( $p < 0.001$ ) lower milk yield (on average -98.3 kg; 95% confidence interval (CI): -116.4 - -80.2) during the first lactation compared to heifers with an SCC  $\leq 100,000$  cell /ml. Heifers with a SCC of  $\leq 200,000$  cells/ml in the first DHI test showed an average milk yield of 7,398.3 kg (ECM) within 305 days and heifers with a SCC  $> 200,000$  cells/ml an average milk yield of 7,302.0 kg (ECM). Heifers with a SCC in the first DHI test of  $> 200,000$  cells/ml also showed a significantly ( $p < 0.001$ ) lower milk yield (on average -96.3 kg; 95% CI: -118.4 - -74.2) in the first lactation compared to heifers with a SCC  $\leq 200,000$  cells/ml (Table 1-4).

The SCS at the time of the first DHI after calving is lower in higher yielding animals as shown in Figure 1.

**Table 1: Milk yield of heifers with a SCC at first DHI test below and above the threshold of 100,000 cells/ml (df: degrees of freedom)**

SCC (cells/ml)	Mean	Standard Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
≤ 100,000	7412,638	26,002	3790,077	7361,658	7463,618
> 100,000	7314,314	26,576	4124,352	7262,212	7366,416

Age at first calving in months was significantly associated with 305 days milk yield. With a mean calving age of the animals studied of 28.3 months, an increase in calving age per month was associated with a decrease in first lactation (305 days) milk yield by an average of 41.7 kg (ECM) ( $p < 0.001$ ; model 1) and 41.6 kg (ECM) ( $p < 0.001$ ; model 2). The average milk yield of the different breeds within the 305 days was 7,586.6 kg (ECM, model 1) and 7,572.8 (ECM, model 2) for German Holstein, 7,181.1 kg (ECM, model 1) and 7,169.8 (ECM, model 2) for Angeln cattle, 7,251.9 kg (ECM, model 1) and 7,238.1 (ECM, model 2) for German red or black pied cattle and 7,434.4 kg (ECM, model 1) and 7,420 (ECM, model 2) for other cattle and crossbreeds, respectively.

A comparison of the mean milk yield of the breeds showed significant differences. German Holstein Heifers showed a significant higher milk yield compared to Angeln cattle (mean difference 405.5 kg (ECM, model 1) and 403 kg (ECM, model 2);  $p < 0.001$ ), to German red or black pied cattle (mean difference 334.7 kg (ECM, model 1) and 334.7 kg (ECM, model 2);  $p < 0.001$ ) and to other cattle and crossbreeds (mean difference 152.2 kg (ECM, model 1) and 152.8 kg (ECM, model 2);  $p < 0.001$ ). Angeln cattle showed a significantly lower milk yield compared to other cattle and crossbreeds (mean difference -253.3 kg (ECM, model 1) and -250.2 kg (ECM, model 2);  $p < 0.001$ ). German red or black pied cattle showed a significantly lower milk yield compared to other cattle and crossbreeds (mean difference -182.5 kg (ECM, model 1) and -181.9 kg (ECM, model 2);  $p < 0.001$ ). The effect of milk loss due to an increased initial SCC did not differ significantly between the breeds in both models.

## Discussion

To the best of our knowledge that is the first study on possible associations of increased SCC in the first DHI after calving and reduced milk

**Table 2: Milk yield of heifers with an SCC at first DHI test below and above the threshold of 200,000 cells/ml (df: degrees of freedom)**

SCC (cells/ml)	Mean	Standard Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
≤ 200,000	7398,306	25,920	3737,831	7347,487	7449,125
> 200,000	7301,982	27,438	4665,392	7248,190	7355,774

yield in the first lactation for German dairy heifers. Heifers which exceed a threshold of 100,000 cells/ml SCC at first DHI test have an average loss of 98.3 kg milk within 305 days compared to heifers that did not exceed this threshold. Heifers that exceed a threshold of 200,000 cells/ml SCC at first DHI test show an average loss of 96.3 kg milk compared to heifers that did not exceed this threshold. Other studies revealed even greater losses of milk yield in the first lactation. Coffey et al. (1986) [6] showed that heifers in 30 dairy farms in Virginia (United States) with an SCC of > 100,000 cells/ml at first DHI test produced about 402 kg less milk in the first lactation compared to heifers with an SCC of < 100,000 cells/ml. De Vlieghe et al. (2005) [7] were also able to show a lower milk yield in heifers with an increased SCC in early lactation. Further studies were able to show that an increased SCC in early lactation is related to an increased SCC in the further course of lactation [6, 27]. In addition, IMI in early lactation increases the risk of a heifer being culled prematurely (28, 29). The reason for the decreased milk yield might be due to the fact that the mammary gland of heifers is still developing [30]. As mentioned earlier, staphylococci infections play an important role in the context of heifer mastitis [14, 17]. Trinidad et al. (1990) [31] were able to show how an infection with staphylococci affects the glandular tissue of heifers. They revealed that the glandular tissue in udder quarters that had been infected with NAS had more connective tissue than in non-infected quarters. Glandular tissue from udder quarters affected by an infection with *S. aureus* showed an increase in the stromal area and a decrease in the luminal area. Infections with both NAS and *S. aureus* resulted in leucocytosis. Looking at these changes in the glandular tissue, it seems obvious that they disrupt the development of the mammary gland and thus have a negative effect on milk production during further lactations. However, the influence of an infection with NAS has not yet been fully clarified. Some studies have shown

**Table 3: Pairwise comparisons of milk yield of heifers with an SCC at first DHI test below and above the threshold of 100,000 cells/ml (df: degrees of freedom; Sig.: significance)**

SCC (cells/ml)	Mean	Standard Error	df	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
≤ 100,000	-	-	-	-	-	-
> 100,000	-98,324	9,239	47283,013	<0.001	-116,433	-80,215

**Table 4: Pairwise comparisons of milk yield of heifers with an SCC at first DHI test below and above the threshold of 200,000 cells/ml (df: degrees of freedom; Sig.: significance)**

SCC (cells/ml)	Mean	Standard Error	df	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
≤ 200,000	-	-	-	-	-	-
>200,000	-96,324	11,275	47258,922	<0.001	-118,423	-74,225

that infections with NAS have no negative impact on milk yield or that infected heifers produce even more milk than uninfected heifers [32; 16]. Piepers et al. (2010) [16] could demonstrate that heifers infected with NAS showed fewer cases of clinical mastitis in the further course of the first lactation. This could be an explanation for the higher milk yield. In connection with this, a protective effect of NAS is discussed [16]. Another possible explanation would be that heifers with a higher milk yield due to their genetic potential are more likely to be affected by IMI with NAS [18]. It becomes clear that so far it cannot be answered whether IMI by NAS influences the milk yield or whether the high milk yield promotes the colonization by NAS. This issue should be addressed in further studies. Even if the importance of NAS and its effects on udder health and milk yield has not yet been conclusively clarified, other pathogens besides NAS are also responsible for heifer mastitis [14, 17]. Our data show a decrease in milk yield with increasing SCC in the first DHI test, which should be avoided in the economic interest of a dairy farm.

The results of the present study also confirm the validity of the threshold of 100,000 cells/ml SCC, which is used in Germany to determine the key figures for describing the udder health situation at herd level. In the Benelux countries and the UK, a threshold of 200,000 cells/ml SCC is usually used [33]. This study shows that the threshold of 100,000 cells/ml can also be used to assess the infection pathology, reduced milk yield and economic losses. It also shows that even if SCC normalized at second DHI test, the SCC increase at first DHI has an influence on the milk production of the first lactation. Therefore, it is very important to prevent intramammary infections in heifers before calving or during the first days of lactation.

The significant differences in milk yield in relation to age at first calving could be due to better management of these dairy farms, which have lower first calving ages. Previous studies have also shown that heifers with a lower first calving age are less likely to be affected by IMI in early lactation and could therefore produce more milk in first lactation [14, 21].

The different milk yields of the breeds examined is not particularly surprising. The influence of genetics on milk yield is generally known [34]. However, the milk loss associated with the increase in the initial SCC was not significantly different between the analysed breeds. This indicated that the effect of reduced milk production, caused by disorders of udder health, was equally evident in all of the breeds examined.

## Conclusions

The present study shows the influence of an increased initial SCC on the milk production of heifers in the first lactation. It becomes clear that heifers with an increased SCC produce less milk, regardless of the breeds examined. The loss of milk production in German dairy heifers is similar when the threshold of 100,000 cells/ml SCC (on average - 98.3 kg ECM) is exceeded compared to exceeding the threshold of 200,000 cells/ml SCC (on average - 96.3 kg ECM). The results show that it is possible to use both thresholds for assessing udder health in individual animals and at herd level. In addition, it becomes clear how important effective preventive measures are for dairy farms in order to monitor the udder health of heifers and, if necessary, to improve it. In this way, economic losses can be avoided. However, the present study only shows the relationship between increased SCC and the loss of milk yield measured in different German breeds of dairy heifers. Future studies must validate further risk factors that influence the udder health of dairy heifers.

## Disclosure of conflicts of interest

The authors declare no conflict of interest.

## Compliance with Ethical Standards

This study has been conducted in compliance with ethical standards.

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