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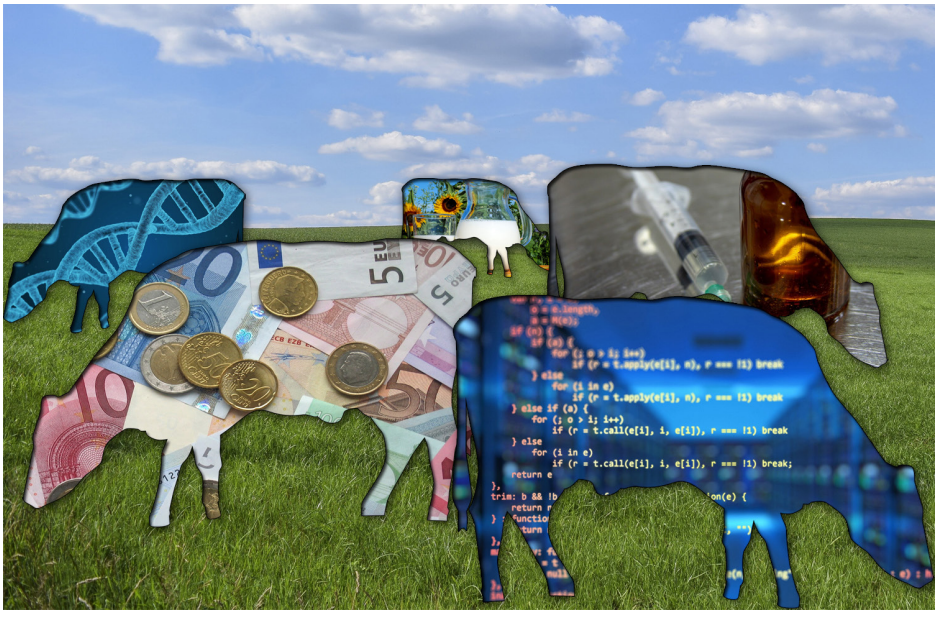
Sensor-based mastitis management in automatic milking system farms

Mastitis management from a data-centric and economic perspective

Johan Hendrikus Bonestroo



Propositions

1. Cows with chronic subclinical mastitis will not cure when the somatic cell count is increased for 4 weeks (this thesis).
2. Costs of chronic mastitis are more important than the costs of clinical mastitis (this thesis).
3. A detection system built on user-detected events never performs better than the users themselves.
4. Insufficient reporting on data cleaning is a major contributor to the replication crisis in social sciences.
5. If dairy farmers worldwide would adopt the operational practices on antibiotic use and feed efficiency of Dutch and Scandinavian dairy farmers, societal problems concerning antimicrobial resistance and farm-related environmental emissions would be solved.
6. Artificial intelligence needs to serve the user and not vice versa.

Propositions belonging to the thesis, entitled

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Johan Hendrikus Bonestroo
Wageningen, 15th of June 2022

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Thesis committee

Promotors
Prof. Dr H. Hogeveen
Personal chair, Business Economics Group
Wageningen University & Research

Dr N. Fall
Associate professor, Department of Clinical Sciences
Swedish University of Agricultural Sciences, Uppsala Sweden

Co-promotors
Dr M. van der Voort
Assistant professor, Business Economics Group
Wageningen University & Research

Prof. Dr U. Emanuelson
Researcher, Department of Clinical Sciences
Swedish University of Agricultural Sciences, Uppsala, Sweden

Industrial supervisor
Dr I.C. Klaas
Director, Dairy Development
DeLaval International AB, Tumba, Sweden

Other members
Prof. Dr I.N. Athanasiadis,Wageningen University and Research
Prof. Dr P.J. Rajala-Schultz, University of Helsinki, Finland
Dr N.C. Friggens, AgroParisTech, France
Dr A.H. Herlin, Swedish University of Agricultural Sciences, Uppsala, Sweden

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Cover: Mastitis from multiple perspectives
(photo: J.H. Bonestroo)

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Sensor-based mastitis management in automatic milking system farms

Abstract

Mastitis, or udder inflammation, is one of the most prevalent and costliest diseases in dairy farming. Automatic milking systems, equipped with sensors measuring mastitis indicators, have been used commercially since the 1990s. The overall objective for this PhD project was to explore the potential applications for a decision support system in automatic milking systems supporting chronic mastitis decision-making. Paper I described that mastitis cases usually recover in somatic cell count within three to four weeks. Paper II found strong non-linearities between milk production and lactate dehydrogenase, somatic cell count, and electrical conductivity, combined with possible actionable thresholds based on the size of milk yield loss. Paper III showed that it was possible to forecast the progression of mastitis. Finally, Paper IV estimated the economic impact of different sensor-based mastitis management strategies to show which strategy tends to decrease the cost of mastitis and chronic mastitis the most. More specifically, it estimated the economic consequences of chronic mastitis cases to show the direct impact of management failure on the economic situation of a dairy farm. This thesis shows that it is possible to support management regarding chronic mastitis with sensors, and it provides the basis for a decision support system. This decision support system would be a system that could tell the farmer which cases of mastitis are chronic, are likely to become chronic, are associated with large milk production loss, and could tell the economic consequences of chronic mastitis cases.

Keywords: Mastitis, udder inflammation, automatic milking system, cow, sensor, chronic, management, progression, milk, production loss

Author's address: Johan Hendrikus Bonestroo, Swedish University of Agricultural Sciences/Wageningen University and Research, Department of Clinical Sciences/Business Economics group, Uppsala/Wageningen, Sweden/The Netherlands

Sensor-based mastitis management in automatic milking system farms

Abstract

Mastit, eller juverinflammation, är en av de vanligaste och mest kostsamma sjukdomarna inom mjölkproduktionen. Automatiska mjölkningssystem (AMS), som kan vara utrustade med sensorer som mäter mastitindikatorer, har använts sedan 1990-talet. Det övergripande målet för det här doktorandprojekt var att utforska potentialen för ett beslutsstödsystem i AMS som stöder beslutsfattande kring kronisk mastit. I artikel I beskrevs att mastitfall som tillfrisknar vanligtvis gör det inom tre till fyra veckor. I artikel II påvisades starka icke-linjära samband mellan mjölkproduktion och LDH, SCC och EC i kombination med möjliga tröskelvärden för åtgärder som baseras på storleken av förlorad mjölkavkastning. Artikel III visade att det var möjligt att förutsäga utvecklingen av mastit på ett bra sätt. Slutligen uppskattades i artikel IV de ekonomiska följderna av olika sensorbaserade strategier för hantering av mastit, för att visa vilken strategi som tenderar att minska kostnaderna för akut och kronisk mastit mest. Mer specifikt uppskattades de ekonomiska konsekvenserna av kronisk mastit för att påvisa den direkta effekten av misslyckad hantering på mjölkgårdens ekonomi.

Den här avhandlingen visar att det är möjligt att med hjälp av sensorer ge beslutsstöd för hantering av kor med kronisk mastit, och den utgör grunden för ett sådant beslutsstödsystem. Detta beslutsstödsystem skulle kunna kan tala om för lantbrukaren vilka mastitfall som är kroniska, vilka fall som sannolikt kommer att bli kroniska, vilka fall som är förknippade med stora förluster i mjölkproduktionen och vilka ekonomiska konsekvenser detta får.

Keywords: Mastit, juverinflammation, automatiskt mjölkningssystem, ko, sensor, kronisk, hantering, progression, mjölk, produktionsförlust

Author’s address: Johan Hendrikus Bonestroo, Swedish University of Agricultural Sciences/Wageningen University and Research, Department of Clinical Sciences/Business Economics group, Uppsala/Wageningen, Sweden/The Netherlands

Dedication

This thesis is dedicated to:

Oma

I miss you, and your constant support in my life-changing decisions continues to empower me to this day.

Life can only be understood backwards; but it must be lived forwards

Søren Kierkegaard

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Bonestroo, J., van der Voort, M., Fall, N., Hogeveen, H., Emanuelson, U., & Klaas, I. C. (2021). Progression of different udder inflammation indicators and their episode length after onset of inflammation using automatic milking system sensor data. Journal of Dairy Science, 104(3), 3458-3473.

II. Bonestroo, J., van der Voort, M., Fall, N., Emanuelson, U., Klaas, I. C., & Hogeveen, H. (2022). Estimating the nonlinear association of online somatic cell count, lactate dehydrogenase, and electrical conductivity with milk yield. Journal of Dairy Science, 105(4), 3518-3529.

III. Bonestroo, J., van der Voort, M., Hogeveen, H., Emanuelson, U., Klaas, I. C., & Fall, N. Forecasting chronic mastitis of an individual cow using automatic milking system sensor data and gradient-boosting classifiers. Accepted in Computers and Electronics in Agriculture.

IV. Bonestroo, J., Fall, N., Hogeveen, H., Emanuelson, U., Klaas, I. C., & van der Voort, M. The costs of chronic mastitis: a simulation study of an automatic milking system farm. Submitted.

The contribution of Johan Hendrikus Bonestroo to the papers included in this thesis was as follows:

I. Involved in formulating research ideas, performed all data processing and analysis, drafted the manuscript, revised the manuscript together with regular feedback from co-authors, and corresponded with the journal.

II. Involved in formulating research ideas, performed all data processing and analysis, drafted the manuscript, revised manuscript together with regular feedback from co-authors, and corresponded with the journal.

III. Involved in formulating research ideas, performed all data processing and analysis, drafted the manuscript, revised manuscript together with regular feedback from co-authors, and corresponded with the journal.

IV. Involved in formulating research ideas, performed all data processing and analysis, drafted the manuscript, and revised manuscript together with regular feedback from co-authors.

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Abbreviations

AMS	Automatic milking system
AUC	Area under the curve
CM	Clinical mastitis
DHI	Dairy herd improvement association
DIM	Days in milk
EC	Electrical conductivity
IMI	Intramammary infection
LDH	Lactate dehydrogenase
MCC	Matthew's correlation coefficient
NAS	Non-aureus staphylococci
OCC	Online cell count
SCC	Somatic cell count
SCM	Subclinical mastitis
SOP	Standard operational procedure

1. Introduction

1.1 Automatic milking systems

Traditionally, cows were milked by hand and later by milking machines requiring manual farmer labor and supervision. However, the workflow changed with the commercial introduction of automatic milking systems (AMS) in 1992 (De Koning, 2010). In an AMS, the cow can be milked completely unsupervised by the farmer, and the cow could determine when she wants to enter the milking robot. When the cow enters the AMS, the teat cups are attached to the teats. The teats of the cow's udder are typically cleaned, and milk ejection is stimulated. The milk begins to flow, and the cow is milked. After the milk flow stops or is close to stopping (i.e., the milk flow reaches a lower limit), the milking is stopped, the milking cups are detached, and the cow is free to go.

Due to the lesser need for human labor, the farmer is no longer present during the milking. This absence may have worsened the detection rate of cow diseases (e.g., mastitis) or other events (e.g., a cow in heat or the start of ovulation). This worsening of the detection rate may explain the initial deteriorating health status of cows after adopting an AMS (Klungel et al., 2000; van den Borne et al., 2021). Different sensors have been developed to detect these diseases or health events without human involvement. Typically, multiple sensors are connected to the AMS to analyze milk and cow behavior. Apart from the mastitis-related sensors, sensors can also be related to fertility, activity, or other events.

When not milking the cow personally, the farmers became dependent on the sensors to give them insight into the cow's health to manage the cow. The farmer should be able to apply sensor-based management on the cows (i.e., using sensor data and information) to make decisions for the cow's health. Mastitis is one of the most important cow health disorders in terms of prevalence and economic cost on dairy farms (Hogeveen et al., 2019). Therefore, mastitis warrants the development of a separate sensor-based management system.

1.2 Mastitis and intramammary infection

Mastitis is an inflammation of one or multiple quarters of a cow's udder (International Dairy Federation, 2011), most often caused by pathogens, which invade through the teat canal, causing an intramammary infection (IMI). Several aspects can characterize mastitis. These aspects of mastitis are discussed below.

1.2.1 Mastitis by clinical signs

Among other classifications, mastitis can be either clinical or subclinical. Clinical mastitis (CM) is defined using visual signs, such as abnormality of milk (i.e., clots or blood in the milk) and a warm or swollen udder (International Dairy Federation, 2011). Various levels are defined for CM. Mild CM cases only have abnormal milk, but the overall condition of the cow is not affected. However, more severe CM is characterized by swelling, redness, increased warmth of the affected udder, and a compromised general condition of the cow (e.g., fever, dehydration, or depression) (International Dairy Federation, 2011; Pinzón-Sánchez and Ruegg, 2011).

Contrary to CM, subclinical mastitis (SCM) is defined using an increase in the inflammatory marker Somatic Cell Count (SCC) without visual symptoms (International Dairy Federation, 2011, 2013). As SCM is not directly observable with the human eye, it can be hard to estimate the severity. The SCC is also used to measure milk quality (International Dairy Federation, 2013). Traditionally, SCC can be measured by a Dairy Herd Improvement Association (DHI) program, where milk samples of cows on participating herds are taken at a monthly frequency and analyzed in a laboratory. Generally, a threshold between 100,000 and 200,000 cells/ml is

recommended to identify cows with SCM (Smith et al., 2001; International Dairy Federation, 2013).

1.2.2 Mastitis by pathogens and transmission modes

To specify the cause of mastitis, bacteriological analysis is used to determine the invading pathogen on species and strain level, to assess prevention or treatment possibilities. Mastitis-causing pathogens are, in most cases, bacteria (Taponen et al., 2017) (e.g., *Staphylococcus aureus*). However, it can also be caused by fungi (Zhou et al., 2013) (e.g., yeasts) or algae (Pieper et al., 2012) (e.g., *Prototheca*). Commonly, mastitis is defined as either contagious or environmental mastitis based on the invading pathogen (International Dairy Federation, 2011). Examples of contagious pathogens are *Staphylococcus aureus* and *Streptococcus agalactiae*. These pathogens can be transferred from living beings to living beings via physical contact or milk. Other pathogens mainly infect cows from the environment (Klaas and Zadoks, 2018). Examples include *Escherichia coli* and *Streptococcus uberis*. However, the distinction between contagious and environmental mastitis is currently being disputed. Some environmental pathogens are shown to be transmitted from cow to cow, and some contagious pathogens can be found in feces (Klaas and Zadoks, 2018).

1.2.3 Mastitis by impact

Mastitis can impact cows in terms of decreased animal welfare (Siivonen et al., 2011), decreased milk production (Hagnestam-Nielsen et al., 2009; Gonçalves et al., 2018b), changed milk composition (e.g., in fat or protein level), (Dos Reis et al., 2013), and increased SCC (De Haas et al., 2004; Dohoo et al., 2011). Mastitis is also being classified as caused by major or minor pathogens (Harmon, 1994). These pathogens are classified by the physical and economic damage they can cause when they infect the mammary gland (Harmon, 1994). Major pathogens would include *Staphylococcus aureus*, Streptococci, enterococci of environmental origin, *Escherichia coli*, and *Klebsiella spp.,* among others (Harmon, 1994). Minor pathogens would include Non-aureus staphylococci (NAS) and *Corynebacterium bovis* (Harmon, 1994).

Besides the impact on cows, mastitis substantially contributes to antibiotic usage on dairy farms in Denmark, Sweden, and the Netherlands (Kuipers et al., 2016; Høg et al., 2019; Växa Sverige, 2020). Veterinary

overuse of antibiotics is linked to antimicrobial resistance, posing a public health risk to society (Speksnijder et al., 2015). As such, a more specific antibiotic treatment protocol for mastitis cases could reduce the usage of antibiotics and could contribute to limiting antimicrobial resistance in society at large.

1.2.4 Mastitis by temporality

When mastitis occurs, the cow can recover, or the cow can remain infected. A cow can obtain acute mastitis where clinical signs are immediately visible (International Dairy Federation, 2011). However, mastitis can also be classified as chronic when the episode continues for an extended period (International Dairy Federation, 2011) which can be clinical and subclinical. Chronic mastitis can increase the milk yield losses relative to mastitis caused by a new infection (Hadrich et al., 2018). However, these milk yield losses may not be significant for cows with chronic SCM caused by minor pathogens (Gonçalves et al., 2020). Moreover, chronic mastitis can cause continuing transmission of pathogens to other cows in the herd (Zadoks et al., 2003) and CM episodes in the future (Swinkels et al., 2005; Steeneveld et al., 2007). More specific standardized definitions of chronic mastitis are lacking. When chronic mastitis is studied, it is commonly defined as having an elevated SCC for the past two to four samplings (St. Rose et al., 2003; Hiitiö et al., 2017) using monthly or bimonthly samples. A more specific definition of chronic mastitis will be needed to study chronic mastitis in detail in the future.

1.3 Management of mastitis

Management of mastitis can be split into preventive management and curative management. Preventive management aims to avoid new mastitis cases with preventive measures (e.g., as studied in Dufour et al. (2011)). Preventive measures can take the form of adequate and frequent cleaning of the milking equipment, using milking gloves, and using teat disinfectant, among other measures (Dufour et al., 2011). Curative management is focused on reducing the impact and duration of ongoing infections using interventions on affected individual cows (as modeled by Steeneveld et al. (2011)). This thesis focuses on the curative management of mastitis.

After mastitis is detected, farmers have six curative management options: (I) further diagnosis to support an intervention decision, (II) treatment with antibiotics, (III) alternative interventions (e.g., increased milking frequency or the use of painkillers), (IV) doing nothing, (V) early dry-off (ending the lactation cycle early), or (VI) culling the affected animal. The selection of animals for treatment or non-treatment is usually based on factors influencing the cure rate. Such factors can include parity, number of quarters infected, the position of the quarter, SCC, mastitis history, duration of infection, pathogen type, and number of colony-forming units (Barkema et al., 2006; Degen et al., 2015; Schmenger and Krömker, 2020). Also, the severity of the symptoms, the general state of the cow, and the state of the herd play a vital role in the intervention decision (Vaarst et al., 2002). In addition, the potential consequences of the intervention decision play a role in the decision on how to intervene, including the expected level of animal welfare after treatment, recovered milk production, and the cost of veterinary treatment (Vaarst et al., 2002; Heikkilä et al., 2012). In summary, cow selection for interventions on mastitis is a complex decision that should be based on various factors.

In mastitis decision-making, farmers can use sensor data to acquire valuable information to improve intervention decisions. Figure 1 describes the Data-Information-Decision mastitis framework (a combination of the frameworks by Rutten et al. (2013) and Kristensen et al. (2016)). The framework is used to structure the theoretical background and the Discussion of the thesis surrounding sensor-based mastitis management. The framework shows how data leads to information, and information can be applied to make and improve farmer decisions. It explains the relationships between Data, Information, and the Decision. In the framework, Data (bottom left pillar) consists of a collection of raw facts (Kristensen et al., 2016), and Information (middle left pillar) is defined as Data processed in a structured manner to offer practicable insight as a basis for decision-making (Kristensen et al., 2016). A Decision (top left pillar) is confined to be a mastitis-related intervention decision in this thesis (e.g., lactational treatment, culling, drying off, or isolating a cow from the herd), which could be based on Information from the cow as well as the herd context (Rutten et al., 2013). The value of Data to Information is dependent on the accurateness and the relevance of the Data to the Information (which can be assessed using variable importance measures in machine learning models, see, e.g., Anglart et al. (2020) and

Naqvi et al. (2022)). The value of Information to a Decision (e.g., a mastitis treatment following a mastitis detection by a mastitis detection algorithm) depends on the accuracy and relevance of the Information to the Decision (Rutten et al., 2013; Rothery et al., 2020).

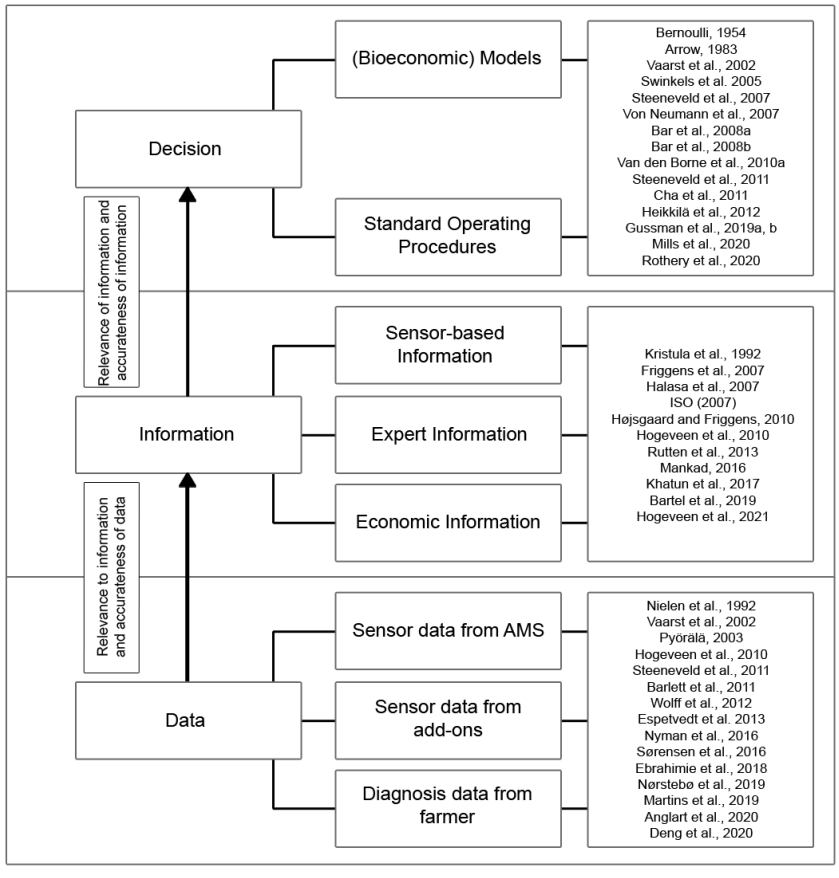

Figure 1. The Data-Information-Decision framework applied to decision-making on mastitis (combination of frameworks by Rutten et al. (2013) and Kristensen et al. (2016)).

1.3.1 Mastitis data

At present, AMS contains sensors to track the indicators to determine the status of the cow and milk. In AMS, sensors can continuously measure disease symptoms and milk composition to detect abnormal milk and signs of mastitis. Pyörälä (2003) and Martins et al. (2019) state the availability of

the following inflammation indicators that are commercially available in AMS to analyze the milk: SCC, color, enzymes in the milk (e.g., N-acetyl-β-D-glucosaminidase or lactate dehydrogenase (LDH)), and electrical conductivity (EC), among others. SCC is a traditional and widely used indicator in mastitis detection. SCC is the measure of cells per milliliter of milk (International Dairy Federation, 2011). Recently, several technologies have been used to estimate SCC within AMS or using AMS add-ons. The accuracy of the sensors has been tested with differing results, where it was shown that cell counters could assess SCC moderately to very well depending on the technology used (Sørensen et al., 2016; Nørstebø et al., 2019; Deng et al., 2020). A color sensor is mainly used to screen the whiteness in the milk (i.e., to detect the presence of blood) and must be used in combination with other sensors as it is not sufficiently relevant on its own (Hogeveen et al., 2010). LDH has been proven to have some ability to detect IMIs causing mastitis, although less so than SCC (Nyman et al., 2016). EC has long been used as an indicator of CM and SCM (Nielen et al., 1992; Hogeveen et al., 2010; Anglart et al., 2020), although EC tends to perform worse than SCC in detecting SCM (Ebrahimie et al., 2018).

Diagnostic data from the farmer and veterinarian is typically used in mastitis information creation and decision-making. The farmer will primarily report the disease data in the management system, including a pathogen diagnosis. This diagnostic data would typically include whether a cow had CM on a given date, possibly with the invading pathogen detected using on-farm or laboratory pathogen analysis (e.g., bacteriological culturing or polymerase chain reaction analysis). However, this data can be problematic due to its possible inconsistency in reporting when used in statistical analyses. More specifically, farmers may miss CM cases or have different thresholds to report them (Vaarst et al., 2002; Espetvedt et al., 2013), leading to underreporting. Other authors have also reported the underreporting of CM cases (Bartlett et al., 2001; Wolff et al., 2012). This underreporting could lead to erroneous data and potentially biased statistical inferences.

1.3.2 Mastitis information

In this thesis, mastitis information could be any information that could be of relevance to mastitis intervention decisions (e.g., knowing if a case is likely to recover in a culling decision). It can be sensor or non-sensor-based (e.g., from experts). However, current sensor-based mastitis research almost solely

consists of mastitis detection algorithm studies, while mastitis information can also help in other mastitis-related decisions. These detection algorithms use data to alarm the farmer on mostly CM for individual cows. Multiple methodologies to detect CM have been tried since 1990, but the accuracy levels are not consistently above the desired level of 99% specificity and at least 80% sensitivity (Hogeveen et al., 2010, 2021; Khatun et al., 2017). Recently, requirements for different accuracy levels for various levels of CM severity have been recommended. It can be expected that more severe cases are more straightforward to detect due to clearer increases in mastitis indicators (Hogeveen et al., 2021), and therefore, higher sensitivity can be expected. An 80% sensitivity and a 99% specificity have been recommended for mild cases. These mild cases would not require immediate detection, while severe cases would need a sensitivity close to 100% and a specificity of 99% within twelve hours (Hogeveen et al., 2021). To express the severity of a mastitis case, researchers have estimated a continuous value of the "degree of mastitis" that is not a dichotomous quantity (Friggens et al., 2007; Højsgaard and Friggens, 2010). In this case, the mastitis value of zero indicates a healthy state, and one indicates severe CM. This "degree of mastitis" approach does not explicitly distinguish between subclinical or clinical cases. The general idea of the approach is to create a continuous mastitis risk variable by using patterns of a combination of mastitis indicator variables. Without setting explicit thresholds on what is and is not mastitis, these values would allow the farmer to derive a list of a predefined number of cows that require attention the most (i.e., are most severely affected). Based on this degree of mastitis, the alert list could provide time-constrained farmers with valuable information in selecting cows to check for CM.

Mastitis information can also be non-sensor-based when it takes the form of expert information. The data of past experiences and knowledge have been processed by human beings who can supply information based on these experiences (Rutten et al., 2013). Expert information from farmers (e.g., visual inspections of the cow's health), herd advisors, other farmers, or veterinarians can also be used to make mastitis decisions. It is essential to mention that farmers may have also developed their own system to transform sensor data into information, albeit unstructured. The transformation from data to information will be less systematic in this case than in mathematical algorithms due to cognitive biases (Mankad, 2016). However, farmer expert information will still be needed in sensor-based mastitis management. This

need is highlighted as the CM detection algorithms proposed in the literature do not consistently achieve the required performance of AMS as stipulated by ISO (2007) (Hogeveen et al., 2010; Khatun et al., 2017).

Another source of information used in mastitis management is economic information (Rutten et al., 2013). Economic information of mastitis in this thesis is defined as the costs (expressed in monetary units) of the consequences of mastitis. More specifically, economic information can be obtained by combining the value of a specific consequence (often represented by a price level, e.g., milk price) with the data on the negative consequence (e.g., production losses in milk yields due to mastitis). These costs can be calculated for a range of consequences, including milk yield production loss, discarded milk, drugs, diagnostics, veterinary services, and labor (Halasa et al., 2007). In decision-making, these costs could help farmers in their mastitis management decisions by evaluating the expected monetary value of intervening versus not intervening (e.g., applying antibiotic treatment, culling, or dry cow treatment). This value of intervening is especially relevant in cases of chronic mastitis, as the expected monetary values of interventions will change dynamically during a chronic episode.

As stated before, research on sensor-based mastitis information that is not focused on mastitis detection is far less common. Nevertheless, mastitis decision-making does not solely consist of detecting cases and treating them. Farmers may also like to evaluate and forecast the progression of an ongoing mastitis case and gain insight into its consequences or impact (e.g., on milk production or in terms of economic costs) to make intervention decisions. These decisions lack sensor-based mastitis management tools. To the author's knowledge, chronic mastitis management applications based on sensors are not explored in the literature. While work on chronic mastitis prediction models has been performed using non-sensor data (Kristula et al., 1992; Bartel et al., 2019), no sensor-based solutions have been proposed to forecast chronic mastitis. Monitoring and forecasting chronic mastitis would allow farmers to intervene earlier when it becomes clear that it is unlikely that a cow will recover. It would allow the initiation of targeted treatment, culling, and drying-off strategies to decrease chronic mastitis. Sensor systems offer opportunities to improve the management of chronic mastitis, but there is a lack of knowledge to implement such a system. These gaps in knowledge would include knowledge on the definition of chronic mastitis,

algorithms to forecast chronic mastitis, and the economic importance of chronic mastitis.

1.3.3 Mastitis decision

Central in sensor-based mastitis intervention management is deciding what to do with a cow, given the available information derived from AMS sensors combined with other available information. The mastitis intervention decision is intricately linked with decision theory and expected utility theory in economics. In an expected-utility framework, the farmer, as a rational agent, is assumed to maximize the expected utility in a choice under uncertainty (Von Neumann et al., 2007). The utility can be derived from farm profit, but possibly also from alternative ends, e.g., reducing antibiotics usage, or improving animal welfare of the animals on-farm, as indicated in farmer interviews (Vaarst et al., 2002). The expected utility of a decision is based on the summed value of all future cow states multiplied by the probability of obtaining those states (Bernoulli, 1954). Sensor-based information can often be used to make informed intervention decisions. Theoretically, the probability distribution of future states conditional on current information might differ from the unconditional probability distribution (Arrow, 1973). The value of this sensor information for decision-making lies in how much the information changes the probability distribution of future cow states relative to the distribution without that information. In other words, the value depends on how much the information changes the uncertainty and expected value of future states. This observation can also be tied to the Value of Information framework. In this framework, the value of information for a specific decision is the difference in expected utility (e.g., profit) between a situation with and without information (Rothery et al., 2020).

Several studies were published using this theoretical background that use bioeconomic models to determine the economically optimal decision for different scenarios. These studies narrow down the expected utility to utility derived from minimizing the economic cost of mastitis. For instance, a dynamic programming approach has been used to optimize management decisions concerning the economic cost of CM (Bar et al., 2008a; b; Cha et al., 2011; Heikkilä et al., 2012). Another technique is Monte Carlo simulation, in which different scenarios can be modeled and the outcome distributions are compared (Van den Borne et al., 2010a; Steeneveld et al.,

2011; Gussmann et al., 2019a; b). Both Monte Carlo simulation and dynamic programming allow these different authors to essentially simulate or optimize different sets of candidate Standard Operating Procedures (SOPs). In this thesis, these SOPs are defined as standardized step-by-step instructions (Mills et al., 2020) performed when a mastitis event is encountered. For instance, when severe CM is detected and confirmed, the SOP describes what action to take (e.g., which medication to give in terms of the type of treatment).

Bioeconomic models in mastitis research primarily do not account for sensor data. In practice, farmers can use sensor data (e.g., EC or SCC) and information (e.g., an alert of a CM detection algorithm) in decision-making. As stated before, sensor data and information can be used for more than solely case detection. For instance, one could potentially determine whether the cow has chronic mastitis (sensor-based information) using sensor data and cull the affected animal based on that information (decision) and avoid possible transmission of pathogens. It would allow users to determine whether the decreased cost of chronic cows would be more than the extra cost of culling. Moreover, better intervention selection would increase animal welfare and recovered milk production (Heikkilä et al., 2012).

It is also important to realize that assessing the cost of a failed recovery or chronic mastitis is important for mastitis decision-making. For chronic mastitis, Steeneveld et al. (2007) showed that antibiotic treatment of chronic mastitis caused by *Streptococcus uberis* was unprofitable, while Swinkels et al. (2005) showed that a 3-day treatment of chronic mastitis caused by *Streptococcus spp.* was profitable. However, these studies did not assess the costs of chronic mastitis at the herd level that controlled for herd dynamics but assessed the benefit of treatment on chronic cases on a cow-by-cow basis for specific pathogens. To the author's knowledge, the specific costs of chronic mastitis at the herd level have not been investigated yet. This estimation would be needed to assess the overall value of chronic mastitis management and information.

2. Objective and aims

The overall objective for this PhD project was to explore the potential applications for a decision support system that includes the course and consequences of chronic mastitis. More specifically,

- Describe the dynamics of sensor data after the first sign of inflammation within a lactation with a focus on the duration of udder inflammation (paper I);

- Estimate the associations of different sensor-related inflammation indicators with milk yield (paper II);

- Develop a sensor-based prediction model that forecasts the future subclinical chronic mastitis status based on past sensor data. (paper III);

- Estimate the economic effects of chronic mastitis on an AMS farm and estimate the economic effects of different sensor-based strategy scenarios on the cost of chronic mastitis (paper IV).

3. Materials and methods

This chapter will provide an overview of the materials and methods used in the thesis, while complete information is found in the respective papers.

3.1 Available data

Table 1 shows an overview of the data used in this thesis. In general, the herds of the study were selected based on the presence of an AMS (VMS series, DeLaval International AB, Tumba, Sweden) and an Online Cell Counter (OCC) (DeLaval International AB, Tumba, Sweden) to measure SCC. In some herds, LDH was also measured using the Herd Navigator (DeLaval International AB, Tumba, Sweden). Data were retrieved from a database of DeLaval International AB. The data was recorded "per milking." The data was retrieved from Western Europe and North America. Furthermore, the study periods were from 2016 to 2019 or 2017 to 2020. Additionally, some variables in the dataset were required for the different papers (e.g., milk diversion or LDH), and henceforth each paper used separate datasets. For Paper I and III, the herds were selected based on whether documentation was available on whether milk from individual cows was diverted from the bulk milk tank to proxy antibiotic treatment. Paper II used a dataset with herds that had measured LDH to study its association with milk yield. These two sets of general requirements formed datasets: dataset A and dataset B. Both datasets consisted of different herds but were not mutually exclusive. Paper IV used the results of Paper I and II and included input from literature and the author's expertise.

Table 1. Overview of data as used in the thesis, including datasets, number of herds, countries, study periods, variables of interest, and selection criteria to determine the dataset

Paper	**I**	**II**	**III**	**IV**
Dataset	Dataset A	Dataset B	Dataset A	Results Paper I and II, author's expertise, and literature sources
Number of herds after preprocessing	15	21	14 (removed 1) [1]	NA
Countries	Belgium, Canada, Germany, the Netherlands, Scotland, and Sweden	Canada, The Netherlands, Finland, and Sweden	Belgium, Canada, Germany, the Netherlands, Scotland, and Sweden	NA
Study period	2016-2019	2017-2020	2016-2019	NA
Variables of interest	EC SCC Milk diversion Days in milk (DIM) Parity	Milk yield SCC EC LDH DIM Parity	A range of variables[2]	SCC Milk yield, Pregnancy DIM Parity
Selection criteria for dataset (A or B)	Presence of milk diversion	Potential presence of LDH	Presence of milk diversion	NA

[1] One herd sampled SCC at a substantially lower rate (once every five days on average) than the other herds and was henceforth removed.

[2] milk yield, milk production speed, standard deviation of quarter ECs, interquarter ratio of quarter ECs, time interval between milkings, blood presence, SCC, DIM, milk diversion, quarter ECs, and parity.

3.2 Data pre-processing

3.2.1 Analyzing the dynamics of sensor data

EC of the milking quarters was used to calculate σ-Conductivity, defined as the standard deviation of the quarter EC within the cow over the total milk produced at each milking. The natural logarithm transformation was applied to σ-Conductivity and SCC to obtain homoscedastic and normally distributed residuals in the statistical analyses. Milking level observations of SCC, σ-Conductivity, and the diverted milk indicator were aggregated to a daily level by taking the maximum of these values on a given day.

The start of a mastitis episode during lactation was defined as the first observation within lactation of an increased SCC, as measured by the OCC higher or equal to 200,000 cells/ml. This start of the mastitis episode was defined as "the initial inflammation" in this study. To counter the possibility of a false-positive initial inflammation detection, the initial inflammation needed to be combined with one or more SCC measurements higher or equal to 200,000 cells/ml. The data used for the analyses included data from four weeks prior to the initial inflammation until twelve weeks after the initial inflammation. This period was defined as the mastitis episode sequence.

Because treatment records were not available from all herds, we used milk diversion from the bulk tank as an approximation of a farmer intervention related to a mastitis episode (Bonestroo et al., 2020, 2021a). As an indication of a farmer intervention in case of mastitis, milk diversion was defined as diversion of milk for at least two consecutive days within the ten days after the initial inflammation.

Recovery from a mastitis episode for an individual cow was defined as having a rolling mean SCC lower than 200,000 (Smith et al., 2001; International Dairy Federation, 2013) for ten consecutive days within the twelve weeks after the initial inflammation in the episode sequence.

The dataset was split into four subsets of cows 1) no diverted milk – no recovery, 2) diverted milk – no recovery, 3) no diverted milk - recovery, and 4) diverted milk – recovery.

3.2.2 Estimating the associations between mastitis indicators and milk yield

A set of variables was created to facilitate statistical analysis. We used milk synthesis rate (kg/hour) as the dependent variable. Each interval between milkings is different in AMS farms, leading to a large variation in time intervals between milkings. (Hogeveen et al., 2001). Therefore, to obtain a comparable milk yield-based measure, we divided the milk yield (in kg per milking) by the time interval between milkings (in hours) to obtain milk synthesis rate. We used online SCC and LDH as independent variables. These two variables were transformed using the natural logarithm (LnSCC and LnLDH). Furthermore, we used the Mean EC of the four quarters as the third independent variable (Mean EC). Mean EC was chosen to compare the milk production loss results to LDH and SCC as it was a cow-level indicator, similar to SCC and LDH. In addition, the subgroup variable "chronicity status" was created to represent whether the cow was chronic or not. A milking day observation was labeled as chronic if a cow had weekly SCC geometric averages equal to or higher than 200,000 cells/ml for a period of four consecutive weeks or more before the current milking day (Bonestroo et al., 2021b) based on available SCC samples. Lastly, we also created a cow lactation variable (*CowLactation*) that combined the unique animal identification number with the parity to identify unique cow lactations.

We aggregated the multiple individual milkings on a given day by using the maximum daily values of LnSCC, LnLDH, Mean EC, and averaged the milk synthesis rate. The daily maximum value was used to capture the severity of the increase. When some values were missing for specific milkings but not for all milkings on specific days, these values were ignored in determining the maximum. When there was no observation of the mastitis indicator at all during a day, no daily maximum value of that day was given. As not all mastitis indicators were always reported, these three datasets differed in the number of observations.

Three subgroups were created and analyzed separately to analyze the association between milk synthesis rate and mastitis indicators for various levels of parity, DIM, and chronicity. The first subgroup was formed according to three DIM levels (5-28, 29-60, and 61-305 DIM). These cut-offs were determined by selecting the median DIM where the day-to-day change in milk synthesis rate was maximal (28 DIM) and where the milk synthesis rate peaked (60 DIM) in our dataset. The second subgroup was

based on parity (first lactation, second lactation, and third or more lactation). The last subgroup was formed according to chronicity (non-chronic and chronic mastitis). The differences in milk synthesis loss in the various parity levels, stages of lactation, and chronicity groups were studied separately using separate regression models.

3.2.3 Predicting mastitis chronicity

The data (e.g., milk yield or interquarter ratio of conductivity) from each milking per day was aggregated to a daily frequency using the mean, minimum, maximum, and standard deviation. After the aggregation to a daily frequency, the daily mean, maximum, and standard deviation of quarter-level conductivity values (e.g., daily mean conductivity of the left-rear quarter) were aggregated to cow-level variables. This aggregation was performed by calculating the mean over daily mean quarter conductivity values and the maximum over daily maximum quarter conductivity values. In addition, we also calculated the standard deviation over daily standard deviations of quarter conductivity values and the standard deviation over daily maximum quarter conductivity values. All variables had to be on cow level as we forecast chronic mastitis on cow level. The remaining quarter-level conductivity variables were not included as input in the forecasting models as they were not reported on cow-level.

A prediction day (i.e., a day on which a prediction of a future state was made) was defined as a day in the lactation with at least a mean SCC higher than or equal to 200,000 cells/ml (International Dairy Federation, 2013) or having an SCC of such a level on one of the four days prior to the day. It is essential to mention that one mastitis case can have multiple prediction days as each day of the episode, a forecast is performed. It would allow the farmer to monitor and forecast during an ongoing episode. For each day on which the future chronic mastitis status was forecasted, we used the data 30 days before the prediction day as input. (i.e., the day on which the forecast was made). The forecasting method could use the feature values of each day during the last 30 days (e.g., MaxIQRConductivity on the 16th day before the prediction day). Moreover, to derive the future chronic mastitis status for each prediction day, 50 days of data after the prediction day were needed (Figure 2). Consequently, each day during lactation with 30 preceding and 50 successive days of data could be a prediction day, given that it had a recent increase in SCC.

Filtering was used to determine a structural decrease in SCC below 200,000 SCC/ml. The future chronic mastitis status on a prediction day was labeled as not chronic if the rolling 20-day mean SCC decreased below 200,000 SCC/ml (0= not chronic mastitis) at least once in the period from the prediction day to 50 days post the prediction day. It was labeled chronic if no structural decrease occurred (1=chronic mastitis). In other words, the label indicates whether the cow would recover (=0) or turn chronic (=1). Suppose a cow had an increase of SCC after a structural decrease in SCC; the cow would be regarded as not chronic (the third example in Figure 2). In these cases, it was impossible to determine whether the new increase in SCC was part of the initial episode or was the start of a new episode based solely on SCC.

To create a training and a validation dataset, we randomly divided the herds in our dataset. Half of the herds were selected for the training set, and the other half entered the validation set. Validation herds were identified as herds 1 until 7, while herds 8 until 14 were designated as training herds. The data from all the training herds were used to fit a prediction model all at once (i.e., the model was trained once using data from all training herds), and data from the validation herds were used to test the model's performance.

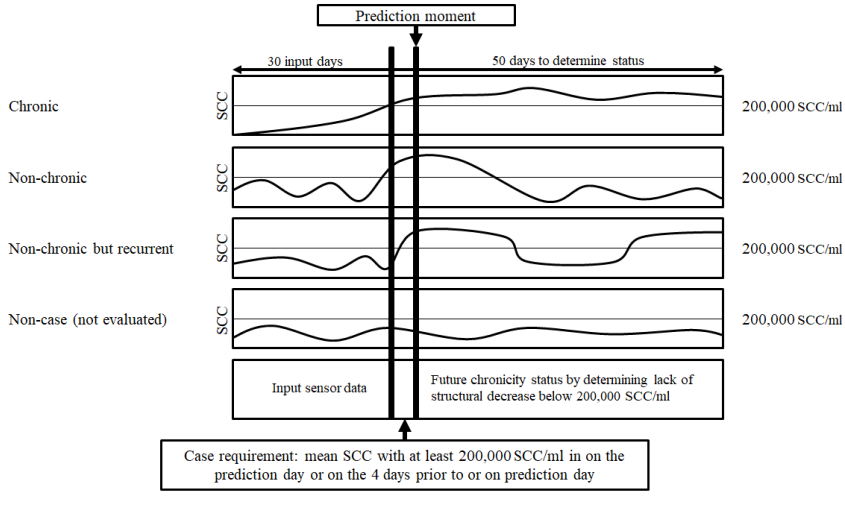

Figure 2. Examples of the prediction task that was performed by the chronicity forecasting model where the label contains the definition of future chronicity.

3.3 Data analysis

3.3.1 Analyzing the dynamics of sensor data

The effects of predictor variables on SCC and σ-Conductivity were analyzed using a multivariable linear mixed model for each subset with *DIM*, *parity,* and *weeks since initial inflammation* as covariates and a random effect of a specific cow lactation (*LactationID)* and a random effect of a specific herd (*HerdID)*. *HerdID* and *LactationID* indicate the identity of the herd and specific cow lactation number for a specific cow (e.g., cow 12 in its second lactation). *Weeks since initial inflammation* was a categorical variable with seventeen levels (once per week from four weeks prior, until twelve weeks after the initial inflammation). *Parity* was a categorical variable coded for primiparous (0) and multiparous cows (1). The models for Y, i.e., SCC or σ-Conductivity, took the following form:

$$Y = Constant + \sum_{i=-4}^{12} week\ since\ alert_i + parity + DIM + random\ intercept\ of\ LactationID\ in\ HerdID + random\ intercept\ of\ HerdID \quad (1)$$

Where i is the week number relative to the week in which the initial inflammation was observed. Estimated marginal means were assessed for the weeks since the initial inflammation while evaluating all other covariates at mean level. Different interactions and quadratic terms were tried, but they had no substantial effect on the estimated marginal means and were therefore left out. Random effects of lactation of a specific cow and herd were included in the models as nested random intercepts (*LactationID* in *HerdID* and *HerdID*), and a first-order autoregressive correlation structure was used. The assumptions of homoscedasticity and normality of residuals were checked using fitted value–residual plots and qq-plots. The linear mixed models were estimated using nlme 3.1-137 (Pinheiro et al., 2019) using Restricted Maximum Likelihood in R 3.5.1 (R Core Team, 2018).

3.3.2 Estimating the associations between mastitis indicators and milk yield

We applied a generalized additive model using the R package mgcv (Wood, 2021) in R 3.6.1 (R Core Team, 2018) to model milk synthesis rate per hour. Milk synthesis rate was estimated as a function of the mastitis indicator and

DIM for each subgroup, respectively (Eq. 2, 3, and 4). *DIM* and *CowLactation* were treated as confounders. Depending on the subgroup that was analyzed, the subgroup value in these equations can take the form of the parity, stage of lactation, or chronicity status. We included a random effect of each cow lactation (*random cow lactation effect*) using the *CowLactation* variable to control for non-independence of observations within cows. Milk synthesis rate was assumed to have a scaled-t distribution rather than a normal Gaussian distribution since it was expected that milk synthesis rate would have more extreme observations than a normal distribution.

$$Milk\ synthesis\ rate = Intercept + Subgroup\ intercept + f_{LnSCC}(LnSCC) * Subgroup + f_{DIM}(DIM) * Subgroup + Random\ cow\ lactation\ effect \quad (2)$$

$$Milk\ synthesis\ rate = Intercept + Subgroup\ intercept + f_{LnLDH}(LnLDH) * Subgroup + f_{DIM}(DIM) * Subgroup + Random\ cow\ lactation\ effect \quad (3)$$

$$Milk\ synthesis\ rate = Intercept + Subgroup\ intercept + f_{Mean\ EC}(Mean\ EC) * Subgroup + f_{DIM}(DIM) * Subgroup + Random\ cow\ lactation\ effect \quad (4)$$

Where f_{DIM} is a non-linear smoothing function modeling the milk synthesis rate over the lactation cycle with a cubic spline basis that was estimated separately for every subgroup. f_{DIM} was not plotted in Results for brevity, but it takes the form similar to a Wood lactation curve found in the literature (Wood, 1967). And where f_{LnSCC}, f_{LnLDH}, and $f_{Mean\ EC}$ are non-linear smoothing functions modelling the association between LnSCC, LnLDH, Mean EC, and milk synthesis rate. To enable the analysis, a baseline was created where the mastitis indicators are not associated with decreases in milk synthesis rate. As such, this study assumed prior to the analysis that a level of 1,000 SCC/ml, 1 U/L LDH, and 4 mS/cm Mean EC would have no effect on milk synthesis rate. These functions are also non-linear smoothing functions with a cubic spline basis. We used the BAM function, which is a generalized additive model with discretization of covariate values that makes it more time and memory efficient when having large datasets (Wood et al., 2017; Wood, 2021). Each of the three models (eq. 2, 3, 4) was estimated separately for each subgroup (parity, stage of lactation, and chronicity). Thus, leading to the fitting of nine models in total (three mastitis indicators by three subgroup variables).

To visualize the associations, we plotted f_{LnSCC}, f_{LnLDH}, and f_{Cond} for each mastitis indicator and each of the subgroups. The value, at which the mastitis indicator started to be negatively associated with milk synthesis rate, was identified as a threshold. This threshold was found by determining the maximum positive milk synthesis rate difference in the partial plot (the highest point) and highlighted in the partial effect plots. After the threshold, further points of potential substantial decreases in milk synthesis rate were described (e.g., whether the line starts to decrease considerably more).

The residuals of all models were checked for normality, homoscedasticity, and autocorrelation using qq-plots fitted values-residual plots and autocorrelation plots. During the analysis, we detected substantial autocorrelations for all models. The autocorrelation problem was solved by adapting the model with an AR1-parameter.

3.3.3 Predicting mastitis chronicity

We used the gradient-boosting trees algorithm as implemented in XGBoost (Chen and Guestrin, 2016) to create a prediction model that forecasts whether the cow would recover (=0) or turn chronic (=1) given that they showed an initial increase in recent daily mean SCC.

The predictive performance of the gradient-boosting trees classifier was compared to that of two default approaches or simple prediction rules: the monthly sampling approach (approach mimicking DHI sampling frequency but using OCC data) and the frequent sampling approach (using all OCC data available). We used sensitivity, specificity, Matthews Correlation Coefficient (MCC), and area under the curve (AUC) to compare the model's forecasting performance with the default approaches. The default approaches are listed below:

- Monthly sampling approach, this approach predicted future chronic mastitis to be present when the SCC was equal to or higher than 200,000 SCC/ml in the evaluation closest to the prediction day and the SCC evaluation furthest away in time in the preceding 30 days relative to the prediction day. The prediction rule predicted chronic mastitis if both SCC samples were higher than 200,000 SCC/ml. The monthly sampling approach mimicked a situation where farmers use monthly SCC data of the previous month and the current month to determine chronic mastitis, common in a non-sensor dairy farm setting.

- Frequent sampling approach, this approach predicted future chronic mastitis when the number of days with 200,000 SCC/ml or higher prior to the prediction day was equal or more than thirteen days in the 30-day input period dataset. This threshold on the number of days was chosen to maximize the sum of sensitivity and specificity to forecast the future chronic mastitis status.

The differences in AUC, MCC, and accuracy between the model predictions and the default approaches were tested using (Welch's) t-tests for unequal variances.

3.4 Simulating the cost of chronicity

We developed and used a stochastic Monte Carlo bioeconomic simulation model to simulate IMI, mastitis, and chronic mastitis. The model also included the consequences of IMI and mastitis in milk production losses and clinical mastitis. In a Monte Carlo simulation, an outcome is simulated dependent on variables that have (random) distributional properties. The model ran a predefined number of times (model iterations), creating different outcomes for every model iteration. The set of outcomes of each model iteration was taken together to form outcome distributions (Dijkhuizen and Morris, 1997; Hogeveen et al., 2019). The outcome distributions were summarized using the 25^{th}, $50^{th,}$ and 75^{th} percentiles.

The model mimicked the daily mastitis situation on a Dutch AMS dairy farm with 100 cow places and with IMIs caused by *Staph. aureus*, *Strep. spp*., Gram-negative bacteria, and non-aureus staphylococci **(NAS)**. The cow-places were simulated every day for seven years, including the burn-in period, for 500 model iterations. This burn-in period was set to two years. Initial experimentation showed that stationarity of ongoing IMI cases occurred around one and a half years, and the number of lactating cows in the herd stabilized in two years. Standard management was implemented regarding antibiotic treatment during lactation and dry-off, and culling. The negative consequences of mastitis were modeled in milk production loss and transmission of contagious bacteria. The negative consequences of mastitis were monetarized by multiplying these consequences with their costs.

The dynamics of IMI in the model were based on literature sources on clinical mastitis incidence rates (Santman-Berends et al., 2015), pathogen populations (Taponen et al., 2017), (contagious) transmissions (Gussmann et

al., 2018; Dalen et al., 2019a), and lactational and dry cow period cure rates (Wilson et al., 1999; Sol et al., 2000; Taponen et al., 2006; Huijps and Hogeveen, 2007; Newton et al., 2008; Halasa et al., 2009; Van den Borne et al., 2010b; Halasa et al., 2010; Fuenzalida and Ruegg, 2019; Swinkels et al., 2021). Simulation of SCC was based on literature sources (Dalen et al., 2019b; Fuenzalida and Ruegg, 2019; Bonestroo et al., 2021b) and SCC data used in Bonestroo et al. (2022). Milk yield simulation was based on an adapted statistical model used by Bonestroo et al. (2022) (see below). Price data was gathered from a range of sources (Lam et al., 2013; Griffioen et al., 2016; Scherpenzeel et al., 2018; Blanken et al., 2019; GD, 2019; Steeneveld et al., 2020).

The model architecture distinguished between a simulated non-transmission IMI case and a modeled transmission IMI case. Both types of cases were used to calculate the incidence rate of IMI, the culling rate, and the incidence rate of clinical mastitis on the farm. The occurrence and the consequences (e.g., actions by the farmer, milk yield losses) of non-transmission cases were directly simulated. Transmission cases were handled differently as the number of such cases was calculated after each model iteration. Transmission cases are defined in this paper as cases that are directly transmitted from infected cows. These transmission cases were determined based on the number of non-transmission cases together with a pathogen-specific transmission rate. This calculation was performed by multiplying the infection days of the non-transmission cases (i.e., days with ongoing infection) of different pathogens with the pathogen-specific transmission rate. The costs of an individual transmission case were considered equal to the average costs of a non-transmission case of the same pathogen in that model iteration.

The simulation model was used to assess the cost of mastitis and chronic mastitis under different sensor-based strategies or SOPs. This assessment was done to analyze the potential value of the SCC sensor in different mastitis decisions, including treating SCM and CM during lactation and the dry cow period and culling. The strategies are outlined in Table 2. The outcomes of each scenario were the total cost of mastitis and chronic mastitis in € per IMI case, the CM and IMI incidence rate in cases per cow year, and the culling rate per cow year.

Table 2. The different sensor-based mastitis strategy scenarios applied in the study.

Strategy	SCC lactational SCM treatment threshold	SCC lactational SCM treatment time threshold	SCC DCT SCM treatment threshold	SCC DCT SCM treatment time threshold	SCC culling treatment threshold	SCC culling treatment time threshold
Default	None	None	150,000	1	200,000	90
Lactational treatment	200,000	14	150,000	1	200,000	90
Dry cow treatment	None	None	150,000	7	200,000	90
Earlier culling	None	None	150,000	1	200,000	45
Earlier culling and more specific dry cow treatment	None	None	150,000	7	200,000	45

4. Results

This chapter will provide an overview of the results used in the thesis, while complete information can be found in the respective papers.

4.1 The dynamics of sensor data

4.1.1 Somatic cell count

Figure 3 shows the estimated marginal means of the SCC from four weeks prior to the initial inflammation to twelve weeks after the initial inflammation. The average levels of SCC increased prior to the initial inflammation in almost all four subsets, apart from the milk diverted – no recovery subset. The average SCC value during the week of the initial inflammation of SCC of the diverted milk subsets was higher than the no diverted milk subsets. At mean level, the diverted milk - recovery subset had above 200,000 SCC/ml (natural logarithm of 200 is 5.298) until approximately one week past the initial inflammation, whereas the no diverted milk - recovery subset was below 200,000 SCC/ml in the week of the initial inflammation (week 0). Moreover, SCC in both diverted milk – recovery and no diverted milk – recovery subsets stabilized approximately three to four weeks after the initial inflammation at a level slightly higher than before the initial inflammation. As expected in the diverted milk - no recovery and the no diverted milk – no recovery subsets, mean SCC remained stable and was on average higher than 200,000 cells/ml after the initial inflammation. This increase persisted throughout the twelve-week time window and was higher than the level before the initial inflammation.

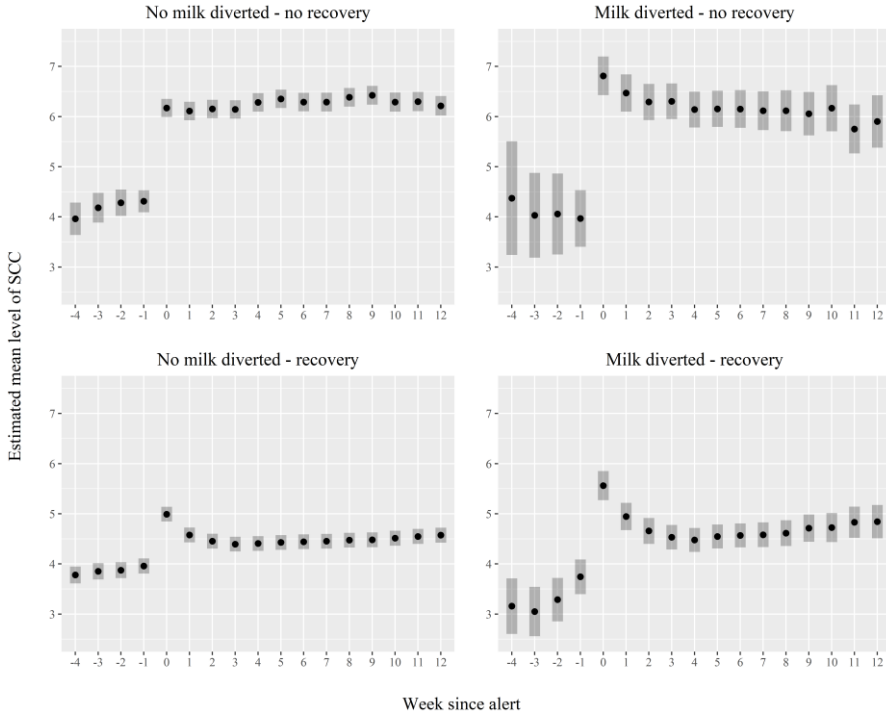

Figure 3. Patterns of SCC measured by online SCC from four weeks before until twelve weeks after the initial inflammation (first time in a lactation where SCC ≥200,000 cells/mL) for four subsets of cows using the estimated marginal effects of linear mixed models with 95% CI of the weekly mean.

4.1.2 Electrical conductivity

Figure 4 shows the estimated marginal means of σ-Conductivity. The average σ-Conductivity increased prior to the initial inflammation in all four subsets. The diverted milk – no recovery subset showed stable σ-Conductivity values above the level before the initial inflammation after the initial inflammation, whereas the diverted milk – recovery subset stabilized in three to four weeks after the initial inflammation but above the estimated level before the initial inflammation. The no diverted milk – recovery subset and the no diverted milk – no recovery did not have a clear increase at the week of the initial inflammation, and it did not have a clear decrease after the week of the initial inflammation. The average σ-Conductivity during the week of the initial inflammation of the diverted milk subsets was higher than the no diverted milk subsets. Interestingly, the recovery pattern of the milk

diverted subsets of σ-Conductivity is similar to the patterns of SCC. However, the relative difference between the peak value at week 0 and the level at which the indicator stabilizes is more negligible.

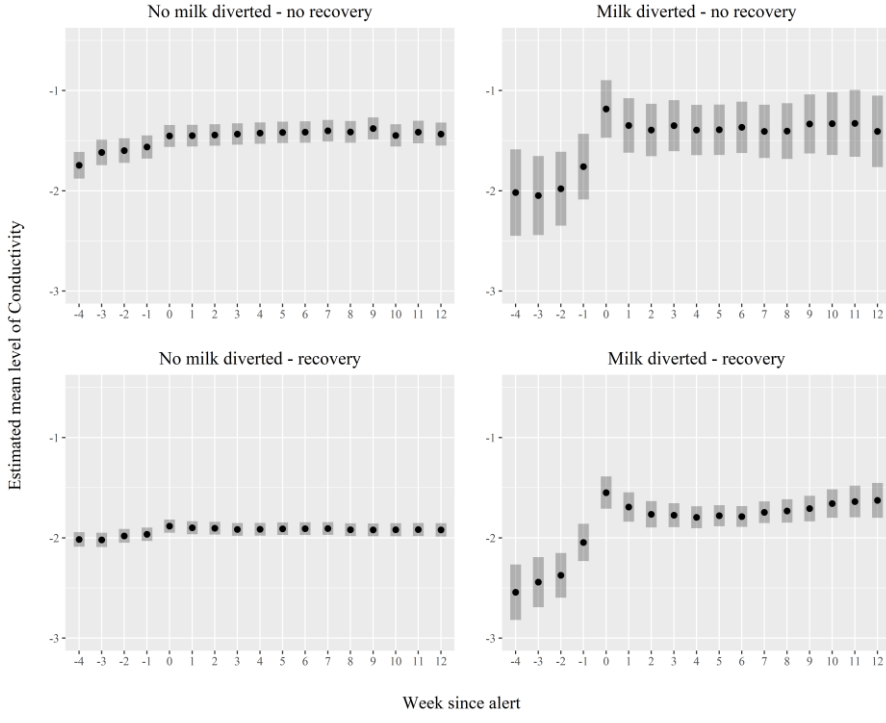

Figure 4. Patterns of σ-Conductivity from four weeks before until twelve weeks after the initial inflammation (first time in a lactation where SCC ≥200,000 cells/mL) for four subsets of cows using the estimated marginal effects of linear mixed models with 95% CI of the weekly mean.

4.2 The associations between mastitis indicators and milk yield

4.2.1 Somatic cell count

Figure 5 provides the visualization of the non-linear association between LnSCC and milk synthesis rate (f_{LnSCC}) and the frequency of LnSCC observations for different parity, stage of lactation, and chronicity classes (Figure 5A, 5B, and 5C). The milk synthesis rate was negatively associated

with LnSCC over a specific threshold. The large dot in Figure 5 marks the point where milk synthesis rate started to decrease, and milk production losses occurred. For most cases, this threshold was approximately 2.5 LnSCC (12,000 SCC/ml) and 3.75 LnSCC (43,000 SCC/ml), while occasional lower and higher thresholds were found in the analysis depending on the analysis of the specific subgroup. Moreover, the milk synthesis rate started to decrease more a second time when LnSCC increased at an increasing speed and non-linearly. This occurred approximately after 5.625 LnSCC/ml (approx. 277,000 SCC/ml) for all subgroups.

Some differences in thresholds and the steepness of the decrease in milk synthesis rate between subgroups were seen. These differences in thresholds were caused by minor differences in the LnSCC and milk synthesis rate association on an overall approximately flat line on the lower levels of LnSCC. Therefore, the differences between thresholds should be interpreted carefully. In Figure 5A, the decrease in milk synthesis rate was steeper at higher parity levels (i.e., older cows showed a steeper decrease in milk synthesis rate). Moreover, the differences in intercept between first parity cows and second parity cows and between first parity cows and third or more parity cows were 0.27, with a standard error of 0.01, and 0.35, with a standard error of 0.01, respectively ($P < 0.01$). Regardless of LnSCC, cows with a higher parity tend to produce significantly more milk. In Figure 5B, no apparent difference in milk synthesis rate in the stage of lactation subgroups could be seen. The difference in intercept between 5-28, 29-60, and 61-305 DIM subgroups was also not significant ($P > 0.05$). In Figure 5C, the chronic subgroups have approximately the same form; the chronic group was steeper in its decrease and was lower than its non-chronic counterpart. The difference in intercepts between the chronic and non-chronic subgroups, indicating long-term effects on milk synthesis rate, were -0.04 with a standard error of 0.01 ($P < 0.01$).

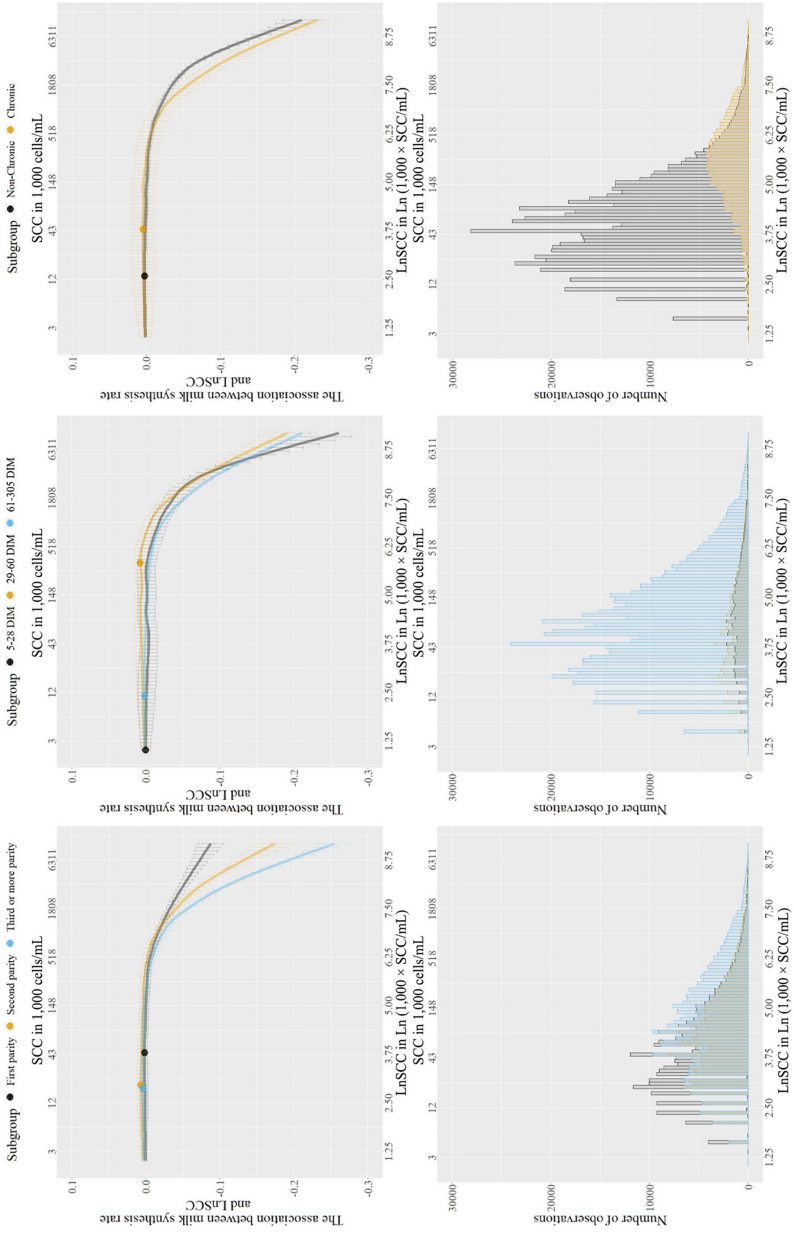

Figure 5. The estimated association between milk synthesis rate and LnSCC and the number of observations for parity, stage of lactation, and chronicity subgroups. The dots indicate that the start of milk synthesis rate decreases and thereby milk production losses increase from that point.

4.2.2 Electrical conductivity

Figure 6 provides a visualization of the non-linear association between Mean EC and milk synthesis rate ($f_{Mean\ EC}$) and the frequency of Mean EC observations for different parity, stage of lactation, and chronicity classes (Figure 6A, 6B, and 6C). Figure 6 indicates that the association between Mean EC and milk synthesis rate was highly nonlinear. In addition, the threshold of milk synthesis rate decrease was within the range of 5.0 to 6.0 mS/cm Mean EC for all subgroups. This threshold was found at a high percentile of the Mean EC distribution compared to LnSCC and LnLDH (see bottom panels in Figures 5 and 7). Mean EC remained negatively associated with milk synthesis rate after 6.0 mS/cm for all parity, stage of lactation, and chronicity subgroups.

Between the subgroups, several differences and similarities could be seen in Figure 6. The differences in thresholds between subgroups were limited as they all fell between 5.0 and 6.0 mS/cm. The differences in the functional forms between subgroups should be interpreted with care as a large section of the decrease in milk synthesis rate was based on a small area of the Mean EC distribution. The limited number of observations explains the increase in milk synthesis rate at 7.5 mS/cm for the second parity subgroup in Figure 6A. In Figure 6A, the milk synthesis rate of the multiparous subgroups decreased more when Mean EC increased than in the first parity subgroups. Furthermore, the differences in intercept between first parity cows and second parity cows and between first parity cows and third or more parity cows were 0.28 with a standard error of 0.01 and 0.36 with a standard error of 0.01 ($P < 0.01$). Regardless of Mean EC, cows with a higher parity significantly produce more milk. In Figure 6B, the milk synthesis rate of the 29-60 and 61-305 DIM subgroups decreased more than the milk synthesis rate of the 5-28 DIM subgroup when Mean EC increased, while the difference in intercepts between the stage of lactation subgroups was not significant ($P > 0.1$). In Figure 6C, the milk synthesis rate of the chronic subgroup decreased more than for the non-chronic subgroup when Mean EC increased. In addition, the chronic mastitis subgroup difference in intercept between chronic cows and non-chronic cows was -0.04 with a standard error of 0.003 ($P < 0.01$). The milk synthesis rate for chronic cows was lower while controlling for the current level of Mean EC.

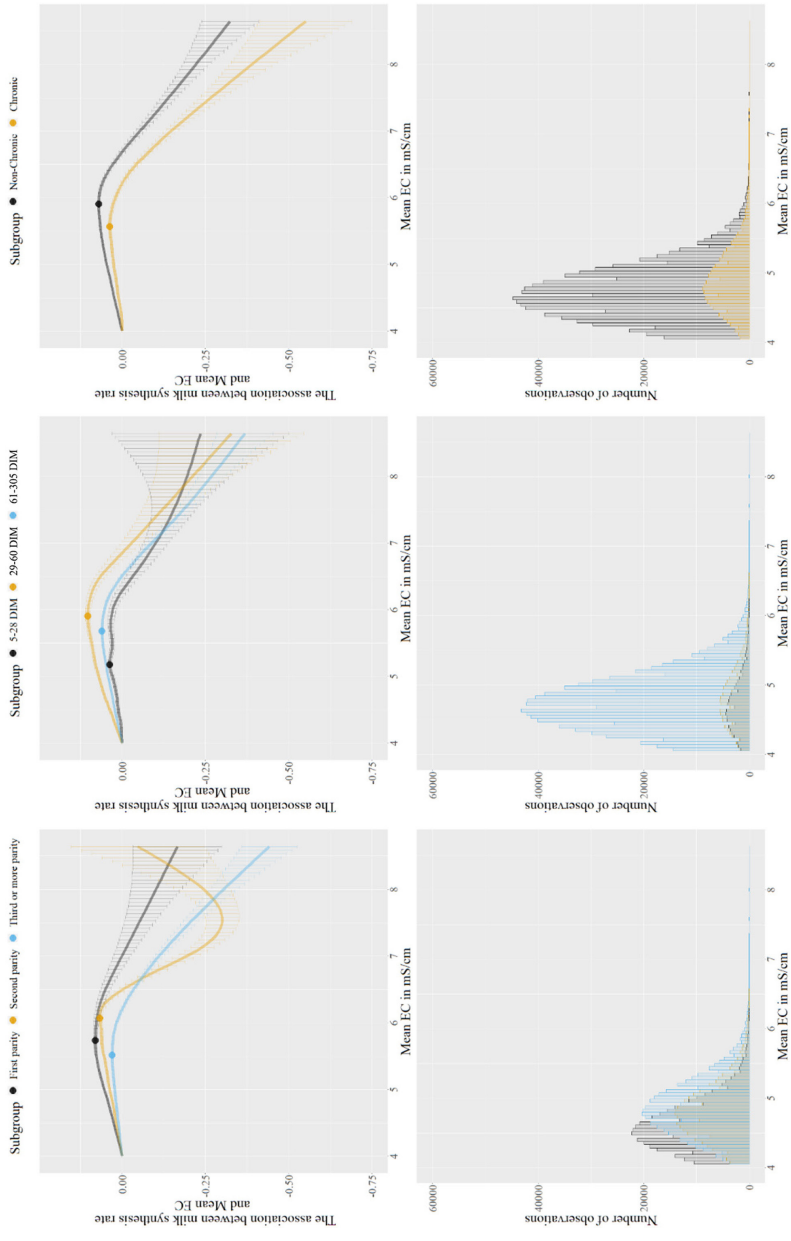

Figure 6. The estimated association between milk synthesis rate and Mean EC and the number of observations for parity, stage of lactation, and chronicity subgroups. The dots indicate that the start of milk synthesis rate decreases, and milk production losses increase from that point.

4.2.3 Lactate dehydrogenase

Figure 7 provides the non-linear association between LnLDH and milk synthesis rate (f_{LnLDH}) and the frequency of LnLDH observations for different parity, stage of lactation, and chronicity classes (Figure 7A, 7B, and 7C). The dots in Figure 7 mark the point where milk synthesis rate started to decrease, and milk production losses increased when LnLDH increased. It can be seen from the results that LnLDH was negatively associated with milk synthesis rate for almost the entire range of LnLDH for most groups. The thresholds ranged from approximately 0 to 3 LnLDH (1 - 20 U/L) for all subgroups. Despite the similarity in the general form and level of the smoothing function, the differences in thresholds are large. The differences in thresholds seem to be caused by minor differences in the shape of the association between LnLDH and milk synthesis rate between the subgroups. In other words, the difference in milk production loss between the thresholds was limited. Nevertheless, the milk synthesis rate decreased noticeably more after approximately 3 LnLDH (20 U/L) in all subgroups.

Several dissimilarities in thresholds and the steepness of the decrease in milk synthesis rate between subgroups were seen. In Figure 7A, multiparous cows showed a larger decrease in milk synthesis rate associated with higher LnLDH than primiparous cows. Even more, the differences in intercept between first parity cows and second parity cows, and between first parity cows and third or more parity cows were 0.27 with a standard error of 0.01 and 0.36 with a standard error of 0.02 ($P < 0.01$), respectively. As such, regardless of LnLDH, cows with a higher parity significantly produce more milk. In Figure 7B, 61-305 DIM observations showed a larger decrease in milk synthesis rate than the 5-28 and 29-60 DIM observations. The stage of lactation subgroup differences in intercept between 5-28, 29-60, and 61-305 DIM subgroups were not significantly different ($P > 0.1$). In Figure 7C, the line of non-chronic cows was slightly lower than the line of chronic cows, but the chronic cow intercept in the model was -0.08 with a standard error of 0.01 ($P < 0.01$). Regardless of current LDH, the milk synthesis rate for chronic cows was lower.

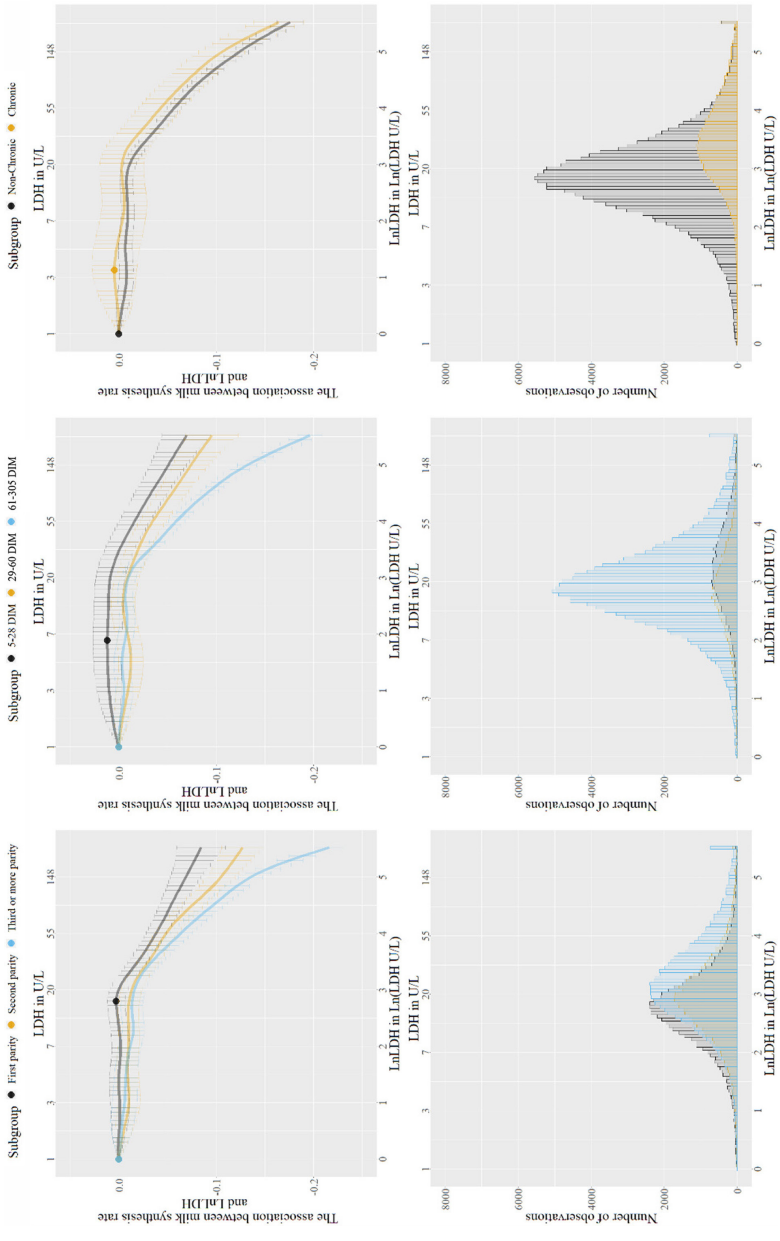

Figure 7. The estimated association between milk synthesis rate and LnLDH and the number of observations for parity, stage of lactation, and chronicity subgroups. The dots indicate that the start of milk synthesis rate decreases and thereby milk production losses increase from that point.

4.3 The prediction of mastitis chronicity

Table 3 presents the different approaches' sensitivity, specificity, MCC, accuracy, and AUC. The chronic mastitis prediction model outperformed the two default approaches on all farms for almost all performance indicators. More specifically, it outperformed them on accuracy (chronic mastitis prediction model: 0.859, frequent sampling approach: 0.833, and monthly sampling approach: 0.809), MCC (chronic mastitis prediction model: 0.694, frequent sampling approach: 0.618, and monthly sampling approach: 0.504), and AUC metrics (chronic mastitis prediction model: 0.944 and frequent sampling approach: 0.910). Using (Welch's) t-tests for unequal variances, we determined that the differences between the default approaches and the model predictions were significant for AUC, and MCC ($P<0.05$) but not for accuracy when compared to the frequent sampling approach ($P>0.05$). The chronic mastitis prediction model also outperformed the other approaches on sensitivity (chronic mastitis prediction model: 0.934, frequent sampling approach: 0.833, and monthly sampling approach: 0.595), but the monthly sampling approach outperformed the other methods on specificity (chronic mastitis prediction model: 0.826, frequent sampling approach: 0.834, and monthly sampling approach: 0.896).

Table 3. The sensitivity, specificity, Matthew's correlation coefficient, accuracy, and Area under Curve (AUC) of the predictions of the model, frequent sampling approach, and monthly sampling approach over seven validation herds using 30 days prior to the prediction day as input.

Herd	Sensitivity	Specificity	Matthew's correlation coefficient	Accuracy	AUC
Model					
Herd 1	0.937	0.798	0.721	0.854	0.938
Herd 2	0.925	0.781	0.677	0.832	0.924
Herd 3	0.940	0.785	0.629	0.821	0.931
Herd 4	0.909	0.842	0.718	0.864	0.945
Herd 5	0.943	0.838	0.762	0.878	0.954
Herd 6	0.934	0.831	0.666	0.853	0.948
Herd 7	0.947	0.909	0.687	0.913	0.969
All herds average	0.934	0.826	0.694	0.859	0.944
Frequent sampling approach					
Herd 1	0.813	0.844	0.652	0.831	0.904
Herd 2	0.833	0.756	0.566	0.783	0.879
Herd 3	0.885	0.774	0.573	0.800	0.912
Herd 4	0.707	0.864	0.573	0.813	0.882
Herd 5	0.825	0.852	0.670	0.842	0.916
Herd 6	0.896	0.838	0.645	0.850	0.933
Herd 7	0.874	0.913	0.649	0.909	0.947
All herds average	0.833	0.834	0.618	0.833	0.910
Monthly sampling approach					
Herd 1	0.553	0.906	0.502	0.764	
Herd 2	0.546	0.862	0.434	0.750	
Herd 3	0.594	0.863	0.449	0.801	
Herd 4	0.585	0.897	0.515	0.795	
Herd 5	0.656	0.903	0.587	0.809	
Herd 6	0.631	0.900	0.531	0.843	
Herd 7	0.598	0.937	0.510	0.900	
All herds average	0.595	0.896	0.504	0.809	

4.4 The cost of chronicity

4.4.1 Mastitis dynamics

The model results indicated a median culling rate was 0.32 with a quartile range of 0.30 to 0.34 per cow year. The median clinical mastitis incidence rate was 0.27 with a quartile range of 0.25 to 0.30 cases per cow year. The median incidence rate of IMI was 1.04 with a quartile range of 0.98 to 1.11 cases per cow year.

4.4.2 Economic results

Total costs of mastitis

Table 4 gives the economic outcomes of the model per IMI case. The median total mastitis costs were € 208 with a quartile range of € 197 to € 225 per IMI case. Most of the costs occurred due to transmission (i.e., transmission cases), culling, and clinical and subclinical milk production losses. Other substantial costs originated from dry cow treatments, lactational treatments, and diverted milk. We could determine that *Staph. aureus* caused the largest share of the total costs of IMI by looking at pathogen-specific economic impact per generic IMI case, followed by NAS, *Strep. spp*., and Gram-negative pathogens.

Table 4. Economic results of the simulation model for the total mastitis costs, and pathogen-specific shares of the total cost (in €) per non-transmission IMI case.

Costs	**1st Qu.**	**Median**	**3rd Qu.**
Total clinical mastitis milk production loss	25.27	28.29	31.77
Total subclinical mastitis milk production loss	21.77	23.06	24.48
Total mastitis culling	21.50	24.55	27.60
Total lactational antibiotics	10.89	11.87	12.85
Total dry cow treatment	10.65	11.33	12.09
Total diagnostics	0.36	0.39	0.42
Total diverted milk	8.82	9.65	10.52
Total clinical mastitis checks	0.37	0.40	0.43
Total extra costs due to transmission	90.76	99.26	110.74
Total mastitis	197.12	208.02	225.27
Pathogen-specific share of the total costs			
Total *Staph. aureus* share	91.46	101.03	113.67
Total Gram-negative pathogens share	8.47	10.67	13.20
Total *Strep. spp.* share	22.79	26.53	30.80
Total NAS share	61.31	67.99	76.28
Total non-pathogen-related share	1.36	1.68	2.11

Costs of chronic mastitis

In Table 5, we present chronic mastitis costs per IMI case and its cost factors. The median total costs due to chronic mastitis were € 104 (50% of the total mastitis costs) per IMI case with a quartile range of € 96 to € 115. The share of chronic mastitis relative to the total mastitis costs was substantial. Unsurprisingly, the costs due to transmission had a large share in the chronic mastitis costs (45% of the total chronic mastitis costs). Culling and milk production losses substantially affected the costs of chronic mastitis as well (culling: 24%, combining subclinical and clinical milk production losses: 16%). Subclinical mastitis production losses were higher than clinical mastitis production losses compared to the share in the total costs of mastitis. On average, *Staph. aureus* had the largest share in the costs of chronic mastitis (70%), followed by NAS (14%), *Strep. spp.* (11%), and Gram-negative pathogens (5%).

Table 5. Economic results of the simulation model for the chronic mastitis costs, and pathogen-specific shares of the chronic cost (in €) per non-transmission IMI case.

Costs	1st Qu.	Median	3rd Qu.
Total chronic mastitis milk production loss due to clinical mastitis	5.61	6.62	7.76
Total subclinical mastitis during ongoing non-spontaneously cured IMI milk production loss	9.93	10.86	12.02
Total chronic mastitis treatments	5.29	6.03	6.83
Total chronic mastitis diagnostics	0.17	0.20	0.23
Total chronic mastitis extra costs due to transmission	41.11	46.81	53.71
Total chronic mastitis diverted milk	4.30	4.89	5.66
Total chronic mastitis culling	21.50	24.55	27.60
Total dry cow treatment	3.60	3.97	4.38
Total chronic mastitis	95.91	104.25	114.95
Pathogen-specific share of costs			
Total chronic *Staph. aureus* share	64.67	72.97	82.33
Total chronic Gram-negative pathogens share	3.04	4.96	6.88
Total chronic *Strep. spp.* share	9.15	11.47	14.11
Total chronic NAS share	12.17	14.57	16.76

4.4.3 Estimating the costs of mastitis for different sensor-based mastitis strategy scenarios

Table 6 indicates the costs of mastitis and chronic mastitis, the IMI and CM incidence rate, and the culling rate for different sensor-based mastitis strategy scenarios. Lactational treatment of SCM based on fourteen days of SCC data leads to more mastitis and chronic mastitis costs but decreased CM and IMI incidence rates and the culling rate relative to the baseline scenario. Dry cow treatment based on seven days of SCC samples rather than one SCC sample led to decreasing mastitis and chronic mastitis costs, CM and IMI incidence rates, and a decreasing culling rate relative to the baseline scenario.

Culling using a 45-day rolling mean of SCC samples rather than a 90-day rolling mean of SCC decreased the mastitis and chronic mastitis costs and the IMI and CM incidence relative to the baseline, but it increased the culling rate. When the earlier culling strategy and specific dry cow treatment strategy were combined, mastitis and chronic mastitis costs were lower than the baseline. It lowered the IMI and CM incidence rate but at the cost of a higher culling rate.

Table 6. The cost (in €) of chronic mastitis, the total cost (in €) of mastitis, clinical mastitis (CM) incidence rate in cases per cow year, IMI incidence rate in cases per cow year, and culling rate per cow year for different sensor strategies. The median and the 25% and 75% quartiles (between brackets) are reported.

Strategy	Cost of chronic mastitis per IMI case	Total cost of mastitis per IMI case	CM incidence in cases per cow year	IMI incidence in cases per cow year	Culling rate per cow year
Baseline	104.25 (95.91 – 114.95)	208.02 (197.12 – 225.27)	0.27 (0.25 – 0.30)	1.04 (0.98 – 1.11)	0.32 (0.30 – 0.34
Subclinical lactational treatment	150.89 (138.94 – 162.86)	335.75 (320.00 – 352.21)	0.22 (0.21 – 0.24)	0.79 (0.74 – 0.83)	0.27 (0.25 – 0.29)
More specific Dry cow treatment	100.56 (90.43 – 110.54)	200.12 (187.65 – 214.05)	0.27 (0.25 – 0.30)	1.04 (0.98 – 1.11)	0.31 (0.30 – 0.33)
Earlier culling	97.28 (90.27 – 105.31)	197.59 (186.27 – 208.71)	0.25 (0.22 – 0.27)	0.88 (0.83 – 0.93)	0.37 (0.35 – 0.39)
Earlier culling and more specific dry cow treatment	94.77 (87.48 – 102.83)	190.26 (179.92 – 203.24)	0.24 (0.22 – 0.27)	0.89 (0.83 - 0.93)	0.37 (0.35 – 0.39)

5. Discussion

5.1 The Data-Information-Decision mastitis framework

In the Introduction, the Data-Information-Decision mastitis framework was put forward to understand the structure of past research on the concept of sensor-based mastitis management. The framework is helpful to obtain a general understanding of the concept. However, it is also helpful to understand the remaining challenges in research and place this thesis's contributions into a broader scientific perspective. The framework structure will guide the Discussion chapter, where the text will address the contributions of the thesis and the remaining challenges in each component of the framework.

5.1.1 Data

Contribution of the thesis

A major contribution of this thesis is that it creates new information based on data from existing sensor technology available on commercial dairy farms. Henceforth, the findings can be implemented directly into practice. Moreover, the methodological approach is general and can obtain mastitis information for different mastitis sensor data. The approach could be applied to study the progression of a new mastitis indicator after initial inflammation, similar to what was done in Paper I. One could estimate the severity of a specific level of a new inflammation indicator by using the estimated milk production loss (Paper II). One could add another indicator into the chronic mastitis forecasting model to increase predictive performance (Paper III). One could also use a similar methodology in creating a bioeconomic model

to simulate a dairy farm with a newly developed sensor (Paper IV). All these approaches could also be used with to-be-developed sensors to acquire new mastitis information in the future.

In Paper IV, the model simulated daily sensor data based on the progression of SCC after a new IMI occurred. To the author's knowledge, this method of simulating daily sensor data has never been used to evaluate potential management strategies in a bioeconomic mastitis model. To simulate the day-to-day variation of SCC, interpolated SCC points were added by points from the day-to-day variation distribution based on general SCC patterns. This idea of simulating sensors could be extended upon by developing methods to capture herd-specific sensor patterns of a specific mastitis indicator and use these extracted sensor patterns in a farm-specific bioeconomic model. Applying these herd-specific sensor patterns would be a step in developing the simulation model as a complete herd-specific digital twin (Pylianidis et al., 2021). This development would allow the farmer to simulate a range of different sensor-based strategies in a context that reflects the herd-specific context even closer, enhancing the practical value of the model.

Challenges

In mastitis research, one of the most considerable remaining challenges with sensor-based data and large datasets is verifying the data quality. The data quality could deteriorate in the case of structural data maintenance issues on-farm. These issues could be mitigated by data cleaning. In the papers in this thesis, data cleaning has mainly been performed by using standards for typical values using thresholds found in literature or by common sense (e.g., a cow cannot get pregnant two times during lactation). In mastitis research, there is a lack of standards to clean data. Researchers use their own methods (which they may or may not describe in publications) to assess and improve data quality. This implicit knowledge adds another layer of complexity to mastitis research. It could lead to misunderstandings between researchers and increase the difficulty for new researchers entering the scientific field.

Another challenge in this thesis was that the available data did not contain bacteriological analysis results. Generally, it is important to keep in mind that the overall focus of this thesis was on mastitis or the degree of inflammation rather than the causal agent. This research focused on sensor-based mastitis management from the farmer's perspective, and the farmer does not know the pathogen at every step of the way (Griffioen et al., 2016).

Farmers tend to use symptoms and consequences to decide on treatment (Vaarst et al., 2002). Furthermore, most applications in this thesis have to do with chronic SCM. Chronic SCM is, by definition, a long-term increase in mastitis indicators (International Dairy Federation, 2011), for which a pathogen specification was not strictly needed. Therefore, bacteriological data would have been beneficial to have but not needed. It could have led to more precise results. For example, it was impossible to assess the progression of different mastitis indicators (Paper I) for differing pathogens, such as performed in Fogsgaard et al. (2015). It was also impossible to assess whether the association between different mastitis indicators and milk yield (Paper II) differed for different pathogens, as done by Gonçalves et al. (2020). Having bacteriology data would have led to more precise results and conclusions concerning the duration and degree of milk loss per pathogen type. Additionally, Paper III did not need bacteriological information as the focus was on predicting a prolonged inflammation and not infection. However, it could have led to a more precise forecast if bacteriological information was used as model input. Bacteriological information was needed in Paper IV, but it could be retrieved from the literature. Nevertheless, for a decision support system, bacteriological information would be necessary to gather a complete overview of the consequences of mastitis in terms of contagious cow-to-cow transmission, as transmission and its mode are highly dependent on pathogen type (see Paper IV).

In general, incomplete data was and will continue to be a problem in future mastitis research. In the current datasets used in this thesis, data was primarily incomplete due to farmers not filling in treatments into their management systems (Bonestroo et al., 2021a) or using risk-based sampling algorithms (i.e., sampling SCC or LDH). Using risk-based algorithms, SCC and LDH tend to be sampled from higher risk milkings (e.g., early in lactation). This tendency could have resulted in an inherent bias of higher SCC or LDH values when observations were reported. This bias would pose a weakness of the dataset used in this thesis. For instance, this would potentially increase the estimated duration in Paper I due to a higher presence of high SCC values. We tried to mitigate the effects of this bias partly by aggregating data per day or week, reducing the amount of incomplete data. Nevertheless, the estimates for milk production loss or decrease in indicators after initial inflammation could be less biased if the dataset included these inflammation indicators for every milking. This increased sampling would

also create more observations, increasing the precision of the parameters estimated in statistical inference. Additionally, in the thesis, farmer-reported treatments were not used as they were unavailable for many studied farms. Instead, milk diversions were used as approximations for treatments, which can be automatically stored. However, milk can be diverted for many reasons and can therefore be unspecific to approximate antibiotic treatment due to mastitis (Bonestroo et al., 2021a). As such, Paper I may have included cases that were not treated for mastitis in the milk diversion subgroup, decreasing the specificity of the conclusions on this subgroup. Nevertheless, milk diversions are consistently reported, while farmer treatment reports may be inconsistent (Vaarst et al., 2002; Espetvedt et al., 2013) and were therefore used in this thesis.

5.1.2 Information

Contribution of the thesis

Paper I contributed to the Information concept in the Data-Information-Decision mastitis framework. Its results can define mastitis chronicity based on inflammation markers measured by sensors. There is an interdependence that can be seen here between the Data concept and the Information concept in the framework. Paper I gives a duration-based threshold for SCM chronicity, which has limited attention in the literature. This limited focus mainly occurred due to the monthly reported DHI SCC samples. Usage of monthly-reported DHI SCC samples caused the minimum threshold to be one month. As a result, researchers used chronicity thresholds of either one or two months (St. Rose et al., 2003). The existence of close-to-daily data instead of monthly data created the possibility for a more precise definition of chronic mastitis, showing this interdependence between Data and Information.

Paper II contributed to the Information concept by estimating the non-linear association between different mastitis indicators and milk yield more precisely. This increased precision is important as it allows for a more exact estimate of milk production loss. The analysis showed that the most substantial losses tend to occur at a higher level of SCC than indicated by other studies (Hortet et al., 1999; Dürr et al., 2008; Hagnestam-Nielsen et al., 2009; Gonçalves et al., 2018a). Others have also found the milk production loss functions of SCC to be non-linear (Dürr et al., 2008; Hagnestam-Nielsen

et al., 2009; Gonçalves et al., 2018a) but still indicated higher milk losses on lower levels of SCC. In Paper II, the associations did show that the milk production loss started to increase after a low level of SCC (e.g., 12,000 SCC/ml) but that it was starting to be substantial at 277,000 SCC/ml. This pattern confirms that the loss is highly non-linear. Farmers in the past have been using milk production loss as an important indicator for mastitis (Vaarst et al., 2002). Milk production loss can make the degree of severity observable and understandable to the farmer, given SCM's lack of physical symptoms. Therefore, the work in Paper II can be used to translate various levels of different mastitis indicators with different unit measures into an estimated milk production loss that is comparable across mastitis indicators.

A major contribution of the thesis was to show the potential value of forecasting mastitis chronicity. In paper III, mastitis chronicity was forecasted for ongoing mastitis cases. In mastitis research, substantial attention was given to the detection of CM (Hogeveen et al., 2010; Jensen et al., 2016; Khatun et al., 2017, 2018). However, sensor technology is not substantially used after the point of detection, while it could also be used to track the progression of ongoing episodes. The contribution of paper III is the introduction of a problem that can be solved with mastitis sensors. The forecasts of mastitis chronicity allow farmers to gain more insight after detecting mastitis. Furthermore, Paper III shows that using a smaller input window of fifteen days had a limited impact on the prediction performance. This limited impact shows that it may be possible to forecast chronicity accurately in early lactation. In any case, forecasting chronic mastitis would allow for a more targeted intervention protocol, which would lead to culling truly chronic cows and reduced usage of antibiotics. The latter is societally relevant in the challenge to limit antimicrobial resistance by limiting the use of antibiotics (Speksnijder et al., 2015).

Challenges

Generally, one of the main remaining challenges in terms of mastitis information is the usage and operationalization of the term chronic mastitis. Multiple efforts have been undertaken to standardize mastitis definitions (International Dairy Federation, 2011). However, the definitions do not define chronic mastitis in detail, apart from indicating that chronic mastitis is an udder inflammation that continues over an extended period (International Dairy Federation, 2011). More specifically, this definition does not specify what mastitis indicator to use (e.g., SCC), how high the

indicator level needs to be (e.g., 100,000-200,000 SCC/ml), and for how long the indicator should be elevated (e.g., three to four weeks of elevated SCC). Suppose a different definition of chronic mastitis was chosen. In that case, the conclusions of this thesis could differ in terms of estimated milk losses (Paper II), the ability to forecast chronic mastitis (Paper III), and the cost of chronic mastitis (Paper IV). A pathway to overcoming this research challenge is applying sensitivity analysis on the chronic mastitis definition. For instance, Paper I used different case definitions in a sensitivity analysis, and one similarly could do a sensitivity analysis on the chronic mastitis definition. However, this does not increase the comparability between study results. A more permanent solution would be that the same chronic mastitis definitions are used across studies, requiring standardization.

Another remaining challenge in transforming data into information is to choose the appropriate method. This thesis applied linear mixed models, generalized additive models, and gradient-boosting trees in Paper I, II, and III. Each of these models could be used interchangeably in the different papers. However, the different papers have different aims (and different pieces of information to obtain), and therefore some methods are more fitting than others. Paper I required an estimate of weekly effects before and after the initial inflammation while controlling for repeated measures from the same cow. Gradient-boosting trees and generalized additive models would achieve this aim. However, a simpler linear mixed model would also achieve this aim and would be preferable from the perspective of parsimony. Estimating non-linear associations between milk yield and mastitis indicators is possible with linear mixed models with polynomial effects. However, the results of Paper II indicated that the non-linear associations are not simply quadratic or cubic. Hence, the linear mixed model results would not reflect the underlying association accurately. Paper III forecasts chronicity status based on past sensor data from the same lactation. A generalized additive or linear mixed model could also have made this prediction. However, these methods would require the interactions between variables to be defined apriori, while a tree-based method does not have this requirement. A tree-based method would also allow for more complex interactions. In summary, these methods were chosen by weighing the aim of the study, the characteristics of the item under study, and the need for interpretability (or parsimony).

5.1.3 Decision

Contribution of the thesis

Paper IV contributes to the Decision concept in the Data-Information-Decision framework as it models the interaction between the decision-maker and the information. The addition of sensor data in bioeconomic models is novel. As implemented in the model, the daily data frequency would allow earlier decision-making on treating and culling cows. Other work has modeled detection systems (i.e., mastitis information) (Van den Borne et al., 2010a), but it did not directly model the mastitis sensor data. Directly modeling sensor data allows different sensor strategies to be tried within the model. The model could be used as a tool to explore different sensor-based mastitis SOPs under the assumption of an economic rational farmer that tries to minimize the cost of mastitis. A selection of potentially cost-minimizing strategies could be made using the simulation model. This selection process is performed on a small scale in Paper IV. However, the resulting strategies should be explored further using different methods before implementing them in practice, as a simulation model will continue to simplify a complex system set in reality.

Another contribution of the thesis is estimating the cost of chronic mastitis. Paper IV focuses on the cost of chronic mastitis, which is a substantial portion of the total cost of mastitis. Past research has primarily focused on the total cost of mastitis, as indicated in Aghamohammadi et al. (2018) and Hogeveen et al. (2019), and how the total cost of mastitis changed due to preventive measures (Aghamohammadi et al., 2018) or intervention strategies (Steeneveld et al., 2007; Van den Borne et al., 2010a; Gussmann et al., 2019a; b). However, an explicit distinction between non-chronic and chronic mastitis costs has not been made so far. This lack of a distinction is surprising as the share of chronic mastitis cost in the total mastitis costs is estimated to be 50% and is related to the failure of current intervention procedures. Chronic mastitis costs indicate the potential value for tools that allow for earlier intervention in chronic mastitis cases (e.g., Paper I and Paper III). Given the importance of chronic mastitis in the cost of mastitis, it obviously would be vital to reduce the cost of chronicity and to look into its largest cost factors. One of the largest cost factors in chronic mastitis is contagious transmission. Interestingly, the size of the cost factor emphasizes the non-directly observed cost of keeping chronic cows in the herd. Limiting transmission has a long tradition as a preventive strategy to combat mastitis

(Hillerton and Booth, 2018). These model results highlight the importance of management options to limit transmission, by the decision, for instance, to clean milking equipment regularly or separate sick from healthy cows. This example shows that the focus on chronic costs is important as it can give insights to farmers and scientists into the cost of inefficiencies in intervention procedures.

Another contribution of this thesis is to explore the potential for sensor-based SOPs. Paper I provides a general estimate on the definition of chronic mastitis using a cut-off duration value for online SCC and EC. This cut-off could be used in an SOP monitoring chronic mastitis. Paper II provides a method to estimate the severity of an SCM case by using milk yield as a proxy, which could be used as an indication to intervene. Paper III extends on Paper I by providing the possibility of individual chronic mastitis SOPs based on the forecasted future state of the cow. Paper IV provides a model where SOPs can be developed on economic principles. More specifically, the model of Paper IV can be used as a selection step to select different sensor-based SOPs prior to testing it out on a farm. Testing different sensor-based strategies can filter out the strategies that will be unprofitable, allowing the most promising candidates to be tested in real-life. Overall, the thesis provides the framework for a structured sensor-based mastitis management decision tool that includes multiple SOPs.

Challenges

The main remaining challenge concerning the farmer's decision-making is its complexity, and that the knowledge on sensor-based decision-making in practice is quite limited. The author could not find specific knowledge on how farmers use mastitis sensors in practice in the academic literature, apart from their use of alerts (Mollenhorst et al., 2012; Hogeveen et al., 2013). Due to this lack of knowledge, the current simulation model focuses on symptom-level and cow-level factors in sensor-based mastitis decision-making and may give a somewhat simplified view of the farmer decision-making using sensors. Sensor-based decision-making can be more complex in practice. For instance, apart from the characteristics of the individual cow, farmers are still dependent on herd-level processes when making cow-level mastitis decisions (Vaarst et al., 2002), such as the inflow of new calves when making culling decisions. In this case, the culling decision might be different when no calves are available, even if the decision-making is sensor-based. Many more complex (herd) factors are crucial in farmer sensor-based decision-

making. Therefore, more research into sensor-based farmer decision-making is needed to incorporate it realistically in the simulation model.

Another remaining challenge is that farmer decision-making in practice will not be focused entirely on cost minimization. Farmers could have different goals, such as supporting biodiversity (Herzon and Mikk, 2007), increasing animal welfare (Vaarst et al., 2002), decreasing the use of antibiotics (Jones et al., 2015), and having enjoyable work (Bergevoet et al., 2004). In economic terms, the farmer may obtain more utility in other activities than in cost minimization (Hansson and Lagerkvist, 2014, 2015). The simulation model in Paper IV was focused on the cost of mastitis and not on antibiotic usage, enjoyment of work, supporting biodiversity, or animal welfare. It restricted the results and conclusions of Paper IV to be monetary. Nevertheless, the model could be expanded upon by using multi-objective optimization (Groot et al., 2012) in the future. In the simulation model, these alternative goal functions could be modeled separately. One may create a farmer utility function by weighing the profit and alternative goal functions based on farmer-specific weights. This utility function would model the trade-offs between profit and alternative goal functions for specific farmers. The simulation models will be made herd-specific and subjective by infusing the farmer-specific importance of goals in the utility function using these weighing methods. It may bridge the gap between bioeconomic models created in academia and farms in practice, allowing farmers to simulate different farming strategies and align them with their preferences.

A practical challenge in mastitis research is to develop tools that farmers actively use. It is problematic if farmers do not actively use provided tools, as these tools would then provide limited value in return. To illustrate this problem, Hogeveen et al. (2013) found that only 3% of the CM alerts led to actions by the farmers. This lack of use could be especially problematic for the model put forth in Paper III as its predictions would barely be used in practice. To this end, mastitis tools should be more farmer-centric to promote their use. The author mentions two alternative pathways to achieve more farmer-centric mastitis tools. The first pathway is to align the mastitis tool with the different types of mastitis decisions farmers may encounter (e.g., different decisions for severe CM, mild CM, or chronic mastitis). An extensive effort to tie different types of required sensor accuracies to different farmer decisions has recently been performed (Hogeveen et al.,

2021), which is a good step in this direction. The second pathway addresses the hard-to-understand reasoning of mastitis decision tools as the cause of the lack of use. When farmers are confronted with a daily alert list with a substantial portion of false alerts with a limited understanding of the model, they lose trust. Unfortunately, most mastitis models tend to use black-box or hard-to-interpret models (e.g., gradient-boosting trees or neural networks) (Hogeveen et al., 2010), where it is hard to understand the reasoning of the model. To increase the understandability of the model, one may apply explainable machine learning techniques to these black-box models. Examples of these explainable machine learning techniques include SHAP (Lundberg and Lee, 2017) or Lime (Ribeiro et al., 2016). Conceptually, the farmer could still understand the algorithm's reasoning if it produced a false alert, avoiding trust issues. Both aligning mastitis tools with the different types of decisions and applying explainable machine learning techniques to black-box models could be part of the solution in increasing the usage of mastitis tools by farmers in practice.

5.2 Mastitis from multiple research perspectives

As the thesis attempted to address mastitis from a veterinary, data science, and economics perspective, it would be vital to address the value of interdisciplinary work in science. Mastitis is seen in a different light by different disciplines. From a veterinary perspective, mastitis is a disease that decreases animal welfare and the proper functioning of the animal (Siivonen et al., 2011; Heikkilä et al., 2018). From an economic perspective, mastitis is a cost factor on dairy farms, and major attention is paid to the consequences of the disease as costs can be attached to them (Halasa et al., 2007). From a data science perspective, mastitis is seen as an event that causes changes in sensor data collected by milking machines and add-ons (Chagunda et al., 2006; Khatun et al., 2019; Hogeveen et al., 2021).

At the crossing of these different perspectives lie the most valuable topics in mastitis research, in the author's opinion. For instance, combining a data science and economics perspective could obtain questions regarding the economic value of sensor data or mastitis detection systems (Van den Borne et al., 2010a). If one combines data science and a veterinary perspective, one could address problems such as measuring animal welfare with sensor data (Silva et al., 2021) or forecast disease outcomes that impede proper

functioning using sensor data (Paper III). If the economic and veterinary perspectives are combined, one could address issues such as determining whether a treatment is cost-effective (Swinkels et al., 2005; Steeneveld et al., 2007; Gussmann et al., 2019b; a). These examples highlight the importance of interdisciplinary work. Mastitis research serves to improve mastitis decision-making of the farmer in practice. In practice, changes in mastitis decision-making affect multiple areas such as animal welfare, farm economics, and public health. The lenses of different disciplines will be needed to assess the impact of a change on these areas and enhance the uptake of mastitis research in practice and in the future.

6. Conclusions

The overall objective for this PhD project was to explore the potential applications for a decision support system that includes the course and consequences of chronic mastitis. The main conclusions are:

- Both mean σ-Conductivity and SCC stabilized three to four weeks for recovered cases after initial inflammation, providing the basis for a sensor-based definition of chronic mastitis. Differences and similarities were identified in mean σ-Conductivity and SCC.
- Negative associations were found between SCC, EC, and LDH and milk yield, which were non-linear and had a similar form between parity groups, at different lactation stages, and at different chronicity statuses of the cow.
- Based on sensor data, the developed prediction model outperformed default approaches that mimic current decision-making based on monthly or more frequently sampled SCC data.
- Using a bioeconomic simulation model, the median cost of chronic mastitis in an AMS farm was € 104 per IMI case, and the median total cost of mastitis was € 208 per IMI case. This share showed the importance of chronic mastitis in the cost of mastitis. It demonstrated that the economic impact of different sensor-based strategies could be assessed using the implemented bioeconomic model.

This thesis shows the potential for a decision support system that monitors and forecasts chronic mastitis and its consequences, which is built on data that is already available on commercial dairy farms.

7. Future perspectives

7.1.1 Data-based improvements

Milk diversion

During the research that procured this thesis, it was noticed that treatment records tend to be incomplete. These incomplete records are a problem when the data is used for research, which is also indicated by others on national levels (Wolff et al., 2012). To combat this problem, the analyses in the thesis have used a proxy variable in the form of milk diversion in Paper I (Bonestroo et al., 2020, 2021a). It is not a perfect proxy to indicate mastitis treatments, as milk may be diverted for other reasons. Even more, milk can be automatically diverted by the system if specific mastitis indicators are too high without intervention from farmers. Nevertheless, it can be made more specific by using multiple days of milk diversion instead of one day (Bonestroo et al., 2021a). Milk diversion holds the opportunity to proxy treatment even if treatment records are not available, and therefore it could be used in future studies involving large sensor-based datasets. In the future, milk diversion could be used in data cleaning to select farms with an adequate recording of treatments. It would allow researchers to remove farms from the dataset that have a substantial number of milk diversions but close to no reported treatments.

7.1.2 Information-based improvements

Herd-specific mastitis models

Paper III proposed a chronic mastitis forecasting model. The model is a general universal model that multiple herds can use. However, it can be expected that making the model herd-specific by adjusting the model to herd-specific parameters (e.g., implicitly learning the non-observed farm pathogen population) would increase the predictive performance. This increase in performance would also hold for CM detection models. One could use transfer learning (Pan and Yang, 2009) (i.e., partly refitting an existing model trained with data from other herds with new data from a new herd) if one switched methodology from a gradient-boosting trees model to a neural network. A transfer learning approach could involve training a general model based on a large set of training herds and fine-tuning the model on the dataset of the herd on which the model is to be used. Transfer learning has been used successfully with image-based or text-based deep learning tasks (Pan and Yang, 2009), where training a model was expensive and the required dataset was large. Transfer learning the models in this manner would potentially create more accurate mastitis models in the future.

Milk production loss monitoring

The milk production loss estimate proposed in Paper II could be extended further to measure the severity of a case of mastitis. A milk production loss model using multiple mastitis indicators as input could be created to measure the severity of an ongoing case in terms of milk production loss. As farmers are familiar with milk production loss (Vaarst et al., 2002), they would be able to interpret it. A single milk production loss measure would be simpler to interpret than four or five mastitis measures with their own units and thresholds.

Additionally, one could extend the milk production loss estimation by forecasting the milk production of the remainder of the lactation cycle after a mastitis case. The system would report the total lactational loss to the farmer. This information would support the farmer in making treatment and culling decisions. Recently, work has been done to forecast lactation curves using deep learning (Liseune et al., 2021). This deep learning work can extend the estimated milk production loss of Paper II into an estimate for a milk production loss for the remainder of the lactation. This information would allow farmers to select cows on their estimated milk yield in the

future, which is one of the most substantial cost factors in mastitis (Paper IV).

Mastitis case forecasting

Paper III proposes a chronic mastitis forecasting model with a binary output (chronic or not chronic). In the future, individual cow case forecasting could be extended to become similar to a weekly weather forecast where the first week, second week, and third week SCC is given with an estimated uncertainty interval. This information could give farmers more precise outputs of what would eventually happen to a case, and the model could simultaneously communicate the uncertainty attached to the prediction. Different indicators could also be used instead of SCC, such as milk production loss or having no CM episodes for a given period, making the tool flexible for different sensor setups. This methodological flexibility would allow a broader group of farmers to perform case forecasting, even when they do not have an SCC sensor.

7.1.3 Decision-based improvements

Research into current sensor-based farmer mastitis decision-making

During the initial stages of the research, it became clear that more insights into current farmer mastitis decision-making are needed. There is some insight into non-sensor-based farmer decision-making (Vaarst et al., 2002) and the influence of farmer attitudes (Jansen et al., 2009) and alternative goals (Hansson and Lagerkvist, 2014, 2015). However, there is a lack of peer-reviewed research on farmers' current sensor-based decision rules. Farmers with an AMS have access to sensor data streams, but it is unknown how farmers currently use sensor data in their decision-making. More information on the current informal SOPs of farmers would make it possible to ensure that solutions that are suggested in the literature fit in practice. These informal SOPs would include which sensor or algorithm value is important to the farmer for which decision and what threshold is being used by the farmer. In the end, the value of sensor-based mastitis management and its decision support systems rely on whether the farmer actively uses it. If the farmer does not use the system as intended, then such a system adds less to no value.

The general approach to sensor-based disease management

This thesis focused on sensor-based management of mastitis and was highly interdisciplinary. It investigated the information that the farmer might need to make a disease decision: 1) defining the measurement characteristics of the disease, 2) detecting the disease, 3) forecasting its recovery, 4) estimating the effects of the disease, and 5) estimating the benefits of intervention. All these aspects need different disciplines to study and fulfill different information needs of the farmer when making disease-related decisions. Although applied to mastitis, this interdisciplinary research approach can be used as a blueprint to support farm management for other diseases.

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Popular science summary

Mastitis in dairy cows is an inflammation in the udder that can reduce animal welfare and can be costly in terms of reduced milk production. Since the 1990s, automatic milking systems have been commercially available where the cow can be milked without human labor. These systems are equipped with sensors that measure the cow's health by analyzing her milk. For instance, these sensors could measure electrical conductivity, the number of immune cells, and activity of enzymes in the milk. Different programs have been developed to use this sensor data to alarm the farmer in the case of mastitis. However, less programs have been developed to help the farmer decide what to do when mastitis is found. This thesis investigates new ways in which existing sensor data can be used to help the farmer in the decision making whether to intervene in a case of mastitis or not.

More specifically, paper I investigated the average duration of mastitis that recover based on sensor data. On average, recovering from mastitis takes 3-4 weeks measured by the commonly used mastitis indicators. After this period, it becomes unlikely that a cow will recover and therefore the farmer may want to treat or remove the cow from the herd.

Paper II investigated the losses of milk production for various value levels of three different sensors (the activity of an enzyme, electronic conductivity, and somatic cell count) that are commonly equipped on automatic milking systems. The results showed that, at low sensor levels, almost no milk production losses were observed. The milk production losses would increase substantially after specific higher sensor values. Using these thresholds, farmers can be alerted when the milk yield production decreases substantially and that action may be needed.

In Paper III, we developed a method to forecast whether a case of mastitis would recover, using an advanced forecasting model and sensor data from

the recent past. The results showed that it was possible to forecast the outcome of the disease, whether it would recover or become chronic. This forecast could help the farmer in the decision to intervene in a mastitis case when it becomes clear that a case does not recover on its own.

Paper IV estimated the economic consequences of non-curing or chronic mastitis cases to show their impact on the economic situation of a dairy farm. The impact was approximately 50% of the total cost of mastitis. This shows that handling chronic mastitis well is important, as it would have a large impact on the total cost of mastitis on the farm.

Paper IV also estimated the economic impact of different sensor-based strategies to manage mastitis. It shows which strategy tends to decrease the cost of mastitis and chronic mastitis the most. The model could be used to test a wide range of sensor-based management strategies to select the most promising strategies before trying them in practice.

This thesis lays the groundwork for a sensor-based decision support program, using different scientific methods, that could inform the farmer on chronic mastitis. Such a program could inform the farmer on when intervention is worthwhile based on the expected outcome and the consequences of the intervention.

Populärvetenskaplig sammanfattning

Mastit är en inflammation som kan uppstå i juvret hos mjölkkor och ha en negativ inverkan på djurvälfärden liksom bondens ekonomi genom förlorade intäkter på grund av minskad mjölkproduktion. Automatiska mjölkningssystem, där kon mjölkas utan att någon aktiv arbetsinsats krävs, har funnits på marknaden sedan 1990 talet. Dessa system är utrustade med sensorer som kan mäta flera olika juverhälsoindikatorer genom att analysera mjölken. För att hitta mastiter har olika modeller som kombinerar data från olika sensorer med information om kon studerats. Emellertid finns det i dagsläget få verktyg som hjälper bonden att ta beslut om vad denne ska göra när en mastit väl har upptäckts av systemet. I denna avhandling undersöktes hur sensordata från automatiska mjölkningssystem kan användas för att utveckla nya sensorbaserade verktyg som kan ge stöd för bonden i beslutsfattande och agerande kring kor med mastit.

I den första artikeln undersöktes den genomsnittliga tiden det tar för en juverinflammation att läka ut av sig själv. Kriterierna för självutläkningen baserades på data från sensorer och det visade sig vara en period mellan 3 och 4 veckor. Efter den perioden är det inte troligt att kon blir frisk och bonden måste i stället behandla henne eller slå ut henne.

I den andra artikeln undersöktes hur tre vanligt förekommande sensorer (som mäter enzymaktivitet, elektrisk konduktivitet samt antalet celler i mjölken) i automatiska mjölkningssystem speglar hur mjölkmängden minskar vid en mastit. Resultatet visade att det inte var stora förluster av mjölk vid låga värden för alla tre typer av sensorer. Däremot kunde vi se att mjölkproduktionen minskade i en ökande takt när sensorerna visade värden över en viss nivå. Denna kunskap är användbar för att indikera för bonden när mjölkförlusterna blir stora ock måste åtgärdas

I den tredje artikeln användes sensordata över en längre tid för att skapa avancerade modeller som kunde förutsäga sannolikheten för ett mastitfall skulle läka ut av sig självt. Resultaten visade att det var möjligt att förutse hur ett mastitfall skulle utvecklas, dvs om mastitfallet skulle självläka eller kvarstå och bli ett så kallat kroniskt mastitfall. Modellen kan därför användas för att fatta beslut om när bonden måste göra något för att kon ska bli bra eller ej.

I den fjärde artikeln skattades de ekonomiska konsekvenserna av de kroniska mastitfallen. Dessa kostnader visade sig uppgå till ungefär 50 % av de totala kostnaderna för mastit. Det betyder att det är minst lika viktigt att ta väl hand om kroniska fall eftersom de har en stor effekt på gårdens ekonomi.

Vidare skattades även den ekonomiska effekten av olika åtgärder kring mastit som baseras på information från sensorer. Detta gjordes för att fastställa vilken typ av strategi som är mest lönsam och minskar kostnaden för mastiter såväl som kronisk mastiter mest. Med en sådan modell kan man utvärdera många olika åtgärder och välja den som har störst positiv effekt redan innan man faktiskt gör något.

Den här avhandlingen lägger grunden för att kunna utveckla beslutsmodeller som baseras på information från sensorer, så kallade sensorbaserade beslutsmodeller. Genom metoder baserade på vetenskap, kan bonden få information om vilka mastitfall som är kroniska, vilka som riskerar att bli kroniska samt vilka fall som ger allvarligast konsekvenser med avseende på mjölkproduktion och ekonomi. En sådan modell kan hjälpa bonden att avgöra när det är lönsamt att vidta åtgärder baserat på förväntade effekter och konsekvenser av åtgärderna.

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Johan Hendrikus Bonestroo
Wageningen School of Social Sciences (WASS)
Completed Training and Supervision Plan

Wageningen School of Social Sciences

Name of the learning activity	Department/Institute	Year	ECTS*
A) Project related competences			
A1 Managing a research project			
WASS Introduction Course	WASS	2018	1
Writing the research proposal	WUR	2018 - 2019	6
Scientific writing	Wageningen In'to Languages	2020	1.8
'Milk diversion in automatic milking systems to estimate incidence of mastitis in the absence of treatments records'	NMC, Orlando, USA	2020	1
'Studying the development of somatic cell count after onset of the inflammation using automatic milking system sensor data'	VEEC, Leiden, the Netherlands	2021	1
Effective and efficient communication in academia and beyond	WGS	2022	0.9
Making Impact: Increasing the relevance of research through science-society interaction (in person)	WGS	2022	1
A2 Integrating research in the corresponding discipline			
Applied economic modelling for the veterinary sciences (MOOC)	UU	2018	3
Big data and machine learning	SLU	2019	3

Risk Analysis and Risk Management in Agriculture: Updates on Modelling and Applications	WASS	2021	3
Understanding & Implementing Bayesian Analyses: from Beginnings to Hierarchical Models	SLU	2020	5

B) General research related competences

B1 Placing research in a broader scientific context

Philosophy of science and research ethics	SLU	2019	4
Information retrieval and methods for scientific communication	SLU	2019	3

B2 Placing research in a societal context

Workshop NMC with farmers and veterinarians and industry professionals 1 morning	NMC	2020	1

Career related competences/personal development			
C1 Employing transferable skills in different domains/careers			
Teaching in Higher Education, basic course	SLU	2022	4.5
Presenting with Impact	Wageningen In'to Languages	2020	1
Teaching Big Data for business decisions	WUR	2019 - 2021	2
Teaching Agricultural Business Economics	WUR	2021	2
Total			**44.2**

*One credit according to ECTS is on average equivalent to 28 hours of study load

I

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Progression of different udder inflammation indicators and their episode length after onset of inflammation using automatic milking system sensor data

John Bonestroo,[1,2,3]* Mariska van der Voort,[3] Nils Fall,[2] Henk Hogeveen,[3] Ulf Emanuelson,[2] and Ilka Christine Klaas[1]
[1]DeLaval International AB, Gustaf De Lavals väg 15, 147 21 Tumba, Sweden
[2]Swedish University of Agricultural Sciences, Department of Clinical Sciences, PO Box 7054, SE-750 07 Uppsala, Sweden
[3]Wageningen University and Research, Business Economics Group, Hollandseweg 1, 6706 KN Wageningen, the Netherlands

ABSTRACT

In automatic milking systems (AMS), sensors can measure cow behavior and milk composition at every milking. The aim of this observational study of previously collected data was to gain insight into the differences in dynamics of udder inflammation indicators between cows that recover and those that do not recover after detection of an initial inflammation. Milk diversion (milk separated from the bulk tank and thus indicating farmer intervention), conductivity, and somatic cell count (SCC) data from 4 wk before the initial inflammation to 12 wk after the initial inflammation were used to analyze 2,584 cases of udder inflammation. An udder inflammation case was defined as an initial observation of SCC ≥200,000 cells/mL as well as 1 additional SCC measurement >200,000 cells/mL within 10 d after the initial case, among other requirements. The data originated from 15 AMS herds in 6 countries. Four subsets of cows were created based on whether milk was diverted after the initial inflammation and whether the udder inflammation cases recovered, using a 10-d rolling average SCC threshold of 200,000 cells/mL and checking whether this rolling mean was below the threshold within 90 d after the initial inflammation as the indication of recovery. This formed the following subsets of cow lactations: milk diverted–recovered, milk diverted–not recovered, no milk diverted–not recovered, no milk diverted–recovered. Thresholds of 100,000 SCC/mL and 300,000 SCC/mL for the definition of case and recovery were also applied in a sensitivity analysis but with no substantial difference in results. Linear mixed models were used for each subset to study the variation in SCC (natural logarithm of SCC divided by 1,000) and σ-conductivity (natural logarithm of standard deviation of quarter conductivities). When observing the fraction of cows with SCC <200,000 cells/mL in the recovery subsets, most cows recovered within 20 d after the initial inflammation. In the recovery subsets, both σ-conductivity and SCC stabilized, mostly within 3 to 4 wk after the initial inflammation. σ-Conductivity stabilized above the pre-onset level in all subsets and did not show a clear increase in the no-milk-diverted subgroups, whereas SCC stabilized closer to the pre-onset level. Overall, this study indicated a cutoff point between nonchronic and chronic changes in indicators 3 to 4 wk after the initial inflammation for SCC and σ-conductivity.

Key words: mastitis, recovery, conductivity, somatic cell count

INTRODUCTION

Mastitis or udder inflammation is a common production disease in dairy herds, causing compromised animal welfare and high but widely varying economic losses (Hogeveen et al., 2019). Early detection and proper treatment of mastitis is of benefit in terms of milk yield, quality of milk, and cow health (Milner et al., 1997). Research on using sensors for mastitis detection has gained attention (Hogeveen et al., 2010), although the prediction of disease progression and duration has garnered almost no attention in the literature.

In automatic milking systems (**AMS**), sensors continuously measure cow behavior and milk composition for the detection of mastitis (Hogeveen et al., 2010). Because of the increasing number of sensors available on dairy farms, additional cow information is available on a daily or per milking level. This high frequency of measurement creates many novel opportunities that were not possible until quite recently. For instance, these high frequency data have the potential to routinely establish patterns of an udder inflammation episode much more precisely than measurements on DHI test

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*Corresponding author: John.bonestroo@delaval.com

days. The time from the point of infection to increased SCC is measured in hours rather than months (Burvenich et al., 1994; Shuster et al., 1996; Kruze et al., 2007; Moyes et al., 2009), and frequent measurement of inflammation indicators is therefore a significant improvement. Having udder inflammation indicator data at every milking could be of high potential benefit for farmers who must decide whether and when to intervene. Farmers could base their decisions on the differences between the patterns of a specific udder inflammation episode and typical patterns of recovered udder inflammation cases. However, practical knowledge of inflammation indicator patterns and the typical inflammation indicator episode duration based on sensor data is lacking. Given that the data are readily available, the potential benefits for farmer decision-making could be large because a potential decision-support system can be widely implemented.

Knowledge about the typical duration and trajectory of an udder inflammation recovery has practical implications. The farmer can decide whether or not to cull a cow when it does not recover after the typical duration of an udder inflammation episode. In addition, definitions of subclinical udder inflammation based on monthly DHI data (e.g., chronicity determined by the past 2 monthly SCC values; as used by St. Rose et al., 2003) are of limited value when a farmer obtains data at every milking. Therefore, specific sensor-based definitions are needed for daily decision-making in sensor-based systems.

Conceptually, udder inflammation recovery or nonchronic udder inflammation can be defined as the cow returning to a healthy state after an udder inflammation episode, as operationalized in terms of SCC in the literature (de Haas et al., 2004). Given that definition, chronicity can be defined as the lack of returning to a healthy state within the period in which recovered cows typically do recover. In the past, researchers used monthly or bimonthly DHI data to study udder inflammation recovery or milk yield losses caused by udder inflammation (Jones et al., 1984; de Haas et al., 2004; Hand et al., 2012). This frequency made it difficult to determine temporal patterns. In contrast, sensors in AMS can measure udder inflammation–related inflammatory indicators, such as conductivity, SCC, and lactate dehydrogenase (LDH), and other milking-related variables (e.g., milk yield, blood presence, and milk flow) at each milking. The analysis of temporal patterns can therefore focus on daily patterns of variables. Fogsgaard et al. (2015) looked at the recovery phase of udder inflammation in general and for different pathogens using AMS data. They concluded that udder inflammation has large effects on milking frequency, LDH activity, and milk yield. However, the patterns of conductivity- and SCC-based measures remain to be explored.

The overarching aim of this observational study was to gain insight into the differences in the progression of inflammation indicators after the initial onset of udder inflammation, as indicated by an increase in SCC between cows that recover and cows that do not recover on commercial dairy farms. More specifically, the study explored sensor measurements of SCC and conductivity in terms of episode length (the time until the inflammation indicator stabilizes; i.e., revolves around a constant mean) after the initial onset of udder inflammation, and whether the level after the initial udder inflammation is equal to that before the initial udder inflammation. This knowledge can be used to build new groundwork for the definitions of chronic and nonchronic udder inflammation cases using daily available sensor data.

MATERIALS AND METHODS

Data Collection

Data of 15 AMS herds located in Belgium, Canada, Germany, the Netherlands, Scotland, and Sweden were retrieved from a database of DeLaval International AB (Tumba, Sweden). The data covered a period from January 4, 2016, to March 14, 2019, although not all farms began reporting on January 4, 2016. The herds were selected based on the presence of an AMS (VMS series, DeLaval International AB) to measure conductivity, an Online Cell Counter (**OCC**; DeLaval International AB) to measure SCC, and having documentation on whether milk from individual cows was diverted from the bulk milk tank. Because this was an observational study using previously collected data, we did not have any information on farmers' approach toward milk diversion. Consequently, we could not control for differences in milk diversion strategies or the diagnostic skills to detect and treat inflammation by the farmer. The average daily milk yield per cow varied between 27.9 and 39.9 kg/d between herds, with a mean of 32.2 kg/d (Appendix Table A1).

The following variables were gathered from the AMS management software and included in this study:

- Milk diversion, defined as whether milk on that day did enter the bulk tank to be sold (1) or not (0). Because the farmer diverted milk away from the bulk tank with consumable milk, the diversion is likely due to an intervention in, for example, a mastitis case (Bonestroo et al., 2020). We used milk diversion as a proxy for farmer intervention and to separate cases where farmers have likely detected and intervened in the case.

- Mean conductivity of the milking quarters during milking. This was used to calculate σ-conductivity, defined as the natural log of the standard deviation of the mean quarter conductivities within cow over the total milk produced at each milking.
- SCC, in 1,000 cells/mL as measured by an OCC.
- DIM.
- Parity, with 2 categories: primiparous (0) and multiparous (1).

The natural logarithm transformation was applied to σ-conductivity and SCC to obtain approximately homoscedastic and normally distributed residuals in the linear mixed model.

Preparation of Data

Milking level observations of SCC, σ-conductivity, and the diverted milk indicator were aggregated to a daily level by taking the maximum value of these values on a given day. Every observation below 10 DIM of every lactation was removed. This was an average of DIM removal thresholds used by other authors (Hand et al., 2012; Dalen et al., 2019).

Below, we define the episodes and their requirements. An overview of these definitions can be seen in Figure 1. The start of an udder inflammation episode during lactation was defined as the first observation within lactation of an increased SCC (as measured by OCC) ≥200,000 cells/mL. This start of the udder inflammation episode was defined as the "initial inflammation" in this study. The data from 4 wk before the initial inflammation and as much as was available until 12 wk after the initial inflammation was used for analyses, and this time period was defined as the "udder inflammation episode sequence." Next, a set of requirements was imposed. First, to counter the possibility of a false-positive initial inflammation detection, the initial inflammation needed to be combined with one or more SCC measurements ≥200,000 cells/mL (Dohoo and Leslie, 1991; Smith et al., 2001) within all measurements taken in the 10 d after the initial inflammation. This 10-d window was chosen because we expected that SCC would be measured on multiple days in the first 10 d after the initial inflammation. It is important to note that the initial inflammation (d 0 in our analysis) remained the first day when SCC increased above or equal to 200,000 cells/mL. Farmers can choose the OCC sampling settings; for example, following the default algorithm of the system or requiring daily measurements of each cow. Lactations without an increased SCC within 10 d after the first initial inflammation were completely removed from the data set, because we could not confirm the start of the udder inflammation episode, and possible later episodes may therefore be part of the same unconfirmed episode. Second, lactation cycles were removed when 80 d or fewer with data were recorded within the first 10 to 100 DIM, to ensure that we had records of at least the start of each selected lactation to minimize the risk that the first initial inflammation that occurred earlier in lactation was not in the data sample.

In total, 7,302 of 7,902 lactations had cases of initial inflammation according to the case definition of an

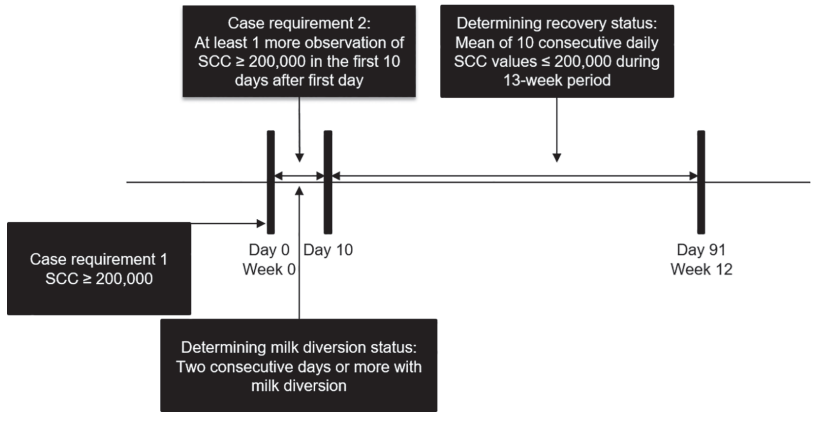

Figure 1. A graphical overview of the case definitions as used in this study. SCC is in cells/mL.

OCC observation ≥200,000 cells/mL. Next, 4,331 of the 7,302 udder inflammation episodes had an additional OCC SCC observation ≥200,000 cells/mL within 10 d after the initial inflammation. Finally, 2,584 udder inflammation episodes of the 4,331 originated from lactation cycles in which more than 80 day-observations during the first 10 to 100 DIM were recorded and thus were retained for analysis.

Because treatment records were not available from all herds, we chose to use milk diversion as an approximation of a farmer intervention related to a mastitis episode (Bonestroo et al., 2020). Milk diversion was defined as diversion of milk for at least 2 consecutive days within the 10 d after the initial inflammation. A period of 2 d was chosen to avoid including automatic milk diversions made by the AMS itself based on sensor thresholds. We assumed that when milk was diverted for at least 2 d within 10 d of the initial inflammation, a cow was confirmed by the farmer as having mastitis and having diverted milk because (a) milk was deemed as not consumable, (b) the cow was treated with antibiotics, or (c) both. If there was no diversion after an episode according to our definition of an udder inflammation episode, we assumed that the farmer did not intervene. Occasionally, some days of some cows with an udder inflammation episode, milking data, and milk diversion data could be missing. The missing values most likely indicated that a cow was milked outside the AMS during an udder inflammation episode. In these cases, we replaced the milk diversion status with the value of the previous day with complete registrations. This was done solely to determine milk diversion status and the imputed version of milk diversion was not further used in the data analysis.

Recovery was defined as a decrease in SCC (measured by the OCC) to a healthy level after an initial increase of SCC to an unhealthy level, as done by de Haas et al. (2004). The threshold between a healthy and an unhealthy level was defined as 200,000 cells/mL (Smith et al., 2001). However, a gray area exists between 100,000 and 199,999 cells/mL, according to the National Mastitis Council (Smith et al., 2001). To evaluate the influence of the chosen threshold, we also tested 100,000 and 300,000 cells/mL in the sensitivity analysis. More specifically, recovery from an udder inflammation episode for an individual cow was defined as the individual cow having a rolling mean SCC <200,000 cells/mL (Smith et al., 2001) for 10 consecutive days within 12 wk after the initial inflammation in the episode sequence. The rolling mean was only calculated when, during the 10-d window, at least 5 d with SCC measurements were available. In the case where fewer than 5 d with SCC measurements were available in the 10-d window, the recovery status was determined as undefined and not regarded as recovered within the 10-d window.

Using the recovery definition and milk diversion status after the initial inflammation, the data set was split into 4 subsets of cows: (1) no diverted milk–no recovery, (2) diverted milk–no recovery, (3) no diverted milk–recovery, and (4) diverted milk–recovery.

Statistical Analysis

The effects of predictor variables on SCC and σ-conductivity were analyzed using a multivariable linear mixed model for each subset with DIM, parity, and weeks since initial inflammation as covariates and a random effect of a specific cow lactation (LactationID) and a random effect of a specific herd (HerdID); HerdID and LactationID indicate the identity of the herd and specific cow lactation number for a specific cow (e.g., cow 12 in its second lactation). Weeks since initial inflammation was a categorical variable with 17 levels (once per week from 4 wk before until 12 wk after the initial inflammation). Parity was a categorical variable coded for primiparous (0) and multiparous cows (1). The analysis used the daily data to estimate the effects of being several weeks before or after initial inflammation to analyze the data (Fogsgaard et al., 2015) to avoid unnecessarily complex models in the number of daily parameters that would need to be estimated.

The models for Y (i.e., SCC or σ-conductivity) took the following form:

$$Y = \text{constant} + \sum_{i=-4}^{12} \left(week\ since\ alert_i\right) + parity + DIM + random\ intercept\ of\ LactationID\ in\ HerdID + random\ intercept\ of\ HerdID,$$

where i is the week number relative to the week in which the initial inflammation was observed. Estimated marginal means were assessed for the weeks since the initial inflammation while evaluating all other covariates at mean level. Different interactions and quadratic terms were tried but they had no substantial effect on the estimated marginal means and were therefore omitted. Random effects of lactation of a specific cow and herd were included in the models as nested random intercepts (LactationID in HerdID and HerdID) and a first-order autoregressive correlation structure was used in line with Fogsgaard et al. (2015). The assumptions of homoscedasticity and normality of residuals were checked using fitted value residual plots and quantile-quantile (qq) plots. The linear mixed models were esti-

mated using nlme 3.1–137 (Pinheiro et al., 2019) using REML in R 3.5.1 (https://www.R-project.org/).

The robustness of the results subject to the exact values for these thresholds described above (Figure 1) was tested in a sensitivity analysis by changing the SCC threshold to 100,000 and 300,000 cells/mL for the recovery definition and case requirements (requirements 1 and 2) in case definition (see Figure 1) separately. We also changed the days in requirement 2 of the case definition from 10 d to 5 and 20 d (see Figure 1). Furthermore, the recovery definition was altered by changing the consecutive days from 10 d to 5 and 20 d during which the rolling mean SCC should be <200,000 cells/mL to determine recovery. The milk diversion status definition was changed from 2 d of milk diversion to 5 consecutive days of milk diversions in the first 10 d after the initial inflammation. Last, we reran the analysis for the 2 herds with the largest number of episodes to explore herd-specific episodes using the default thresholds and compared results with the full data set.

RESULTS

Descriptive Analyses

We analyzed 2,584 episode sequences from 15 herds. Table 1 displays descriptive statistics per herd for cows according to our definition of udder inflammation. The herds varied greatly in terms of proportions of days with diverted milk, duration of milk diversion, mean daily milk yield, median day of initial inflammation, mean SCC, number of lactations, and number of observations. Figure 2 shows the progression of the fraction of cows <200,000 cells/mL per day for the 4 subsets after the start of the episode up to 90 d after the start of the episode. For example, the recovery fraction in Figure 2 at d 10 after the initial inflammation was 68% of the cows in the no diverted milk–recovery subset; that is, cows that had an SCC observation <200,000 cells/mL. As expected, in the nonrecovery subsets (no diverted milk–no recovery and diverted milk–no recovery), the fraction remained low because, per the subset definition given in Material and Methods, the cows in this subset did not have 10 consecutive days with a mean SCC <200,000 cells/mL. In both recovery subsets (no diverted milk–recovery and diverted milk–recovery), the fraction increased substantially during the first 20 to 30 d after the initial inflammation, up to 65 to 80% of the cows in the respective subsets. The recovery fraction of the no diverted milk–recovery subset increased substantially faster than its diverted milk–recovery counterpart. Extra descriptive analysis on general herd information and descriptive analysis per subset are presented in Appendix Tables A1 and A2.

Linear Mixed Model Analyses

Somatic Cell Count. Somatic cell count in the week of the initial inflammation (i.e., week since initial inflammation = 0) was significantly higher than in most other weeks in all subsets (Table 2). However, the subset no diverted milk–no recovery was different, because the mean SCC at the week of the initial inflammation was not significantly higher than the weeks after the week of the initial inflammation. The standard deviation of the cow lactation random effect was larger than the standard deviation of the herd random effect for all SCC subset models, indicating a larger variation in the residuals between cows than between herds.

Figure 3 shows the estimated marginal means of the SCC from 4 wk before the initial inflammation to 12 wk after the initial inflammation. At mean level, the diverted milk–recovery subset had >200,000 cells/mL (natural logarithm of 200 is 5.298) at approximately 1 wk past the initial inflammation whereas that of the no diverted milk–recovery subset was <200,000 cells/mL in the week of the initial inflammation. Moreover, SCC in both the diverted milk–recovery and no diverted milk–recovery subsets stabilized approximately 3 to 4 wk after the initial inflammation at a level slightly higher than that before the initial inflammation. As expected in the diverted milk–no recovery and no diverted milk–no recovery subsets, mean SCC remained stable and was, on average, >200,000 cells/mL after the initial inflammation throughout the 12-wk time window and higher compared with the level before initial inflammation. The average levels of SCC increased before the initial inflammation in all subsets except in the diverted milk–no recovery subset. Last, the average SCC value during the week of the initial inflammation of SCC of the diverted-milk subsets was higher than that of the no-diverted-milk subsets.

σ-Conductivity. Results from the multivariable analysis of σ-conductivity are presented in Table 2. σ-Conductivity in the week of initial inflammation was significantly different from that in most other weeks after the initial inflammation in all subsets, except for several weeks after initial inflammation in the no diverted milk–no recovery subset. However, even in the 3 other subsets, the difference in the later weeks was less substantial than in the SCC subsets due, in part, to an increase in standard errors of the weekly coefficients. The standard deviation of the cow lactation random effect was larger than that of the herd random effect for all subsets, indicating greater variation in the residuals between cows than between herds.

Table 1. Descriptive statistics of the variables under study in the data set of cows with an udder inflammation episode according to our definition of udder inflammation in the selected herds

Herd number	Mean milk diversion proportion[1]	Mean duration of consecutive milk diversion (d)	Mean milk yield (kg/d)	Median DIM of initial inflammation after 10 DIM	Mean σ-conductivity[2]	Mean SCC[3]	Mean days between OCC samples[3]	No. of lactations	No. of milking days
1	0.01	2.79	36.35	23	−1.84	4.58	1.39	152	15,717
2	0.01	6.34	36.56	18	−1.73	4.82	1.50	468	45,611
3	0.04	2.22	38.41	23	−1.74	4.73	2.28	225	22,632
4	0.04	6.10	48.31	13	−1.90	4.79	1.16	176	17,209
5	0.05	6.08	38.34	11	−1.90	4.63	1.33	126	11,878
6	0.06	2.55	35.65	12	−1.66	5.30	1.76	227	21,821
7	0.02	3.37	42.03	15	−1.89	4.80	2.06	450	44,364
8	0.08	3.14	42.72	11	−1.81	4.77	1.46	141	13,448
9	0.03	5.27	36.21	13	−1.92	4.38	1.25	84	8,198
10	0.01	4.72	32.68	23	−1.85	4.74	1.28	164	16,266
11	0.01	6.55	40.93	12	−1.87	4.72	1.59	89	8,337
12	0.04	4.21	34.06	13	−1.68	5.32	3.02	72	6,763
13	0.01	8.29	39.19	12	−2.01	4.02	1.32	98	9,403
14	0.02	6.79	35.49	27	−1.82	4.39	1.21	86	8,808
15	0.03	2.38	36.18	11	−1.82	4.81	1.48	26	2,428
Mean	0.03	4.72	38.21	15.80	−1.83	4.72	1.61	172.27	16,858.87
SD	0.02	1.92	3.98	5.49	0.09	0.32	0.50	129.04	12,698.72
Minimum	0.01	2.22	32.68	11	−2.01	4.02	1.16	26	2,428
Maximum	0.08	8.29	48.31	27	−1.66	5.32	3.02	468	45,611

[1]Diverted milk proportion = number of milkings with diversion/total number of observed milkings.

[2]σ-Conductivity = natural logarithm of the standard deviation between quarter conductivities as measured by the automatic milking system. It can be seen that σ-conductivity is negative as the natural logarithm of a value between 0 and 1 is negative.

[3]SCC = natural logarithm of the SCC as measured by Online Cell Counter (OCC; DeLaval International AB, Tumba, Sweden).

Figure 4 shows the estimated marginal means of σ-conductivity. As expected, the diverted milk–no recovery subset showed stable σ-conductivity values above the level after the initial inflammation, whereas the diverted milk–recovery subset stabilized in 3 to 4 wk after the initial inflammation, but above the estimated level before the initial inflammation. The no diverted milk–recovery and the no diverted milk–no recovery subsets did not show a clear increase in the week of initial inflammation and did not have a clear decrease after the week of initial inflammation. The average σ-conductivity increased before initial inflammation in all 4 subsets. The average σ-conductivity during the week of the initial inflammation of the diverted-milk subsets was higher than that of the no-diverted-milk subsets.

Overall. Somatic cell count and σ-conductivity had similar patterns in the estimated marginal means across subsets. However, in the recovery subsets, SCC stabilized relatively closer to the level before initial inflammation than σ-conductivity. Furthermore, σ-conductivity in the no milk diverted–recovery and the no milk diverted–no recovery subset had a less clear pattern than SCC. The residuals for the 4 subset models for both SCC and σ-conductivity were approximately normally distributed and homoscedastic, although the negative residuals at lower fitted values formed a pattern of diagonal lines in the fitted values residuals plot where no pattern should be present, possibly because of sensor measurement error. We estimate that this concerned approximately 1% of the milking-day observations, assuming that every measurement below ln(50) SCC with a standardized residual of −2 is subject to this measurement error.

In the sensitivity analysis, we applied different SCC thresholds to define initial inflammation and the recovery. We also applied different thresholds for milk diversion duration, the maximum number of days between the initial inflammation and milk diversion, and the maximum number of days between the initial inflammation and second SCC measurement ≥200,000 cells/mL. In the recovery definition, we changed the number of input days to compute the mean. Overall, the results of the sensitivity analysis remained similar to the original results. More specifically, changing the SCC threshold to 100,000 cells/mL (300,000 cells/mL) in the initial inflammation definition resulted in a slightly larger (similar) initial increase in recovery fraction after the initial inflammation. The estimated marginal means of SCC and σ-conductivity showed a slightly lower (higher) peak at wk 0. However, the duration until stabilization remained between 3 and 4 wk. When we changed the SCC threshold to 100,000 cells/mL (300,000 cells/mL) in our recovery definition, it resulted in a higher (similar) fraction of cows below 200,000 cells/mL in the recovery subgroups in the recovery fraction analysis. This change in our recovery definition also resulted in a slightly lower (higher) level at which SCC and σ-conductivity stabilized. However, the duration until stabilization remained between 3 and 4 wk in all plots. Changing the number of consecutive diversion days from 2 to 5 did not substantially change the recovery fraction or the estimated marginal means of SCC and σ-conductivity. We changed the maximum

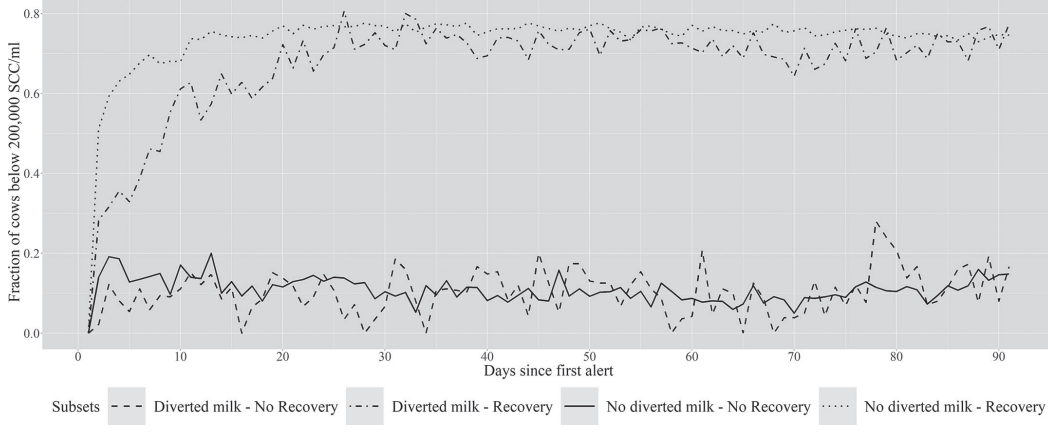

Figure 2. Progression of online SCC after initial inflammation (day = 0, first time in a lactation where SCC ≥200,000 cells/mL) in 4 subsets of cows as the fraction of cows with SCC <200,000 cells/mL relative to all cows in their respective subset from d 0 to 90.

Table 2. Estimated regression coefficients, standard deviation (SD) of the herd, cow, and residual random effects, first-order autocorrelation, and the number of herds, lactations, and observations from the SCC (cells/mL) and σ-conductivity models for 4 subsets of cows[1]

	SCC				σ-Conductivity			
	No recovery		Recovery		No recovery		Recovery	
Item	No diverted milk	Diverted milk	No diverted milk	Diverted milk	No diverted milk	Diverted milk	No diverted milk	Diverted milk
Week relative to first initial inflammation (SCC ≥200,000 cells/mL)								
−4	−2.209***	−2.438***	−1.213***	−2.403***	−0.292***	−0.833***	−0.132***	−0.993***
−3	−1.989***	−2.780***	−1.140***	−2.510***	−0.163***	−0.862***	−0.136***	−0.893***
−2	−1.890***	−2.754***	−1.117***	−2.273***	−0.146***	−0.795***	−0.097***	−0.825***
−1	−1.860***	−2.843***	−1.034***	−1.817***	−0.110***	−0.574***	−0.081***	−0.497***
0	−0.061	−0.341***	−0.415***	−0.615***	0.003	−0.164**	−0.016*	−0.144***
1	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
2	−0.020	−0.521***	−0.539***	−0.902***	0.010	−0.209**	−0.020*	−0.217***
3	−0.029	−0.506***	−0.598***	−1.029***	0.020	−0.166*	−0.032***	−0.225***
4	0.111*	−0.671***	−0.585***	−1.083***	0.029	−0.209*	−0.031**	−0.246***
5	0.181***	−0.657***	−0.563***	−1.014***	0.036	−0.207*	−0.026*	−0.230***
6	0.117*	−0.661***	−0.550***	−0.991***	0.039	−0.182†	−0.025*	−0.238***
7	0.117*	−0.694***	−0.540***	−0.979***	0.052†	−0.223†	−0.024†	−0.196*
8	0.213***	−0.695***	−0.519***	−0.947***	0.040	−0.219†	−0.035*	−0.183*
9	0.253***	−0.754***	−0.512***	−0.846***	0.075*	−0.149	−0.037*	−0.159
10	0.117*	−0.644**	−0.480***	−0.835***	0.005	−0.146	−0.035*	−0.109
11	0.127*	−1.059***	−0.444***	−0.730***	0.039	−0.142	−0.034†	−0.089
12	0.043	−0.909**	−0.419***	−0.717***	0.019	−0.222	−0.037*	−0.078
Other covariates								
DIM	−0.001*	0.004	−0.002***	−0.007**	0.001***	0.002	0.0001	−0.004**
Parity	0.145	0.439†	0.276***	0.566***	0.383***	0.495*	0.201***	0.434***
Constant	6.184***	6.379***	5.000***	5.682***	−1.750***	−1.527***	−1.994***	−1.540***
Random effect SD								
Herd	0.219	0.199	0.251	0.302	0.061	0.0004	0.102	0.045
Lactation	0.496	0.471	0.586	0.551	0.458	0.431	0.414	0.474
Residual	0.819	0.904	0.98	1.059	0.476	0.509	0.477	0.548
Residual AR(1)	0.377	0.35	0.300	0.466	0.447	0.432	0.385	0.504
Observations	14,240	2,617	133,290	11,037	23,766	3,876	184,140	14,430
Number of herds	15	11	15	15	15	11	15	15
Number of lactations	288	49	2,068	179	262	44	1,923	166

[1]Using the recovery definition and milk diversion status after the initial inflammation, the data set was split into 4 subsets of cows: (1) no diverted milk–no recovery, (2) diverted milk–no recovery, (3) no diverted milk–recovery, and (4) diverted milk–recovery.

†, *, **, and *** indicate significance of coefficient at $P = 0.1$, 0.05, 0.01, and 0.001, respectively, against the null hypothesis that the coefficient is equal to 0 and the alternative hypothesis that the coefficient is not equal to 0.

period between the initial inflammation and the second increased SCC observation equal to or higher than 200,000 cells/mL from 10 d to 5 and 20 d, which did not cause substantial differences in the estimated marginal means of SCC or σ-conductivity. However, the recovery fraction of the no diverted milk–recovery subset increased faster during the initial days after the initial inflammation but again plateaued after approximately 3 to 4 wk. Changing the maximum period between the initial inflammation and milk diversion from 10 d to 5 or 20 d did not substantially alter the recovery fraction results or the estimated marginal means of SCC or σ-conductivity. We changed the recovery period over which a SCC mean was computed, from 10 d to 5 and 20 d, which resulted in no substantial differences in the estimated marginal means of SCC or σ-conductivity, although the no-recovery subsets attained a higher recovery fraction when the recovery period was set to 5 d. Two herds with the largest number of selected episodes were also analyzed separately to explore herd-specific episode durations (data not shown). The confidence intervals of the weekly estimates increased substantially and it was hard to determine when the pattern would stabilize because of the limited number of observations. Taking this substantially increased uncertainty into account, we observed that the herd-specific episode durations until stabilization were approximately equal to 3 to 4 wk after the initial inflammation in both herds for SCC; that is, as found in the overall population. However, for the σ-conductivity analysis in 1 of the 2 individual herd data sets, we could not determine the same duration of 3 to 4 wk that we were able to determine in our main results. We observed no decrease of σ-conductivity after the initial inflammation and the confidence interval was very large.

DISCUSSION

In this study, we aimed to gain insight into the differences in udder inflammation indicators after an initial inflammation, as measured by AMS sensors, between cows that recover and cows that do not recover. Because this is one of the first studies to describe the duration of an udder inflammation episode based on

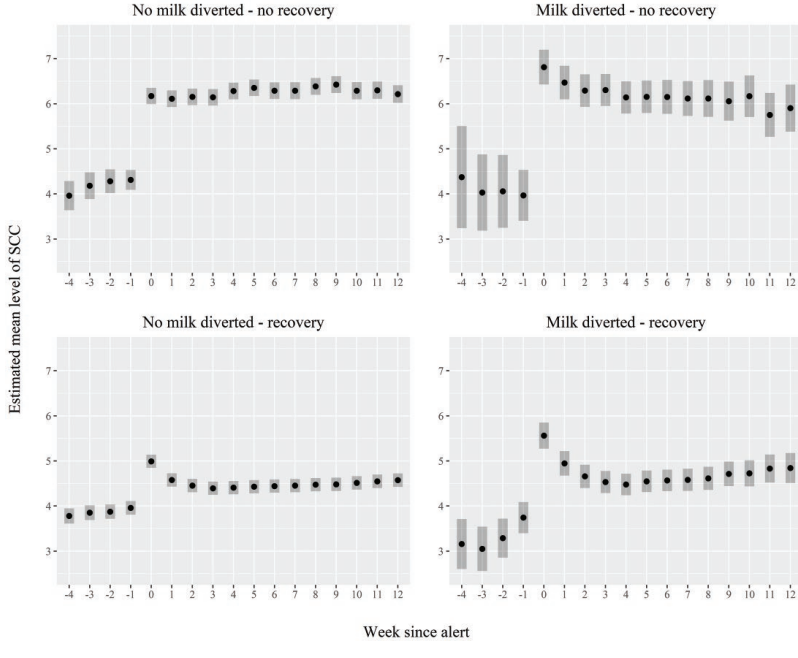

Figure 3. Patterns of SCC measured by online SCC from 4 wk before until 12 wk after the initial inflammation (first time in a lactation where SCC ≥200,000 cells/mL) for 4 subsets of cows using the estimated marginal effects of linear mixed models with 95% CI of the weekly mean.

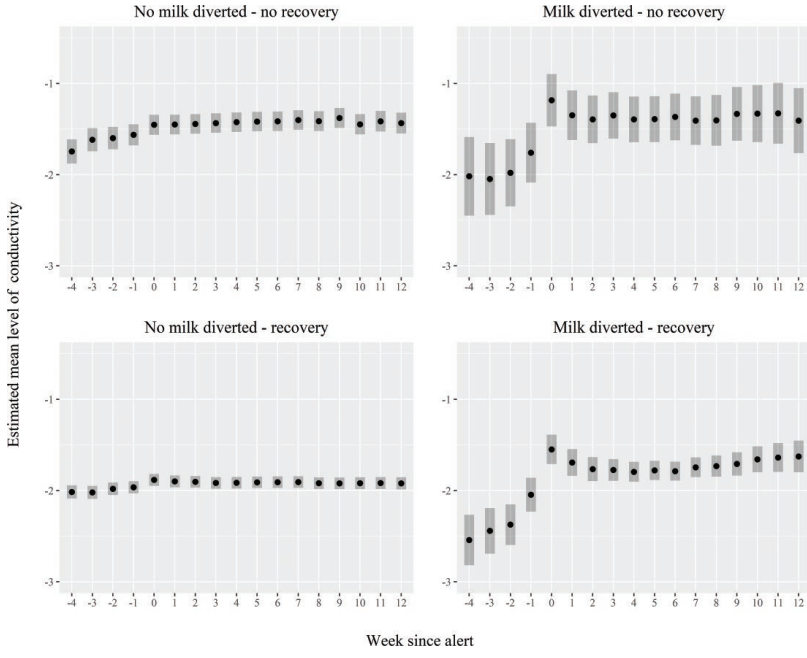

Figure 4. Patterns of σ-conductivity from 4 wk before until 12 wk after the initial inflammation (first time in a lactation where SCC ≥200,000 cells/mL) for 4 subsets of cows using the estimated marginal effects of linear mixed models with 95% CI of the weekly mean. It can be seen that σ-conductivity is negative because the natural logarithm of a value between 0 and 1 is negative.

daily sensor data, there was no standardized manner by which to define recovery or evaluate results. Our first major contribution is to show that it is possible to analyze the dynamics of inflammation indicators and gain insight into these dynamics using routinely available sensor and other data. Because farmers worldwide use similar sensors and management software, this creates interesting research and development opportunities as well as future practical applications. Second, our results showed that the mean of σ-conductivity and SCC stabilized, at most, 3 to 4 wk after the initial inflammation, above the level that occurred before the initial inflammation, depending on the inflammation indicator as SCC stabilized closer to the pre-onset level than did σ-conductivity. However, we also found that there was only a limited increase in σ-conductivity in both no-milk-diverted subsets. This could be due to the SCC-based case definition. Another case definition (that includes conductivity or a conductivity-based measure) would change the pattern in these subsets (data not shown). The observed recovery pattern would, in some cases, depend on the variables used in the case definition. It is important to consider that these are means, and substantial natural variation occurs in SCC and σ-conductivity; we observed a sizable residual variation compared with the size of the variation in residual herd- and cow-effects (Table 2). Nørstebø et al. (2019) argued that the normal variation of the SCC could cause high variability of OCC measurements. We observed that the estimated marginal mean value of σ-conductivity and SCC in the week of the initial inflammation was generally higher for diverted-milk subsets than for no-diverted-milk subsets. This most likely indicates a higher severity of the cases where farmers intervened by diverting the milk.

To date, no definitions of recovered and nonrecovered (i.e., chronic udder inflammation episodes) based on daily AMS measurements are available. Our findings showed distinctive mean patterns for both σ-conductivity and SCC during the course of an udder inflammation episode. Based on these mean SCC and σ-conductivity patterns, we suggest a cutoff point of 3 to 4 wk after initial inflammation to discriminate between chronic and recovered cases of udder inflam-

mation. Pinzón-Sánchez and Ruegg (2011) reported that 58.2% of the cows with clinical mastitis (which is different from our SCC-based case definition) returned to an SCC <200,000 cells/mL within 21 to 55 d after treatment based on DHI SCC, which is within the range of our findings. Somatic cell count and conductivity can be affected by factors other than mastitis. Harmon (1994) indicated that, aside from infection status, parity, stress, age, season, and stage of lactation can affect the variation in SCC. Other factors that may influence conductivity are temperature, stage of lactation, and milk composition (Nielen et al., 1992).

The use of sensors allowed us to study the dynamics of udder inflammation episodes on a large set of cows with daily measures; we analyzed 2,584 episodes almost daily for 90 d after the initial inflammation. In comparison, Francoz et al. (2017) mention 40 experimental treatment trials (out of 41 total trials summarized), studying treatments other than conventional antibiotics, that used a data sample consisting of, at most, 258 cows over, at most, 60 d after an onset of udder inflammation. Without sensor data, and other than carrying out expensive data collection schemes, the dynamics of mastitis could only be studied using DHI data, which has a bimonthly or monthly test frequency. A major disadvantage of using large observational data rather than smaller detailed observational or experimental data is that information on relevant factors may be missing. In our case, these would be data on bacteriology, clinical severity scores (if clinical signs were observed), or farmer criteria for initiating milk diversion and mastitis treatments as several other studies report (see Francoz et al., 2017, for examples). In terms of bacteriology, inflammation patterns can differ between different pathogens (Fogsgaard et al., 2015) or can be more associated with certain pathogens (de Haas et al., 2004) and could be used as the onset of an episode. Moreover, scoring the severity of clinical mastitis, if clinical mastitis was observed, could have given more insights into farmer decision-making and effects of mastitis severity on the progression and chances of recovery. Because farmer criteria for initiating treatments were not available, differences in farmer treatment decision-making (Espetvedt et al., 2013) could have influenced our results. However, the standard deviation in the random herd effect was low compared with that of the random cow lactation effect, indicating limited herd effects on average (e.g., due to a difference in treatment protocol) compared with the cow effect. Nonetheless, we could not completely control for the differences between herds, as we did not have access to the treatment protocols or background information on cases of the farms in our sample. Missing data on important factors is one inherent weakness of analyzing observational data retrospectively. Nevertheless, the extensive usage of observational data sets procured by DHI associations in research has led to insightful results on the general udder health status of herds as well as the association between milk production and SCC in the past (Tyler et al., 1989; Dohoo and Morris, 1993; Hand et al., 2012). Observational data sets can be used to describe general patterns. Therefore, we argue that large data sets with less detailed data can be used to explore and describe general patterns and associations in a larger population, and this type of observational study could be the first step to future research using more detailed but smaller data sets to study these general patterns in more detail.

Mean σ-conductivity stabilized above the level before initial inflammation, whereas mean SCC stabilized close to the level before initial inflammation. Furthermore, mean σ-conductivity showed a less substantial increase in the week of initial inflammation than SCC. Conductivity and SCC measures, as used in this study, are distinct udder inflammation indicators that are medium to highly correlated when transformed appropriately (Nielen et al., 1992). This is caused by both indicators measuring related but distinct processes associated with inflammation (Viguier et al., 2009); SCC in milk is largely the result of an activated immune response when PMN are released into the milk to engulf the pathogen. Then, apoptosis occurs and somatic cells can be found in the milk. Differences in conductivity occur through tissue damage and breaching of the blood–milk barrier. Tissue damage can also be caused by the PMN themselves as well as by the pathogen (Zhao and Lacasse, 2008). We hypothesize that the tissue damage remains even after an episode, causing a lasting weak point in the blood–milk barrier and affecting conductivity. Therefore, it can be expected that mean SCC and σ-conductivity would not share exactly the same pattern.

In this study, we focused on the progression of inflammation indicators after an initial inflammation and we assumed that SCC (measured by OCC) and standard deviation (σ) of conductivity are relevant to measure this progression. We did not aim to assess the diagnostic quality of SCC or conductivity, as this has already been studied (Nielen et al., 1992; Dalen et al., 2019); in addition, the diagnostic quality of OCC SCC was studied by Nørstebø et al. (2019) by comparing it with DHI SCC. They found a mean correlation of 0.82 between SCC measured by the OCC and SCC as measured in a DHI laboratory. Fadul-Pacheco et al. (2018) also reported a high mean correlation coefficient of 0.91, ranging from 0.84 to 0.98 between herds, for OCC measurements and SCC as measured in a DHI laboratory. Interestingly, there were differences in accu-

racy reported for 4 farms, but high agreement between SCC measured by OCC and SCC measured by a DHI laboratory remained. Given that SCC measurements by OCC have similar test performance as DHI SCC, frequent or even daily measurements enable detailed investigations of the onset and course of inflammation indicators compared with monthly or bimonthly DHI SCC measurements. In this study, we developed a specific conductivity measure, standard deviation (σ)-conductivity, which is similar to the variation of quarter conductivities measures as used by Anglart et al. (2020). The diagnostic quality of conductivity was discussed in the meta review of Nielen et al. (1992), in which raw conductivity and relative differences were compared across different studies using different gold standards (SCC-based, California Mastitis Test, Wisconsin Mastitis Test, and IMI). They found that measures using raw conductivity levels had a median specificity of 91% and median sensitivity 57%), whereas measures based on the difference in conductivity between quarters had a median specificity of 96% and median sensitivity of 79%). This supports the use of a conductivity measure that looks at differences between quarters. We chose the natural logarithm of the standard deviation of quarter conductivity specifically because it resulted in homoscedastic and normally distributed residuals in our statistical analyses.

Treatment with antibiotics can have a large effect on the udder inflammation recovery of a cow (Barkema et al., 2006). However, the data set used in this study did not contain detailed treatment records and milk diversions were used as a proxy for farmer intervention because farmers will divert milk when they find the milk unfavorable for sale or consumption. This could be to avoid a high bulk tank SCC, to avoid milk with antibiotic residues in the bulk tank, or milk diversion during an alternative treatment. Milk diversion is relatively untested and might not be as precise as farmer treatment records, which is one limitation of this study. This study is exploratory in nature, utilizing data from a very large number of cows, and we argue therefore that it is useful to use a novel, possibly less precise, but widely available variable in AMS data sets. The threshold was set to 2 consecutive days when milk was diverted within 10 d after the initial inflammation. A typical duration of milk diversions in relation to antibiotic treatment may vary between and within herds due to differences in required milk withdrawal times between different antibiotic drugs and treatment regimens. In an economic simulation study, Steeneveld et al. (2011) used a 5-d milk withdrawal time for the shortest antibiotic treatment course. Using 2 consecutive days of milk diversion rather than 5 consecutive days of milk diversion might be too strict, but it was used to ensure that no treated cases entered the no-diverted-milk subsets. Short milk diversion periods could represent cases in which farmers determined that the milk was not suitable for human consumption, but decided not to treat the animal with antibiotics based on the visual appearance or sensor data. Nevertheless, the milk diversion and initial inflammations were happening in approximately the same time window (Appendix Figure A1).

In our research, we made use of SCC to perform a first screening of a potential onset of an udder inflammation episode, which we required to be followed up by at least one more observation of SCC $\geq$200,000 cells/mL within 10 d of the initial inflammation. Confirmation of an IMI by the presence of an udder pathogen was not feasible in our study because the participating farmers did not regularly collect milk samples for bacteriology. Potentially different farmer thresholds for bacteriology would have resulted in a different frequency and timing of bacteriological testing and thus would have biased our results. Instead, we analyzed the udder inflammation indicators an AMS farmer, or any farmer using the OCC system, would monitor. From a practical point of view, a farmer wants to know how long a case typically takes from the first moment of detection, here by a sensor system, to a possible recovery of udder inflammation. Therefore, our results show the progression of udder inflammation indicators from the onset detected by the system until 90 d after the initial inflammation. Nevertheless, defining onsets of inflammation solely on robotic sensor data is a significant limitation in our study. Future studies with more refined definitions based on nonrobotic reference data such as farmer-confirmed clinical observations or identification of udder pathogens would be useful to add to the results of this study.

A set of thresholds was used on milk diversions, SCC, and number of days after the initial inflammation to define the episode using SCC and the number of consecutive days to determine recovery (Figure 1). The robustness of the results subject to the exact values for these thresholds was tested in a sensitivity analysis. The different set of thresholds did change the number of episodes that would be in each subset. However, our results were mostly robust to different thresholds.

The analysis as applied and the recovery definitions as defined focused on analyzing single episodes of udder inflammation. From the perspective of sensors, it can be hard to distinguish a new flare-up due to a new IMI from recurrent udder inflammation due to a remaining IMI. Therefore, we chose to focus on the first flare-up or episode. Nevertheless, when we changed the recovery duration threshold from 10 d with a mean <200,000 cells/mL to 20 d with a mean <200,000 cells/mL, which

can include the time for extra flare-ups, it did not affect the duration estimate.

During analysis, we encountered a data issue because the negative residuals at lower fitted values formed a pattern of diagonal lines in the fitted values residuals plot where no pattern should be present. A closer investigation indicated that these values would have an improbably low SCC value (e.g., 1,000 cells/mL), and we attribute this to measurement error of the sensor. This behavior of the OCC has been reported in the literature (Nørstebø et al., 2019). Nevertheless, OCC values are highly correlated with DHI SCC observations (Nørstebø et al., 2019) so they can be used as an adequate measurement. Overall, we argue that this had a limited effect due to the relatively small number of these observations compared with the total number of observations.

Practically, farmers could use the knowledge of the typical duration threshold of 3 to 4 wk from an initial inflammation to a healthy state as an indication of when to reevaluate the udder health status of the cow and effects of any interventions. When a cow persists with high SCC or σ-conductivity values for longer than 3 to 4 wk after the initial inflammation, recovery will most likely not occur, at least not within the studied time period of 12 wk after the initial inflammation. Further research is necessary to determine the course of chronic udder inflammation in cows that did not recover during the study period and appropriate follow-up intervention. However, the severity of clinical signs should always be the most important factor in the intervention decision because of animal welfare concerns and may justify recurrent treatment. In addition, IMI status and specific bacteriological information should always be used to determine the type of intervention.

The results of this study represent an important step toward understanding differences in SCC and conductivity from the start of an udder inflammation episode and over the course of 12 wk. By including herds from different geographic regions and countries, we covered a wide range of different management styles represented within AMS herds.

CONCLUSIONS

We identified differences and similarities in mean σ-conductivity and SCC after initial inflammation as defined using SCC. In subsets of cows that recovered, both mean σ-conductivity and SCC stabilized 3 to 4 wk, after the initial inflammation. Therefore, the time point of 3 to 4 wk after the initial inflammation may be regarded as a threshold to discriminate between nonchronic and chronic udder inflammation and to help farmers in their intervention decisions. Nevertheless, differences were observed between mean σ-conductivity and SCC. Duration of an udder inflammation episode and differences in temporal patterns between sensors after initial inflammations are affected by a large range of other cow, pathogen, and treatment factors and need more research. Generally, combining AMS data with milk diversion data seems to be a promising approach to analyze temporal patterns of udder inflammation and to explore differences between nonchronic and chronic udder inflammation.

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ORCIDS

John Bonestroo https://orcid.org/0000-0001-8815-6357
Mariska van der Voort https://orcid.org/0000-0003-0503-259X
Nils Fall https://orcid.org/0000-0001-5597-2358
Henk Hogeveen https://orcid.org/0000-0002-9443-1412
Ulf Emanuelson https://orcid.org/0000-0001-7889-417X
Ilka Christine Klaas https://orcid.org/0000-0002-1397-8505

APPENDIX

Table A1. Descriptive statistics of the variables under study in the data set of both the selected cows with an udder inflammation episode according to our definition of an inflammation case and not-selected cows in the selected herds from January 4, 2016, to March 14, 2019

Herd no.	No. of milking days	Mean diverted milk fraction[1]	Mean milk yield (kg/d)	Mean SCC[2] ($\times 10^3$ cells/mL)	No. of OCC samples	No. of lactations	Mean days between OCC samples	Fraction of primiparous cows
1	98,162	0.01	31.90	184.05	60,326	469	1.63	0.32
2	442,836	0.01	30.84	225.56	242,438	2,121	1.83	0.39
3	159,131	0.03	34.26	188.07	54,328	673	2.93	0.34
4	112,357	0.02	39.85	216.58	83,666	491	1.34	0.28
5	72,465	0.03	32.27	209.22	53,937	376	1.34	0.33
6	130,334	0.05	29.37	333.46	54,012	519	2.41	0.37
7	222,373	0.03	37.38	234.58	85,733	1,323	2.59	0.43
8	88,503	0.05	36.97	210.23	58,050	500	1.52	0.42
9	51,891	0.02	30.14	203.89	38,692	224	1.34	0.42
10	111,233	0.01	30.18	261.84	39,360	440	2.83	0.23
11	59,894	0.01	32.75	174.88	36,513	277	1.64	0.29
12	62,423	0.01	29.61	371.76	9,758	307	6.40	0.29
13	57,207	0.01	32.45	142.02	42,231	289	1.35	0.26
14	57,549	0.01	30.82	134.08	30,076	273	1.91	0.34
15	12,876	0.05	28.84	277.93	9,254	138	1.39	0.35
Mean	115,948.93	0.02	32.51	224.54	59,891.60	561.33	2.16	0.34
SD	103,800.74	0.02	3.27	65.05	55,004.34	513.33	1.30	0.06
Minimum	12,876	0.01	28.84	134.08	9,254	138	1.34	0.23
Maximum	442,836	0.05	39.85	371.76	242,438	2,121	6.40	0.43

[1]Diverted milk fraction = number of diverted observations/total number of observations.

[2]SCC = SCC as measured by Online Cell Counter (OCC; DeLaval International AB, Tumba, Sweden).

Table A2. Descriptive statistics of the variables under study in the data set of the selected cows with an udder inflammation episode according to our definition of an inflammation case per subset

Subset	No. of milking days	Diverted milk fraction[1]	Mean milk yield (kg/d)	Mean SCC[2] ($\times 10^3$ cells/mL)	No. of OCC samples	No. of lactations	Average days between OCC samples	Fraction of primiparous cows
No diverted milk, no recovery	27,003	0.05	40.59	6.25	14,240	288	1.90	0.14
Diverted milk, no recovery	4,615	0.17	39.66	6.41	2,617	49	1.76	0.11
No diverted milk, recovery	204,344	0.02	38.47	4.54	133,290	2,068	1.53	0.35
Diverted milk, recovery	16,921	0.12	37.84	4.81	11,037	179	1.53	0.35

[1]Diverted milk fraction = number of diverted observations/total number of observations; no diverted milk subsets have a nonzero mean value because of milk diversions after the 10 d after the initial inflammation (first time in a lactation where SCC $\geq$200,000 cells/mL).

[2]SCC = SCC as measured by Online Cell Counter (OCC; DeLaval International AB, Tumba, Sweden).

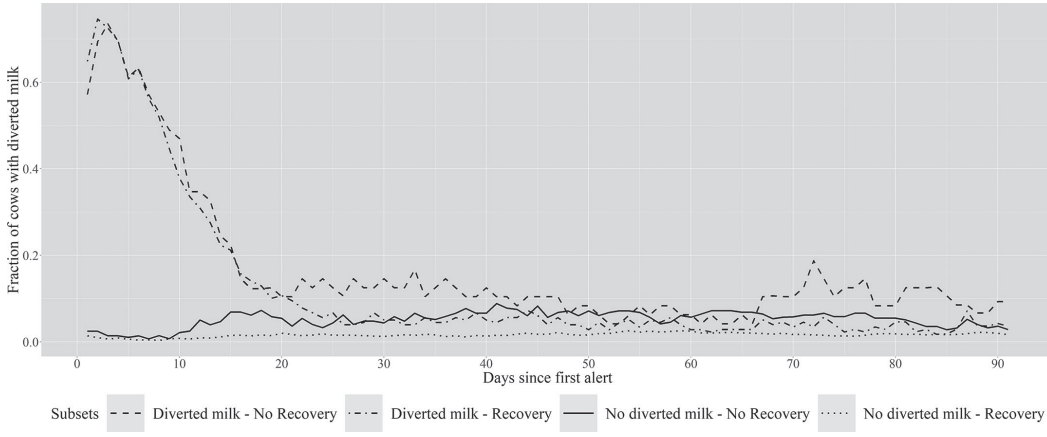

Figure A1. Progression of milk diversions after the initial inflammation (day = 0, first time in a lactation where SCC ≥200,000 cells/mL) in 4 subsets of cows from d 0 to 90. The figure shows the fraction of cows with diverted milk over all recorded cows in the recovery and no recovery subsets after the initial inflammation (first time in a lactation where SCC ≥200,000 cells/mL). In the recovery cases, milk diversions and recoveries were well aligned. This suggests that the farmer is also inclined to think that these cows are recovered and therefore the farmer allows their milk to be placed in the bulk tank again. In the nonrecovery case, the diverted milk fraction showed larger variation than in the recovered subset after 20 d after the initial inflammation. In these cases, the initial inflammation and the apparent intervention were less aligned than in the recovered cases. In the case of recovery as well as nonrecovery, a clear peak of diverted milk fraction could be seen in the first 20 d after the initial inflammation. This also makes sense as the sum of days of antibiotic treatments and the subsequent necessary period of milk diversion usually last between 5 and 10 d. Some interventions may have been started later, which could prolong the period of increased diverted milk fraction to 20 d.

II

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Estimating the nonlinear association of online somatic cell count, lactate dehydrogenase, and electrical conductivity with milk yield

John Bonestroo,[1,2,3]* Mariska van der Voort,[2] Nils Fall,[2] Ulf Emanuelson,[2] Ilka Christine Klaas,[1] and Henk Hogeveen[3]
[1]DeLaval International AB, Gustaf De Lavals väg 15, 147 21 Tumba, Sweden
[2]Swedish University of Agricultural Sciences, Department of Clinical Sciences, PO Box 7054, SE-750 07 Uppsala, Sweden
[3]Wageningen University and Research, Business Economics Group, Hollandseweg 1, 6706 KN Wageningen, the Netherlands

ABSTRACT

Reduction of milk yield is one of the principal components in the cost of mastitis. However, past research into the association between milk yield and mastitis indicators is limited. Past research has not been based on online or in-line daily measurements and has not fully explored nonlinearity and the thresholds at which milk yield starts to decrease. In dairy herds with automated milking systems equipped with sensors, mastitis indicators of individual cows are measured on an intraday frequency, which provides unprecedented avenues to explore such effects in detail. The aim of this observational study was primarily to investigate the nonlinear associations of lactate dehydrogenase (LDH), electrical conductivity (EC), and somatic cell count (SCC) with milk yield at various stages of lactation, parity, and mastitis chronicity status (i.e., whether the cow had SCC ≥200,000 SCC/mL for the last 28 d). We also investigated thresholds at which mastitis indicators (LDH, EC, and SCC) started to be negatively associated with milk yield. We used data from 21 automated milking system herds measuring EC and online SCC. Of these herds, 7 of the 21 additionally measured online LDH. We operationalized milk yield as milk synthesis rate in kilograms per hour. Applying a generalized additive model, we estimated the milk synthesis rate as a function of the 3 mastitis indicators for 3 different subgroups based on parity, stage of lactation, and mastitis chronicity. Partial dependence plots of the mastitis indicators were used to evaluate the milk synthesis rate to study if the milk synthesis rate was associated with mastitis indicators at a specific level. Results showed that milk synthesis rate decreased with increasing SCC, LDH, and EC, but in a nonlinear fashion. The thresholds at which milk synthesis rate started to decrease were 2.5 LnSCC (12,000 SCC/mL) to 3.75 LnSCC (43,000 SCC/mL), 0 to 1 LnLDH (1−2.7 U/L), and 5.0 to 6.0 mS/cm for EC. Additionally, another substantial decrease of milk synthesis rate was observed at thresholds of 5.625 LnSCC (277,000 SCC/mL) and 3 LnLDH (20 LDH U/L) but not for EC. Having chronic mastitis decreased milk synthesis rate in all models. The identified nonlinearities between mastitis indicators and milk synthesis rate should be incorporated in statistical models for more accurate estimations of milk loss due to mastitis.

Key words: mastitis, correlation, generalized additive model, milk loss

INTRODUCTION

Mastitis is one of the most important diseases on commercial dairy farms, and the costliest consequence of it is loss of milk production (Hogeveen et al., 2019), both in subclinical (72% of the subclinical mastitis cost) as well as in clinical mastitis cases (48% of the clinical mastitis cost; Aghamohammadi et al., 2018). To support farmer decision-making regarding udder health, insight into the milk production losses due to mastitis is important. Milk production losses due to subclinical mastitis can be estimated and linked to the level of a specific mastitis indicator (e.g., milk loss of 1 kg/d with 200,000 SCC/mL).

Many milking systems have electrical conductivity (**EC**) sensors and could be equipped with a range of sensors that measure mastitis indicators of individual cows on an intraday frequency, such as SCC and lactate dehydrogenase (**LDH**). The higher the mastitis indicator, the more severe the inflammation is in the udder, resulting in a larger milk yield loss (Hortet et al., 1999; Hagnestam-Nielsen et al., 2009).

Somatic cell count is the most widely studied mastitis indicator for estimating losses of milk yield. Measuring SCC in DHI tests or in an experimental setting showed that milk yield is negatively associated with increasing SCC with greater losses at higher SCC (Jones et

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*Corresponding author: John.bonestroo@delaval.com

al., 1984; Hortet et al., 1999; Hagnestam-Nielsen et al., 2009). Previous studies found or suggested a negative effect on milk yield at thresholds of 148,000 SCC/mL (Tyler et al., 1989), 100,000 SCC/mL (Halasa et al., 2009), 50,000 SCC/mL (Hortet and Seegers, 1998), 7,400 SCC/mL (Dürr et al., 2008), and 12,400 SCC/mL (Gonçalves et al., 2018). A significantly reduced milk yield due to subclinical chronic mastitis was found by Hadrich et al. (2018).

Electrical conductivity can also be used as an indicator of inflammation of the mammary gland (International Dairy Federation, 2011). A few studies found a negative association between EC and milk production or milk loss (Oshima et al., 1990; Nielen et al., 1993). Similar to EC, LDH is also less well studied in relation to milk yield. Nyman et al. (2014) found that milk yield was negatively associated with LDH, analyzed using milk samples in a laboratory, together with other cow factors (such as DIM and parity).

Although the studies mentioned used a multitude of different approaches and data collection protocols, they did not use data from online sensors of LDH and SCC that are used in commercial dairy farms. Moreover, all these studies assume a form of relationship between milk yield and the mastitis indicator beforehand using linear models (Hagnestam-Nielsen and Østergaard, 2009), logarithmic transformations (Green et al., 2006; Dürr et al., 2008; Hagnestam-Nielsen and Østergaard, 2009), or a combination of linear segments (Dürr et al., 2008; Gonçalves et al., 2018). However, the exact form, which could be a combination of effects (e.g., linear, exponential, and cyclical), is less studied. By choosing a functional form beforehand, the researcher might be limited by the inflexibility of the chosen parametric functional form to model these effects. In addition to the functional form, the threshold of SCC, LDH, and EC, at which a negative association with milk yield can be seen, has been studied to a limited extent. In the case of SCC, various thresholds have been suggested (Tyler et al., 1989; Hortet et al., 1999; Dürr et al., 2008; Halasa et al., 2009; Gonçalves et al., 2018), whereas EC and LDH thresholds based on milk loss have not been studied.

The primary aim of this observational study was to investigate the nonlinear associations of LDH, EC, and SCC with milk yield at differing stages of lactation, parity, and chronicity status during cow lactation. Chronicity status in this study is defined as a mean increase of SCC above 200,000 SCC/mL for at least 28 d. A second aim was to investigate thresholds at which mastitis indicators start to be negatively associated with milk yield. To achieve these aims, we applied a generalized additive model on milk yield, LDH, SCC, EC, and DIM to estimate milk synthesis rate over cow lactation cycles, and to assess the association between these mastitis indicators and milk synthesis rate.

MATERIALS AND METHODS

Herd Selection and Data

Data were available in a central DeLaval database from 21 dairy herds from Canada, the Netherlands, Finland, and Sweden with automated milking systems (**AMS**; VMS series, DeLaval International AB) that measured EC and were equipped with an online cell counter (**OCC**; DeLaval International AB) to measure SCC. Farms were selected from the database on the presence of an OCC. Of these 21 herds with OCC, 7 also were equipped with a DeLaval Herd Navigator (DeLaval International AB) to measure LDH. Data were retrieved from a database of DeLaval International AB for the period from January 2017 to April 2020. The majority of the herds kept Holstein cows. The number of lactating cows ranged from 66 to 603 cows, with a mean of 194 cows. The 305-d herd average milk yield ranged from 5,712 to 11,979 kg, with an average of 9,870 kg. These data were reported per milking and included the herd identification, cow identification, DIM, time of milking, milk yield, SCC, LDH, and the EC. For SCC and LDH, a sampling algorithm based on the risk of mastitis determined when a sample was taken (Mazeris, 2010). We could not check the setting of the algorithm in the farms. However, we did observe fewer samples of SCC and LDH than number of milkings in the analyzed data set.

Data Cleaning and Preparation

As a first step in the analyses, several data cleaning and data preparation activities were undertaken. All cow lactations had missing data as not all mastitis indicators were measured at every milking. The raw data set consisted of 5,990,883 milkings from 6,372 cows. Milkings outside the range of 5 to 305 DIM were discarded (removed 735,977 milkings). Furthermore, quarter EC values outside of the range from 3 to 10 mS/cm were set to "not available" (in 53,015 milkings) as done by Anglart et al. (2020). To ensure that the decreases in milk yield were not due to teat blinding (not milking 1 or more udder quarters during lactation), milkings with quarter milk yields equal to 0 were removed (removed 640,958 milkings). We used milk synthesis rate (see below), assuming an approximately linear relationship between time interval and milk yield. This was not the case for short- and long-time intervals (Hogeveen et al., 2001); therefore, we removed them. As such, records where the time interval between the current milking

and the previous milking was outside of the range of 4 to 24 h were discarded (removed 66,044 milkings). Records on days where the number of milkings for a cow was equal or greater than 5 were discarded (removed 11,577 milkings). This resulted in a data set of 4,536,637 milkings from 7,352 lactations from 5,805 cows from 21 herds.

A set of variables was created to facilitate statistical analysis. We used milk synthesis rate (kg/h) as the dependent variable. Each interval between milkings is different in AMS farms, leading to a large variation in time intervals between milkings (Hogeveen et al., 2001). Therefore, to obtain a comparable milk yield–based measure, we divided the milk yield (in kg/milking) by the time interval (in h) between milkings to obtain milk synthesis rate. We used online SCC and LDH as independent variables. These 2 variables were transformed using the natural logarithm (LnSCC and LnLDH). Furthermore, we used the mean EC of the 4 quarters as the third independent variable (mean EC). Mean EC was chosen to compare the milk loss results for LDH and SCC, as it was a cow-level indicator, similar to SCC and LDH. In addition, the subgroup variable "chronicity status" was created to represent whether the cow was chronic or not. A milking day observation was labeled as chronic if a cow had weekly SCC geometric averages equal or higher than 200,000 cells/mL for a period of 4 consecutive weeks or more before the current milking day (Bonestroo et al., 2021) based on available SCC samples. Last, we also created a cow lactation variable (CowLactation) that combined the unique animal identification number with the parity to identify unique cow lactations.

We aggregated the multiple individual milkings on a given day by using the maximum daily values of LnSCC, LnLDH, mean EC, and averaged the milk synthesis rate (reducing the data from 4,536,637 milkings to 1,687,508 milking days). The daily maximum value was used to capture the severity of the increase. When some values were missing for specific milkings but not for all milkings on specific days, these values were ignored in determining the maximum. When there was no observation of the mastitis indicator at all during a day, no daily maximum value of that day was given. Lactations with less than 100 SCC day observations or on average 1 SCC sample per 3 d (to allow determination of the chronicity status based on SCC throughout the lactation) were discarded. This requirement allowed us to define chronic observations anywhere in the lactation. We chose 100 d as a threshold because a lower value (e.g., 29 d) would only let us define observations coming from chronic cows very sparingly, whereas using a requirement of higher number of samples limited the data set to such a substantial amount that we had little data left. Lactations with less than 100 d of observations of a specific mastitis indicator were also discarded in the data set for that indicator-specific model. For each of the 3 mastitis indicators, a separate data set was created. Because not all mastitis indicators were always reported, these 3 data sets differed in number of observations. The selection steps reduced the data further from 1,687,508 milking days to 788,572 milking days of the SCC data set (4,516 lactations and 3,352 cows and 21 herds), 179,335 milking days of the LDH data set (1,394 lactations and 1,116 cows and 7 herds), and 1,146,320 milking days of the mean EC data set (4,515 lactations and 3,350 cows and 21 herds).

To analyze the association between milk synthesis rate and mastitis indicators for different levels of parity, DIM, and chronicity, 3 subgroups were created and analyzed separately. The first subgroup was formed according to 3 DIM levels (5–28, 29–60, and 61–305 DIM) as multiple authors have found differences in milk loss between stages of lactation (Hagnestam-Nielsen et al., 2009; Gonçalves et al., 2018). These cut-offs were determined by selecting the median DIM where the day-to-day change in milk synthesis rate was maximal (28 DIM) and where the milk synthesis rate peaked (60 DIM) in our data set. The second subgroup was based on parity (first lactation, second lactation, and third or more lactation) as it can be expected that multiparous cows, which give more milk, will have a higher milk loss when subclinical mastitis occurs (Dürr et al., 2008; Hagnestam-Nielsen et al., 2009; Gonçalves et al., 2018). The last subgroup was formed according to chronicity (nonchronic and chronic mastitis) as cows with chronic mastitis tend to have higher milk losses (Hadrich et al., 2018). The differences in milk synthesis loss in the various levels of parity, stage of lactation, and chronicity were studied separately using separate regression models, as discussed in the next section.

Statistical Analysis

We applied a generalized additive model using the R package mgcv (Wood, 2012) in R 3.6.1 (https://www.r-project.org/) to model milk synthesis rate per hour. Milk synthesis rate was estimated as a function of the mastitis indicator and DIM, for each subgroup, respectively (Eq. 1, 2, and 3). The DIM and CowLactation were treated as confounders. Depending on the subgroup that was analyzed, the subgroup value in these equations can take the form of the parity, stage of lactation, or chronicity status. A generalized additive model is an extension of a general linear model where the dependent variable can depend linearly on unknown smoothing functions in combination with normal regression coefficients and random effects (as

used in the general linear mixed model). The smooth functions can be fitted with data and can have any form (e.g., linear, quadratic, plateauing, or a combination of them). The function form does not have to be predefined, allowing a very flexible estimation of the association between a mastitis indicator and milk synthesis rate. Last, we included a random effect of each individual cow lactation (random cow lactation effect) using the CowLactation variable to control for nonindependence of observations. In generalized additive models, different link functions can be used to model the relation between the dependent variable and the independent variables, as it is an extended general linear model. Milk synthesis rate was assumed to have a scaled-t distribution rather than a normal Gaussian distribution because it was expected that milk synthesis rate would have more extreme observations than a normal distribution. We used the following models:

$$\begin{aligned}\text{Milk synthesis rate} &= \text{intercept} + \text{subgroup intercept} \\ &+ f_{LnSCC}(\text{LnSCC}) \times \text{subgroup} + f_{DIM}(\text{DIM}) \\ &\times \text{subgroup} + \text{random cow lactation effect}, \quad [1]\end{aligned}$$

$$\begin{aligned}\text{Milk synthesis rate} &= \text{intercept} + \text{subgroup intercept} \\ &+ f_{LnLDH}(\text{LnLDH}) \times \text{subgroup} + f_{DIM}(\text{DIM}) \\ &\times \text{subgroup} + \text{random cow lactation effect}, \quad [2]\end{aligned}$$

$$\begin{aligned}\text{Milk synthesis rate} &= \text{intercept} + \text{subgroup intercept} \\ &+ f_{Mean\ EC}(\text{mean EC}) \times \text{subgroup} + f_{DIM}(\text{DIM}) \\ &\times \text{subgroup} + \text{random cow lactation effect}, \quad [3]\end{aligned}$$

where f_{DIM} is a nonlinear smoothing function modeling the milk synthesis rate over the lactation cycle with a cubic spline basis that was estimated separately for every subgroup. The f_{DIM} was not plotted in the results for brevity, but it takes the form similar to a Wood lactation curve as found in literature (Wood, 1967), and where f_{LnSCC}, f_{LnLDH}, and $f_{Mean\ EC}$ are a nonlinear smoothing function modeling the association between LnSCC, LnLDH, and mean EC with milk synthesis rate. To enable the analysis, we used a baseline where the mastitis indicators were not associated with decreases in milk synthesis rate. As such, this study assumed, before the analysis, that a level of 1,000 SCC/mL, 1 U/L of LDH, and 4 mS/cm mean EC would have no effect on milk synthesis rate. These levels were close to the minimally observable levels, and were chosen due to the low thresholds found and proposed for SCC (Dürr et al., 2008; Gonçalves et al., 2018). These assumptions were needed to estimate f_{LnSCC}, f_{LnLDH}, and $f_{Mean\ EC}$. These functions are also nonlinear smoothing functions with a cubic spline basis. We used the BAM function, which is a generalized additive model with discretization of covariate values that makes it more time and memory efficient when having large data sets (Wood, 2012, 2017). Each of the 3 models (Eq. 1, 2, and 3) were estimated separately for each subgroup (parity, stage of lactation, and chronicity), thus leading to the fitting of 9 models in total (3 mastitis indicators times 3 subgroup variables).

To visualize the associations, we plotted f_{LnSCC}, f_{LnLDH}, and $f_{Mean\ EC}$ for each mastitis indicator and each of the subgroups. The value of the mastitis indicator, at which it started to be negatively associated with milk synthesis rate, was identified as a threshold. This point was found by determining the maximum positive milk synthesis rate difference in the partial plot (the highest point in the plot) and was highlighted in the partial effect plots. Further points of potential substantial decreases in milk synthesis rate start, after this initial threshold, will be described by how the rate of the decrease changes abruptly (e.g., whether the line starts to decrease considerably more).

The residuals of all models were checked for normality, homoscedasticity, and autocorrelation using qq-plots, fitted values-residual plots, and autocorrelation plots. During the analysis, we detected substantial autocorrelation for all models. The autocorrelation problem was solved by adapting the model. The BAM function used in the mgcv library (Wood, 2012) does not allow to estimate a first order autoregressive (**AR1**) residual structure, but it does allow for a predefined AR1 parameter. Consequently, we allowed an AR1 residual structure by first estimating a model without an AR1 parameter, then estimating the residual autocorrelation at the first lag, and inserting that value as the AR1 parameter when fitting the final model using autocorrelation functions of the R package itsadug (Van Rij et al., 2017). The inclusion of the AR1 structure reduced the autocorrelation problem to an insubstantial level.

RESULTS

Descriptive Statistics

The number of AMS per herd ranged from 1 to 9 with a mean of 2.7. After the data selection process, the number of cows analyzed in each herd varied with a mean of 159, a minimum of 22, and a maximum of 512. Two herds had an especially small number of lactations because we required 100 SCC-day observations per lactation for all lactations, SCC were not sampled

every day, and only data on completed lactations from mid-2018 were available. The mean parity across herds was 2.42 with a standard deviation of 0.36, a herd parity mean minimum of 1.63, and a herd parity mean maximum of 3.22. The mean milk synthesis rate was 1.47 kg/h across herds with a standard deviation of 0.17, with a herd mean minimum of 0.99 and a herd mean maximum of 1.74.

In the SCC data set, the mean LnSCC across herds was 4.39 (80,640 SCC/mL) with a standard deviation of 0.45, a herd mean minimum of 3.58 (35,873 SCC/mL), and a herd mean maximum of 5.30 (200,337 SCC/mL). In the LDH data set, the mean LnLDH across herd means was 3.07 (21.54 U/L) with a standard deviation of 0.24, a herd mean minimum of 2.83 (16.95 U/L), and a herd mean maximum of 3.54 (34.47 U/L). In the EC data set, the mean EC across herds was 4.66 mS/cm with a standard deviation of 0.22, a herd mean minimum of 4.28 mS/cm, and a herd mean maximum of 5.09 mS/cm.

Generalized Additive Model Analyses

SCC Results. Figure 1 provides the nonlinear association between LnSCC and milk synthesis (f_{LnSCC}) and the frequency of LnSCC observations for different parity, stage of lactation, and chronicity classes (Figure 1–C). Table 1 summarizes the results of the regression models. The milk synthesis rate was negatively associated with LnSCC over a specific threshold. The large dot in Figure 1 marks the point on the line where milk synthesis rate started to decrease, and thereby milk losses occurred. For most cases, this threshold was approximately between 2.5 LnSCC (12,000 SCC/mL) and 3.75 LnSCC (43,000 SCC/mL), whereas occasional lower and higher thresholds were found in the analysis depending on subgroup. Moreover, the milk synthesis rate started to decrease even further a second time when LnSCC increased, and at an increasing speed and nonlinearly. This occurred approximately after 5.625 LnSCC/mL (~277,000 SCC/mL) for all subgroups.

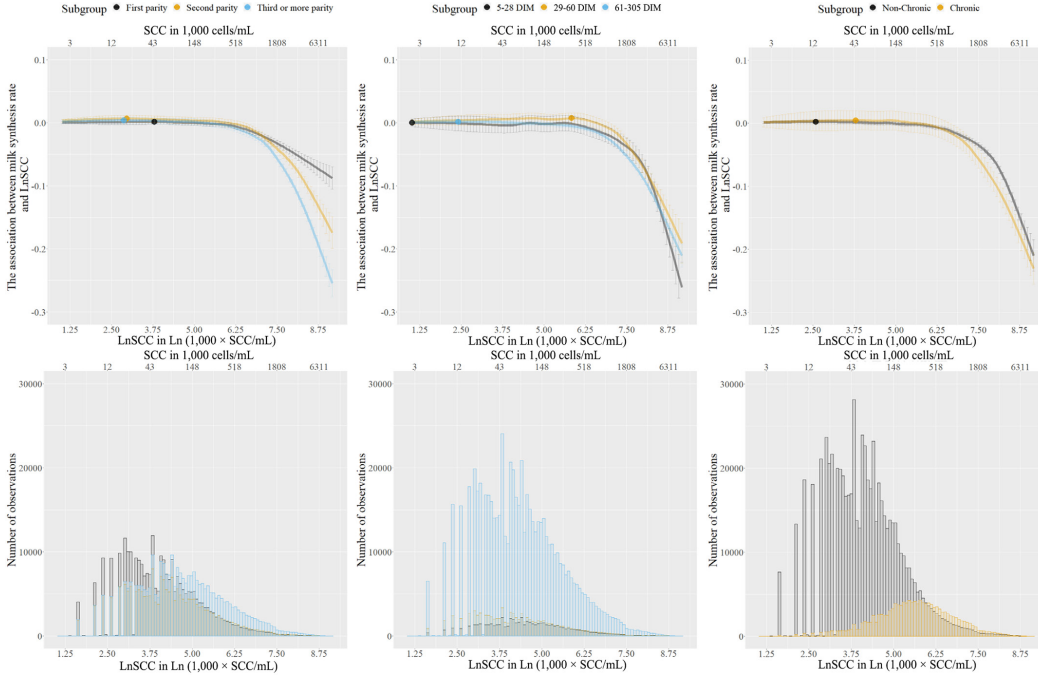

Figure 1. Estimated associations between milk synthesis rate and LnSCC and number of observations for parity, stage of lactation, and chronicity subgroups. The dots indicate that the start of milk synthesis rate decreases, and thereby milk losses increase from that point.

Table 1. Regression summary of somatic cell count analysis[1]

Item	Parity groups model	Coefficient (SE)	Chronicity model	Coefficient (SE)	Stage of lactation model	Coefficient (SE)
Parametric terms						
	Intercept	1.27 (0.01)***	Intercept	1.48 (0.005)***	Intercept	0.99 (5.84)
	Intercept second parity	0.27 (0.01)***	Intercept chronicity	−0.04 (0.01)***	Intercept of DIM 29–60	0.68 (5.86)
	Intercept ≥third parity	0.35 (0.01)***			Intercept of DIM 61–305	0.52 (5.84)
Smoothing terms		EDF (Ref. df)		EDF (Ref. df)		EDF (Ref. df)
	DIM of first parity	32.66 (36.87)[2]***	DIM of nonchronic	36.35 (38.60)***	DIM of 5–28	3.94 (3.99)***
	DIM of second parity	33.11 (37.16)***	DIM of chronic	17.14 (21.67)***	DIM of 29–60	3.95 (4.58)***
	DIM of ≥third parity	34.68 (37.97)***			DIM of 61–305	9.59 (12.26)***
	LnSCC of first parity	7.34 (8.61)***	LnSCC of nonchronic	12.97 (14.58)***	LnSCC of 5–28 DIM	9.71 (11.36)***
	LnSCC of second parity	10.02 (11.56)***	LnSCC of chronic	11.86 (13.82)***	LnSCC of 29–60 DIM	9.71 (11.36)***
	LnSCC of ≥third parity	12.26 (13.79)***			LnSCC of 61–305 DIM	12.26 (14.01)***
Adjusted R^2		0.81		0.78		0.78

[1]EDF = estimated degrees of freedom; Ref. df = reference degrees of freedom.
[2]Significance of smoothing terms can be determined by a method explained in detail in Wood (2013).
***$P < 0.0001$.

Some differences in thresholds and the steepness of the decrease in milk synthesis rate between subgroups were seen. These differences in thresholds were caused by minor differences in the LnSCC and milk synthesis rate association on an overall approximately flat line on the lower levels of LnSCC. Therefore, the differences between thresholds should be interpreted carefully. In Figure 1A, the decrease in milk synthesis rate was steeper at higher levels of parity (indicating an increasing decrease in milk synthesis rate in older cows) of LnSCC for the multiparous subgroups. Moreover, the parity subgroup differences in intercept between first-parity cows and second-parity cows and first-parity cows and ≥third-parity cows were 0.27, with a standard error of 0.01, and 0.35, with a standard error of 0.01, respectively ($P < 0.01$). Regardless of LnSCC, cows with a higher parity tended to produce more milk. In Figure 1B, no clear difference in milk synthesis rate in the stage of lactation subgroups could be seen, and the difference in intercept between 5 to 28, 29 to 60, and 61 to 305 DIM subgroups was also not significant ($P > 0.05$). In Figure 1C, the chronic subgroups had approximately the same form; the chronic group was steeper in its decrease and was lower than its nonchronic counterpart. The difference in intercepts between the chronic and nonchronic subgroups, indicating long-term effects on milk synthesis rate, was −0.04 with a standard error of 0.01 ($P < 0.01$).

LDH Results. Figure 2 provides the nonlinear association between LnLDH and milk synthesis rate (f_{LnLDH}) and the frequency of LnLDH observations for different parity, stage of lactation, and chronicity classes (Figure 2A–C). Table 2 summarizes the results of the regression models. The dot in Figure 2 marks the point where milk synthesis rate started to decrease, and thereby milk losses increased when LnLDH increased. It can be seen from the results that LnLDH was negatively associated with milk synthesis rate after the threshold for all parity, stage of lactation, and chronicity subgroups. The thresholds ranged from approximately 0 to 3 LnLDH (1–20 U/L) for all subgroups. Despite the similarity in the general form and level of the smoothing function, the differences in thresholds were large. The differences in thresholds seemed to be caused by minor differences in the shape of the association between LnLDH and milk synthesis rate between the subgroups. In other words, the difference in milk loss between the thresholds was limited. Nevertheless, the milk synthesis rate decreased noticeably more after approximately 3 LnLDH (20 U/L) in all subgroups.

Several dissimilarities in thresholds and the steepness of the decrease in milk synthesis rate between subgroups were seen. In Figure 2A, multiparous cows showed a larger decrease in milk synthesis rate as-

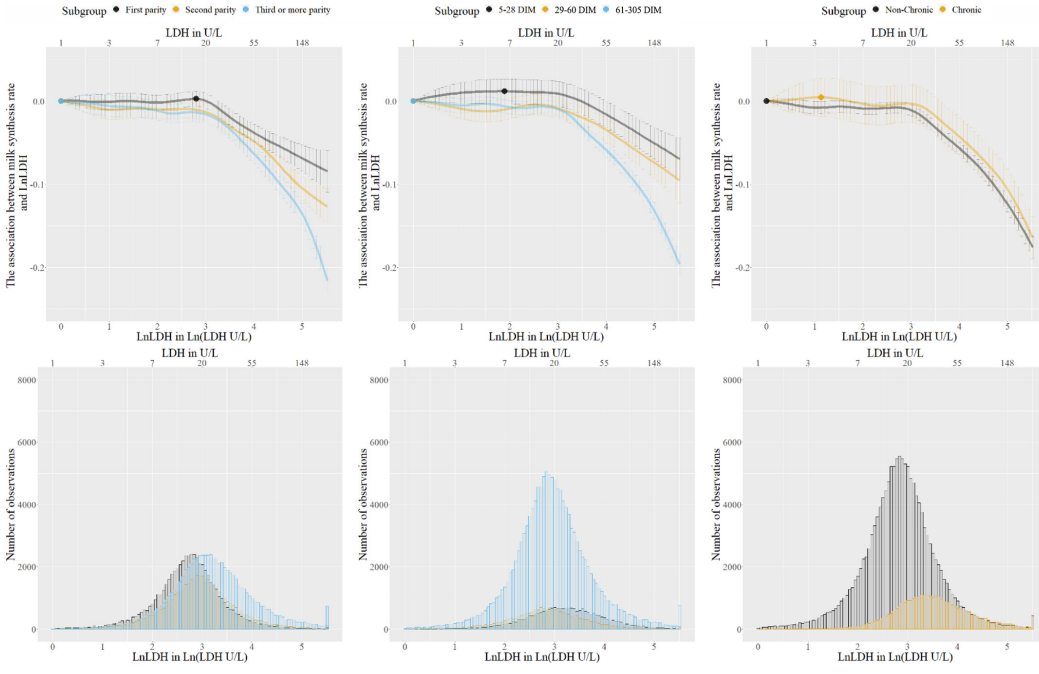

Figure 2. Estimated associations between milk synthesis rate and LnLDH and number of observations for parity, stage of lactation, and chronicity subgroups. The dots indicate that the start of milk synthesis rate decreases, and thereby milk losses increase from that point. Occasionally, the orange dot is covered by the blue dot in the parity and the stage of lactation subgroups. LDH = lactate dehydrogenase.

sociated with higher LnLDH than primiparous cows. Even more, the parity subgroup differences in intercept between first-parity cows and second-parity cows and first-parity cows and ≥third-parity cows were 0.27 with a standard error of 0.01 and 0.36 with a standard error of 0.02 ($P < 0.01$), respectively. Regardless of LnLDH, cows with a higher parity produced significantly more milk. In Figure 2B, 61 to 305 DIM observations showed a larger decrease in milk synthesis rate than the 5 to 28 and 29 to 60 DIM observations. The stage of lactation subgroup difference in intercept between 5 to 28, 29 to 60, and 61 to 305 DIM subgroups were not significantly different ($P > 0.1$). In Figure 2C, the line of nonchronic cows was slightly lower than the line of chronic cows, but the chronic cow intercept in the model was −0.08 with a standard error of 0.01 ($P < 0.01$). Regardless of current LDH, the milk synthesis rate for chronic cows was lower.

Mean EC Results. Figure 3 provides the nonlinear association between mean EC and milk synthesis ($f_{Mean\ EC}$) and the frequency of mean EC observations for different parity, stage of lactation, and chronicity classes (Figure 3A–C). Table 3 summarizes the results of the regression models. Figure 3 indicates that the association between mean EC and milk synthesis rate was highly nonlinear. In addition, the threshold of milk synthesis rate decrease was within the range of 5.0 to 6.0 mS/cm mean EC for all subgroups. This threshold was found at a high percentile of the mean EC distribution compared with LnSCC and LnLDH (see bottom panels in Figure 2 and 3). Mean EC remained negatively associated with milk synthesis rate after the threshold for all parity, stage of lactation, and chronicity subgroups.

Between the subgroups, several differences and similarities could be seen in Figure 3. The differences in thresholds between subgroups were limited as they all fell between 5.0 and 6.0 mS/cm. The differences in the function forms between subgroups should be interpreted with care as a large section of the decrease in milk synthesis rate was based on a small area of the mean EC distribution. The limited number of observations explained the increase in milk synthesis rate at 7.5 mS/cm for the second-parity subgroup in Figure 3A. In Figure 3A, the milk synthesis rate of the multiparous

Table 2. Regression summary of lactate dehydrogenase (LDH) analysis[1]

Item	Parity groups model	Coefficient (SE)	Chronicity model	Coefficient (SE)	DIM model	Coefficient (SE)
Parametric terms						
	Intercept	1.26 (0.01)***	Intercept	1.49 (0.01)***	Intercept	2.03 (3.58)
	Intercept second parity	0.27 (0.02)***	Intercept chronicity	−0.08 (0.01)***	Intercept of DIM 29–60	−0.45 (3.62)
	Intercept ≥third parity	0.36 (0.02)***			Intercept of DIM 61–305	−0.53 (3.57)
Smoothing terms		EDF (Ref. df)		EDF (Ref. df)		EDF (Ref. df)
	DIM of first parity	22.08 (27.81)***	DIM of nonchronic	28.74 (34.31)***	DIM of 5–28	3.56 (3.85)***
	DIM of second parity	24.93 (30.77)***	DIM of chronic	13.35 (17.23)***	DIM of 29–60	3.42 (4.09)***
	DIM of ≥third parity	26.66 (32.39)***			DIM of 61–305	7.11 (9.19)***
	LnLDH of first parity	9.07 (11.13)***	LnLDH of nonchronic	11.20 (13.42)***	LnLDH of 5–28 DIM	5.63 (7.17)***
	LnLDH of second parity	9.53 (11.66)***	LnLDH of chronic	8.93 (10.93)***	LnLDH of 29–60 DIM	6.79 (8.54)***
	LnLDH of ≥third parity	12.51 (14.73)***			LnLDH of 61–305 DIM	12.00 (14.23)***
Adjusted R^2		0.77		0.74		0.73

[1]EDF = estimated degrees of freedom; Ref. df = reference degrees of freedom.
[2]Significance of smoothing terms can be determined by a method explained in detail in Wood (2013).
***$P < 0.0001$.

subgroups decreased more when mean EC increased than in the first-parity subgroups. Furthermore, the parity subgroup differences in intercept between first-parity cows and second-parity cows and first-parity cows and ≥third-parity cows were 0.28 with a standard error of 0.01 and 0.36 with a standard error of 0.01, respectively ($P < 0.01$). Regardless of mean EC, cows with a higher parity produced significantly more milk. In Figure 3B, the milk synthesis rate of the 29 to 60 and 61 to 305 DIM subgroups decreased more than the milk synthesis rate of 5 to 28 DIM subgroup when mean EC increased, whereas the difference in intercepts between the stage of lactation subgroups was not significant ($P > 0.1$). In Figure 3C, the milk synthesis rate of the chronic subgroups decreased more than for the nonchronic subgroup when mean EC increased. In addition, the chronic mastitis subgroup differences in intercept between chronic cows and nonchronic cows was −0.04 with a standard error of 0.003 ($P < 0.01$). Milk synthesis rate for chronic cows was lower when controlling for the current level of mean EC.

DISCUSSION

Our aim was to investigate the association between milk synthesis rate and online LDH, EC, and SCC at differing stages of lactation, parity, and chronicity statuses. As a second aim, we wanted to investigate the differences in thresholds at which online LDH, mean EC, and SCC levels start to be associated negatively with milk synthesis rate. We found strong nonlinearities after a linear phase in the association between milk synthesis rate and different subgroups. Estimating the nonlinearity correctly would lead to a more accurate estimation of milk loss. In past research, the resulting nonlinear functions of the associations between the studied mastitis indicators with milk yield have not been found in this detail (Dürr et al., 2008; Gonçalves et al., 2018). Each of these mastitis indicators is tied to a connected, but dissimilar, mechanism in the immune response of a cow (Viguier et al., 2009), which may be the explanation for the differences we see. An immune response leads to increases in SCC in the milk when polymorphonuclear neutrophils, white blood cells, are released into the milk to engulf the pathogen. Next, apoptosis occurs, and somatic cells can be found in the milk. Differences in EC occur by tissue damage and breaching of the blood-milk barrier (Viguier et al., 2009). The LDH, on the other hand, is released in the milk when a pathogen is engulfed and killed by a polymorphonuclear neutrophil (Viguier et al., 2009).

This study looked at differences in associations between parity, stage of lactation, and chronicity subgroups. We found nonlinear as well as linear charac-

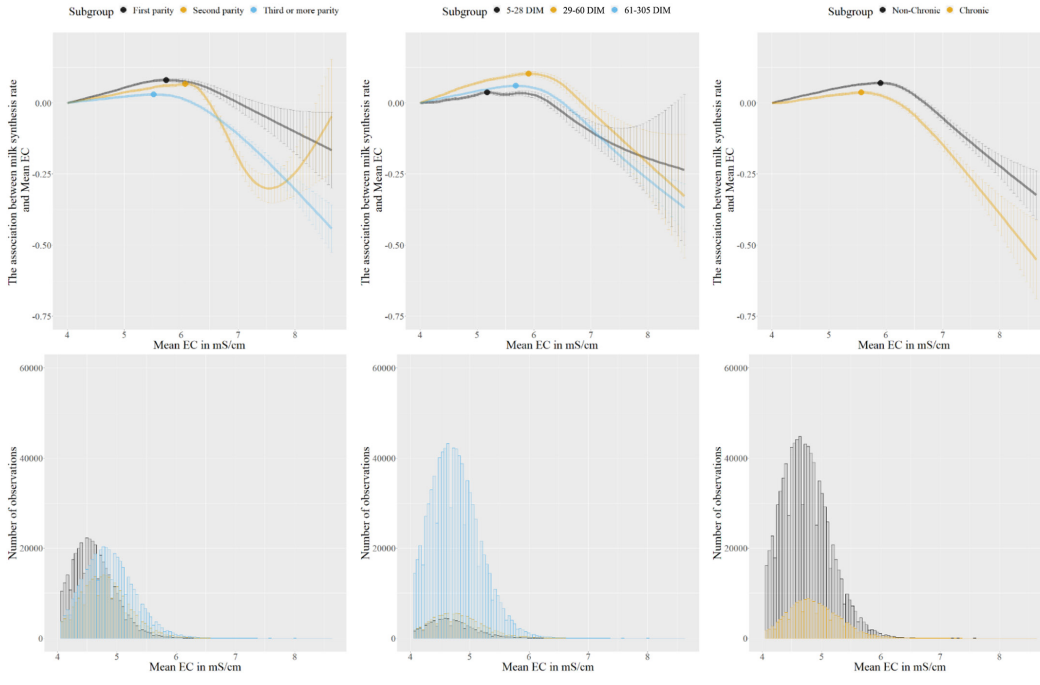

Figure 3. Estimated associations between milk synthesis rate and mean electrical conductivity (EC) and number of observations for parity, stage of lactation, and chronicity subgroups. The dots indicate that the start of milk synthesis rate decreases, and thereby milk losses increase from that point.

teristics in the association between milk synthesis rate and LDH, SCC, and mean EC. Typically, a linear phase with no clear association or a proportionally small decreasing association would be followed by a nonlinear phase where a quadratic decrease (i.e., decrease at an increasing speed) could be seen. This justified the use of nonlinear models for LDH, SCC, and EC measures, and supported the use of the threshold model in Dürr et al. (2008) and Gonçalves et al. (2018) wherein SCC has no effect on milk yield up to a certain threshold, and a negative effect on milk yield after the threshold. After reaching this threshold, milk synthesis rate would decrease at an increasing rate, and this is also reported by others (Jones et al., 1984; Hagnestam-Nielsen et al., 2009; Hand et al., 2012). The milk synthesis difference was higher for the multiparous subgroups than their primiparous counterparts for all mastitis indicators. This is also seen in Hagnestam-Nielsen et al. (2009) and Hand et al. (2012) in the case of SCC. Multiparous cows often have a higher milk production; therefore, the losses can be greater. For the stage of lactation subgroups, no clear differences were seen in the SCC models; however, the milk synthesis rate in the 5 to 28 and 29 to 60 DIM subgroups decreased less than 61 to 305 DIM in the LnLDH models, and the 29 to 60 and 61 to 305 DIM subgroup decreased more than 5 to 28 DIM for mean EC. In contrast to our SCC results, Hagnestam-Nielsen et al. (2009), Dürr et al. (2008), and Gonçalves et al. (2018) reported increasing milk losses related to SCC with increasing DIM, although this relationship could also be parabolic. These conflicting results could be caused by the limited number of DIM subgroups in our study. The results of the chronic subgroups are difficult to compare with results of other researchers due to differences in the definition and operationalization of mastitis chronicity. Nevertheless, Hadrich et al. (2018) also found that milk losses increased when the number of past consecutive observations with a higher SCC increased.

We found substantial variation in thresholds for the onset of milk loss between the different subgroups in all SCC models as well as the LDH models, which was

Table 3. Regression summary of mean electrical conductivity (EC)[1]

Item	Parity groups model	Coefficient (SE)	Chronicity model	Coefficient (SE)	DIM model	Coefficient (SE)
Parametric terms						
	Intercept	1.25 (0.01)***	Intercept	1.45 (0.004)***	Intercept	1.25 (5.55)
	Intercept second parity	0.28 (0.01)***	Intercept chronicity	−0.04 (0.003)***	Intercept of DIM 29–60	0.33 (5.57)
	Intercept ≥third parity	0.36 (0.01)***			Intercept of DIM 61–305	0.23 (5.55)
Smoothing terms		EDF (Ref. df)		EDF (Ref. df)		EDF (Ref. df)
	DIM of first parity	32.81 (36.96)[2]***	DIM of nonchronic	36.16 (38.54)***	DIM of 5–28	3.93 (3.98)***
	DIM of second parity	32.98 (37.06)***	DIM of chronic	16.37 (20.65)***	DIM of 29–60	4.05 (4.70)***
	DIM of ≥third parity	34.17 (37.71)***			DIM of 61–305	9.28 (11.76)***
	Mean EC of first parity	10.37 (12.21)***	Mean EC of nonchronic	13.42 (15.12)***	Mean EC of 5–28 DIM	10.67 (12.30)***
	Mean EC of second parity	15.63 (16.97)***	Mean EC of chronic	11.64 (13.62)***	Mean EC of 29–60 DIM	10.38 (12.05)***
	Mean EC of ≥third parity	11.64 (13.53)***			Mean EC of 61–305 DIM	12.96 (14.43)***
Adjusted R^2		0.80		0.76		0.76

[1]EDF = estimated degrees of freedom; Ref. df = reference degrees of freedom.
[2]Significance of smoothing terms can be determined by a method explained in detail in Wood (2013).
***$P < 0.0001$.

driven by minor differences in overall similar functional forms. Nevertheless, similar low thresholds (mostly 2.5 LnSCC or 12,000 SCC/mL) for SCC were found compared with milk loss thresholds reported in the past (7,400–12,400 SCC/mL; Dürr et al., 2008; Gonçalves et al., 2018). We found some occasional deviation from 2.5 LnSCC between parity groups and stage of lactations, which is also found by Gonçalves et al. (2018). Other research pointed toward a dilution effect of SCC on milk yield (Green et al., 2006), and the dilution effect may cause an overestimation of the milk yield loss with increasing SCC. Due to the nonparametric nature of generalized additive models, the threshold was not a single parameter in the model, and we could not estimate the uncertainty of the threshold, whereas other authors report occasional substantial uncertainty in thresholds (Dürr et al., 2008; Gonçalves et al., 2018). Moreover, parametric piecewise models may have difficulty converging when finding complex (quadratic) parametric functions (Gonçalves et al., 2018). Nevertheless, we would argue that, if one were interested in only the threshold, one should fit a parametric piecewise model in which the threshold is a parameter. It would give a more precise description of the threshold together with an estimation of the uncertainty of the threshold.

This study had several limitations that may constrain the conclusions drawn. The disadvantage of observational secondary data, as used in this study, is that one cannot control all factors (e.g., farmer decision-making, availability of data, and LDH or SCC sampling algorithm) that can influence the association between inflammation indicators and milk production, potentially reducing the internal and external validity. Additionally, we did not have LDH data for all farms, reducing the generalizability of the LDH results relative to the SCC or mean EC results. In terms of farmer decision-making, farmers may have decided to retain high-producing cows with chronic mastitis more than lower-producing cows with chronic mastitis. This may cause a bias in the data where chronic cows are less affected by higher values of the mastitis indicators, reducing generalizability. The (automated) sampling strategy for LDH or SCC was partly based on the risk of having mastitis. Hence, cows that were sampled were more likely to have udder problems, even when they had low levels of mastitis indicators, potentially reducing the estimated milk loss (i.e., the difference between a cow with a low SCC and the cow with a high SCC) and generalizability. Furthermore, the time intervals between milkings differed substantially. We assumed that the effect of time interval on milk yield was linear by calculating the milk synthesis rate as milk yield divided by the time interval. However, milk yield

is nonlinearly associated with time interval (Hogeveen et al., 2001), especially during the first hours of the time interval between milkings. We have attempted to solve this issue by excluding observations with extreme time intervals between milkings of less than 4 h. Also, we took the daily average of milk synthesis rate, reducing the influence of short or long milking intervals. Therefore, milk synthesis rate can be regarded as an adequate measurement of a cow's milk production capacity. The accuracy of SCC, LDH, and EC to measure udder inflammation may differ between indicators and between the same indicator measured by equipment from different brands, but it should be inconsequential with regard to the estimates of milk yield losses associated with the indicators because we are not claiming to assess milk yield losses due to (subclinical) mastitis. Nevertheless, for the OCC, Nørstebø et al. (2019) showed that the correlation between laboratory SCC and OCC was 0.82. The research results regarding the ability of LDH to detect mastitis of Chagunda et al. (2006) and Friggens et al. (2007) were integrated in the development of the Herd Navigator system and hence can be used by farmers. The EC is a commonly used mastitis indicator (Nielen et al., 1992), but to our knowledge, the diagnostic properties of EC specific to a DeLaval system are not reported. In variable creation, we decided to create the cow-level mean EC variable by taking the mean of the quarter conductivity values rather than using the quarter conductivity values. This decision was made to make the estimated cow-level milk yield losses comparable to the losses associated with SCC and LDH. Nevertheless, the availability of quarter level EC, in combination with quarter level milk yield, would make it possible to estimate the milk losses due to increased EC at the quarter level, which would improve the accuracy of the estimates. That possibility, however, was beyond the scope of this article.

The overall statistical properties of the models were acceptable. The statistical fit of the SCC, LDH, and mean EC models was good in that the adjusted R^2 of the models ranged from 0.78 to 0.81, 0.73 to 0.77, and 0.76 to 0.80, respectively (see Tables 1, 2, and 3). Without an adjustment for autocorrelation, we saw substantial autocorrelation in all models, and this affected the estimated associations between the mastitis indicators and milk synthesis rate. In our models, we have attempted to correct for autocorrelation by allowing an AR1-correlation structure. This resulted in a decrease in autocorrelation to less than approximately 0.2 on all lags, although the autocorrelation did not completely disappear. Nevertheless, the autocorrelation was minimal; therefore, we think that our estimates were not substantially influenced. No large deviations from the assumptions of homoscedasticity and normality could be seen using qq-plots and fitted versus residuals plots.

CONCLUSIONS

We found that the negative associations between SCC, EC, and LDH with milk yield were nonlinear and had a similar function form between parity groups, at different lactation stages, and at different chronicity statuses of the cow but occasionally differed in their level of decrease. Nevertheless, multiparous cows incurred larger milk losses than primiparous cows, whereas the effect of stage of lactation differed between indicators. Chronicity had a negative association with milk synthesis rate. In contrast to mean EC, milk synthesis rate started to decrease substantially more for SCC and LDH at higher SCC (277,000 SCC/mL) and LDH (20 U/L) levels. The study highlighted the nonlinearities that exist in the associations between different mastitis indicators that can be useful to more accurately predict mastitis-related milk loss.

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ORCIDS

John Bonestroo https://orcid.org/0000-0001-8815-6357
Mariska van der Voort https://orcid.org/0000-0003-0503-259X
Nils Fall https://orcid.org/0000-0001-5597-2358
Ulf Emanuelson https://orcid.org/0000-0001-7889-417X
Ilka Christine Klaas https://orcid.org/0000-0002-1397-8505
Henk Hogeveen https://orcid.org/0000-0002-9443-1412

III

Forecasting chronic mastitis of an individual cow using automatic milking system sensor data and gradient-boosting classifiers

John Bonestroo,[*†‡], Mariska van der Voort[‡], Henk Hogeveen[‡], Ulf Emanuelson[†], Ilka Christine Klaas[*], Nils Fall[†]

[*] DeLaval International AB, Gustaf De Lavals väg 15, 147 21 Tumba, Sweden
[†] Swedish University of Agricultural Sciences, Dep't Clinical Sciences, POB 7054, SE-750 07 Uppsala, Sweden
[‡] Wageningen University and Research, Business Economics Group, Hollandseweg 1, 6706 KN Wageningen, the Netherlands

Abstract

Although most of the losses due to mastitis per case in dairy production are estimated to be caused by clinical cases, subclinical cases, especially chronic, can also be problematic due to milk production losses and the risk of transmission of pathogens. Knowing which subclinical mastitis cases will become chronic at an early stage would help intervene in these cases. Automatic milking systems (**AMS**) can collect data on mastitis indicators such as conductivity, Somatic cell count (**SCC**), and blood in the milk for each milking. This study aimed to develop a sensor-based prediction model that forecasts the chronicity in subclinical mastitis cases after an initial increase in SCC using SCC, conductivity, blood in the milk, parity, milk diversion, time interval between milkings, milk yield, and days in milk (**DIM**). We used sensor data from 14 European and North American dairy farms (with herd sizes of lactating cows ranging from 55 to 638 cows) with an AMS and an Online Cell Counter, measuring SCC. A 200,000 SCC/ml threshold has been used to distinguish cows with subclinical mastitis from healthy cows. We used gradient-boosting trees and sensor data to forecast whether the SCC would structurally decrease below 200,000 SCC/ml in the 50 days after the day at which the prediction was performed. Data from 30 and 15 days prior to the day where the forecast was made was used. The model was trained on data from seven randomly selected dairy farms from the dataset. The data of the remaining seven dairy farms were used to estimate the predictive performance. These results were compared with two approaches that simulate how farmers would diagnose chronic mastitis with simple prediction rules based on close-to-daily SCC (frequent sampling approach) and less frequent monthly SCC (monthly sampling approach). We used accuracy, Matthew's correlation coefficient (**MCC**), and area under the curve (**AUC**) as metrics to assess the forecasting performance of the chronic mastitis prediction model. On average, the forecasting model, using 30 days of sensor data prior to the day of prediction, outperformed the approaches according to the accuracy (model accuracy: 0.859, Frequent sampling approach accuracy: 0.833, and monthly sampling approach accuracy: 0.809), MCC (model MCC: 0.694, frequent sampling approach MCC: 0.618, and monthly sampling approach MCC: 0.504), and AUC (model AUC: 0.944 and frequent sampling approach AUC: 0.910) metrics. The results also indicate that shortening the input requirement from 30 days of prior sensor data to 15 days had a limited effect on the model's performance. Overall, this study shows that it is possible to predict the future chronic mastitis status with high accuracy using past sensor data and machine learning models.

Keywords: mastitis, chronic, sensor, cow, forecast

Introduction

Most of the economic losses due to mastitis in dairy production are estimated to be caused by clinical cases when estimated per case (Huijps et al., 2008). Nevertheless, subclinical cases, especially chronic or long-term, can also be problematic due to milk production losses (Aghamohammadi et al., 2018) and the risk of transmission of pathogens (Swinkels et al., 2005). Subclinical mastitis is rarely treated during lactation on most dairy farms as it is not recommended (Krömker and Leimbach, 2017). However, some cases may develop into chronic subclinical mastitis, which can be defined as a case where a long-term increased SCC is not expected to cure spontaneously during lactation (St. Rose et al., 2003; Bonestroo et al., 2021). Chronic subclinical mastitis leads to prolonged periods of milk loss and an increased risk of pathogen transmission. At the onset of and during subclinical mastitis, it would be helpful to distinguish between cases expected to cure spontaneously quickly and cases that develop into chronic subclinical mastitis. In other words, it

would be helpful to forecast chronic subclinical mastitis so that early intervention is possible (i.e., culling, early dry-off, or antibiotic treatment).

Despite the considerable number of studies on the sensor-based detection of clinical mastitis (Rutten et al., 2013; Jensen et al., 2016; Khatun et al., 2018), a smaller amount of research has been done into sensor-based detection of subclinical mastitis (Polat et al., 2010; Khatun et al., 2019). As stated before, subclinical mastitis is not commonly treated during lactation and, therefore, subclinical mastitis detection may be regarded as less applicable than clinical mastitis detection. However, prospective forecasting of chronic subclinical mastitis is now possible due to a clear definition of chronic subclinical mastitis of three to four weeks (Bonestroo et al., 2021) and the availability of data collected frequently from on-farm sensor systems.

Sensor systems can measure mastitis indicators such as conductivity, somatic cell count (**SCC**), and blood in the milk daily. Being more frequent than commonly performed monthly Dairy Herd Improvement (**DHI**) SCC sampling and testing, these high-frequency indicators could be used to obtain insight into udder health over time. The benefits of more frequent sampling would include a higher diagnostic performance to detect a case and potentially forecast the outcome of such a case.

Therefore, this study aimed to develop a sensor-based prediction model that forecasts the future subclinical chronic mastitis status based on past sensor data. The model-based gradient-boosting trees approach was compared to two approaches representing the performance achieved with simple prediction rules on monthly sampled data and daily SCC data alone. The effect of using input data from a shorter period in the model on the predictive performance was explored using data from 30 days and 15 days prior to the moment of forecasting.

Methods

Data

For this study, we used data from 14 herds from Belgium, Canada, France, Sweden, and the Netherlands, with herd sizes of lactating cows ranging from 55 to 638 cows. The data was retrieved from a central database of DeLaval International AB (Tumba, Sweden). Herds with an online cell counter (**OCC**) (DeLaval OCC, DeLaval International AB, Tumba, Sweden) using an automatic milking system (DeLaval VMS series, DeLaval International AB, Tumba, Sweden) were selected. The OCC is an add-on to the AMS that measures the commonly used SCC in the milk to assess the degree of udder inflammation. The OCC was validated against laboratory SCC, resulting in a high correlation (0.82-0.86) with laboratory SCC (Sørensen et al., 2016; Nørstebø et al., 2019).

Besides SCC data, the AMS collected data on the conductivity of the milk per quarter of the udder (in mS/cm), the occurrence of blood in the milk (using an RGB sensor), as well as milk yields (in kg). The data was recorded in different time intervals for different herds, but all herds started to record in 2016 or 2017, and the average time recorded per herd was 2.8 years, with a minimum of 1.4 years and a maximum of 4.2 years. The data was reported in a "per milking" frequency. This data included cow identification number, herd identification number, milk yield in kilograms, blood presence (binary variable indicating the presence of blood), SCC, days in milk (**DIM**), milk diversion (the action of diverting the milk away from the consumable milk bulk tank into a sink), 4 quarter conductivities throughout the milking, and parity (i.e., the lactation number of the cow). We calculated the time interval between milkings in hours and the standard deviation of quarter conductivity values (see Appendix A). In addition, we also calculated the interquarter ratio of quarter conductivity values (the highest quarter conductivity value divided by the lowest quarter conductivity value) and milk production rate (milk yield in kilograms divided by the time interval between milkings in hours). An overview of the data can be seen in Table 1. We selected cows for which we had the data from the start of the lactation (at least one milking reported in the first 10 DIM). Furthermore, we removed all milking days that had a between-milking interval shorter than 3 and longer than 24 hours because the milk-yield-based variables are misrepresented for milkings outside this range (Hogeveen et al., 2001).

Training and validation datasets

To create a training and a validation dataset, we randomly divided the herds in our dataset. Half of the herds were selected for the training set, and the other half entered the validation set. Validation herds were identified as herds 1 to 7, while herds 8 to 14 were designated as training herds. The data from all the training herds were used to fit a prediction model all at once (i.e., the model was trained once using data from all training herds), and data from the validation herds were used to test the model's performance.

Table 1. *An overview of the variables per milking used in the study*

Herd	Milkings with milk diversion	Mean time between SCC samples	Mean parity	Milkings with blood detected	Mean SCC	Mean STDConductivity	Mean IQRConductivity	Mean milk yield	Mean time interval
1	0.02	1.94	2.37	0.01	134.01	0.15	1.07	11.82	8.91
2	0.01	2.54	2.22	0.00	185.66	0.15	1.07	11.46	8.99
3	0.02	4.32	2.31	0.06	155.75	0.16	1.08	13.61	9.57
4	0.03	1.45	2.96	0.01	158.88	0.14	1.07	15.93	9.73
5	0.05	2.11	2.40	0.08	206.66	0.16	1.08	13.92	10.67
6	0.04	4.20	2.22	0.01	282.69	0.17	1.09	10.36	8.54
7	0.04	3.52	2.00	0.06	191.91	0.15	1.07	15.56	10.08
8	0.05	2.80	2.04	0.01	227.58	0.15	1.07	13.41	8.74
9	0.03	2.66	2.03	0.01	228.80	0.14	1.07	10.39	8.33
10	0.02	3.51	3.19	0.05	178.89	0.15	1.08	10.48	8.31
11	0.01	2.21	2.57	0.02	130.65	0.13	1.07	11.30	8.37
12	0.02	2.66	2.69	0.01	134.55	0.13	1.07	11.58	8.60
13	0.01	2.22	2.59	0.02	89.14	0.13	1.07	11.43	8.94
14	0.04	2.90	2.15	0.01	317.83	0.17	1.09	11.63	9.74

Data pre-processing

All data processing and case predicting were performed in Python 3.7. The data (e.g., milk yield, interquarter ratio of conductivity) from each milking per day was aggregated to a daily frequency using the mean, minimum, maximum, and standard deviation functions. After the aggregation to a daily frequency, the daily mean, maximum, and standard deviation of quarter-level conductivity values (e.g., daily mean conductivity value of the left-rear quarter) were aggregated to cow-level variables. This aggregation was performed by calculating the mean over daily mean quarter conductivity values and the maximum over daily maximum quarter conductivity values. In addition, we also calculated the standard deviation over daily standard deviations of quarter conductivity values and the standard deviation over daily maximum quarter conductivity values. A description of the aggregated variables in the dataset with their calculation is given in Appendix A. All variables had to be on cow level as we forecast chronic mastitis on cow level. The remaining quarter-level conductivity variables were not included as input in the forecasting models as they were not reported on cow-level.

Case definition

A prediction day (i.e., a day on which a prediction of a future state was made) was defined as a day in the lactation with at least a mean SCC higher than or equal to 200,000 cells/ml (International Dairy Federation, 2013) or having an SCC of such a level on 1 of the 4 days prior to the day. It is essential to mention that one mastitis case can have multiple prediction days as each day of the episode, a forecast is performed. It would allow the farmer to monitor and forecast during an ongoing episode. For each day on which the future chronic mastitis status was forecasted, we used the data 30 days before the prediction day as input. Referring back to Appendix A, the feature values of each day during the last 30 days (e.g., MaxIQRConductivity on the 16th day before the prediction day) could be used by the forecasting method. Moreover, to derive the future chronic mastitis status for each prediction day, 50 days of data after the prediction day were needed (Figure 1). Consequently, each day during lactation with 30 preceding and 50 successive days of data could be a prediction day, given that it had a recent increase in SCC.

Labeling of chronic mastitis cases

Filtering was used to determine a structural decrease in SCC below 200,000 SCC/ml. The future chronic mastitis status on a prediction day was labeled as not chronic if the rolling 20-day mean SCC decreased below 200,000 SCC/ml (0= not chronic mastitis) at least once in the period from the prediction day to 50 days post the prediction day. It was labeled chronic if no structural decrease occurred (1=chronic mastitis). In other words, the label indicates whether the cow would recover (=0) or turn chronic (=1). When the SCC is consistently above 200,000 SCC/ml across the whole 50-day period in the future, it is chronic (the top example in Figure 1), and when the SCC decreases structurally below 200,000 SCC/ml in the 50 days after a prediction day, it is not

chronic (the second example from the top in Figure 1). If a cow had an increase of SCC after a structural decrease in SCC, the cow was regarded as not chronic (the third example in Figure 1). In these cases, it was impossible to determine whether the new increase in SCC was part of the initial episode or the start of a new episode. The 20-day rolling window was chosen to ensure that a case recovered long-term and not just for a few days. The 50 days post prediction day were chosen based on the approximate chronic cut-off of Bonestroo et al. (2020) of 4 weeks or approximately 30 days plus the rolling 20-day window (20+30=50 days). Because SCC is sampled using a risk-based sampling strategy in the OCC, SCC was not sampled every day. As such, we required at least 10 SCC measurements in the rolling 20-day window to calculate the rolling mean. If no rolling mean could be calculated at all, future chronic mastitis could not be determined, and the observation was discarded.

Different input periods

Besides the default 30-day input period, we also pre-processed the data for a 15-day input period to evaluate the effect of different input periods on the forecasting performance. A shorter input period using less information would allow users to forecast chronic mastitis earlier in lactation.

These different pre-processing steps resulted in 59,541 chronic mastitis prediction days and 107,702 not chronic prediction days using a 30-day input period and 63,362 chronic mastitis prediction days and 118,808 not chronic prediction days using a 15-day input period. A longer input period results in fewer cases to be forecasted as it requires more days with measured sensor data. Table 2 shows the number of cow lactations and prediction days per herd for both input period categories.

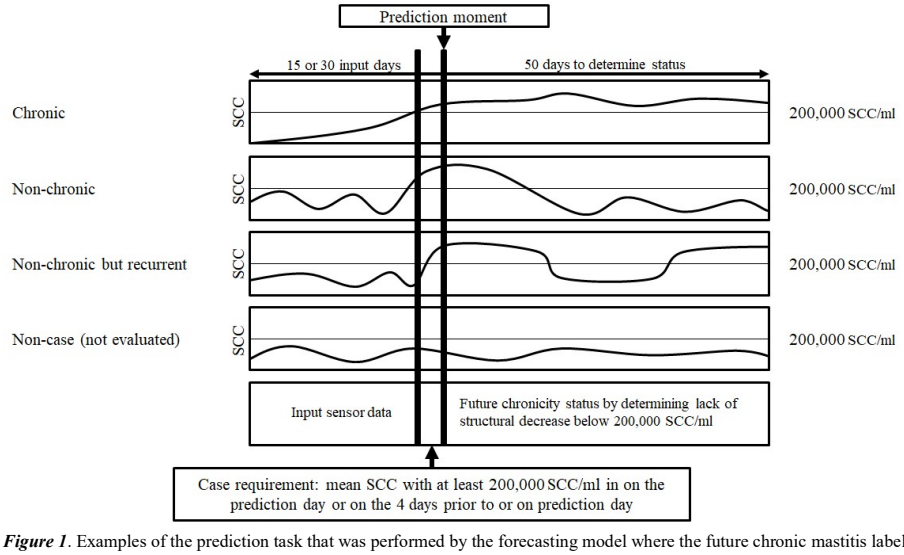

Figure 1. Examples of the prediction task that was performed by the forecasting model where the future chronic mastitis label is created by determining whether the rolling 20-day mean of daily mean SCC decreased below 200,000 SCC/ml (0= not chronic mastitis) or not (1=chronic mastitis) at least once in the period from the prediction day to 50 days post the prediction day

Gradient-Boosting Classification Trees

We used the gradient-boosting trees algorithm as implemented in XGBoost (Chen and Guestrin, 2016) to create a prediction model that forecasts whether the cow would recover (=0) or turn chronic (=1), using all features in Appendix A for every input day (from the prediction day to 29 days before the prediction). We chose gradient-boosting trees as it can deal with missing values, which can be frequent (Hogeveen et al., 2010). In addition, past work on clinical mastitis detection with boosting and bagged trees showed good results (Kamphuis et al., 2010) using similar sensor data. Essentially gradient-boosting trees (Friedman, 2001) use boosting with decision trees. In boosting, a combination of decision trees is made by sequentially building several decision trees from the data to minimize the classification error. In short, a first decision tree is fitted on the training data and a classification error is computed using the loss function (log loss function in our case). To minimize the classification error, the second decision tree uses the residual classification error of the first decision tree, and the third decision tree uses the residuals of the second decision tree, and so forth until the upper limit of the number of trees is reached. Each tree will give a prediction on a log(odds) scale. The final prediction of the

model is the sum of the predictions of each decision tree multiplied by a pre-defined learning rate. Lastly, the final prediction in log(odds) is transformed to a prediction in probability by using the inverse (logit) link function. Sequentially using the residual allows later decision trees to compensate for errors of the earlier decision trees. To address the class imbalance between the number of chronic and healthy prediction days, we set the positive class weight parameter (scale_pos_weight) to be equal to the ratio between the positive and the negative samples (i.e., chronic and non-chronic prediction days) in the training dataset. This was 0.653 in the 30-day input period dataset and 0.635 in the 15-day input period dataset.

Table 2. *Herd descriptive statistics in terms of the number of cow lactations, the number of prediction days per input period*

Herd	Herd type	Cow lactations for 30-day input dataset	Prediction days for 30-day input dataset	Cow lactation for 15-day input dataset	Prediction days for 15-day input dataset
1.	Validation	326	19,375	355	21,030
2.	Validation	326	10,689	345	11,681
3.	Validation	295	26,244	307	28,301
4.	Validation	212	11,142	228	12,410
5.	Validation	196	12,983	200	13,647
6.	Validation	151	8,398	158	9,070
7.	Validation	36	896	45	1,221
8.	Training	233	9,434	243	10,081
9.	Training	245	16,543	259	17,791
10.	Training	210	12,566	222	13,857
11.	Training	545	19,704	595	22,286
12.	Training	137	8,439	140	9,009
13.	Training	118	6,292	129	6,920
14.	Training	127	4,538	131	4,866

Hyperparameter optimization

Gradient-boosting trees have several hyperparameters (or settings) that can be optimized. An explanation of the specific hyperparameters can be found in Chen and Guestrin (2016). The data cannot directly estimate these hyperparameters as they must be set before the learning process (i.e., the number of trees in a gradient-boosting trees classification model must be set beforehand). Therefore, these hyperparameters require optimization. To determine the optimal hyperparameter set, we sampled 100 hyperparameter combinations from the statistical distributions in Table 3. We used seven-fold random search cross-validation in the programming library scikit-learn (Pedregosa et al., 2011) to choose the optimal hyperparameter combination.

In short, we separated the training dataset into seven folds based on the herd identification number to ensure that every herd occupied 1 fold. This separation was done to ensure that the herd-specific performance mimics the model's situation in a new herd (Hogeveen et al., 2010). Next, 6 folds of data were used to train a gradient-boosting trees model with a specific set of hyperparameters randomly sampled from the distributions described in Table 3. A possible hyperparameter combination might be 0.02 learning rate, 5 minimum child weight, 2 maximum depth of each tree, 0.4 fraction of variables considered for each tree, and 75 number of trees. It could be any value as described by the distributions with a likelihood dependent on the type of distribution. This random search procedure has been proven to work well relative to a grid search (Bergstra and Bengio, 2012). Subsequently, sample predictions in the form of prediction probabilities were made, using the unused fold as a test fold. These probabilities were compared with the label (i.e., whether the case was going to be chronic or not in the future) using the area under the curve (**AUC**) metric. The AUC was implemented using the roc_auc_score function in scikit-learn (Pedregosa et al., 2011). The roc_auc_score function calculates the area under the receiver operating characteristic (**ROC**) curve. This procedure was iterated seven times, with each fold being the test fold once for every hyperparameter combination. The average AUC over the test folds was the final score of the hyperparameter combination.

The above procedure was done for 100 randomly sampled hyperparameter combinations. We chose the optimal hyperparameter combination where the model maximized the average AUC. This procedure was solely done to attain the optimal hyperparameter set on the training dataset. We trained a model with the optimal hyperparameters using all the training data from the training herds. This model was subsequently validated using data from the validation dataset.

***Table 3**. The hyperparameter space that is explored in the random hyperparameter optimization by using 100 combinations of hyperparameters*

Hyperparameter	Distribution
Learning rate	Log uniform(0.01-1.0)
Minimum child weight	Uniform(1-20)
Maximum depth of each tree	Uniform(1-15)
Fraction of variables considered for each tree	Uniform(0.01-1.0)
The number of trees	Uniform(50-200)

Validation

The predictions of the model take the form of class probabilities. The predictions and the labels in the validation dataset were compared using the AUC, Matthew's correlation coefficient (**MCC**), accuracy, sensitivity, and specificity per herd. Accuracy, sensitivity, specificity, and MCC (Eq. 1, 2, 3, 4) were calculated using a probability threshold for predicted future chronic mastitis statuses. These statuses were created on the predicted probability using a threshold that maximizes Youden's index ($\text{sensitivity} + \text{specificity} - 1$), weighting false positives and false negatives equally. After the model was trained, a model probability threshold of 0.18 and 0.16 in the 30-day and 15-day input period categories was estimated to maximize Youden's index. Henceforth, these thresholds were used for their respective input period categories.

$$MCC = \frac{tp \times tn - fp \times fn}{\sqrt{(tp + fp)(tp + fn)(tn + fp)(tn + fn)}} \quad (1)$$

$$Accuracy = \frac{tp + tn}{tp + tn + fp + fn} \quad (2)$$

$$Sensitivity = \frac{tp}{tp + fn} \quad (3)$$

$$Specificity = \frac{tn}{tn + fp} \quad (4)$$

Where tp is the number of true positives, tn is the number of true negatives, fp is the number of false positives, and fn is the number of false negatives. AUC was estimated as indicated in the hyperparameter optimization section.

The predictive performance of the gradient-boosting trees classifier was compared to that of two default approaches, the monthly sampling approach (approach mimicking DHI sampling frequency but using OCC data) and the frequent sampling approach (using all OCC data available).

- Monthly sampling approach, this approach predicted future chronic mastitis to be present when the SCC was equal to or higher than 200,000 SCC/ml in the evaluation closest to the prediction day and the SCC evaluation furthest away in time in the preceding 30 days relative to the prediction day (Figure 1). The prediction rule predicted chronic mastitis if both SCC samples were higher than 200,000 SCC/ml. The monthly sampling approach mimicked a situation where farmers use monthly SCC data of the previous month and the current month to determine chronic mastitis, common in a non-sensor dairy farm setting.
- Frequent sampling approach, this approach predicted future chronic mastitis when the number of days with 200,000 SCC/ml or higher prior to the prediction day was equal to or more than 13 days in the 30-day input period category (7 days in the 15-day input period category). This threshold on the number of days was chosen to maximize Youden's index to forecast the future chronic mastitis status.

Comparing different approaches based on different metrics allowed us to determine whether the increase in predictive performance was due to more complex models or more frequent SCC samples. The AUC could not be computed for the monthly sampling approach as this approach results in a class prediction and not a continuous value. The differences in AUC, MCC, and accuracy between the model predictions and the default approaches were tested using (Welch's) t-tests for unequal variances on herd-specific performance measures. Accuracy, MCC, and AUC were selected for the statistical tests. This decision was made as they take all classified cases into account, while specificity (no tp or fn) and sensitivity (no tn or fp) do not.

Table 4. *The sensitivity, specificity, Matthew's correlation coefficient, accuracy, and Area under Curve (AUC) of the model predictions and the frequent and monthly sampling approaches over 7 validation herds using 30 days prior to the prediction day as input*

Herd	Sensitivity	Specificity	Matthew's correlation coefficient	Accuracy	AUC
Model					
Herd 1	0.937	0.798	0.721	0.854	0.938
Herd 2	0.925	0.781	0.677	0.832	0.924
Herd 3	0.940	0.785	0.629	0.821	0.931
Herd 4	0.909	0.842	0.718	0.864	0.945
Herd 5	0.943	0.838	0.762	0.878	0.954
Herd 6	0.934	0.831	0.666	0.853	0.948
Herd 7	0.947	0.909	0.687	0.913	0.969
All herds	0.934	0.826	0.694	0.859	0.944
Frequent sampling approach					
Herd 1	0.813	0.844	0.652	0.831	0.904
Herd 2	0.833	0.756	0.566	0.783	0.879
Herd 3	0.885	0.774	0.573	0.800	0.912
Herd 4	0.707	0.864	0.573	0.813	0.882
Herd 5	0.825	0.852	0.670	0.842	0.916
Herd 6	0.896	0.838	0.645	0.850	0.933
Herd 7	0.874	0.913	0.649	0.909	0.947
All herds	0.833	0.834	0.618	0.833	0.910
Monthly sampling approach					
Herd 1	0.553	0.906	0.502	0.764	
Herd 2	0.546	0.862	0.434	0.750	
Herd 3	0.594	0.863	0.449	0.801	
Herd 4	0.585	0.897	0.515	0.795	
Herd 5	0.656	0.903	0.587	0.809	
Herd 6	0.631	0.900	0.531	0.843	
Herd 7	0.598	0.937	0.510	0.900	
All herds	0.595	0.896	0.504	0.809	

Results

Using the previous 30 days as input to predict future chronic mastitis

A chronic mastitis forecasting model was trained and validated. Given automatically-collected sensor data, the farmer would gain insight into the probable end of a case and use sensor data structurally. Table 4 presents all approaches' sensitivity, specificity, MCC, accuracy, and AUC. The chronic mastitis prediction model outperformed the two default approaches on all farms for almost all performance indicators. More specifically, it outperformed them on accuracy (chronic mastitis prediction model: 0.859, frequent sampling approach: 0.833, and monthly sampling approach: 0.809), MCC (chronic mastitis prediction model: 0.694, frequent sampling approach: 0.618, and monthly sampling approach: 0.504), and AUC metrics (chronic mastitis prediction model: 0.944 and frequent sampling approach: 0.910). Using (Welch's) t-tests for unequal variances, we determined that the differences between the default approaches and the model predictions were significant for AUC, and MCC ($P<0.05$) but not for accuracy when compared to the frequent sampling approach ($P>0.05$). The chronic mastitis prediction model also outperformed the other approaches on sensitivity (chronic mastitis prediction model: 0.934, frequent sampling approach: 0.833, and monthly sampling approach: 0.595), but the monthly sampling approach outperformed the other methods on specificity (chronic mastitis prediction model: 0.826, frequent sampling approach: 0.834, and monthly sampling approach: 0.896).

Using the previous 15 days as input to predict future chronic mastitis

Table 5 provides the different approaches' sensitivity, specificity, accuracy, MCC, and AUC. In this case, the monthly sampling approach could not be applied, as it requires at least 30 input days. In this case, the model outperformed the approach in sensitivity, MCC, accuracy, and AUC but not in specificity. Using (Welch's) t-tests for unequal variances, we determined that the differences between the default approach and the model predictions were significant for AUC and MCC ($P<0.05$) but not for accuracy when compared to the frequent sampling approach ($P>0.05$). When comparing the results of differing input periods, we saw no substantial differences between them. The decrease in performance was limited due to decreasing the input period from 30 days to 15 days.

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Table 5*. The sensitivity, specificity, Matthew's correlation coefficient, accuracy, and Area under Curve (AUC) of the model predictions, and the frequent sampling approach over 7 validation herds using 15 days prior to the prediction day as input. It was not possible to use the monthly sampling approach using the 15-day input*

Herd	Sensitivity	Specificity	Matthew's correlation coefficient	Accuracy	AUC
Model					
Herd 1	0.931	0.791	0.705	0.846	0.931
Herd 2	0.915	0.781	0.663	0.827	0.922
Herd 3	0.931	0.774	0.605	0.809	0.914
Herd 4	0.905	0.836	0.704	0.858	0.938
Herd 5	0.933	0.834	0.746	0.871	0.947
Herd 6	0.930	0.833	0.660	0.853	0.941
Herd 7	0.912	0.887	0.625	0.890	0.964
All herds	0.922	0.820	0.673	0.851	0.937
Frequent sampling approach					
Herd 1	0.840	0.824	0.653	0.830	0.901
Herd 2	0.870	0.777	0.617	0.809	0.896
Herd 3	0.884	0.769	0.561	0.794	0.899
Herd 4	0.666	0.878	0.556	0.810	0.862
Herd 5	0.842	0.837	0.665	0.839	0.914
Herd 6	0.840	0.852	0.617	0.850	0.919
Herd 7	0.855	0.900	0.611	0.895	0.946
All herds	0.828	0.834	0.612	0.832	0.905

Discussion

This study is the first study that uses on-farm sensor data to predict future chronic mastitis. We developed a prediction model and compared it with the monthly and frequent sampling approaches using predictive performance. The significantly higher performance of the model compared to the performance of the approaches showed the potential value of future chronic mastitis prediction models based on sensor data. The results show that this model would have value for farmers in forecasting chronicity. These points are strengthened because farmers may not need to invest in extra sensor technology to gather these forecasts. Limited research has been published on future chronic mastitis forecasting. Bartel et al. (2019) created two chronic mastitis prediction models for healthy and unhealthy cows, using non-sensor DHI data and generalized additive models. They reported an AUC of 0.779 and 0.868 for the unhealthy and healthy cow models. Although we built only one model to classify healthy and chronic cows, our reported AUC was larger. Nevertheless, these studies cannot be directly compared as they used a different chronic mastitis definition using diverse data types.

To keep the comparison between approaches fair, we have chosen an equal weighting between misclassification types by optimizing Youden's index. Preferring specificity to sensitivity would not be fair to the monthly sampling approach. The consequences of misclassification differ between false positives and false negatives in chronic mastitis forecasting. Chronic cases classified as

not chronic have more time in the herd, while more data is gathered that could lead to a correct prediction in the end. However, this cow may infect other cows in this period. On the other side, misclassification of a not chronic case as a chronic case can lead to culling, which can be costly. In that case, the farmer incurs unnecessary culling costs, and it leads to an unnecessary loss of life. One may argue that unnecessary culling is more costly than keeping a chronic cow in the herd or vice versa and make the prediction algorithm more sensitive to either class. However, our primary aim was to compare the model to the approaches, and the monthly sampling approach does not allow us to adapt it and make it cost-sensitive. Therefore, we have refrained from making the model cost-sensitive.

Several limitations constrained the study and its results. We based our future chronic mastitis definition on a long-term increased SCC without a period where SCC was below 200,000 cells/ml. Chronic mastitis itself is not well-defined in the literature. As SCC is a primary indicator for inflammation (International Dairy Federation, 2011) and DHI SCC has been used to indicate chronic mastitis (St. Rose et al., 2003), we argue that SCC is a primary candidate to operationalize chronic mastitis. However, one could also have used conductivity to define chronic mastitis, but the conductivity thresholds of healthy versus sick cows are less well defined and accepted than the thresholds of SCC (Smith et al., 2001; International Dairy Federation, 2013). In the end, we recorded 50 days of SCC measurements after the prediction day and determined whether, within the 50 days, there was a 20-day period where the mean SCC was lower than 200,000 SCC/ml. Another limitation was that we required more than 10 non-missing observations to calculate a rolling mean when we labeled cases. Whether a value is missing may also be dependent on the sampling strategy. The sampling strategy is based on the mastitis risk assessment on the OCC sampling algorithm. This may cause structural missing values as it depends on the decision of the sampling algorithm and may bias the labels to be definable when the cow is indeed chronic and indefinable when a cow is not. It should be emphasized that the limitations do not make the model invalid from a practical perspective, as farmers would detect chronic cases that they would not have detected (or detected later) without any additional cost.

We have made several choices concerning the model and training process. In the hyperparameter optimization, we used random search with herd-based cross-validation and gradient-boosting trees. We used gradient-boosting trees as they tended to work well with tabular AMS sensor data (Kamphuis et al., 2010) and natively supports missing values. Other models might perform better than gradient-boosting trees. However, this study aimed to investigate the possibility of developing a future chronic mastitis prediction model and not to find the best performing model. In hyperparameter optimization, we could not do an exhaustive search, but the results in this paper still show the added value of a future chronic mastitis prediction model. We also used a herd-based split between training and validation datasets to avoid the model learning herd-specific characteristics that might increase the predictive performance. This herd-based split mimics the algorithm's performance when deployed on a new farm. Different farm management strategies or pathogen populations might cause herd-specific associations. The performance of such a validated model might then be disappointing when the model is deployed on a new farm. Therefore, a more conservative herd-based cross-validation strategy should be preferred when testing on-farm detection or prediction models. However, in a practical application, the current proposed model can be extended to be herd-specific by using the herd data to retrain the model partly (i.e., by applying transfer learning). Although interesting, this was outside the scope of this paper and henceforth was not performed.

We have trained the chronic mastitis prediction model using data from 15 and 30 days prior to the prediction day as model input. The predictive performance decreased when the number of input days decreased. However, the differences were minor (e.g., 0.944 to 0.937 average AUC). This limited decrease in predictive performance with a decreasing input period indicates that it might be possible to predict future chronic mastitis with only a small number of input days prior to the prediction day. If a small number of input days are required, it becomes possible to predict future chronic mastitis early in lactation. Predicting chronic mastitis early in lactation is valuable as mastitis is most prevalent in that stage of lactation (Nyman et al., 2007). Predicting which case may turn into a chronic case, with or without treatment, would be helpful in decision-making at this stage. Future research could investigate possibilities of early lactation chronic mastitis forecasting.

Conclusion

The developed prediction model based on sensor data outperformed default approaches that mimic current decision-making based on monthly or more frequently sampled SCC data. Decreasing the input period from 30 to 15 days only had a limited effect on the model's predictive performance. An accurate prediction of future chronic mastitis could alarm farmers of potentially chronic cows in the future, resulting in earlier and potentially more beneficial interventions, such as treatment and more targeted culling. In the end, this supports sensor-driven decision-making concerning chronic cows.

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Potential conflicts of interest

John Bonestroo and Ilka C. Klaas are employed by DeLaval International AB. Mariska van der Voort, Nils Fall, Ulf Emanuelson, and Henk Hogeveen have no conflict of interest to report.

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Appendix

Appendix A. Overview of the features as used in the prediction model in this study.

Table A1*. Overview of the features and their definitions as used in the study*

Feature name	Explanation
DIM	The DIM of the day.
MeanYield	The mean of the milk yields from different milkings on a day.
MaxYield	The maximum of the milk yields from different milkings on a day.
MinYield	The minimum of the milk yields from different milkings on a day.
STDYield	The standard deviation of the milk yields from different milkings on a day.
TotalYield	The sum of the milk yields from different milkings on a day
MeanIQRConductivity	The mean of the ratio between the quarter with the highest conductivity and the lowest conductivity for a milking over all milkings on a day.
MaxIQRConductivity	The maximum of the ratio between the quarter with the highest conductivity and the lowest conductivity for a milking over all milkings on a day.
MinIQRConductivity	The minimum of the ratio between the quarter with the highest conductivity and the lowest conductivity for a milking over all milkings on a day.
STDIQRConductivity	The standard deviation of the ratio between the quarter with the highest conductivity and the lowest conductivity for a milking over all milkings on a day.
MeanSTDConductivity	The mean of the standard deviation between the mean conductivities measured between the four quarters of all milkings on a certain day.
MaxSTDConductivity	The maximum of the standard deviation between the mean conductivities measured between the four quarters of all milkings on a certain day.
MinSTDConductivity	The minimum of the standard deviation between the mean conductivities measured between the four quarters of all milkings on a certain day.
STDSTDConductivity	The standard deviation over the standard deviation between the mean conductivities measured between the four quarters of all milkings on a certain day.
MeanTimeInterval	The mean time between milkings on a day.
MaxTimeInterval	The maximum time between milkings on a day.
MinTimeInterval	The minimum time between milkings on a day.
STDTimeInterval	The standard deviation time between milkings on a day.
MeanMilkRate	The mean milk production in kilograms per hour on a day.
MaxMilkRate	The maximum milk production in kilograms per hour on a day.
MinMilkRate	The minimum milk production in kilograms per hour on a day.
STDMilkRate	The standard deviation of milk production in kilograms per hour on a day.
MeanSCC	The mean SCC in ml on a day.
MaxSCC	The maximum SCC in ml on a day.
MinSCC	The minimum SCC in ml on a day.
STDSCC	The standard deviation of SCC in ml on a day.
MeanBlood	The share of milkings that had a detection of blood in the milk on a day.
MaxBlood	The maximum of blood detections in the milk on a day (whether there was any blood in the milkings on a given day).
MinBlood	The minimum of blood detections in the milk on a day (whether there was any no blood milking on a given day).
STDBlood	The standard deviation of milkings that had a detection of blood in the milk on a day.
Treatment duration	A number indicating the start of a treatment where milk was diverted for several days in the future.

Feature name	Explanation
Parity	The parity of the cow at the time of milking.
MeanOverallConductivity	The mean of the daily mean quarter conductivities on a day.
MaxOverallConductivity	The maximum of the daily maximum quarter conductivities on a day.
STDOverallConductivity	The standard deviation of the daily standard deviations of quarter conductivities on a day.
STDMaxOverallConductivity	The standard deviation of the daily maximum quarter conductivities on a day.

IV

The costs of chronic mastitis: a simulation study of an automatic milking system farm

John Bonestroo,[*†‡], Nils Fall[†], Henk Hogeveen[‡], Ulf Emanuelson[†], Ilka Christine Klaas[*], and Mariska van der Voort[‡]

[*] DeLaval International AB, Gustaf De Lavals väg 15, 147 21 Tumba, Sweden
[†] Swedish University of Agricultural Sciences, Dep't Clinical Sciences, POB 7054, SE-750 07 Uppsala, Sweden
[‡] Wageningen University and Research, Business Economics Group, Hollandseweg 1, 6706 KN Wageningen, the Netherlands

Abstract

Mastitis is a production disease in dairy farming that causes economic losses. Especially chronic mastitis (i.e., mastitis cases continuing longer than 28 days) can substantially affect the risk of transmission of intramammary infections (**IMI**) and total milk production losses. Insights into the impact of chronic mastitis on production and farm economics are needed to guide chronic mastitis decision-making. We aimed to estimate the costs of chronic mastitis with a Monte Carlo simulation model in which the costs of chronic mastitis were estimated as part of the total mastitis costs. The model simulated milk yields, IMI dynamics, somatic cell count (**SCC**), and pregnancy status on an average Dutch dairy farm with 100 cow places over 7 years. The model was parameterized using information from the literature and actual sensor data from automatic milking system (**AMS**) farms. The daily subclinical milk production losses were modeled using a generalized additive model and sensor data. Transmission of IMI was modeled as well. Model results indicated median total costs of mastitis of € 208 per generic IMI case (i.e., a weighted average of all pathogens). The most substantial cost factors were the extra mastitis cases due to transmission, culling, and milk production losses. Other significant costs originated from dry cow treatments and diverted milk. The model also indicated median total costs due to chronic mastitis of € 104 (50% of the total mastitis costs). The share of chronic mastitis relative to the total mastitis costs was substantial. Transmission of contagious bacteria had the largest share among the chronic mastitis costs (45% of the costs of chronic cases). The large share of chronic mastitis costs in the total mastitis costs indicates the economic importance of these mastitis cases. The results of the study point to the need for future research to focus on chronic mastitis and reducing its presence on the dairy farm.

Keywords: Monte Carlo, simulation model, mastitis, chronic, automated milking system

Introduction

Mastitis is a common production disease in dairy farming, causing compromised animal welfare and high economic losses (Hogeveen et al., 2019). Mastitis can manifest itself for a brief period with a quick recovery, but it can also manifest itself for a prolonged period and become chronic. Chronic mastitis is defined as a long-term episode of mastitis (International Dairy Federation, 2011), with somatic cell counts (**SCC**) elevated for at least 3 to 4 weeks (Bonestroo et al., 2021). The longer the case takes, the less likely a chronic case will recover (Bonestroo et al., 2021). Chronic mastitis cases can have a substantial effect on the number of transmissions of intramammary infections (**IMIs**) (Zadoks et al., 2003; Swinkels et al., 2005a) and the total milk production losses (Hadrich et al., 2018).

In automatic milking systems (AMS), sensors continuously measure milk composition to detect mastitis (Hogeveen et al., 2010). In herds participating in dairy herd improvement associations (DHI), detecting chronic mastitis is based upon milk recording where SCC of the last two or three months is used (St. Rose et al., 2003). Because of the more frequent measurements, chronic mastitis can be defined more precisely based on on-farm SCC sensors of high accuracy (Nørstebø et al., 2019), allowing us to study chronic mastitis in more detail.

Frequent online measurements of SCC enable timely and accurate detection and improve the management of chronic mastitis. An essential aspect in developing the management of chronic mastitis is to get insights into its consequences. Therefore, it is vital to gain insight into the costs of chronic mastitis. To give an example from

another application, to support transition cow management, Rollin et al. (2015) studied the costs of clinical mastitis in the early stage of lactation to estimate the economic importance of the dry cow period. One could similarly indicate the economic importance of chronic mastitis. For chronic mastitis, Steeneveld et al. (2007) showed that antibiotic treatment of chronic mastitis caused by *Streptococcus uberis* was unprofitable, while Swinkels et al. (2005b) showed that a 3-day treatment of chronic mastitis caused by *Streptococcus spp.* was profitable. Both studies emphasized the importance of herd and cow characteristics (e.g., bacterial flora or cow history) in determining the profitability of treating chronic mastitis. However, both studies used monthly DHI SCC measurements to define chronic mastitis. Moreover, these studies did not assess the costs of chronic mastitis at the herd level but assessed the benefit of treatment on chronic cases on a cow-by-cow basis. To our knowledge, the specific costs of chronic mastitis on the herd level have never been investigated. Therefore, a preferred tool to estimate the costs of chronic mastitis would be a herd-based bioeconomic model. In the past, herd-based bioeconomic model studies have focused on the overall costs of (subclinical) mastitis (Halasa et al., 2009a; Down et al., 2013) with limited attention paid to the costs of chronic mastitis.

This study aimed to estimate the costs of chronic mastitis on an AMS farm. The costs of chronic mastitis were defined as the costs from the start of the episode until its end. These costs were estimated by a Monte Carlo simulation model. The model was parameterized to represent a typical Dutch AMS farm. To our knowledge, no other herd-level bioeconomic simulation models have been applied to estimate chronic mastitis costs, and therefore a new model was developed.

Method

Model overview

We developed and used a stochastic Monte Carlo bioeconomic simulation model to simulate IMI, mastitis, and chronic mastitis. The model also included the consequences of IMI and mastitis in milk production losses and clinical mastitis. In a Monte Carlo simulation, an outcome is simulated dependent on variables that have (random) distributional properties. The distributional properties for the variables in our simulation model are given in Table A1 and Table A2 in Appendix B. The model ran a predefined number of times (model iterations), creating different outcomes for every model iteration. The set of outcomes of each model iteration was taken together to form outcome distributions (Dijkhuizen and Morris, 1997; Hogeveen et al., 2019). The outcome distributions were summarized using the 25th, 50^{th,} and 75th percentiles.

The model mimicked the daily mastitis situation on a Dutch AMS dairy farm with 100 cow places and with IMIs caused by *Staph. aureus*, *Strep. spp*., Gram-negative bacteria, and non-aureus staphylococci **(NAS)**. The cow-places were simulated every day for 7 years, including the burn-in period, for 500 model iterations. This burn-in period was set to 2 years. Initial experimentation showed that stationarity of ongoing IMI cases occurred around 1.5 years, and the number of lactating cows in the herd stabilized in 2 years. Standard management was implemented regarding antibiotic treatment during lactation and dry-off, and culling. The negative consequences of mastitis were modeled in milk production loss and transmission of contagious bacteria. The negative consequences of mastitis were monetarized by multiplying these consequences with their costs.

The dynamics of IMI in the model were based on literature sources on clinical mastitis incidence rates (Santman-Berends et al., 2015), pathogen populations (Taponen et al., 2017), (contagious) transmissions (Gussmann et al., 2018; Dalen et al., 2019a), and lactational and dry cow period cure rates (Wilson et al., 1999; Sol et al., 2000; Taponen et al., 2006; Huijps and Hogeveen, 2007; Newton et al., 2008; Halasa et al., 2009b; Van den Borne et al., 2010; Halasa et al., 2010; Fuenzalida and Ruegg, 2019; Swinkels et al., 2021). Simulation of SCC was based on literature sources (Dalen et al., 2019b; Fuenzalida and Ruegg, 2019; Bonestroo et al., 2021) and SCC data used in Bonestroo et al. (2022). Milk yield simulation was based on an adapted statistical model used by Bonestroo et al. (2022) (see below). Price data was gathered from a range of sources (Lam et al., 2013; Griffioen et al., 2016; Scherpenzeel et al., 2018; Blanken et al., 2019; GD, 2019; Steeneveld et al., 2020).

The model architecture distinguished between a simulated non-transmission IMI case and a modeled transmission IMI case. Both types of cases were used to calculate the incidence rate of IMI, the culling rate, and the incidence rate of clinical mastitis on the farm. The occurrence and the consequences (e.g., actions by the farmer, milk yield losses) of non-transmission cases were directly simulated. Transmission cases were handled differently as the number of such cases was calculated after each model iteration. Transmission cases are defined in this paper as cases that are directly transmitted from infected cows. These transmission cases were determined based on the number of non-transmission cases together with a pathogen-specific transmission rate. This calculation was performed by multiplying the infection days of the non-transmission cases (i.e., days with ongoing infection) of different pathogens with the pathogen-specific transmission rate. The costs of an individual transmission case were considered equal to the average costs of a non-transmission case of the same pathogen in that model iteration.

Model details

The model parameters are described in Table A1 and A2 in Appendix B.

Initial state

An initial state of the cows at the start of the model in terms of the days in milk (**DIM**), pregnancy status, and parity had to be initiated prior to running the model. To initiate DIM, we used a discrete uniform distribution from 1 to 365 days, where cows in cow places were considered to be in lactation from 1 to 305 days and in the dry period from 306 to 365 days. To initiate the parity of cows at the start of the model run, we used a distribution of parities (Poisson with a mean of 2). At the start of the model, the pregnancy start status of the cow was initiated. A DIM at which a cow in the cow place would become pregnant was sampled for every cow place using a DIM pregnancy distribution (e.g., cow place 5 will get pregnant at 70 DIM, see Pregnancy section). The model started on day 1 of the simulation model with no IMIs in the herd and all lactations ending at 305 DIM. We used a burn-in period of two years to stabilize the number of cow places with an active IMI and the number of lactating cows per day in the herd and remove the effect of the initial state of the herd on the results.

Pregnancy

To simulate the pregnancy of each cow in a cow place in the model, we used a zero-inflated Poisson distribution (i.e., a Poisson distribution with a specific probability of a 0 value) that modeled the DIM at which a cow in the cow place started to be pregnant. If the current DIM was larger than the DIM of the start of pregnancy, the cow in the cow place was regarded as pregnant. If the DIM of the start of pregnancy for a cow in a cow place was 0, the cow in the cow place would never become pregnant during the current lactation. Whenever the DIM was equal to 1 for a cow place in the lactation, the start of pregnancy DIM was resampled for the new lactation. The cow would start her dry cow period 210 days after pregnancy start. Pregnancy was used to determine whether the cow occupying the cow place would be culled for fertility problems. The cow in the cow place was culled when the DIM became equal to or larger than 250, and the cow in the cow place was not pregnant. Culling made the cow place empty and, after a replacement period, which was sampled from a Poisson distribution, a cow of parity 1 was introduced.

IMI dynamics

To model the onset of a non-transmission IMI episode, we used a probability of IMI occurrence for every DIM (see Appendix A for the method to obtain the probability), similar to Østergaard et al. (2005). Only single IMIs were considered in the simulation model, as not enough data or information from the literature was available to model the consequences of the interactions of multiple IMIs. The base probability of obtaining an IMI was adjusted when cows had an IMI earlier in the same lactation by a factor of 1.2 (increased probability) (Østergaard et al., 2005). The probability was also adjusted when the cow in the cow place received dry cow treatment at the end of the preceding dry period by a factor of 0.61 (decreased probability during the first 21 days of the new lactation) (Halasa et al., 2009c). If an IMI-free cow in the cow place obtained an IMI, the pathogen was determined by a multinomial distribution based on pathogen share in the IMI pathogen population (i.e., *Staph. aureus*, Gram-negative pathogens, *Strep. spp.*, and NAS).

Spontaneous recovery from non-transmission IMI was modeled with a pathogen-specific probability. If the cow in the cow place was determined to have spontaneously recovered from the IMI, the time to recovery was drawn from a pathogen-specific duration distribution (Table A1). If the cow in the cow place did not recover spontaneously, IMI status was set to the specific pathogen for the remainder of the model run. This IMI status could subsequently change due to interventions (further described in the Measures section below).

Pathogen transmission

Transmission in this study is strictly constricted to the transmission of contagious bacteria and not environmental bacteria. The transmission of pathogens between cows was calculated for the whole herd at the end of each model iteration. The number of infection days during the model iteration of each pathogen was multiplied by a pathogen-specific transmission parameter (expected number of transmission cases per infected day) based on Dalen et al. (2019a). These cases were summed, forming the total number of transmission cases (Equation 1).

$$N_{transmission} = \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} \beta_i \, Case_{i,j,k} \qquad (1)$$

where $N_{transmission}$ is the number of transmission cases caused by all pathogens, I = (1,2,3,4) for *Staph. aureus*, Gram-negative pathogens, *Strep. spp.*, and NAS, J = (1,… Number of cow places), K = (3*365 days,…7*365 days), β_i is the transmission parameter for pathogen i, and $Case_{i,j,k}$ is a binary parameter indicating an active IMI (1=True, 0=False) for pathogen i at cow place j for day k.

Clinical mastitis

The occurrence of clinical mastitis for non-transmission cases was based on the IMI status of the cow in the cow place. The model specified two ways an IMI episode could turn into a clinical mastitis episode. Firstly, at the beginning of an IMI episode, it had a pathogen-specific probability to turn into a clinical mastitis episode. The number of days with clinical signs was sampled from a statistical distribution and simulated if this event occurred. Secondly, for cow places with an IMI but no clinical mastitis signs (subclinical mastitis), it was simulated whether these cows in cow places would start a new clinical episode (a flare-up) with a pathogen-specific probability. More specifically, at the start of a new subclinical episode (i.e., at the start of a new IMI episode or when previous clinical signs have subsided), there was a pathogen-specific probability of a flare-up at a later date during the same IMI episode. When there was going to be a future flare-up, each day during the remaining episode had an equal probability of being the day of the flare-up. Whether an episode turned clinical from the start or due to a flare-up, the duration of the clinical episode was sampled from a clinical mastitis duration distribution (Table A1).

The non-transmission IMI incidence rate of primiparous and multiparous cows was changed so that the overall clinical mastitis incidence rate for the years after the burn-in was close to 0.28 per cow year (calibration). This value is the median clinical mastitis incidence rate reported in the Netherlands (Santman-Berends et al., 2015). Each clinical mastitis episode was assigned a clinical mastitis severity class (mild, moderate, or severe) according to a pathogen-specific probability distribution (Østergaard et al., 2005; Oliveira et al., 2013).

A conceptual clinical mastitis detection process was implemented. The clinical mastitis detection process was modeled with a sensitivity and a specificity parameter, see Table A1. Specificity was interpreted as a probability of a true negative for a random healthy cow in a cow place, and where $1 - \text{specificity}$ was the probability of a false negative. Sensitivity was interpreted as a probability of a true positive for a random sick cow in a cow place. It was determined whether clinical mastitis was detected for every day and cow place. If the cow in a cow place had clinical mastitis, the probability that the clinical mastitis was detected was equal to the sensitivity parameter. If the cow in the cow place had no clinical mastitis, the probability that the clinical mastitis detection process showed that the cow in the cow place had clinical mastitis was equal to $1 - \text{specificity}$.

Milk yield

To simulate milk yield and milk yield losses due to mastitis for non-transmission cases, we used DIM, SCC, parity status, and SCC history to estimate the daily milk synthesis rate (kg/hour). This simulation was performed using the Generalized Additive Model (**GAM**) proposed in Bonestroo et al. (2022). The adapted model differed from the published model in using the SCC history variable rather than the chronicity group variable. The SCC history variable was defined as the number of days with SCC higher or equal to 200,000 SCC/ml divided by 7 days. It was included to reflect the additive nature of the effect of chronic mastitis on the milk yield (Hadrich et al., 2018). Milk production losses due to subclinical mastitis were calculated by taking the effect of SCC and SCC history on milk synthesis rate from the milk yield GAM (Bonestroo et al., 2022) each day. The milk production rate and losses per hour were multiplied by 24 hours to obtain the daily milk yield. This daily milk yield was combined with the treatment status and clinical mastitis milk loss to determine diverted milk for clinical mastitis cases.

The base clinical mastitis milk production losses were a percentage of milk production over the remainder of the lactation, and this parameter was set to 5% (Hortet and Seegers, 1998; Seegers et al., 2003). This base reduction of daily milk yield was adjusted for clinical mastitis severity using severity class multipliers, adapted from Oliveira et al. (2013). These multipliers ensured that milk production losses due to mild clinical mastitis were lower than those of severe clinical mastitis.

Apart from clinical mastitis milk production losses during the same lactation, milk yield losses incurred by clinical mastitis in any of the previous lactations were estimated to be 3.3%, adapted from the results of Bar et al. (2008).

Somatic cell count

The SCC for non-transmission cases was simulated at the start of an IMI episode and depended on if the cow in the cow place would recover from the IMI episode. The SCC for recovered and non-recovered cases was handled separately.

At the start of recovering cases, SCC data points were sampled from the pathogen-specific SCC distributions for the start point (day 1 of the episode), mid point (day halfway of the episode), and end point of the IMI episode (the last day of the episode). Next, the data points between the starting, mid, and end-point were interpolated using a cubic spline (i.e., a nonlinear piecewise third-order polynomial) (Figure 1). An SCC variation distribution was added to the cubic-spline-interpolated SCC values to simulate the day-to-day variation in SCC. This SCC variation distribution was fitted using actual data used in Bonestroo et al. (2022). It was parameterized as a normal distribution using the difference between an individual SCC sample and the 7-day rolling average of SCC during lactation, resulting in a Normal(0,0.8) distribution. The distribution estimated was similar to the variation distribution used in Østergaard et al. (2005). For the non-recovered cases, SCC samples

were equal to a pathogen-specific constant of SCC for non-recovering cases. These samples were combined with samples of the distribution that mimic day-to-day variation used for the recovered cases using addition (SCC values + day variation in SCC).

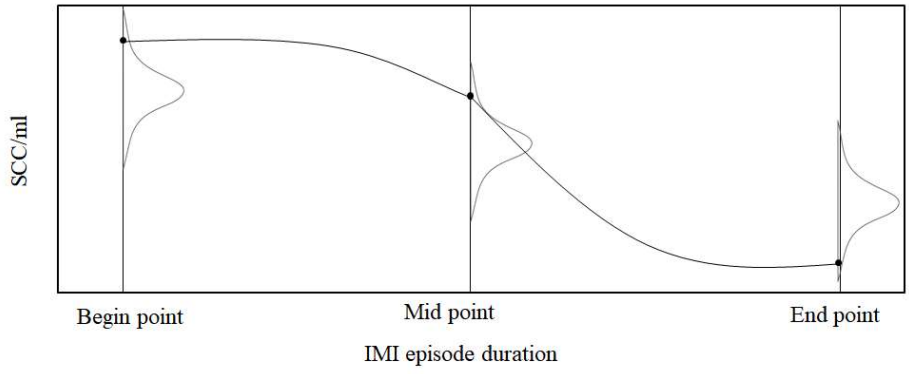

Figure 1. The process of sampling SCC measurements (the dots) at the start point, mid point, and end point and interpolating the points in between the dots (the line) during a recovering IMI episode (points and line are only an example).

For non-IMI SCC measurements, a similar approach was taken as in Østergaard et al. (2005). Using the dataset from Bonestroo et al. (2022), an SCC lactation curve was estimated in a GAM using SCC (dependent variable) and DIM (independent variable) with a random effect on cow lactation level. The fitted values for 1 to 305 DIM were obtained and used in the simulation to reflect that SCC tends to be higher in early lactation (Østergaard et al., 2005). If the lactation was longer than 305 DIM, the SCC level was set to the SCC level at 305 DIM. When the SCC at the end of an IMI episode would be higher than the SCC of a healthy cow with the same DIM, the highest SCC was used for the remainder of the cow's life. Additional variation from samples of the SCC variation distribution was added to these non-IMI samples by using addition. These final values were used as the non-IMI SCC measurements in the model.

Measures

Standard mastitis measures or interventions were simulated based on pre-set generic decision rules grounded on SCC, DIM, and clinical mastitis data. Each measure also had a pre-specified outcome (stochastic in the case of in-lactation or dry cow treatment) on the state of the cow. The decision rules were initiated for every day in the model:

- Clinical mastitis which is detected but was not treated with antibiotics during the last 50 days: treatment with antibiotics
- Rolling median SCC higher than 200,000 SCC/ml for 90 days and not pregnant: cull
- Last day of lactation and having an SCC higher than 150,000 SCC/ml: dry cow treatment

All other combinations of SCC, DIM, and clinical mastitis resulted in no mastitis-related actions. A lactational treatment had a pathogen-specific probability of resulting in recovery from IMI. If the cow in the cow place was simulated to recover after lactational treatment, the duration of the IMI episode was simulated. Otherwise, the IMI status remained the same.

In the Netherlands, standard practice is to initiate dry cow treatment based on the last known SCC without bacteriological testing (KNMvD, 2018). As such, we modeled the initiation of dry cow treatment based on an SCC cut-off. A current IMI, on the last day of lactation, had a pathogen-specific probability of recovering after dry cow treatment. If the cow in the cow place did not have an IMI, the treatment was still performed. If a cow did have an IMI but was not treated in the dry period, the cow would have a pathogen-specific probability of spontaneously recovering during the dry cow period. If the cow in the cow place recovered, the IMI status was reset at the beginning of the next lactation.

Lastly, the possibility of mortality or culling for non-mastitis-or-fertility reasons was modeled for each simulation day. This possibility was modeled as a constant probability for each cow place and simulation day. This culled cow was treated as a culled case without mastitis-related costs.

Calculation of incidence rates and culling rates

Economic outcomes are divided by the number of IMI cases to express outcomes per case of IMI. However, the IMI and clinical mastitis incidence rate were expressed

per cow years at risk, while the culling rate was expressed as per cow (Equations 2, 3, and 4). In Equation 3, we assumed that the transmission cases had the same proportion of clinical mastitis as the non-transmission cases. Henceforth, the fraction of transmission cases relative to the non-transmission cases is multiplied by the non-transmission clinical mastitis cases. This multiplication would approximate the number of clinical mastitis cases for the transmission cases. Dividing the non-transmission and transmission clinical mastitis cases by the number of cow years at risk would give the clinical mastitis incidence rate per cow year. Cow years at risk are defined as the total number of IMI-free days divided by 365 days. The overall culling rate was calculated similarly (Equation 4).

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$$Incidence_{IMI} = \frac{IMI\ Cases_{nt} + IMI\ Cases_{transmission}}{Number\ of\ cow\ years\ at\ risk} \quad (2)$$

$$Incidence_{CM} = \frac{CM\ Cases_{nt} + CM\ Cases_{nt}(\frac{IMI\ Cases_{transmission}}{IMI\ Cases_{nt}})}{Number\ of\ cow\ years\ at\ risk} \quad (3)$$

$$Culling\ rate_{All} = \frac{Total\ cullings_{nt} + Mast.cullings_{nt}(\frac{IMI\ Cases_{transmission}}{IMI\ Cases_{nt}})}{Number\ of\ cow\ years} \quad (4)$$

where $Incidence_{IMI}$ is the IMI incidence rate per cow year, $IMI\ Cases_{nt}$ is the number of the non-transmission IMI cases, $IMI\ Cases_{transmission}$ is the number of the IMI cases that were expected based on the non-transmission IMI cases (see Pathogen transmission section), $Incidence_{CM}$ is the clinical mastitis incidence rate per cow year, $CM\ Cases_{nt}$ is the number of non-transmission clinical mastitis cases, $Culling\ rate_{All}$ is the overall culling rate that includes non-transmission and transmission cases, $Total\ cullings_{nt}$ is the number of non-transmission cullings, and $Mast.cullings_{nt}$ is the number of non-transmission cullings due to mastitis.

Economic calculations

In the economic calculations, we estimated the costs of mastitis as the net effect on farm profit based on the non-economic outcomes. These mastitis costs included the costs of milk production losses, treatment, culling, labor, and contagious transmission. Equations 5 to 15 give the calculations for the economic outcomes. All outcomes and costs were calculated per pathogen (i) for all cow places (j) and all days (k) and summed up across all pathogens to form the total costs of mastitis. More specifically, I = (1,2,3,4,5) for *Staph. aureus*, Gram-negative pathogens, *Strep. spp*., NAS, and no pathogen respectively, J = (1,… Number of cow places), and K = (3*365 days,…7*365 days).

$$Cost_{milk\ loss\ SCM,i} = \sum_{j=1}^{J}\sum_{k=1}^{K} subclinical\ milk\ loss_{i,j,k} * (milk\ price - feed\ correction) \quad (5)$$

$$Cost_{milk\ loss\ CM,i} = \sum_{j=1}^{J}\sum_{k=1}^{K} clinical\ milk\ loss_{i,j,k} * (milk\ price - feed\ correction) \quad (6)$$

On the costs of different factors, $Cost_{milk\ loss\ SCM}$ are the costs of subclinical mastitis milk production losses, $Cost_{milk\ loss\ CM}$ are the costs of clinical mastitis milk production losses, $Cost_{milk\ diversion}$ are the costs of milk diversion, $Cost_{treatment}$ are the costs of lactational antibiotic treatment due to mastitis, $Cost_{diagnostics}$ are the costs of laboratory analysis to find underlying IMI, $Cost_{DCT}$ are the costs of applying dry cow treatment, $Cost_{labor\ che}$ are the labor costs of checking clinical mastitis alerts, $Cost_{culling}$ are the costs of culling, $N_{IMI\ cases}$ is the total number of non-transmission cases, and $N_{transmission}$ is the total number of transmission cases. $Cost_{transmission}$ are the total costs of contagious transmission. All costs were also expressed as the pathogen-specific share of the overall chronic mastitis costs.

For the costs of diagnostics, it was assumed that 15% of the lactational treatments are carried out in combination with bacteriology (diagnostic proportion), adapted from Griffioen et al. (2016). For the costs of lactational treatment, it was assumed that 5% of the treatments required a veterinarian (proportion veterinary treatment) (Lam et al., 2013).

$$Cost_{diagnostics,i} = \sum_{j=1}^{J}\sum_{k=1}^{K} Lactational\ treatment_{i,j,k} * diagnostic\ price * diagnostic\ proportion \quad (7)$$

$$Cost_{diverted\ milk,i} = \sum_{j=1}^{J}\sum_{k=1}^{K} Diverted\ milk_{i,j,k} * (milk\ price - feed\ correction) \quad (8)$$

$$Cost_{lactational\ treatment,i} = \sum_{j=1}^{J}\sum_{k=1}^{K} Lactational\ treatment_{i,j,k} * (lactational\ treatment\ price + labor\ lactational\ treatment * labor\ wage * (1 - proportion\ involvement\ veterinarian) + labor\ lactational\ treatment * veterinarian\ wage * proportion\ involvement\ veterinarian) \quad (9)$$

$$Cost_{labor\ che\ ,i} = \sum_{j=1}^{J}\sum_{k=1}^{K} Lactational\ treatment_{i,j,k} * labor\ farmer\ check * labor\ wage \quad (10)$$

$$Cost_{DCT,i} = \sum_{j=1}^{J}\sum_{k=1}^{K} Dry\ cow\ treatment_{i,j,k} * (dry\ cow\ treatment\ price + labor\ dry\ cow\ treatment * labor\ wage) \quad (11)$$

$$Cost_{culling,i} = \sum_{j=1}^{J}\sum_{k=1}^{K} culled\ due\ to\ mastitis_{i,j,k} * culling\ cost \quad (12)$$

$$Cost_{mastitis\ without\ transmission,i} = Cost_{milk\ loss\ SCM,i} + Cost_{milk\ loss\ CM,i} + Cost_{diverted\ milk,i} + Cost_{lactational\ treatment,i} + Cost_{diagnostics,i} + Cost_{DCT,i} + Cost_{labour\ checks,i} + + Cost_{culling,i} \quad (13)$$

$$Cost_{transmission} = \sum_{i=1}^{I} \frac{Cost_{mastitis\ without\ transmission,i}}{N_{IMI\ cases,i}} * N_{transmission,i} \quad (14)$$

$$Cost_{mastitis} = Cost_{transmission} + \sum_{i=1}^{I} Cost_{mastitis\ without\ transmission,i} \quad (15)$$

Calculation of costs of chronicity

In this study, we specifically investigated the costs due to chronic mastitis, and therefore, the simulation model output associated with chronic cases was used. Days of ongoing chronic cases were identified by an IMI duration of more than 28 days combined with a 28-day SCC mean of more than 200,000 SCC/ml (Bonestroo et al., 2021). When identified as a chronic case, the initial stage (i.e., the 27 days up to the identification) was also designated as chronic. The milk production losses due to subclinical and clinical mastitis, diverted milk, diagnostics, contagious transmission, culling, and the number of treatments on these days were also identified as consequences of chronic mastitis.

Special attention needed to be paid to identifying the transmission of chronic mastitis cases in the model. Equation 1 was adapted to ensure that only the transmission of more prolonged cases (4 weeks or longer IMI and high SCC) was included in the calculation of the chronic mastitis costs by the addition of the $Chronic_{i,j,k}$ variable (Equation 16).

$$N_{transmission\ chronic} = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} \beta_i\, Case_{i,j,k} Chronic_{i,j,k} \quad (16)$$

where $N_{transmission\ chron}$ is the number of transmission cases caused by all IMIs during chronic IMI episodes, I = (1,2,3,4) for *Staph. aureus*, Gram-negative pathogens, *Strep. spp*., and NAS, respectively, J = (1,… Number of cow places), K = (3*365 days,…7*365 days), β_i is the transmission parameter for IMI i, $Case_{i,j,k}$ is a binary parameter indicating an active IMI (1=True, 0=False) for IMI i at cow place j for day k, and $Chronic_{i,j,k}$ is a binary parameter indicating whether the active IMI is chronic (current episode active for more than 4 weeks, 1=True, 0=False) for IMI i at cow place j for day k.

The costs during a chronic mastitis episode were indicated as chronic costs. As such, Equations 5 until 11 were adapted for chronic mastitis (as in Equation 16). and these costs would include: the costs of subclinical and clinical milk production losses, diverted milk costs, the lactational treatment costs, the labor costs of checking clinical mastitis alerts, dry cow treatment costs, and the diagnostic costs. Culling costs due to mastitis were set equal to the culling cost due to mastitis (Equation 12). The number of transmissions during chronic episodes was multiplied by the average costs of a mastitis case caused by each pathogen to obtain the costs due to extra transmissions. All previously mentioned chronic mastitis costs were summed to obtain the total chronic mastitis costs. All outcomes and costs were calculated per pathogen as well and expressed as the pathogen-specific share of the overall chronic mastitis costs.

Validation and sensitivity analysis

The model was validated using multiple validation strategies as proposed by Sargent (2010). Firstly, the model was validated using extreme condition tests. We used extreme scenarios to determine whether the model resulted in expected behavior (e.g., setting IMI incidence rate or transmission to 0 and estimating no mastitis or transmission-related costs). Secondly, traces were used as individual cow places were monitored throughout the simulation to determine consistency, similar to Gussmann et al. (2018). Thirdly, external validation was performed. To assess whether the model reflected the average Dutch dairy farm, we compared the culling rate of the model with the Dutch average for dairy farms. The cullings were in line with the reported Dutch average culling rate (0.3 cullings per cow year) (Nor et al., 2014).

Lastly, a sensitivity analysis was performed by changing lactational cure rates after treatment for all pathogens, dry cow treatment cure rates for all pathogens, and the transmission parameters for all pathogens, decreasing and increasing them by 20%. The sensitivity analysis parameters were chosen as they were expected to have the most considerable effects on mastitis costs.

Results

Mastitis dynamics

The model results indicated a median culling rate was 0.32 with a quartile range of 0.30 to 0.34 per cow year. The median clinical mastitis incidence rate was 0.27 with a quartile range of 0.25 to 0.30 cases per cow year. The median incidence rate of IMI was 1.04 with a quartile range of 0.98 to 1.11 cases per cow year.

Economics

Total costs of mastitis

Table 1 gives the economic outcomes of the model per IMI case. The median total mastitis costs were € 208 with a quartile range of € 197 to € 225 per IMI case. Most of the costs occurred due to transmission (i.e., transmission cases), culling, and clinical and subclinical milk production losses. Other substantial costs originated from dry cow treatments, lactational treatments, and diverted milk. We could determine that *Staph. aureus* caused the largest share of the total costs of IMI by

looking at pathogen-specific economic impact per generic IMI case, followed by NAS, *Strep. spp*., and Gram-negative pathogens.

Costs of chronic mastitis

In Table 2, we present chronic mastitis costs per IMI case and its cost factors. The median total costs due to chronic mastitis were € 104 (50% of the total mastitis costs) with a quartile range of € 96 to € 115 per IMI case. The share of chronic mastitis relative to the total mastitis costs was substantial. Unsurprisingly, the costs due to transmission had a large share in the chronic mastitis costs (45% of the total chronic mastitis costs). Culling and milk production losses substantially affected the costs of chronic mastitis as well (culling: 24%, combining subclinical and clinical milk production losses: 16%). Subclinical mastitis production losses were higher than clinical mastitis production losses compared to the share in the total costs of mastitis. On average, *Staph. aureus* had the largest share in the costs of chronic mastitis (70%), followed by NAS (14%), *Strep. spp.* (11%), and Gram-negative pathogens (5%).

Sensitivity analysis

In the sensitivity analysis, we assessed the effects of decreasing and increasing the transmission rate of all pathogens with 20%, lactational cure rates for all pathogens with 20%, and dry cow treatment cure rates for all pathogens with 20%. The median (25% and 75% quartiles) of total mastitis costs and the chronic mastitis costs are reported in Table 3. The results show that changing the dry cow treatment parameters had the most influence on the total and chronic mastitis costs. Changing the dry cow treatment parameters was followed by changing the transmission and lactational cure parameters.

Table 1. *Economic results of the simulation model for the total mastitis costs, and pathogen-specific shares of the total cost (in €) per non-transmission IMI case.*

Costs	1st Qu.	Median	3rd Qu.
Total clinical mastitis milk production losses	25.27	28.29	31.77
Total subclinical mastitis milk production losses	21.77	23.06	24.48
Total mastitis culling	21.50	24.55	27.60
Total lactational antibiotics	10.89	11.87	12.85
Total dry cow treatment	10.65	11.33	12.09
Total diagnostics	0.36	0.39	0.42
Total diverted milk	8.82	9.65	10.52
Total clinical mastitis checks	0.37	0.40	0.43
Total extra costs due to transmission	90.76	99.26	110.74
Total mastitis costs	197.12	208.02	225.27
Pathogen-specific share of the total costs			
Total *Staph. aureus* share	91.46	101.03	113.67
Total Gram-negative pathogens share	8.47	10.67	13.20
Total *Strep. spp.* share	22.79	26.53	30.80
Total Non-Aureus Staph. share	61.31	67.99	76.28
Total non-pathogen-related share[1]	1.36	1.68	2.11

[1] Costs due to culling cows with high SCC with no pathogen or treating cows with no pathogen

Table 2. *Economic results of the simulation model for the chronic mastitis costs, and pathogen-specific shares of the chronic cost (in €) per non-transmission IMI case.*

Costs	1st Qu.	Median	3rd Qu.
Total chronic mastitis milk production loss due to clinical mastitis	5.61	6.62	7.76
Total subclinical mastitis during ongoing non-spontaneously cured IMI milk production losses	9.93	10.86	12.02
Total chronic mastitis treatment	5.29	6.03	6.83
Total chronic mastitis diagnostics	0.17	0.20	0.23
Total chronic mastitis extra costs due to transmission	41.11	46.81	53.71
Total chronic mastitis diverted milk	4.30	4.89	5.66
Total chronic mastitis culling	21.50	24.55	27.60
Total chronic dry cow treatment	3.60	3.97	4.38
Total chronic mastitis costs	95.91	104.25	114.95
Pathogen-specific share of costs			
Total chronic *Staph. aureus* share	64.67	72.97	82.33
Total chronic Gram-negative pathogens share	3.04	4.96	6.88
Total chronic *Strep. spp.* share	9.15	11.47	14.11
Total chronic Non-Aureus Staph. share	12.17	14.57	16.76

Table 3. *The median (25% and 75% quartiles) economic results of the sensitivity analysis of increasing (+20%) and de*creasing (*-20%) the transmission rate for all pathogens, the lactational cure rate after treatment for all pathogens, and the dry cow treatment cure rate for all pathogens.*

	Transmission parameters- 20%	Transmission parameters +20%
Total mastitis costs per case (in €)	189.39 (177.66 – 201.23)	229.41 (213.89 – 244.74)
Total costs due to chronic mastitis per case (in €)	94.36 (86.76 – 102.45)	113.55 (103.81 – 123.91)
	Lactational cure rate parameters –20%[1]	Lactational cure rate parameters +20%[1]
Total mastitis costs per case (in €)	226.94 (211.60 – 241.58)	194.50 (180.26 – 207.50)
Total costs due to chronic mastitis per case (in €)	118.92 (108.64 – 128.49)	90.71 (81.28 -100.71)
	Dry cow treatment cure rate parameters – 20%[1]	Dry cow treatment cure rate parameters +20%[1]
Total mastitis costs per case (in €)	243.53 (225.80 – 260.41)	184.37 (172.95 – 195.55)
Total costs due to chronic mastitis per case (in €)	124.28 (112.75 – 136.58)	88.88 (80.50 – 95.85)

[1] If the resulting lactational or dry cow treatment cure rate was above 100%, it was capped at 100%

Discussion

This study is not the first study on mastitis costs, but it contains several unique features adding to our knowledge of the economic impact of mastitis. Simulation modeling has been frequently used to assess mastitis costs in the past (Hogeveen et al., 2019), but it did not distinguish between non-chronic and chronic mastitis. This study is the first that used a simulation model that estimates the costs of chronic mastitis on a herd basis. Another unique feature of the model was its use of GAM models to model daily milk yields and SCC levels in detail based on real-life milking data. The effect of subclinical mastitis on milk yield was estimated by associating SCC with milk yield in the GAM model. The model results show that chronic mastitis caused a large share of the total costs of mastitis. The chronic mastitis

costs mainly were caused by *Staph. aureus* due to its low cure rate.

This study's median total costs of mastitis of € 208 per IMI can be compared to other simulation model studies focusing on Dutch dairy farms. Others have calculated the costs of clinical and subclinical cases separately. Huijps et al. (2008) estimated the total costs of € 210 per clinical case and € 53 to € 72 for a subclinical case for an average Dutch dairy farm. Van Soest et al. (2016) estimated a total cost of € 301 per clinical case for an average Dutch dairy farm. Without the transmission cost factor, we would estimate the total costs of mastitis per case to be € 109 per IMI case, based on a combination of clinical and subclinical cases. The estimated costs of IMI in this study were between the costs of clinical and subclinical cases in the other studies. Therefore, the estimated total cost of mastitis was in line with previous results.

The total costs of (chronic) mastitis could be attributed to different cost factors. As such, it was possible to estimate the most crucial factors in mastitis costs. The transmission was found to be an important factor, and others have found comparable results (Halasa et al., 2009a; Down et al., 2013; Gussmann et al., 2018). Culling was also an important cost factor, which others found as well (Huijps et al., 2008; Halasa et al., 2009a; Aghamohammadi et al., 2018). Milk production losses were also an important cost factor. Nevertheless, the cost estimates of milk yield losses found in our study were lower than reported by van Soest et al. (2016), Huijps et al. (2008), and Aghamohammadi et al. (2018). This difference can be explained by the different methods of calculating subclinical mastitis milk production losses. We used a GAM model that found a nonlinear association between SCC and milk yield that is difficult to estimate with a linear model (Bonestroo et al., 2022). This GAM model estimated limited subclinical milk losses until 277,000 SCC/ml. As such, previous studies may have overestimated subclinical milk production losses at lower levels of SCC. To further investigate this, we have fitted a linear mixed model with SCC and SCC^2 together with a random cow effect explaining milk yield and used it in the simulation model instead of a GAM model (data not shown). In that case, the subclinical milk production losses were similar to those reported in Aghamohammadi et al. (2018). Therefore, it is likely that the estimated non-linearity of the GAM model provided lower estimations of subclinical milk production losses at lower levels of SCC.

In the model, we used a limited IMI pathogen population of 4 pathogen classes and assumed that a cow could only have one specific IMI ongoing at the time. This simplification was made due to the limited information available on other pathogens and the effects of having multiple pathogens at once on SCC and milk production. For the limited pathogen population, similar approaches were taken by others in the past (Østergaard et al., 2005; Halasa et al., 2009a). Nevertheless, the model could be adapted to add more pathogens to the pathogen population of the herd or the possibility to have multiple IMIs at once.

We used a large set of input variables to estimate the costs of chronic mastitis. Although we have tried to get a consistent set of input parameters, these were from multiple trials with multiple herds under various production circumstances. This variety in sources increased the uncertainty of the parameters in the current model. The model was calibrated to model an average Dutch herd. However, farm and regional differences will still exist on individual farms, and therefore differences in the costs of mastitis will also exist between farms. Nevertheless, the current model can be adapted to account for regional and price differences in the future.

We simplified farmer behavior in the model to (sensor-based) decision rules and tied it to an action (e.g., antibiotic treatment or culling). In practice, farmer decision-making is complex. For instance, modeling the culling behavior of farmers is difficult as there are many reasons for culling cows. Bascom and Young (1998) indicated that mastitis is the primary reason in 15% for the culling, but in 5% and 2% of culled cows as secondary or tertiary reasons. Therefore, it was difficult to model culling decisions in detail and likewise for the other decisions (e.g., when to apply antibiotic treatment). However, we would argue that this simplified approach is valuable. It allows the model user to estimate the cost of mastitis and chronic mastitis for different (sensor-based) decision rules relatively swiftly. Different sensor-based strategies can be operationalized relatively simply. These simple strategies can be focused on specific changes in procedures in this manner. As such, these simplified decision rules could be used to form economics-based and sensor-based farm procedures by firstly experimenting with these rules in a simulation model before applying them in practice.

We decided to simplify transmission in the simulation model due to the uncertainty of transmission of contagious bacteria in AMS systems. The transmission was calculated after each model iteration of the simulation model instead of using a susceptible-infected-recovered framework, as done by Halasa et al. (2009a). The costs of transmission cases were estimated by multiplying the expected transmission cases by the pathogen-specific average costs of a mastitis case. Limited data were available on the transmission of several IMIs on a farm with AMS, making the estimation of transmission uncertain. It can be expected that transmission rates on AMS farms are lower than on conventional farms due to automated cleaning and disinfection procedures in milking robots. Currently, only Dalen et al. (2019a), Skarbye et al. (2021), and Deng et al. (2021) recently reported transmission rates with current robots. It was only studied on either 1 or 2 farms for 4 to 17 months for a limited number of pathogens. Other studies that measure transmission on AMS systems were unknown to the authors. We used the results of Dalen et al. (2019a) as they covered

the most pathogens. The differences in these studies indicate that the transmission rates vary substantially between farms and have a large confidence interval. Due to this uncertainty and variety, we wanted to separate the economic effect of contagious transmission. The model would still return adequate estimates for the rest of the cost factors for non-transmission cases, even if the transmission rate was highly uncertain. Nevertheless, the considerable influence of transmission on the cost of mastitis can be seen in the results, and this is also found by others (Halasa et al., 2009a; Down et al., 2013). Our results confirm that extra costs due to transmission are a substantial factor in the total costs of mastitis and chronic mastitis.

We chose to use an adapted definition of mastitis chronicity during modeling based on Bonestroo et al. (2021). An IMI case was deemed chronic if it lasted longer than 4 weeks in IMI and elevated SCC. A definition with a longer minimum duration would lower chronic mastitis costs. However, Bonestroo et al. (2021) supply the only daily-SCC measurement-based chronic mastitis duration threshold to our knowledge. We used it as it was the only study available.

This study highlights the economic importance of chronic mastitis in the total costs of mastitis. Failure to completely recover early in the IMI episode was responsible for a substantial portion of the total costs of mastitis. The costs of chronic cases are more focused on the failure costs: the failure of a cure with and without treatment. Efforts have been made to assess the efficiency and effectiveness of treating chronic mastitis (St. Rose et al., 2003; Swinkels et al., 2005b; Steeneveld et al., 2007) for specific IMIs. Nevertheless, clinical mastitis has traditionally been focussed on in lactational treatment (Pyörälä, 2009). We estimated that 45% of the costs of chronic mastitis are due to the transmission of contagious bacteria. Therefore, one of the most effective strategies to decrease the costs of chronic mastitis would be finding chronic cows and isolating them or improving milking procedures to reduce the risk of transmission during milking. Knowing the importance of chronic cases' costs helps us understand the potential benefits of better chronic mastitis prevention and management. With the current sensor technology, it may be possible to identify cows with chronic IMI episodes and possibly even predict cows that are expected to become chronic, allowing for identification and resulting interventions at an earlier stage.

Conclusion

Our results show that the median costs of chronic mastitis were € 104 and that the median total costs of mastitis were € 208. The share of the costs of chronic mastitis relative to the total mastitis costs was 50%, showing the importance of chronic mastitis in the total costs of mastitis. The underlying causes of the costs of chronic mastitis mainly were the transmission of IMIs of ongoing cases, culling, and milk production losses. In terms of pathogen impact, *Staph. aureus* caused the largest share of the costs of chronic mastitis. As current developments in the application of sensors enable better identification of chronic mastitis, prevention and management of chronic mastitis should get more attention in the future.

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Potential conflicts of interest

John Bonestroo and Ilka C. Klaas are employed by DeLaval International AB. Mariska van der Voort, Nils Fall, Ulf Emanuelson, and Henk Hogeveen have no conflict of interest to report.

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Appendix

Appendix A. The estimation of the probability of obtaining IMI

A logistic Generalized Additive Model (GAM) was fitted that predicted whether the SCC was higher than 200,000 for the first time during an episode (dependent variable) based on DIM (independent variable). The start of the episode was determined by checking whether the mean SCC of the next 25 days was higher than 200,000 SCC/ml. As we did not have access to the probability of being infected for every DIM, we have used the SCC-based GAM to obtain a proxy for these probabilities based on increased SCC, see Figure 1. A probability was predicted for each DIM between 1 and 305. These probabilities were normalized to be summed up to 1 (the area under the curve in Figure 1). This probability of increased SCC was used as a proxy for a probability of IMI. The probability of IMI for each DIM was calculated by multiplying these normalized values with the incidence rates of IMI for primiparous and multiparous cows. For DIM after 305. i.e., for longer lactations, the probability of infection was assumed to be equal to the probability of infection at 305 DIM. For each time step and IMI-free cow, the probability of an IMI was retrieved from these "curves."

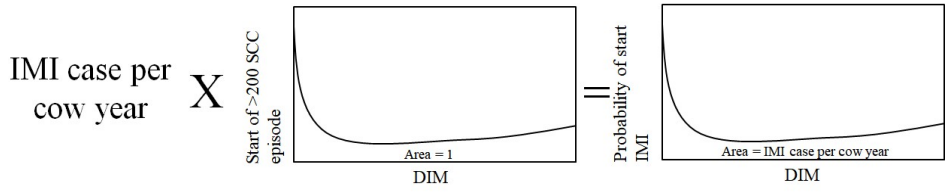

Figure A1. The process of acquiring the incidence rate distribution of intramammary infections (IMI) based on the predefined IMI incidence rate parameter to indicate the level of IMI on a herd level.

Appendix B. The model parameters with references

Table A1. *The model parameters in the simulation model with their distribution or value and their sources*

Model parameter	Distribution or value	Reference
Incidence rate non-transmission cases of IMI for primiparous cows	0.28 per cow year	Tuned to obtain a model-reported incidence rate close to reported in Santman-Berends et al. (2015)
Incidence rate non-transmission cases of IMI for multiparous cows	0.504 per cow year	Tuned to obtain a model-reported incidence rate close to reported in Santman-Berends et al. (2015)
Daily probability of culling due to other reasons than fertility or mastitis	Uniform(1, $1.145^{1/365}$-1)	Adapted from Nor et al. (2014) and Bascom and Young (1998)
Day in milk at the initiation of the model for cow	Uniform(min = 1, max = 365) where 306-365 is in the dry cow period	Initial initialization
Parity at the initiation of the model for cow	Poisson(mean = 2) +1	Initial initialization
Day in milk of pregnancy distribution	Zero-inflated Negative Binominal distribution (mean = 123.72, sigma = 0.28, probability = 0.05)	Based on a dataset used in Bonestroo et al. (2022) with the addition of pregnancy information. The mean DIM of pregnancy is similar to Berends et al. (2008).
Event IMI	Bernoulli(probability = a DIM-specific probability)	Adapted from a DIM-specific probability based on the first occurrence of high SCC in the dataset used in Bonestroo et al. (2022) multiplied by the incidence rate (Appendix A), similar to Østergaard et al. (2005)
Specific IMI occurrence given IMI occurrence	Multinomial(P.aureus = 0.29, P.g.negative = 0.05, P.strep = 0.17, P.NAS = 0.49)	Adapted from Taponen et et al. (2017)
Lactational IMI self-recovery *Staph. aureus*	Bernoulli(probability = 0.19)	Van den Borne et al. (2010)
Lactational IMI self-recovery Gram-negative	Bernoulli(probability = 0.51)	Fuenzalida and Ruegg (2019)
Lactational IMI self-recovery *Strep. spp.*	Bernoulli(probability = 0.66)	Wilson et al. (1999)
Lactational IMI self-recovery NAS	Bernoulli(probability = 0.46)	Taponen et al. (2006)
Lactational treatment IMI recovery *Staph. aureus* given no self-recovery	Bernoulli(probability = 0.52-0.19)	Sol et al. (2000) and Van den Borne et al. (2010)
Lactational treatment IMI recovery Gram-negative given no self-recovery	Bernoulli(probability = 0.70-0.51)	Fuenzalida and Ruegg (2019)
Lactational treatment IMI recovery *Strep. spp.* given no self-recovery	Bernoulli(probability = 0.83-0.66)	Wilson et al. (1999)
Lactational treatment IMI recovery NAS given no self-recovery	Bernoulli(probability = 0.86-0.46)	Taponen et al. (2006)
Duration *Staph. aureus* IMI if cured	Poisson(mean = 30)	Sol et al., (2000)
Duration Gram-negative IMI if cured	Poisson(mean = 18)	Based on the median in the survival graph in Fuenzalida and Ruegg (2019)
Duration *Strep. spp.* IMI if cured	Poisson(mean = 37.5)	Average of both groups in Zadoks et al. (2003)
Duration NAS IMI if cured	Poisson(mean = 28)	Adapted from Valckenier et al. (2021) by combining transient and persistent IMI classes for all NAS (assuming that transient IMI can take 14 days)
Clinical mastitis given underlying *Staph. aureus* on first day of IMI	Bernoulli(probability = 0.17)	Swinkels et al. (2005a)
Clinical mastitis given underlying Gram-negative on first day of IMI	Bernoulli(probability = 0.85)	Hogan and Smith (2003)
Clinical mastitis given underlying *Strep. spp.* on first day of IMI	Bernoulli(probability = 0.3)	Swinkels et al. (2005b)
Clinical mastitis given underlying NAS on first day of IMI	Bernoulli(probability = 0.08)	Todhunter et al. (1993)

Model parameter	Distribution or value	Reference
Flare-up during the same IMI episode of *Staph. aureus*	Bernoulli(probability = 0.27)	Adapted from Wente et al. (2020)
Flare-up during the same IMI episode of Gram-negative	Bernoulli(probability = 0.10)	Adapted from Wente et al. (2020)
Flare-up during the same IMI episode of *Strep. spp.*	Bernoulli(probability = 0.19)	Adapted from Wente et al. (2020)
Flare-up during the same IMI episode of NAS	Bernoulli(probability = 0.02)	Adapted from Wente et al. (2020)
Clinical mastitis episode duration	Poisson(mean = 6.11)	Nash et al. (2002)
Clinical mastitis severity class, given *Staph. aureus*	Multinomial(Mild = 0.53 Moderate = 0.47 Severe = 0.00)	Oliveira et al. (2013)
Clinical mastitis severity class, given Gram-negative	Multinomial(Mild = 0.32 Moderate = 0.35 Severe = 0.33)	Adapted from Oliveira et al. (2013) by averaging all gram-negative pathogens
Clinical mastitis severity class, given *Strep. spp.*	Multinomial(Mild = 0.61 Moderate = 0.38 Severe = 0.01)	Oliveira et al. (2013)
Clinical mastitis severity class, given NAS	Multinomial(Mild = 0.61 Moderate = 0.37 Severe = 0.02)	Oliveira et al. (2013)
Base clinical mastitis milk production losses remainder lactation	5%	Seegers et al. (2003)
Milk production losses multiplier of mild clinical mastitis	-3.7/-5.1 = 0.725	Oliveira et al. (2013), assuming the differences between mild and moderate hold for the remainder of the lactation
Milk production losses multiplier of moderate clinical mastitis	-5.1/-5.1 = 1	Oliveira et al. (2013)
Milk production losses multiplier of severe clinical mastitis	-11.2/-5.1 = 2.196	Oliveira et al. (2013), assuming the differences between severe and moderate hold for the remainder of the lactation
SCC at the beginning of a *Staph. aureus* IMI episode	Triangular(a = ln(281), b = ln(430), c= ln(355))	Adapted from Dalen et al. (2019a), where the minimum and maximum are based on the 95% confidence interval
SCC at the mid-point of a *Staph. aureus* IMI episode	Triangular(a = ln(100), b = ln(200), c= ln(150))	Adapted from Dalen et al. (2019a) and the general pattern in Bonestroo et al. (2021)
SCC at the end-point of a *Staph. aureus* IMI episode	Triangular(a = ln(50), b = ln(150), c= ln(100))	Adapted from Dalen et al. (2019a) and the general pattern in Bonestroo et al. (2021)
SCC at the beginning of a Gram-negative IMI episode	Triangular(a = ln(1600), b = ln(2500), c= ln(2000))	Adapted from Fuenzalida and Ruegg (2019) quarter weekly SCC, we assumed a healthy SCC of 50,000 SCC/ml in the other quarters and assuming equal MY for all quarters and averaging the SCC for all quarters

Model parameter	Distribution or value	Reference
SCC at the mid-point of a Gram-negative IMI episode	Triangular(a = ln(300), b = ln(800), c= ln(500))	Adapted from Fuenzalida and Ruegg (2019) quarter weekly SCC, we assumed a healthy SCC of 50,000 SCC/ml in the other quarters and assuming equal MY for all quarters and averaging the SCC for all quarters and Bonestroo et al. (2021)
SCC at the end-point of a Gram-negative IMI episode	Triangular(a = ln(50), b = ln(150), c= ln(100))	Adapted from Fuenzalida and Ruegg (2019) quarter weekly SCC, we assumed a healthy SCC of 50,000 SCC/ml in the other quarters and assuming equal MY for all quarters and averaging the SCC for all quarters and Bonestroo et al. (2021)
SCC at the beginning of a *Strep. spp.* IMI episode	Triangular(a = ln(160), b = ln(430), c= ln(300))	Adapted from Dalen et al. (2019a), where the minimum and maximum are based on the 95% confidence interval
SCC at the mid-point of a *Strep. spp.* IMI episode	Triangular(a = ln(100), b = ln(200), c= ln(150))	Adapted from Dalen et al. (2019a) and the general pattern in Bonestroo et al. (2021)
SCC at the end-point of a *Strep. spp.* IMI episode	Triangular(a = ln(50), b = ln(150), c= ln(100))	Adapted from Dalen et al. (2019a) and the general pattern in Bonestroo et al. (2021)
SCC at the beginning of a NAS IMI episode	Triangular(a = ln(50), b = ln(350), c= ln(170))	Adapted from Dalen et al. (2019a), where the minimum and maximum are based on the 95% confidence interval calculated using a weighted standard deviation of the NAS IMIs
SCC at the mid-point of a NAS IMI episode	Triangular(a = ln(50), b = ln(200), c= ln(150))	Adapted from Dalen et al. (2019a) and the general pattern in Bonestroo et al. (2021)
SCC at the end-point of a NAS IMI episode	Triangular(a = ln(50), b = ln(150), c= ln(100))	Adapted from Dalen et al. (2019a) and the general pattern in Bonestroo et al. (2021)
SCC non-recovery constant of *Staph. aureus* IMI episode	Ln(355)	Adapted from the reported mean in Dalen et al. (2019a)
SCC non-recovery constant of Gram-negative IMI episode	Ln(2000)	Adapted from Fuenzalida and Ruegg (2019a) quarter weekly SCC, we assumed a healthy SCC of 50,000 SCC/ml in the other quarters and assuming equal MY for all quarters and averaging the SCC for all quarters
SCC non-recovery constant of *Strep. spp.* IMI episode	Ln(300)	Adapted from the reported mean in Dalen et al. (2019a)
SCC non-recovery constant of NAS IMI episode	Ln(170)	Adapted from the reported mean in Dalen et al. (2019a), where a weighted mean was taken of all NAS IMIs
SCC healthy (IMI free)	Function dependent on DIM	-
Day-to-day variation SCC	Normal(0,0.80)	Based on SCC data from Bonestroo et al. (2022)
Milk yield	Based on a GAM model[1]	Bonestroo et al. (2022)
Clinical mastitis detected given clinical mastitis	Bernoulli(probability = 0.40)	Author's expertise
Clinical mastitis detected given no clinical mastitis	Bernoulli(probability = 1 - 0.99)	ISO (2007)
Dry cow treatment recovery *Staph. aureus*	Bernoulli(probability = 0.77)	Halasa et al. (2009b)
Dry cow treatment recovery Gram-negative	Bernoulli(probability = 0.90)	Halasa et al. (2010)
Dry cow treatment recovery *Strep. spp.*	Bernoulli(probability = 0.89)	Halasa et al. (2009b)
Dry cow treatment recovery NAS	Bernoulli(probability = 0.81)	Newton et al. (2008)
Untreated dry cow period recovery *Staph. aureus*	Bernoulli(probability = 0.44)	Halasa et al. (2009b)
Untreated dry cow period recovery Gram-negative	Bernoulli(probability = 0.60)	Huijps and Hogeveen (2007)
Untreated dry cow period recovery *Strep. spp.*	Bernoulli(probability = 0.47)	Halasa et al. (2009b)

Model parameter	Distribution or value	Reference
Untreated dry cow period recovery NAS	Bernoulli(probability = 0.76)	Adapted from the minor pathogens for untreated low SCC group in Swinkels et al. (2021)
Replacement period where cow spot was empty after culling	Poisson(Lamba = 15)	Author's expertise
Labor farmer clinical mastitis check cow	0.10 hours per activity	Author's expertise
Labor lactational treating cow	0.50 hours per activity	Author's expertise
Labor dry cow treatment cow	0.50 hours per activity	Author's expertise
Expected number of extra transmissions per infection day (transmission parameter beta) for *Staph. aureus*	0.0093 new infections per infected day	Dalen et al. (2019b)
Expected number of extra transmissions per infection day (transmission parameter beta) for Gram-negative	0.0001 new infections per infected day	Gussmann et al.(2018)
Expected number of extra transmissions per infection day (transmission parameter beta) for *Strep. spp.*	0.003 new infections per infected day	Dalen et al. (2019b)
Expected number of extra transmissions per infection day (transmission parameter beta) for NAS	0.007452 new infections per infected day	A weighted average of NAS in Dalen et al. (2019b)

[1]A Generalized Additive Model (GAM) that models the association of SCC and DIM with milk yield using a nonlinear function

Table A2. *The economic prices in the simulation model with their value and their sources.*

Economic price	Value	Source
Milk price	€ 0.36 per kg	Blanken et al. (2019)
Feed cost correction milk price	€ 0.09 per kg	Adapted from Blanken et al. (2019)
Labor wage	€ 15.40 per hour	Adapted from Blanken et al. (2019)
Laboratory diagnostic price	€ 10.00 per test	Authors' expertise
Laboratory diagnostic use proportion	15%	Adapted from Griffioen et al. (2016)
Involvement of veterinarian in treatment	5%	Lam et al. (2013)
Dry cow treatment costs	€ 11.00 per treatment	Scherpenzeel et al. (2018)
Lactational treatment	€ 35.00 per treatment	Lam et al. (2013)
Veterinarian price	€ 128.40 per hour	GD (2019)
Culling costs based on the slaughter value of the culled cow (revenue) and the opportunity costs of the replacement heifer (costs)	€ 297.00 per first-parity cow, € 212.00 per second-parity cow, € 186.00 per third-or-more-parity cow	Steeneveld et al. (2020)

Appendix C. The convergence of the number of IMI cases and number of lactating cows

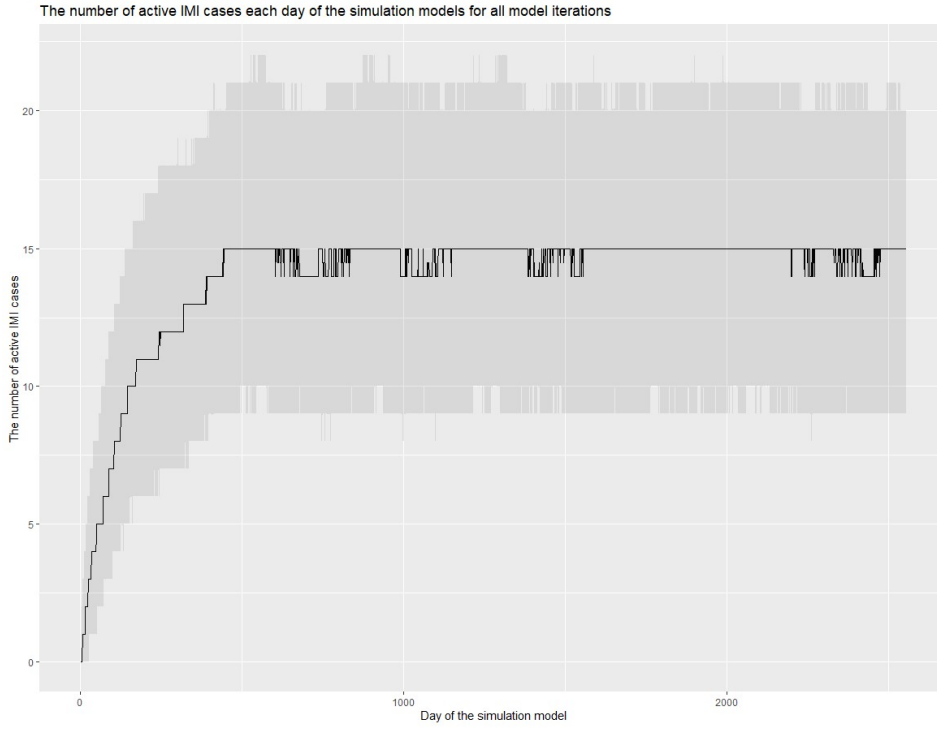

Figure A2. The median (black line) and the 95% quantile interval (shaded area) of the number of active cases (y-axis) over the number of simulated days in the simulation model.

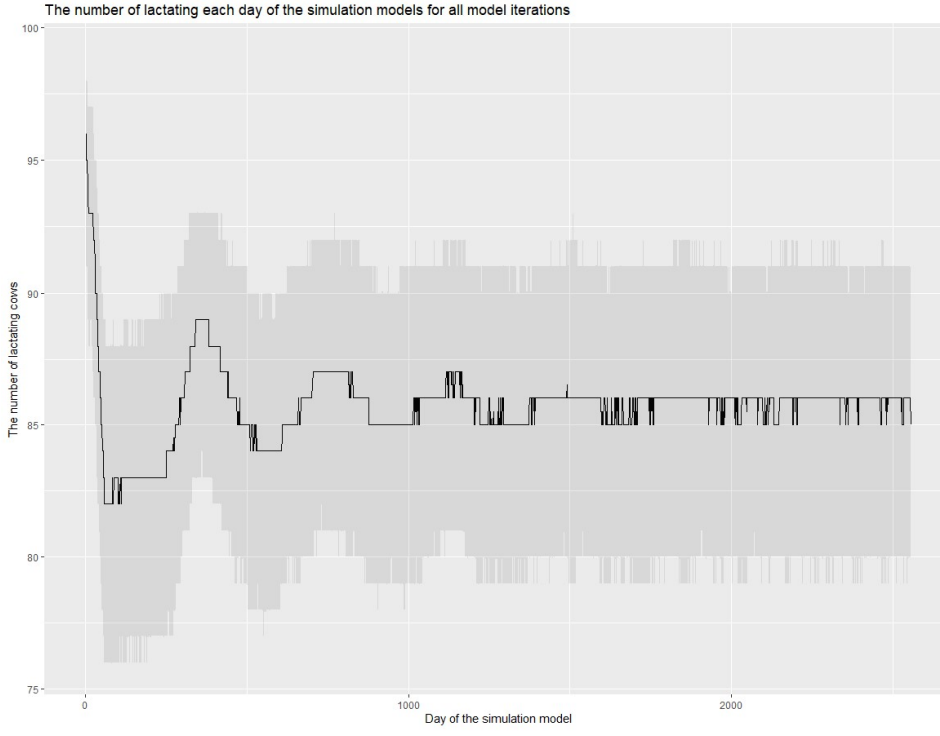

Figure A3. The median (black line) and the 95% quantile interval (shaded area) of the number of lactating cows (y-axis) *over the* number of simulated days in the simulation model.

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2022:16

Mastitis, or udder inflammation, is one of the most prevalent and costliest diseases in dairy farming. Automatic Milking systems (AMS), potentially equipped with sensors measuring mastitis indicators, have been used since the 1990s. The objective for this PhD project was to explore the potential for a decision support system in AMS supporting chronic mastitis decision-making. This thesis shows that it is possible to support management regarding chronic mastitis with sensors.

Johan Hendrikus Bonestroo received his bachelor's degree in Business and Consumer Studies and master's degree in Management, Economics, and Consumer studies at Wageningen University and Research in the Netherlands.

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