Aus dem Institut für Tierzucht und Haustiergenetik

Professur für Tierzüchtung

der Justus-Liebig-Universität Gießen

Towards a deeper understanding of genetics of dermatitis digitalis in dairy cows through the consideration of housing characteristics, climate and barn emissions in alternative statistical modelling approaches

INAUGURAL-DISSERTATION

zur Erlangung des Doktorgrades (Dr. med. vet.)

im Fachbereich Veterinärmedizin der Justus-Liebig-Universität Gießen

vorgelegt von

NIKLAS SÖLZER

TIERARZT

aus Kassel

Gießen 2025

Mit Genehmigung des Fachbereiches Veterinärmedizin der

Justus-Liebig-Universität Gießen

Dekan: Prof. Dr. Dr. Stefan Arnhold

1. Gutachter: Prof. Dr. Sven König

2. Gutachter: Prof. Dr. Christine Wrenzycki

Tag der Disputation:

19. November 2025

This work was supported by a scholarship from the H. Wilhelm Schaumann Foundation.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percent
305-d	305 days
aiv	Air volume in the barn
barn	Barn characteristics (latent variable)
BTA	*Bos taurus* (autosome)
BT	Bedding temperature
bp	Base pair position
CFI	Calving to first insemination
CIN	Calving interval
cm	Centimetre
CM	Clinical Mastitis
C:N	Carbon:Nitrogen
DD	Dermatitis Digitalis
DIM	Days in milk
ebv	Estimated breeding value
EM	Endometritis
FA	Foot angle
FY	Fat yield
GEBV	Genomic estimated breeding value
GEN	Genetic (latent variable)
GWAS	Genome-wide association study
G-matrix	Genomic relationship matrix
h^2	Heritability
hyg	Hygiene score
HS	Heat stress
HYP	Interdigital Hyperplasia
indiv	Individual
kb	Kilobase
kg	Kilogram
km	Kilometre
LSmeans	Last-squares-means
m	metre
m^2	Square metre
M-stage	Mortellaro-stage

mo	Month
MY	Milk yield
NR90	Non return at day 90
pBF	Significance level according to Bonferroni
pCD	Significance threshold
ppm	Parts per million
PY	Protein yield
QTL	Quantitative trait loci
RH	Relative humidity
rg	Genetic correlation
RV	Rear leg rear view
SCC	Somatic cell count
SCS	Somatic cell score
SE	Standard Error
SEM	Structural equation model
SNP	Single-nucleotide polymorphism
SU	Sole ulcer
SV	Rear leg side view
sys	Housing system
T	Temperature
THI	Temperature humidity index
WS	Wind speed

ZUSAMMENFASSUNG

Klauenerkrankungen stellen eine der größten Abgangsursachen für Milchkühe aus dem Produktionssystem dar. Aufgrund der sich oft anschließenden Lahmheiten im Bestand gehen die Futteraufnahme und damit nicht zuletzt auch die Milchleistung der betroffenen Tiere zurück. Diese indirekten Kosten werden durch die ebenfalls zurückgehende Fruchtbarkeit betroffener Tiere weiter erhöht. Hinzu kommen die direkten Kosten durch tierärztliche Behandlungen, Medikamentengaben und einen höheren Pflegebedarf der lahmenden Tiere. Eine der häufigsten Erkrankungen in diesem Bereich mit weltweitem Vorkommen ist Dermatitis digitalis, meist besser als Mortellaro'sche Erkrankung bekannt. Bei Dermatitis digitalis (**DD**) handelt es sich um eine multifaktorielle Erkrankung, bei welcher sowohl die Haltungsumwelt als auch die Genetik der Tiere eine Rolle spielt. So wurden in den vergangenen Jahren bereits einige Studien zur Aufklärung der genetischen Hintergründe von Klauenerkrankungen im Allgemeinen und für DD im Speziellen publiziert. In diesem Zusammenhang wurden Genom-weite Assoziationsstudien (**GWAS**) durchgeführt und bereits potentielle Kandidatengene, die in Verbindung mit Klauenerkrankungen stehen könnten, annotiert. Was jedoch bisher unklar blieb, ist die Frage wie spezifische Umweltbedingungen auf die Schätzung von Varianzkomponenten und GWAS einwirken. Gerade die klimatischen Bedingungen haben sich in den letzten Jahren verändert und werden auch zukünftig einen immer größeren Einfluss auf Produktivität und Wohlbefinden der Rinder haben. Ein bereits wissenschaftlich erprobtes Tool stellt in diesem Zusammenhang der Temperatur-Luftfeuchtigkeit Index (**THI**) dar. So beginnt für Rinder der Hitzestress (**HS**) ab einem THI > 68. Durch die breite Datenverfügbarkeit von Wetterstationen in der Nähe von Betrieben kann so ein entsprechendes Management erfolgen. Um hier mögliche Zusammenhänge mit der Klauengesundheit identifizieren zu können, wurden im ersten Teil dieser Studie Single-nucleotide polymorphism (**SNP**) x Hitzestress (**HS**) Interaktionen für Klauenerkrankungen analysiert. Hierbei zeigte sich das bestimmte SNPs und deren annotierten Kandidatengene nur unter HS oder thermoneutralen Bedingungen von Bedeutung waren. Im zweiten Teil dieser Studie wurde der Aspekt Interaktionseffekte nochmals aufgegriffen. Hier wurden für drei gebildete DD-Merkmale in den Haltungssystemen Liegeboxenlaufstall und Kompostierungsstall, SNP x Haltungssystem Interaktionen geschätzt. Hier zeigten sich allerdings sehr ähnliche genetische Parameter für dieselben Merkmale in verschiedenen Umwelten, sowie vernachlässigbare Genotyp x Haltungssystem Interaktionen. Dies deutet daraufhin, dass das Haltungssystem nur geringe Auswirkungen auf die genetischen Hintergründe einer DD-Erkrankung hat. Da die Rinderhaltung sich zum einen auf die negativen Folgen der Klimaveränderungen einstellen muss, zum anderen als Mitverursacher des Klimawandels genannt wird, sollten sich auch diese Aspekte im Studiendesign wieder finden. Daher wurden zum einen Klimaeinflüsse (Windgeschwindigkeit,

Temperatur und Luftfeuchte) erfasst, zum anderen auch die Emissionen von Ammoniak, Distickstoffmonoxid, Kohlenstoffdioxid und Methan im Stall gemessen. Hierfür erfolgte der Bau eines eigenen "Klimagasmesswagens". Dieser ermöglichte es die Klimaeinflüsse, sowie die Emissionen zeitgleich an verschieden Bereichen im Stallgebäude zu erfassen. Im dritten Teil dieser Studie sollten die Effekte der Haltungsumwelt, Kuh-Phänotypen und Genomik in einen übergeordneten Gesamtzusammenhang gebracht werden. Dieser Ansatz wurde in der Tierzucht bisher sehr selten und mit Bezug auf eine DD-Erkrankung noch gar nicht verfolgt. Für diesen Lösungsweg stellte man Strukturgleichungsmodelle (**SEM**) auf. Diese Modelle können sowohl messbare als auch nicht messbare Variablen in die Analysen mit einbeziehen und fanden bisher meist in der Psychologie Anwendung. In dieser Studie ermöglichen die aufgestellten Modelle die simultane Berücksichtigung von Umwelt- und Genetik-Effekten. Die SEM dieser Studie zeigten, dass die Haltungsumwelt einen größeren Einfluss auf eine DD-Erkrankung, als beispielsweise genetische Parameter hat. Zusammenfassend lässt sich feststellen, dass die in dieser Studie gemachten Erkenntnisse dazu beitragen, die genetischen Hintergründe von Klauenerkrankungen im Allgemeinen und von DD im Speziellen besser verstehen zu können. Ebenfalls leisten die gemachten Erkenntnisse einen wichtigen Beitrag zum besseren Verständnis und zur weiteren Aufklärung von Genotyp-Umwelt-Interaktionen.

Um in das Thema der Dissertation allgemein einzuleiten, wird in **Kapitel 1** die Erkrankung DD näher beleuchtet. Hierbei wird zum einen auf das allgemeine Krankheitsgeschehen mit Klinik, Therapie und Prävention eingegangen, zum anderen werden aber auch wirtschaftliche Aspekte und die Vielschichtigkeit möglicher Erkrankungsursachen genannt. Im weiteren Verlauf geht es um die Haltungssysteme konventioneller Liegeboxenlaufstall und Kompostierungsstall. Das Kapitel endet mit verschiedenen Möglichkeiten der Modellierung und den bisherigen Erkenntnissen zur Zucht auf Krankheitsresistenz.

Kapitel 2 verfolgt das Ziel einer tieferen genomischen Analyse der drei Klauenerkrankungen DD, Hyperplasia interdigitalis (**HYP**) und Sohlengeschwüre (**SU**), sowie deren genetische Assoziation zu anderen wichtigen Zuchtzielmerkmalen. Abschließend werden mögliche SNP x Hitzestress Interaktionen für Klauenerkrankungen analysiert. Das Studiendesign umfasste hier 17.264 genotypisierte, erstlaktierende Holstein Friesian Kühe, welche in 50 Herden in Nordost-Deutschland standen. Die Heritabilitäten der drei Klauenerkrankungen wurden über lineare und Schwellenwertmodelle geschätzt und lagen bei 0,04 und 0,08 für DD, 0,03 und 0,10 für SU und 0,03 und 0,23 für HYP. Die genetischen Korrelationen, welche in bivariaten linearen Modellen geschätzt wurden, zeigten sich mit den ausgewählten Fundamentmerkmalen durchweg positiv. Dies verdeutlicht indirekt die Notwendigkeit zur Selektion auf Fundamentmerkmale zur Verbesserung der Klauengesundheit. Genetische Korrelationen mit anderen Zuchtzielmerkmalen zeigten verminderte Fruchtbarkeit, schlechtere

Eutergesundheit und Produktivität bei erkrankten Kühen. Zwischen den Klauenerkrankungen fanden sich genetische Korrelationen, die einen näheren genetischen Zusammenhang vermuten lassen. Weiterhin wurden krankheitsspezifische Kandidatengene und genetische Assoziationen basierend auf den umliegenden SNPs, welche sich teilweise von den genetischen Korrelationen unterschieden geschätzt. Für die SNP x Hitzestress Interaktionen wurden signifikante SNPs auf Bos Taurus Autosome (**BTA**) 2,4,5,7,8,9,13,22,25 und 28 identifiziert. Die Ergebnisse lassen Gen-spezifische Mechanismen auf die Klauenerkrankungen nur in spezifischen Umwelten vermuten.

In **Kapitel 3** wurde eine detaillierte Phänotypisierung der Klauenerkrankung DD in den zwei Haltungsumwelten (konventioneller Liegeboxenlaufstall und Kompostierungsstall) durchgeführt, um mögliche Genotyp x Haltungssystem Interaktionen zu ermitteln. Insgesamt umfasste der Datensatz 2.980 Beobachtungen für die drei gebildeten DD-Merkmale (DD-erkrankt, DD-akut und DD-chronisch) von 1.311 Holstein-Friesian und 399 Fleckvieh-Simmental Kühen. Hier lagen für 926 Kühe, Daten für die genomischen Studien vor. Von den Kühen standen 899 im Kompostierungsstall (1.530 Beobachtungen) und 811 im konventionellen Liegeboxenlaufstall (1.450 Beobachtungen). Die Krankheitsprävalenz lag hier im Liegeboxenlaufstall höher als im Kompostierungsstall. Die Heritabilitäten über den gesamten Datensatz lagen bei 0,16 für DD-erkrankt, 0,14 für DD-akut und 0,11 für DD-chronisch. Hierbei war ein leichter Anstieg der Heritabilitäten und genetischen Varianzen bei der Haltungsumwelt Liegeboxenlaufstall im Vergleich zum Kompostierungsstall zu erkennen. Genetische Korrelationen zwischen den gleichen DD-Merkmalen in den unterschiedlichen Haltungsumwelten lagen nahe 0,80, dies widerlegt offensichtliche Genotyp x Haltungssystem-Interaktionen. Die genetischen Korrelationen zwischen den drei DD-Merkmalen bewegten sich zwischen 0,58 und 0,81. Die SNP Haupteffekte und SNP x Haltungssystem-Interaktionen wurden über genomweite Assoziationsstudien geschätzt. Für DD-erkrankt und DD-akut wurden hier einige gemeinsame Kandidatengene identifiziert. Die Gene hatten direkte oder indirekte Effekte auf Krankheitsresistenz oder immunologische Prozesse. Für die SNP x Haltungssystem Interaktionen wurden für DD-erkrankt und DD-akut die Gene *ASXL1* und *NOL4L* annotiert.

Kapitel 4 analysiert tiefergehend die Effekte aus Haltungsumwelt, Kuh-Phänotypen und Genomik auf die in Kapitel 3 behandelten DD-Merkmale. In linearen Modellen wurden die relevantesten Haltungscharakteristika analysiert. Die Last-squares-means für die Infektionshäufigkeit waren allgemein im Kompostierungsstall kleiner als im Liegeboxenlaufstall. Die genomweiten Assoziationsstudien zeigten ähnliche Manhattan-Plots für DD-erkrankt und DD-akut und jeweils ähnliche potentielle Kandidatengene. Fünf SNPs waren signifikant assoziiert entweder mit DD-akut und DD-erkrankt oder mit DD-chronisch und

DD-erkrankt. Diese signifikanten SNPs wurden im Weiteren mit phänotypischen und genetisch geschätzten Zuchtwerten für die DD-Merkmale, sowie mit Produktionsdaten und Haltungsumweltfaktoren in Strukturgleichungsmodellen in Beziehung gesetzt. Hier zeigte sich, dass die Haltungsumwelt einen größeren Einfluss auf ein DD-Infektionsrisiko hatte als beispielsweise genetische Parameter.

Abschließend werden einige wichtige Aspekte dieser Arbeit in **Kapitel 5** noch einmal zusammenhängend diskutiert. Hier werden besonders genomische Aspekte von Klauenerkrankungen, Einflüsse der Haltungsumwelt und wirtschaftliche Gewichte von Klauenerkrankungen im Gesamtzuchtwert betrachtet.

SUMMARY

Claw disorders are one of the main reasons for the culling of dairy cows. Due to the lameness that often follows in the herd, the feed intake and, not least, the milk yield of the affected animals decrease. These indirect costs are further increased by the also decreasing fertility of affected animals. In addition, there are the direct costs of veterinary treatment, medication and the need for more care of the lame animals. One of the most frequent claw disorders in this area, occurring worldwide, is dermatitis digitalis, usually better known as Mortellaro's disease. Dermatitis digitalis (**DD**) is a multifactorial disease in which both the housing environment and the genetics of the animals play a role. In recent years, several studies have been published to clarify the genetic background of claw disorders in general and DD in particular. In this context, genome-wide association studies (**GWAS**) have been conducted and potential candidate genes associated with claw disorders have been annotated. However, it is still unclear how specific environmental effects influence the estimation of variance components and GWAS. Climatic conditions in particular have changed in recent years and will continue to have an increasing impact on the productivity and welfare of cattle in the future. The Temperature-Humidity Index (**THI**) is a scientifically proven tool in this context. Heat stress (**HS**) for cattle begins at a THI > 68. The wide availability of data from weather stations near farms enables appropriate management. In order to identify possible correlations with claw health, SNP x heat stress (**HS**) interactions for claw disorders were analysed in the first part of this study. This showed that specific SNPs and their annotated candidate genes were only significant under HS or thermoneutral conditions. In the second part of this study, the aspect of interaction effects was taken up again. Here, SNP x housing system interactions were estimated for three DD traits in the housing systems of cubicle housing and compost bedded pack barn. However, very similar genetic parameters were found for the same traits in different environments. As well as negligible genotype x housing system interactions. This indicates

that the housing system has only a minor effect on the genetic evaluation of a DD disease. Since cattle farming must adapt to the negative consequences of climate change, on the one hand, and is mentioned as a contributor to climate change, on the other, these aspects should also be reflected in the study design. Therefore, on the one hand, climate influences (wind speed, temperature and humidity) were recorded, and on the other hand, the emissions of ammonia, nitrous oxide, carbon dioxide and methane were measured in the barn. A special 'climate gas trolley' was built for this purpose. Using this, it was possible to measure the effects of climate and emissions at the same time in different areas of the barn. In the third part of this study, the effects of the housing environment, cow phenotypes and genomics were placed in an overall context. This approach has been used very rarely in animal breeding to date and not at all in relation to a DD disorder. Structural equation modelling (**SEM**) was used to address this issue. These models can incorporate both measurable and non-measurable variables into the analyses and have mostly been used in psychology until now. In this study, the models allow for the simultaneous consideration of environmental and genetic effects. The SEM showed that the housing environment has a greater influence on DD than genetic parameters, for example. In summary, the results of this study contribute to a better understanding of the genetic background of claw disorders in general and DD in particular. The results also provide an important contribution to a better understanding and further clarification of genotype-environment interactions.

To provide a general introduction to the subject of the dissertation, **Chapter 1** will take a closer look at the disease DD. On the one hand, the general course of the disease with its clinical symptoms, therapy and prevention is discussed, but on the other hand, economic aspects and the complexity of possible causes of the disease are also mentioned. The following part of the chapter deals with the housing systems of conventional cubicle housing and compost bedded housing. The chapter ends with various possibilities for modelling and the current knowledge on breeding for disease resistance.

Chapter 2 aims at a deeper genomic analysis of the three claw disorders DD, hyperplasia interdigitalis (**HYP**) and Sole ulcer (**SU**), as well as their genetic association with other important breeding goal traits. Finally, possible SNP x heat stress interactions for claw disorders are analysed. The study design included 17,264 genotyped, first-lactating Holstein Friesian cows from 50 herds in northeast Germany. The heritabilities of the three claw disorders were estimated using linear and threshold models and were 0.04 and 0.08 for DD, 0.03 and 0.10 for SU and 0.03 and 0.23 for HYP. Genetic correlations estimated in bivariate linear models were consistently positive with the selected conformation traits. This indirectly indicates the need for selection on conformation traits to improve claw health. Genetic correlations with other breeding goal traits showed reduced fertility, poorer udder health and

productivity in diseased cows. Genetic correlations were observed between the claw disorders, suggesting a closer genetic relationship. Furthermore, disease-specific candidate genes and genetic associations based on the surrounding SNPs were estimated, which in some cases differed from the genetic correlations. For the SNP x heat stress interactions, significant SNPs were identified on BTA 2,4,5,7,8,9,13,22,25 and 28. The results suggest gene-specific mechanisms for claw disorders only in specific environments.

In **Chapter 3**, a detailed phenotypic characterisation of the claw disorder DD in the two housing environments (conventional cubicle barn and compost bedded pack barn) was carried out to determine possible genotype x housing system interactions. In total, the data set comprised 2,980 observations for the three traits DD-sick, DD-acute and DD-chronic from 1,311 Holstein-Friesian and 399 Simmental cows, 926 of these animals were available for the genomic studies. In total 899 cows were housed in the compost bedded pack barn (1,530 observations) and 811 were housed in the conventional cubicle barn (1,450 observations). The disease prevalence was higher in the cubicle barn than in the compost bedded pack barn. The heritabilities over the entire data set were 0.16 for DD-sick, 0.14 for DD-acute and 0.11 for DD-chronic. A slight increase in heritabilities and genetic variances was observed in the housing environment of the cubicle barn compared to the compost bedded pack barn.

Genetic correlations between the same DD traits in the different housing environments were close to 0.80, indicating obvious genotype x housing system interactions. The genetic correlations between the three DD traits ranged from 0.58 to 0.81. The SNP main effects and SNP x housing system interactions were estimated using genome-wide association studies. Some common candidate genes were identified for DD-sick and DD-acute. The genes had direct or indirect effects on disease resistance or immunological processes. The genes *ASXL1* and *NOL4L* were annotated for the SNP x housing system interactions for DD-sick and DD-acute.

Chapter 4 analyses the effects of the environmental factors, cow phenotypes and genomics in more detail for the DD traits discussed in Chapter 3. The most relevant housing characteristics were analysed in linear models. The last-squares means for the infection probability were generally lower in the compost bedded pack barn than in the cubicle barn. The genome-wide association studies showed similar Manhattan plots for DD-sick and DD-acute and similar potential candidate genes in each case. Five SNPs were significantly associated with either DD-acute and DD-sick or with DD-chronic and DD-sick. These significant SNPs were then related to phenotypic and genetic estimated breeding values for the DD traits, as well as to production data and housing environmental factors in structural equation models. This showed that the housing environment had a greater influence on the risk of DD infection than genetic parameters, for example.

Finally, some important aspects of this work are discussed again in **chapter 5**. In particular, genomic aspects of claw disorders, the influence of the housing environment and the economic weight of claw disorders in the total breeding value are considered here.

CHAPTER 1

General Introduction

1 Introduction

In addition to mastitis and fertility disorders, lameness is one of the major causes of culling in dairy herds worldwide (Booth et al., 2004). Among the claw diseases, dermatitis digitalis (**DD**) is one of the most important, as DD is not only highly prevalent worldwide, but has also a significant economic impact on farms (Klitgaard et al., 2014; Solano et al., 2016). DD is a multifactorial disease (Read and Walker, 1994). In addition to the involvement of certain bacteria, primarily of the genus Treponema ssp., the housing, feeding and genetics of the animals play a decisive role (Döpfer et al., 2012; Solano et al., 2017). In order to better understand the deeper connections between cattle genetics and DD, several studies have already been published in recent years. So the pedigree-based heritabilities for DD were in the small to moderate range, between 7.3% and 14.2% (König et al., 2005; Schöpke et al., 2015; van der Linde et al., 2010), and in the range of 5% to 36.7% when genomic relationship matrices were used. In further studies, genome-wide association studies (**GWAS**) were conducted and potential candidate genes associated with DD were identified (Naderi et al., 2018; Kopke et al., 2020). What has not yet been done is to include specific environmental conditions and how these affect the estimation of variance components and GWAS. In particular, due to changing climatic conditions, external influences such as heat stress (**HS**) and its effects on productivity and health will become increasingly important in cattle breeding (Polsky and von Keyserlingk, 2017). Brügemann et al. (2013) and Gernand et al. (2019) especially identified effects of HS directly after calving on genetic parameter estimates for low heritability functional traits, and hypothesized HS effects on genetic mechanisms. In such context, consideration of high-throughput genomic marker data combined with innovative statistical modeling approaches allows deeper insights with possibilities to infer SNP marker x HS interactions (Halli et al., 2021). In the first part of this study, SNP x HS interactions for claw disorders were estimated. This showed that certain SNPs and their annotated candidate genes were only significant under HS or thermoneutral conditions. In genomic evaluations, data from farmers or claw trimmers are frequently employed for the purposes of assessing claw diseases. These data are typically structured in a straightforward manner and can be accessed in large numbers via herd management software (Zwald et al., 2014). In particular with regard to more complex diseases, such as DD with specific acute and chronic stages, the disease pathogenesis is challenging to describe using only binary diagnoses (Döpfer et al., 1997). Schöpke et al. (2015) established a scoring system for the different DD stages for quantitative genetic analyses. This scoring is much more complex and requires more time and effort, but it provides higher heritabilities compared to binary diagnoses (Schöpke et al., 2015).

A more detailed modelling of environmental effects is crucial in the context of more precise phenotyping in order to gain a deeper understanding of possible genomic mechanisms (Yin and König, 2018). This aspect and the fact that lameness in conventional cubicle barns (**CON**) is often favoured by the concrete floor (Somers et al., 2005; Kester et al., 2014) led to a focus on novel housing systems such as the compost bedded pack barn (**CBPB**). This housing system has the potential to combine a high level of animal welfare, productivity and the reduction of greenhouse gas emissions (Leso et al., 2020; Petersen, 2018). In the second part of this study, three DD traits were analysed in the two housing systems CON and CBPB to identify possible genotype x housing system interactions. It showed very similar genetic parameters for the same traits in different environments and negligible genotyp x housing system interactions, this indicate only minor effects on genetic evaluation for DD due to housing system particularities. In addition to the effects of climate change, cattle farming is also confronted with the role of co-polluter (Statham et al., 2017). In the experimental set-up of this study, on the one hand, the external influences (wind speed, temperature and humidity) were recorded, and on the other hand, the concentrations of ammonia, carbon dioxide, nitrous oxide and methane in the barn were measured using a specially developed ‘climate gas trolley’. Inferring the complex interplay among genetics, housing conditions, climate parameters, cow emissions and individual cow characteristics (production level, overall health status) via standard mixed model applications might be a challenge. Possible confounding effects as well as mutual recursive or causal relationships among response variables and between traits and effects hamper applications of standard mixed model theory (Rehbein et al., 2013). For taking into account mutual trait relationships and to assess effects of parameters which are not directly measurable, structural equation models (**SEM**) have been suggested (Gana and Broc, 2019). SEM are standard analytical tools in social sciences and psychology, because they are able to measure causal relationships and to represent complex causal processes via measurable (manifest) and non-measurable (latent) variables (Bielby and Hauser 1977; Hair et al., 2014). With regard to animal health analyses, Detilleux et al. (2013) applied SEM to infer risk factors and tolerance mechanisms for bovine mastitis infections. Wagner et al. (2023) used an SEM approach to detangle causal relationships among environmental and genetic factors on udder health. The SEM of this study showed that the housing environment has a greater influence on DD than genetic parameters, for example. In summary, the results of this study contribute to a better understanding of the genetic background of claw disorders in general and DD in particular. The results also provide an important contribution to a better understanding and further clarification of genotype-environment interactions.

1.1 Dermatitis digitalis

Dermatitis digitalis (DD) is a claw disorder which occurs in cattle as well as in sheep and goats. In general, claw disorders are divided into infectious and non-infectious disorders (Dhakal et al., 2015). DD is an infectious disorder, because it usually causes acute or chronic inflammation of the skin (dermis; Kopke, 2019). In today's dairy cattle farming, DD is one of the most important claw disorders alongside with other disorders such as phlegmon, interdigital hyperplasia, white line defects, bunion ulcers and laminitis.

1.1.1 Distribution and significance

DD was first named in Italy in 1974 by Cheli and Mortellaro, which is why the name Mortellaro's disease is still widely used in Europe. The name strawberry disease comes from the strawberry-like appearance of the lesions (Cheli and Mortellaro, 1974; Weiss and Teifke, 2007). Today, the disorder is a worldwide problem in cattle farming due to its severity, contagiousness and the limited effect of therapeutic measures (Evans et al., 2016). Prevalences of between 10.5% and 55% are described for individual animals and up to 83% at herd level (Somers et al., 2005; Holzhauer et al., 2012b). DD occurs in cows as well as in heifers, bulls, calves and beef cattle (Sullivan et al., 2013; Gomez et al., 2014b and 2015b). Animals kept in loose housing are more likely to be affected than those kept on pasture or tethered (Dirksen, 2006). The highest prevalence is described in young cattle, newly housed heifers or during initial outbreaks in previously naive herds; DD usually occurs in connection with unhygienic housing conditions (Read and Walker, 1998). Links with non-healing lesions in the claw and udder area are suspected (Evans et al., 2011; Clegg et al., 2016b). Secondary infection in cases of interdigital hyperplasia or claw sole ulcers has also been described (Holzhauer et al., 2008a; Blowey, 2008). The annual economic losses in the European Union and the United States are calculated at 133 US dollars per diseased animal, with an incidence rate of 25% at over 1.1 billion US dollars (Zinicola et al., 2015b). For a farm with 65 cows, costs are estimated at 1,517 US dollars (Bruijnis et al., 2015). The largest share of costs here is attributable to veterinary treatment and medication (Cha et al., 2010). As diseased animals usually show an increased risk of disease outbreaks in subsequent lactations, further costs are incurred (Döpfer et al., 2018; Gomez et al., 2015b). Further consequences are a reduced general state of health, reduced milk yield and fertility (Gomez et al., 2015 a and b; Bicalho et al., 2007). The influence of DD on milk yield is still being discussed, with Gomez et al. (2015b) describing significant milk yield losses in young cows. It is also assumed that higher milk yields lead to a higher susceptibility to claw disorders (Koenig et al., 2005; Onyiro et al. 2008). In addition to the points mentioned, there are significantly lower conception rates, lower

conception probabilities and longer times to successful conception (Bicalho et al. 2007; Gomez et al., 2015b). Among other things, lameness and severe, protracted and recurring illnesses significantly increase the risk of early exit from the herd (Bicalho et al., 2007; Bruijnis et al., 2012). Animal welfare is negatively affected by DD infection, which manifests itself in defence reactions when touching the painful lesions, frequent lifting and shaking of the affected limb, prolonged lying times and reduced movement (Hernandez et al., 2001; Bruijnis et al., 2012). Due to the high incidences in herds, DD has a major impact on animal welfare alongside other claw diseases such as sole haemorrhages or dermatitis interdigitalis (Bruijnis et al., 2012; Solano et al., 2016).

1.1.2 Etiology

The reasons for DD are still not fully understood (Krull et al., 2014). As DD is geographically widespread, highly contagious and antibiotic therapy can lead to a cure, it was assumed to be an infectious disease with a bacterial cause (Döpfer et al., 1997; Read and Walker, 1998). It is currently assumed that DD is a multifactorial disease with polymicrobial involvement (Krull et al., 2014; Zinicola et al., 2015 a and b). The gram-negative, anaerobic to microaerophilic spirochetes of the genus Treponema in particular appear to have a major influence on the disease (Weiss and Teifke, 2007; Evans et al., 2014). Further species have been detected intralesionally: *Campylobacter spp, Tissierella spp, Dichelobacter nodosus, Fusobacterium necrophorum, Porphyromonas spp, Guggenheimella bovis, Candidatus Amoebophilus asiaticus, Mycoplasma spp, Moraxella spp.* and *Aerococcus spp.* (Döpfer et al., 1997; Capion et al., 2012; Rasmussen et al., 2012; Krull et al., 2014, Zinicola et al., 2015b; Nielsen et al., 2016). The involvement of fungi and viruses has not yet been proven (Brandt et al., 2011; Krull et al., 2014). To this day, the direct pathological role of the bacteria involved remains unclear (Hargis and Ginn, 2009; Straubinger, 2015). The majority of bacteria appear to be localised in the superficial layers of the skin (Nielsen et al., 2016). The species *Sphearophorus necrophorus, Fusobacterium necrophorum* and *Dichelobacter nodosus* are responsible for macerating the skin (Nuss and Steiner, 2004).

1.1.3 Dermatitis digitalis as a factor disease

The multifactorial causes of DD can be categorised into animal factors, management and environmental factors. Animal factors are the breed and individual predisposing factors, such as genetic predisposition (Rodriguez-Lainz et al., 1996 and 1999; Holzhauer et al., 2006). The genotype may have a decisive influence on the occurrence and course of the disease in the individual animal (Schöpke et al., 2015). For example, Döpfer et al. (2004) already showed

that there are cows that do not get sick despite a high incidence of disease in the herd, while other animals repeatedly show lesions. The sequencing of the bovine genome in particular, as well as the now widespread high-throughput genotyping in combination with genome-wide association studies, could detect genetic variants that are associated with specific traits and diseases (Kopke, 2019). The identification of these markers in association with claw diseases allows a better understanding of the genetic architecture and development of these diseases (van der Spek et al., 2015).

Other influences are parity, stage of lactation, as well as age at first calving and calving season (Read and Walker, 1998; Somers et al., 2005; Krull et al., 2016b). In terms of management, inadequate and lack of claw care, damp and unhygienic housing conditions with prolonged contact of the distal limbs with faeces and urine are particularly important influencing factors (Somers et al., 2005; Cramer et al., 2008). In terms of environmental factors, high levels of precipitation during grazing in autumn and winter and excessively dry soils can lead to microtraumatisation (Rodriguez-Lainz et al., 1996 and 1999). Other factors described include a lack of grazing, excessive stocking rates, poor cubicle design, a lack of cubicles and unbalanced feeding (Somers et al., 2005; Onyiro et al., 2008; Holzhauer, 2012b). Increased prevalence of DD can also occur in under- and over-conditioned animals (BCS < 2.5 or >3.0; Schöpke et al., 2013). Other factors include the purchase of diseased animals as well as the presence of other claw diseases such as dermatitis interdigitalis, hyperplasia or phlegmons (Somers et al., 2005; Holzhauer, 2006).

1.1.4 Clinic

The disease occurs immediately proximal to the coronary band, at the border to the interdigital space, soft bunion as a circumscribed inflammation of the bunion skin (Read and Walker, 1998; Holzhauer et al., 2008 a). As the disease progresses, DD can spread to the corneal capsule and the interdigital space; rarely does the disease primarily affect the interdigital space or the front of the foot (Blowey, 2005; Solano et al., 2017). A tenfold increase in prevalence was detected on the hind legs compared to the front legs (Cramer et al., 2008). The early lesions are usually only observed incidentally and show circumscribed epithelial loss with adherent detritus in the bunion furrow, as well as a white to yellowish discolouration of the skin (Kopke, 2019). Later, a 1-5 cm large, sharply defined, slightly raised, round or oval focus with an erosive or proliferative character develops above the coronary band, which is usually covered with a grey-white, foul-smelling coating (Weiss and Teifke, 2007). In some case´s there is loss of skin and necrosis of the epidermis, while the neighbouring hairs are conspicuously long and protrude. A whitish border forms at the edge of the lesions and the neighbouring skin appears to be raised in the shape of a wall. The focus spreads both in terms of area and depth, and the

bumpy, blood-red granulation tissue that forms give´s the rise to the strawberry-like appearance (Weiss and Teifke, 2007). DD can spread to the sole dermis or occur on the dewclaws (Rodrigues et al., 2010; Gomez et al., 2012). In the chronic form, massive growths and occasional keratinisation occur; due to the parakeratosis over the dermal papillae, this form is also referred to as papillomatous or verrucous (Read and Walker, 1998; Weiss and Teifke, 2007). As the chronic stages can relapse into acute stages, once infected animals pose a potential risk to the entire herd (Berry, 2012). In severe cases, infections of deeper structures can occur, including phlegmonous inflammation of the entire bale area (Weiss and Teifke, 2007). Clinically, affected animals often present with mild to moderate lameness of the supporting leg and hyperflexion of the distal limb (Cheli and Mortellaro, 1974). Animals with advanced DD show longer lying times and consume less feed, which subsequently leads to milk yield losses (Gomez et al., 2015 a and b, Bicalho et al., 2007). Occasionally there are severe movement disorders, severe lameness due to deep-seated diseases can also be associated with disorders of general condition (Kopke, 2019). Affected animals do not necessarily have to show signs of lameness; a rhythmic shift in weight from one leg to the other can often be observed (Krull et al., 2016b).

1.1.5 Improved disease monitoring through more precise description

As chronic lesions essentially influence the dynamics of DD, categorisation systems have been established to record the stages and progression of the disease (Döpfer et al., 2008; Krull et al., 2014). The simple classification of affected and unaffected cows has been replaced by more specific schemes to describe macroscopic changes in the skin as well as the cow's pain perception and response to treatment (Döpfer et al., 1997; Laven and Hunt, 2002; Krull et al., 2014 and 2016b). A further subdivision into active and inactive or early and late lesions is also proposed. Based on macroscopic findings of DD lesions, one of the most important categorisation systems was described by Döpfer et al. (1997) and refined by Berry et al. (2012). This system has been used in numerous studies investigating DD (Gomez et al., 2014b and 2015a; Schöpke et al., 2015; Zinicola et al., 2015b). The system defines six stages, the so-called M-stages, of the disease. The stages are part of the disease cycle: M0 defines healthy skin with no signs of previous lesions, M1 describes the onset of the disease, which begins with local ischaemia with discolouration and loss of the epithelium, as well as swelling and hyperaemia in the area surrounding the lesion. Macroscopically, there is a small focal red/grey demarcated lesion, which is < 2cm in diameter and is not painful. The loss of the epithelium and the damage to the keratinocytes lead to an erosive and later proliferative stage M2. This in turn shows a large active ulcerative red/grey lesion > 2cm in diameter, which is painful for the animals. M3 describes the healing centre with a scab-like surface. This scab-like dry brown surface is also visible macroscopically, which is typically present after treatment and is not

painful. The chronic stage appears as a hyperkeratotic or proliferative form M4. This is characterised by dyskeratosis or irregular brown/black proliferative hyperkeratotic growths, which are not painful for the animal (Kopke, 2019). Within these chronic stages, new foci can flare up again M4.1, which are now painful again (Döpfer et al., 1997; Berry et al., 2012). The transitions between the individual lesions can last from several days to years (Krull et al., 2014 and 2016b). For the control and better prevention of DD in the herd, repeated categorisation of the herd at short intervals therefore appears to be useful (Döpfer et al., 2012).

1.1.6 Therapy and prevention

For the therapy of DD, a distinction is made between treatment of individual animals and treatment at the herd level. The type of therapeutic approach depends on the occurrence and severity of the disease in each individual case. Treatment options include: surgical treatment, systemic antibacterial therapy, local antibacterial therapy, local antiseptic therapy and herd treatments with claw baths (Kopke, 2019). Surgical removal of the affected tissue is considered obsolete due to the pain caused, delayed healing, poorer success rate and possible complications (Read and Walker, 1998). Systemic antibacterial therapy with penicillin and ceftiofur has been used effectively, but is not very useful at the herd level due to the waiting time for milk and eatable tissue (Read and Walker, 1998). As DD is present in many herds with high prevalence, it is advisable to apply prevention and control throughout the herd (Evans et al., 2016). The best way to do this is through effective footbathing strategies and localised treatment of individual animals. Before applying localised substances, the claws should be cleaned (also during the footbath) and the hair in the ball area should be clipped and dried. After repeated application of antibacterial, anti-inflammatory or antiseptic substances as an application or spray treatment, a loose bandage should be applied for two to three days (Holzhauer et al., 2012a; Sullivan et al., 2013). Local treatment is in any case simpler, cheaper and more effective than a claw bath, but involves more work in identifying and treating affected animals (Evans et al., 2016). In addition to the type and duration of medication, the success of the therapy depends on the anatomical localisation of the lesion, the timing of the treatment, the elimination of favourable factors and animal-specific criteria (Döpfer et al., 2008). Early controlled treatments show rapid and complete healing. If the treponemas have already formed a reservoir in the skin, recurrence rates are high (Kopke, 2019). Recurrence rates of up to 50% and the occurrence of new lesions after the treatment has ended have been described (Read and Walker, 1998; Berry et al., 2012). Since heifers affected by DD also show increased DD lesions later in life, preventive measures should already start at calf/heifer age (Döpfer et al., 2008; Gomez et al., 2015b). Problem animals/groups can be identified via monitoring systems and treated locally and with effective footbath strategies (Kopke, 2019). Regular follow-up

checks should be carried out at the transition to and during lactation to avoid potential outbreaks at the herd level (Döpfer et al., 2012; Schöpke et al., 2015). Effective prophylaxis focuses in particular on the predisposing factors. These include the creation of species-appropriate and hygienic husbandry conditions, quarantine when purchasing animals, purchasing only from DD-free herds, and correct and regular claw care. When feeding the animals, it is important to feed them according to their nutritional needs and performance, as this can lead to a suppression of the immune system (Gomez et al., 2014b; Kopke, 2019).

1.2 Housing systems

The housing system has a strong impact on animal health and welfare (Fregonesi et al., 2009; Kester et al., 2014). Conventional cubicle barns, which are the standard housing system used for decades in the dairy industry, enable the best possible hygiene by separating manure and urine from the lying area however, at the expense of animal welfare (Bewley et al., 2017; Petersen, 2018). In addition, the high proportion of liquid manure contributes to greenhouse gas emissions (Petersen, 2018). New freewalk housing systems, such as the compost-bedded pack barns, grow in attraction due to better animal welfare, longevity and health (Leso et al., 2020).

1.2.1 Structure and differences between compost-bedded pack barns and cubicle barns

Conventional cubicle barns (**CON**) typically have a feed alley with a solid floor alleys with slatted concrete surfaces and individual resting areas (Leso et al., 2020). The lying area can vary between deep bedded and synthetic mattresses. A wide variety of bedding materials can be used in the deep bedded areas. Sand, straw, sawdust and solids from recycled manure are used most often. They usually provide good comfort, but are very labor-intensive and require a lot of bedding material (Bewley et al., 2017). Synthetic mattresses, on the other hand, reduce labor intensity and material consumption, but reduce lying comfort and increase hock lesions and lameness (Cook et al., 2004). Manure and urine are excreted onto the concrete slatted floor, where it is trodden through the slats or moved away using a scraper. If this concrete floor is not roughened, it can become very slippery for the cows (Albright, 1995).

The system of compost-bedded pack barns (**CBPB**) was developed in Virginia in the 1980s (Wagner, 2002). This housing system attracted increasing interest worldwide in the last 15 years. In Europe, the first CBPB were established in 2009/2010 (Leso et al., 2020).

The CBPB also contains a feeding corridor with a solid floor (Bewley et al., 2017) and access to water (Janni et al., 2007). The rest of the area consists of an open bedded pack where the animals can move and rest. The open bedded pack area consists of a mixture of organic bedding and cattle excrement. This area is cultivated one to three times per day to get fresh manure and air into the pack, allowing for an aerobic composting process (Leso et al., 2020). This mixture also has the advantage that it is solid and already has undergone a composting process enabling it to be used directly for soil application (Bewley et al., 2017). However, the area per cow in the compost-bedded pack barn is greater than in the cubicle barn or straw yard (Bewley et al., 2017; Leso et al., 2020). Depending on various factors, such as climate, bedding, pack management and cow characteristics, between 7.4 - 15m^2/cow are required (Leso et al., 2020). The pack depth varies between 20 cm and 1m, depending on pack management and bedding use (Leso et al., 2020). Galama et al. (2014b) were able to show that around 50 cm are required to maintain sufficient heat in the pack to support the composting process.

As evaporation occurs on the surface, more surface area per cow results in dryer bedding, reduced use of bedding material and therefore lower running costs (Leso et al., 2020). Another factor influencing the drying rate of the pack is the heat development within the pack (Smits and Aarnink, 2009). Ideally, the temperature at a depth of 15 to 30 cm should be between 43.3°C and 65°C (Janni et al., 2007; Bewley et al., 2013). For good cultivation of the pack, the manure level should ideally be between 40 and 65% (Janni et al., 2007; Black et al., 2013).

Climate, especially air conditions, also have a high impact on the drying rate (Eckelkamp et al., 2016a). In warm, dry and windy regions, the drying rate is higher and therefore the consumption of bedding is lower. In cold and humid regions, on the other hand, the drying rate is limited. Therefore, more space per cow is needed to achieve a higher drying rate (Smits and Aarnink, 2009). In addition, the outside temperature plays a central role in the processing of the pack. In warm temperatures, more frequent processing of the pack leads to an increase in temperature of up to 10°C and thus a better composting process (Black et al., 2013). At cold temperatures, more frequent processing leads to a significant loss of temperature in the pack. Therefore, in this case processing should be reduced (Galama, 2014a; Leso et al., 2020). With regard to the weather, the building design should also consider the structural orientation of the barn in order to optimize utilization of natural ventilation (Gooch, 2008).

Manure removal is usually done every six months to once a year, then the composting process starts all over again (Barberg et al., 2007a). The time to add fresh bedding is essential for a well-functioning system, clean animals and healthy udders. It should be added before the pack sticks to the animals (Barberg et al., 2007a; Janni et al., 2007). There is a wide variety of bedding materials worldwide. In Kentucky, for example, green, kiln-dried shavings or soy hulls

mixed with sawdust are used (Black et al., 2013). In Italy, a mixture of sawdust and wood shavings is used (Leso et al., 2013). As well as in Brazil, where rice straw or coffee husks are also used (Mota et al., 2017). Whereas in the Netherlands wood chips used (Galama et al., 2011).

1.2.2 Effects of the housing system on animal welfare

Animal health and welfare significantly depend on the housing system and management (Fregonesi et al., 2009; Kester et al., 2014). Particularly the lying time affects rumination time and consequently milk production (Schirmann et al., 2012). Uncomfortable barns have a major impact, particularly in cubicle barns. Poorly designed lying areas result in less lying time and more standing time on slatted floors, influencing rumination, milk production and claw health (Fregonesi et al., 2009; Kester et al., 2014). Additionally, the soil has an effect on walking performance and thus on total behavior. Especially in the case of non-roughened concrete floors, the heat behavior changes and thus affects the fertility rate (Frankena et al., 2009; López-Gatius, 2012). In Fregonesi et al. (2009), it was observed that the animals spent significantly more time in the open pack than in the cubicle barn when given a free choice between cubicle barn and straw yard. The animals showed synchronized lying behavior, as well as longer lying times and thus an increased rumination time in straw yard (Fregonesi and Leaver, 2001). However, the disadvantages of the straw yard were shown by a higher degree of soiling of the animals and lower udder health. The somatic cell count and the incidence of clinical mastitis was significantly higher in straw yards than in cubicle barns (Fregonesi and Leaver, 2001).

Compost-bedded pack barns were developed to improve cow comfort and longevity (Janni et al., 2007) and to provide more natural living conditions for the animals (Endres and Barberg, 2007). The pack management should be hygienic and offer the cows a comfortable lying area (Leso et al., 2020).

Due to the shorter standing times on concrete surfaces, cows suffer significantly less diseases, when compared to cubicle barns (Bewley et al., 2017). Various studies have shown that animals in compost-bedded pack barns have significantly reduced lameness and hock lesions compared to cubicle barns (Fulwider et al., 2007; Lobeck et al., 2011; Ofner-Schröck et al., 2015). Due to the softer bedding, the animals rest longer, stand more frequently on soft ground and lie down more often than in cubicle barns (Haley et al., 2001).

The animals' cleanliness and udder health depend primarily on the management (Black et al., 2013; Eckelkamp et al., 2016b). Wet bedding can stick to the cows and lead to dirty animals with an increased risk of mastitis and a longer preparation time of the udders before milking

(Black et al., 2013). Especially, manure levels (Eckelkamp et al., 2016a) and the frequency of pack preparation have a major impact (Janni et al., 2007; Damasceno, 2012).

Udder health in compost bedded pack barns is discussed controversially. Lobeck et al. (2011) found a higher somatic cell count (**SCC**) in compost-bedded pack barns than in cubicle barns. An increased proportion of *Klebsiella spp.* in the pack, as well as an increased prevalence in the udder, can occur with intensive use of wood shavings and sawdust (Janni et al., 2007). In contrast, Eckelkamp et al. (2016b), determined no significant differences between the two systems in SCC and mastitis prevalence. Other studies even showed a lower herd mastitis infection prevalence (Barberg et al., 2007b), a lower SCC (Fulwider et al., 2007) and higher milk production as a result of the lower stress level of the animals in compost-bedded pack barns (Black et al., 2013; Borchers, 2018). However, optimized bedded pack conditions and management are essential for udder and cow hygiene (Fávero et al., 2015; Albino et al., 2018). Nevertheless, no relationship was detected between the SCC and the count of bacteria in the pack (Shane et al., 2010).

1.3 Breeding for disease resistance

Since the process of domestication, farmers have been engaged in efforts to adapt their animals to changing environmental influences and diseases (Morris, 2007). The financial burden imposed by diseases on the livestock industry is considerable and pervasive, affecting all aspects of the production system (Bishop and Wooliams, 2014). The total costs associated with disease are estimated to account for approximately 20% of sales in the production system of industrialised countries, and in the direct livestock industry, the total estimated costs of disease are estimated to account for a significant proportion of sales, ranging from 35% to 50% (Bennett et al., 2005). However, it should be noted that estimating these costs is a highly complex undertaking. There is the question of whether the costs are direct, indirect, or immaterial. Who is affected by the disease and can the disease affect other species, is there even a zoonotic potential for humans (Perry and Grace, 2009). Nicholas (2005) roughly divides diseases into two types. On the one hand, diseases from the outside, which are caused by bacteria, viruses, parasites and feed toxins, and diseases from the inside, which are genetically determined. However, it can also be observed that there is a great degree of variability in the occurrence of diseases in animals. This variability therefore also appears to be genetically determined (Bishop et al., 2002) and is often referred to as disease resistance. The concept of resistance can be most effectively conceptualised from an ecological perspective, specifically in relation to the interaction between the host and the pathogen species (Grenfell

and Dobson, 1995). It can be defined as the ability of the host to exert some degree of control over the pathogen cycle (Bishop and Stear, 2003; Bishop, 2012).

Disease resistance can be observed in two distinct forms: firstly, as a consequence of the existence of breed differences in susceptibility to a given disease, and secondly, as a result of the inheritance of disease resistance within a given breed (Morris, 2007). In addition to genetic selection within a breed, other aspects of disease prophylaxis also need to be considered. These include herd management, herd protection (e. g. vaccination, treatment), utilisation of natural resistance and the general implementation of genetically resistant breeds and cross-breeding (Morris, 2007). In this context, the costs and time required to achieve disease resistance vary. Especially in the case of diseases that occur less frequently, progress towards achieving resistance is significantly slower than in the case of common diseases, as selection and testing can be performed differently (Morris, 2007).

To date, diseases caused by an external agent have been combated through the administration of vaccines or antibiotic treatments. In light of concerns pertaining to drug residues and the rising prevalence of antibiotic and antiparasitic resistance (Bishop and Wooliams, 2014), there is a growing interest in breeding for disease resistance. Achieving success in this domain would be particularly advantageous in the context of mastitis and lameness, given that these are the two most costly diseases in the dairy production system (Heringstad et al., 2003; Berry et al., 2011).

Nevertheless, classical genetic evaluations are unable to fully account for the complexities of breeding for improved disease resistance. On the one hand, recorded phenotypes are frequently inaccurate or the phenotype cannot be quantified in both sexes (e. g. mastitis). Moreover, a considerable time interval elapses before the performance of an adult animal can be evaluated (from calf to heifer). There are frequent antagonistic and unfavourable genetic correlations between traits of interest, such as health and milk production. Additionally, there can be interactions between genotype and environment, as the environment influences the expression of an animal's genes (Berry et al., 2011). By combining SNP genotyping with traditional genetic evaluations, a genomic estimated breeding value (**GEBV**) can be generated (van Raden et al., 2009). One of the biggest challenges in breeding for improved animal health or disease resistance remains access to accurate phenotypes of health traits. This may be accomplished by the development of inexpensive tests (e. g., tuberculin tests) or the creation of biomarkers that can be obtained from numerous animals at relatively low costs (Berry et al., 2011). In addition, not only the host genome should be genotyped, but also that of the pathogen, in order to determine how they interact with each other and to develop prophylactic strategies (Berry et al., 2011). “Lastly, breeding for disease resistance is a multidisciplinary activity. Our own experiences strongly reinforce the need for widespread dialogue between geneticists and animal health experts, and the need to incorporate concepts from disease

biology and epidemiology into animal genetics, and vice versa. Both communities must understand the goal and techniques used by the other community" (Bishop and Wooliams, 2014, p. 197).

1.4 Modelling options for detecting effects between variables, between variables and traits

To approach the subject slowly, we should first look at the definition of the individual terms: model (from the Latin modulus, meaning measure or standard); a representation, illustration, reproduction of an object that emphasises the features considered to be essential. The aim is to create a simplified image of reality, whereby the important thing is what you want to achieve with this model or what it is used for.

Modelling then describes the development, formation or production of a model in the scientific sense, the representation of empirical objects, phenomena or physical processes in order to be able to understand, define, quantify, visualise or simulate a certain part. The variables (as a variable size), the traits (observable property, e. g. phenotypic expression) and effects (an effect that simply arises or can also be deliberately brought about) then flow into the modelling. Various models will be described in more detail below.

One possible option would be to set up mixed models. This involves checking whether a dependent variable is influenced by one or more independent factors. Independent factors are usually categorical, but can also be numerical. 'Mixed' here stands for the presence of both fixed and random factors, i.e. factors whose influence on the dependent variables interest the researchers (fixed factors), as well as those that are highly likely to exert an influence on the dependent variable, but whose influence is of no interest to the researchers (Reubold and Harrington, 2015). This type of modelling is increasingly replacing univariate (split-plot in time) or multivariate ANOVA models (Wang and Gonnewardene, 2004), as it is able to analyse data with hierarchical or nested structures. This is the case when data are grouped into units that may be grouped into higher units. The individual observations or units are usually at the lowest level. The mixed models now allow a more precise modelling of the relationships between the variables, since observations are not considered to be independent of each other in principle, but the model recognises that the variations exist both within and between groups (Hox et al., 2017). Another advantage of this modelling is that repeated observations can be included. Even unbalanced data is not a problem for mixed models; the influence of the group is taken into account in the model according to the group size. Another advantage is that missing data is not a problem either. Disadvantages become apparent with smaller numbers of observations, where the model is unstable and does not converge (Reubold and Harrington, 2018).

Particularly in animal and plant breeding, there is often feedback or recursive relationships between phenotypes, which are represented in many biological processes. Standard models cannot reflect these relationships (Gianola and Sorensen, 2004). Turner and Stevens (1959) tried to illustrate this with feedback relationships between the concentrations of CO_2 in the air (A) and in the alveoli of the lungs (C), as well as the depth of respiration (D). A influences C; in turn C and D have a feedback relationship. Further recursive relationships can be shown in offspring-parent regression and for genetic and environmental correlations between traits (Gianola and Sorensen, 2004). The special feature of recursive models is that all causal effects go in one direction and that disturbances are uncorrelated. Thus, y_{i1} affects y_{i2}, but the latter variable has no effect on y_{i1} (Gianola and Sorensen, 2004). An example is the maternal-effects model proposed by Falconer (1965) and examined by Koerhuis and Thompson (1997). This model postulate that the phenotype of an offspring is affected by the phenotype of its dam. In pigs, for instance, it is known that females born in larger litters tend to produce smaller litters. Gianola and Sorensen (2004) have illustrated a figure for a "cyclically recursive" model, which is also shown here (Figure 1). "Here, the causal relationship modeled is $Y_1 \rightarrow Y_2 \rightarrow Y_3 \rightarrow Y_1 \rightarrow \ldots$. For instance, consider a hypothetical situation where Y_1, Y_2 and Y_3 are phenotypic values in sibships of size 3, with the subscript indicating birth order. It may be that the chain on influences is such that the older sib (with phenotype Y_1) affects the second sib (Y_2) and so on, with the loop closing via an influence of the youngest on the oldest sib" (Gianola and Sorensen, 2004).

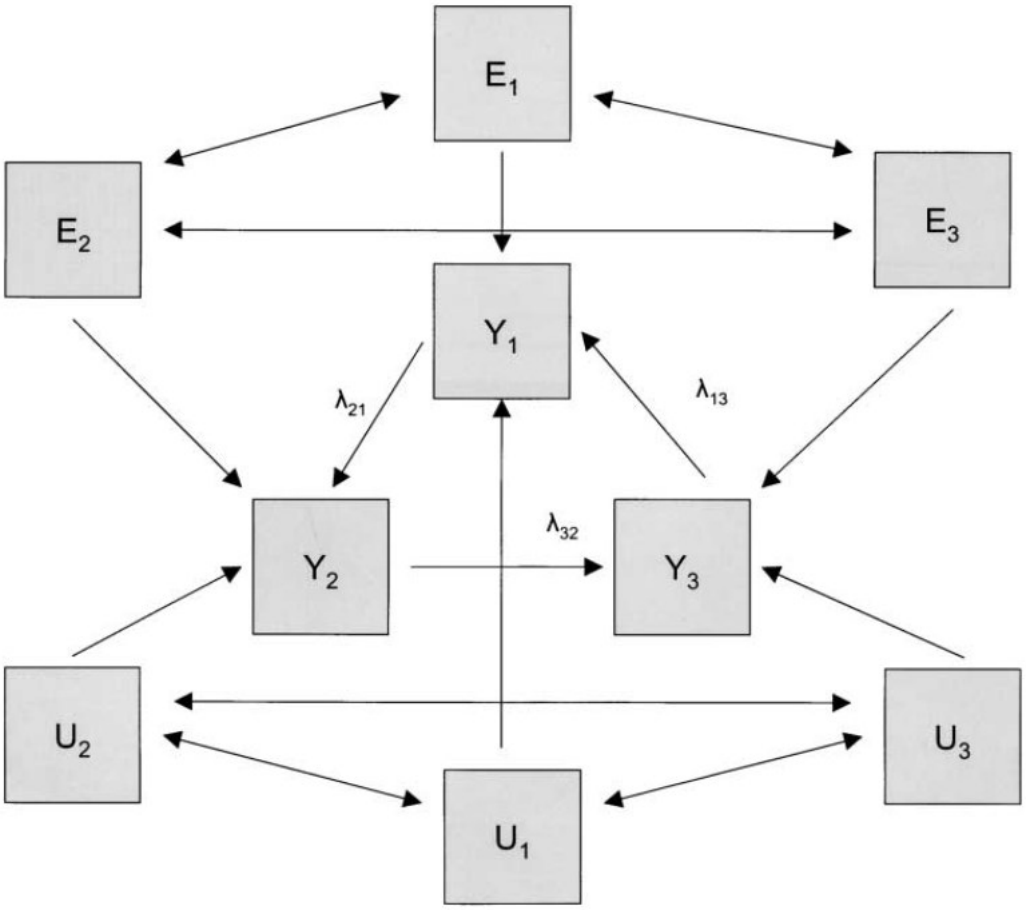

Figure 1.: Fully recursive model for three variables: Y_1, Y_2 and Y_3 are the phenotypic values; U_1, U_2 and U_3 are additive genetic effects acting on this system; E_1, E_2 und E_3 are residual effects. A single-headed arrow (*e.g.*, $A \rightarrow B$) indicates that variable *A* affects variable *B*. Double-headed arrows denote correlations between pairs of variables. λ_{ij} indicates the rate of change of variable *i* with respect to variable *j.* (Gianola and Sorensen, 2004).

Finally, structural equation models (**SEM**) will be discussed in this section. These are able to empirically test theoretical statements about complex cause-and-effect relationships (Geiser, 2010; Fuchs, 2011). SEMs can be used to check whether a theoretical model matches observed data and show whether and how theoretical models are appropriate. However, it is not used to test the classic null hypothesis (Urban and Mayerl, 2014). A special feature of SEM is that it allows a clear distinction between manifest (measurable) and latent (non-measurable) variables (Fuchs 2011; Urban and Mayerl, 2014). Thus, measurement errors can be taken into account in the analysis by using latent variables. Furthermore, correlations can be estimated more correctly than is possible in correlation, regression and path analyses (Geiser, 2010). SEM therefore enables multivariate analyses of causal models, in which a distinction is also made between independent (exogenous) variables and dependent (endogenous) variables. It is also possible to estimate direct, indirect and total effects (Urban and Mayerl, 2014). So far, SEMs have been less used in animal breeding. Peñagaricano et al. (2015) used SEM to

analyse possible causal relationships between growth and meat quality traits in pigs. Detilleux et al. (2012) tried to model risk factors for subclinical and clinical mastitis using SEMs. Wagner et al. (2023) wanted to better identify environmental and genetic influences on somatic cell count and mastitis pathogens using SEMs.

1.5 Aims of the study

The present study deals with the genetic analysis of DD and its interaction with the housing environment in dairy cows of the Holstein Friesian and Simmental breeds in the housing systems cubicle housing and compost bedded pack barn. In addition to the DD scores, animal, performance and environmental parameters were recorded on the participating farms in order to analyse them in genetic statistical models. In GWAS SNP main effects, SNP x housing system interactions and structural equation models were used to analyse the influence of animal, performance and environmental data on DD disease. The chapters of this study have the following aims:

I. **Chapter 2** should provide a comprehensive genomic analyses for DD, HYP, and SU including the estimation of heritabilities based on genomic relationship matrices, the estimation of SNP-marker effects for DD, HYP, and SU and the annotation of potential candidate genes from models considering or ignoring possible HS interactions, and the estimation of genetic correlations based on genomic relationship matrices between DD, HYP, and SU with breeding goal traits reflecting health, female fertility, and productivity, and of respective correlations based on SNP effects with focus on the identified candidate gene segments.

II. **Chapter 3** conduct a comprehensive genetic and genomic analyses for novel traits derived from DD scoring including the estimation of genetic parameters in a single-step approach separately for the overall, CON, and CBPB datasets, and GWAS and the annotation of potential candidate genes for main and interaction effects with the discrete housing system considering the phenotypic records from genotyped cows.

III. **Chapter 4** will analyse the CBPB environment and its various influencing factors. Afterwords the collected parameters in connection with the DD diagnosis which were also recovered. In the end the parameters from the housing environment, cow-related and genetic aspects should be analyse in SEM for a possible DD disease.

References

Albright J.C. 1995. Flooring in dairy cattle facilities: International Conference on Animal Behavior and the Design of Livestock and Poultry Systems, Indianapolis, IN. Northeast Regional Agricultural Engineering Service, Cooperative Extension, Ithaca, NY.:168–182.

Bennett, R. and J. Ijpelaar. 2005. Updated estimates of the costs associated with thirty four endemic livestock diseases in Great Britain: a note. J. Agric. Econ. 56:135-144. DOI:10.1111/j.1477-9552.2005.tb00126.x.

Berry, D. P., M. L. Bermingham, M. Good, and S. J. More. 2011. Genetics of animal health and disease in cattle. Ir. Vet. J. 64:1-10. DOI:10.1186/2046-0481-64-5.

Berry, S. L., D. H. Read, T. R. Famula, A. Mongini, and D. Doepfer. 2012. Long-term observations on the dynamics of bovine digital dermatitis lesions on a California dairy after topical treatment with lincomycin HCl. Vet. J. 193:654–58. DOI:10.1016/j.tvjl.2012.06.048.

Bewley, J. M., J. L. Taraba, D. McFarland, P. Garrett, R. Graves, B. Holmes, D. Kammel, J. Porter, J. Tyson, S. Weeks, and P. Wright. 2013. Guidelines for Managing Compost Bedded-Pack Barns. The Dairy Practices Council, Ritchboro, PA.

Bewley, J. M., L. M. Robertson, and E. A. Eckelkamp. 2017. A 100-Year Review: Lactating dairy cattle housing management. Journal of dairy science 100(12):10418–10431. https://doi.org/10.3168/jds.2017-13251.

Bicalho, R. C., F. Vokey, H. N. Erb, and C. L. Guard. 2007. Visual locomotion scoring in the first seventy days in milk: impact on pregnancy and survival. J. Dairy Sci. 90:4586–91. DOI: 10.3168/jds.2007-0297.

Bielby, W. T. and R. M. Hauser. 1977. Structural equation models. Annual review of sociology. 3:137-161.

Bishop, S. C., J. Chesnais, and M. J. Stear. 2002. Breeding for disease resistance: issues and opportunities.

Bishop, S. C. and M. J. Stear. 2003. Modeling of host genetics and resistance to infectious diseases: understanding and controlling nematode infections. Vet. Parasitol. 115:147-166. DOI:10.1016/S0304-4017(03)00204-8.

Bishop, S. C. 2012. A consideration of resistance and tolerance for ruminant nematode infections. Front. in Genet. 3:168. DOI:10.3389/fgene.2012.00168.

Bishop, S. C. and J. A. Woolliams. 2014. Genomics and disease resistance studies in livestock. Livest. Sci. 166:190-198. DOI:10.1016/j.livsci.2014.04.034.

Blowey, R. W. 2008. Cattle lameness and hoofcare: An illustrated guide. 2. Aufl. Ipswich, United Kingdom: Old Pond.

Brandt, S., V. Apprich, V. Hackl, R. Tober, M. Danzer, C. Kainzbauer, C. Gabriel, C. Stanek, and J. Kofler. 2011. Prevalence of bovine papillomavirus and Treponema DNA in bovine digital dermatitis lesions. Vet. Microbiol. 148:161–67. DOI: 10.1016/j.vetmic.2010.08.031.

Bruegemann, K., E. Gernand, U. U. von Borstel and S. König. 2013. Application of random regression models to infer the genetic background and phenotypic trajectory of binary conception rate by alterations of temperature × humidity indices. Livestock Science, 157:389-396.

Bruijnis, M. R. N., B. Beerda, H. Hogeveen, and E. N. Stassen. 2012. Assessing the welfare impact of foot disorders in dairy cattle by a modeling approach. Animal. 6:962–70. DOI:10.1017/S1751731111002606.

Capion, N., M. Boye, C. T. Ekstrom, and T. K. Jensen. 2012. Infection dynamics of digital dermatitis in first-lactation Holstein cows in an infected herd. J. Dairy Sci. 95:6457–64. DOI: 10.3168/jds.2012-5335.

Cha, E., J. A. Hertl, D. Bar and Y. T. Groehn. 2010. The cost of different types of lameness in dairy cows calculated by dynamic programming. Prev. Vet. Med. 97:1–8. DOI:10.1016/j.prevetmed.2010.07.011.

Cheli, R. and C. M. Mortellaro. 1974. La Dermatite digitale del bovino. Proceedings of the 8th Int. Conf. Diseases of Cattle; Milan, Italy.

Clegg, S. R., S. D. Carter, J. P. Stewart, D. M. Amin, R. W. Blowey, and N. J. Evans. 2016b. Bovine ischaemic teat necrosis: a further potential role for digital dermatitis treponemes. Vet. Rec. 178:71. DOI:10.1136/vr.103167.

Cook, N. B., T. B. Bennett, and K. V. Nordlund. 2004. Effect of free stall surface on daily activity patterns in dairy cows with relevance to lameness prevalence. Journal of dairy science 87(9):2912–2922. https://doi.org/10.3168/jds.S0022-0302(04)73422-0.

Cramer, G., K. D. Lissemore, C. L. Guard, K. E. Leslie, and D. F. Kelton. 2008. Herd- and cow-level prevalence of foot lesions in Ontario dairy cattle. J. Dairy Sci. 91:3888–95. DOI: 10.3168/jds.2008-1135.

Detilleux, J., L. Theron, J. M. Beduin, and C. Hanzen. 2012. A structural equation model to evaluate direct and indirect factors associated with a latent measure of mastitis in Belgian dairy herds. Preventive veterinary medicine, 107:170-179.

Detilleux, J., L. Theron, J. N. Duprez, E. Reding, M. F. Humblet, V. Planchon, C. Delfosse, C. Bertozzi, J. Mainil und C. Hanzen. 2013. Structural equation models to estimate risk of infection and tolerance to bovine mastitis. Genetics Selection Evolution, 45:1-7.

Dhakal, K., F. Tiezzi, J. S. Clay, and C. Maltecca. 2015. Short communication: Genomic selection for hoof lesions in first- parity US Holsteins. J. Dairy Sci. 98:3502–07. DOI: 10.3168/jds.2014-8830.

Döpfer, D., A. Koopmanns, F. A. Meijer, I. Szakáll, Y. H. Schukken, W. Klee, R. B. Bosma, J. L. Cornelisse, A. J. van Asten, and A. A. ter Huurne. 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis. Vet Rec. 140:620–23.

Döpfer, D., R. M. van Boven and M.C.M de Jong. 2004. A mathematical model for the dynamics of digital dermatitis in groups of cattle to study the efficacy of group-based therapy and prevention strategies. Proceedings of the 12th Int. Conf. Production Diseases in Farm Animals; Lansing, MI. Wageningen: Wageningen Academic Publishers; 2006.

Döpfer, D., T. B. Bennett, and N. B. Cook. 2008. Dynamics of Digital Dermatitis Infection Spread in a Large Freestall Housed Wisconsin Dairy Herd. Proceedings of the 15th Int. Symp. and 7th Conf. Lameness in Ruminants; 2008 June 9-13; Kuopio, Finland. Kuopio: Savonia University of Applied Sciences.

Döpfer, D., K. Anklam, D. Mikheil, and P. Ladell. 2012. Growth curves and morphology of three Treponema subtypes isolated from digital dermatitis in cattle. Vet. J. 193:685–693.

Eckelkamp, E. A., J. L. Taraba, K. A. Akers, R. J. Harmon, and J. M. Bewley. 2016a. Sand bedded freestall and compost bedded pack effects on cow hygiene, locomotion, and mastitis indicators. Livestock Science 190:48–57. https://doi.org/10.1016/j.livsci.2016.06.004.

Eckelkamp, E. A., J. L. Taraba, K. A. Akers, R. J. Harmon, and J. M. Bewley. 2016b. Understanding compost bedded pack barns: Interactions among environmental factors, bedding characteristics, and udder health. Livestock Science 190:35–42. https://doi.org/10.1016/j.livsci.2016.05.017.

Evans, N. J., R. W. Blowey, D. Timofte, D. R. Isherwood, J. M. Brown, R. Murray, R. J. Paton, and S. D. Carter. 2011. Association between bovine digital dermatitis treponemes and a range of 'non-healing' bovine hoof disorders. Vet. Rec. 168:214. DOI:10.1136/vr.c5487.

Evans, N. J., J. M. Brown, R. Scholey, R. D. Murray, R. J. Birtles, C. A. Hart, and S. D. Carter. 2014. Differential inflammatory responses of bovine foot skin fibroblasts and keratinocytes to digital dermatitis treponemes. Vet. Immunol. Immunopathol. 161:12–20. doi:10.1016/j.vetimm.2014.05.005.

Evans, N. J., R. D. Murray, and S. D. Carter. 2016. Bovine digital dermatitis: Current concepts from laboratory to farm. Vet. J. 211:3–13. DOI:10.1016/j.tvjl.2015.10.028.

Falconer, D. S. 1965. Maternal effects and selection response. Genetics Today. Pages 763-774.

Frankena, K., J. G. C. J. Somers, W. G. P. Schouten, J. V. van Stek, J. H. M. Metz, E. N. Stassen, and E. A. M. Graat. 2009. The effect of digital lesions and floor type on locomotion score in Dutch dairy cows. Preventive Veterinary Medicine 88(2):150–157. https://doi.org/10.1016/j.prevetmed.2008.08.004.

Fregonesi, J. A., M. A. G. von Keyserlingk, C. B. Tucker, D. M. Veira, and D. M. Weary. 2009. Neck-rail position in the free stall affects standing behavior and udder and stall cleanliness. Journal of dairy science 92(5):1979–1985. https://doi.org/10.3168/jds.2008-1604.

Fregonesi, J. A., and J.D. Leaver. 2001. Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. Livestock Production Science 68(2-3):205–216. https://doi.org/10.1016/S0301-6226(00)00234-7.

Fuchs, A. 2011. Methodische Aspekte linearer Strukturgleichungsmodelle. Ein Vergleich von Kovarianz-und Varianzbasierten Kausalanalyseverfahren (No. 2/2011). Research papers on marketing strategy.

Gana, K., and G. Broc. 2019. Structural equation modeling with lavaan.

Geiser, C. 2010. Datenanalyse mit Mplus: Eine anwendungsorientierte Einführung. Springer eBook Collection Humanities, Social Science. VS Verlag für Sozialwissenschaften, Wiesbaden.

Gernand, E., S. König and C. Kipp. 2019. Influence of on-farm measurements for heat stress indicators on dairy cow productivity, female fertility, and health. Journal of dairy science. 102:6660-6671.

Gianola, D. and D. Sorensen. 2004. Quantitative genetic models for describing simultaneous and recursive relationships between phenotypes. Genetics. 167:1407-1424.

Gomez, A., N. Bernardoni, J. Rieman, A. Dusick, R. Hartshorn, D. H. Read, M. T. Socha, N. B. Cook, and D. Doepfer. 2014b. A randomized trial to evaluate the effect of a trace mineral premix on the incidence of active digital dermatitis lesions in cattle. J. Dairy Sci. 97:6211–22. DOI:10.3168/jds.2013-7879.

Gomez, A., N. B. Cook, J. Rieman, K. A. Dunbar, K. E. Cooley, M. T. Socha, and D. Döpfer. 2015a. The effect of digital dermatitis on hoof conformation. J. Dairy Sci. 98:927–36. DOI:10.3168/jds.2014-8483.

Gomez, A., N. B. Cook, M. T. Socha, and D. Döpfer. 2015b. First-lactation performance in cows affected by digital dermatitis during the rearing period. J. Dairy Sci. 98:4487–98. DOI:10.3168/jds.2014-9041.

Gooch, C. 2008. Dairy freestall barn design—A Northeast perspective 9th Annual Fall Dairy Conference, Cornell University College of Veterinary Medicine and Cornell PRO-DAIRY

Program, Ithaca, NY. 9th Annual Fall Dairy Conference, Cornell University College of Veterinary Medicine and Cornell PRO-DAIRY Program, Ithaca, NY:1–12.

Grenfell, B. T. and A. P. Dobson. 1995. Ecology of infectious diseases in natural populations (Vol. 7). Cambridge University Press.

Hair, Jr, F. J., M. Sarstedt, L. Hopkins and G. V Kuppelwieser. 2014. Partial least squares structural equation modeling (PLS-SEM) An emerging tool in business research. European business review. 26:106-121.

Halli, K., S. F. Vanvanhossou, M. Bohlouli, S. König and T. Yin. 2021. Identification of candidate genes on the basis of SNP by time-lagged heat stress interactions for milk production traits in German Holstein cattle. PLoS One. 16:10

Hargis, A. M. and P. E. Ginn. 2009. Haut. In: MacGavin MD, Zachary JF, Hrsg. Pathologie der Haustiere: Allgemeine, spezielle und funktionelle Veterinärpathologie. 1. Aufl. München: Urban & Fischer in Elsevier.

Heringstad, B., G. Klemetsdal, and T. Steine. 2003. Selection responses for clinical mastitis and protein yield in two Norwegian dairy cattle selection experiments. J. Dairy Sci. 86:2990-2999. DOI:10.3168/jds.S0022-0302(03)73897-1.

Hernandez, J., J. K. Shearer, and D. W. Webb. 2001. Effect of lameness on the calving-to-conception interval in dairy cows. J. Am. Vet. Med. Assoc. 218:1611–14. https://doi.org/10.2460/javma.2001.218.1611.

Holzhauer, M., C. Hardenberg, C. J. Bartels, and K. Frankena. 2006. Herd- and cow-level prevalence of digital dermatitis in the Netherlands and associated factors. J. Dairy Sci. 89:580–88.

Holzhauer, M., C. J. M. Bartels, D. Döpfer, and G. van Schaik. 2008a. Clinical course of digital dermatitis lesions in an endemically infected herd without preventive herd strategies. Vet. J. 177:222–30. DOI:10.1016/j.tvjl.2007.05.004.

Holzhauer, M., D. Doepfer, J. de Boer, and G. van Schaik. 2008b. Effects of different intervention strategies on the incidence of papillomatous digital dermatitis in dairy cows. Vet. Rec. 162:41–46.

Holzhauer, M., C. J. M. Bartels, C. Bergsten, M. M. J. van Riet, K. Frankena, and T. J. G. M. Lam. 2012a. The effect of an acidified, ionized copper sulphate solution on digital dermatitis in dairy cows. Vet. J. 193:659–63. DOI:10.1016/j.tvjl.2012.06.049.

Holzhauer, M., B. Brummelman, K. Frankena, and T. J. G. M. Lam. 2012b. A longitudinal study into the effect of grazing on claw disorders in female calves and young dairy cows. Vet. J. 193:633–38. DOI:10.1016/ j.tvjl.2012.06.044.

Janni, K. A., M. I. Endres, J. K. Reneau, and W. W. Schoper. 2007. Compost dairy barn layout and management recommendations. Appl. Eng. Agric. 23:97–102. https://doi .org/10.13031/2013.22333.

Kester, E., M. Holzhauer and K. Frankena. 2014. A descriptive review of the prevalence and risk factors of hock lesions in dairy cows. The Veterinary Journal. 202:222-228. https://doi.org/10.1016/j.tvjl.2014.07.004.

Klitgaard, K., M. W. Nielsen, M. L. Strube, A. Isbrand, W. D. Al-Medrasi, M. Boye, and T. K. Jensen. 2016. Potential bacterial core species associated with digital dermatitis in cattle herds identified by molecular profiling of interdigital skin samples. Vet. Microbiol. 186:139–49. DOI:10.1016/j.vetmic.2016. 03.003.

Koerhuis, A. N. M. and R. Thompson. 1997. Models to estimate maternal effects for juvenile body weight in broiler chickens. Genetics Selection Evolution, 29:225-249.

Kopke, G. 2019. Genomic and genetic-statistical analysis on susceptibility to dermatitis digitalis in Holstein cattle. PhD Thesis. Leipzig University, Germany.

Kopke, G., K. Anklam, M. Kulow, L. Baker, H. H. Swalve, F. B. Lopes, G. J. M. Rosa and D. Döpfer. 2020. The identification of gene ontologies and candidate genes for digital dermatitis in beef cattle from a genome-wide association study. International Journal of Veterinary Science and Research. 6:027-037.

Koenig, S., A. R. Sharifi, H. Wentrot, D. Landmann, M. Eise, and H. Simianer. 2005. Genetic parameters of claw and foot disorders estimated with logistic models. J. Dairy Sci. 88:3316–3325. https:// doi.org/10 .3168/jds.S0022-0302(05)73015-0.

Krull, A. C., J. K. Shearer, P. J. Gorden, V. L. Cooper, G. J. Phillips, and P. J. Plummer. 2014. Deep sequencing analysis reveals temporal microbiota changes associated with development of bovine digital dermatitis. Infect. Immun. 82:3359–73. DOI:10.1128/IAI.02077-14.

Krull, A. C., J. K. Shearer, P. J. Gorden, H. M. Scott, and P. J. Plummer. 2016b. Digital dermatitis: Natural lesion progression and regression in Holstein dairy cattle over 3 years. J. Dairy Sci. 99:3718–31. DOI:10.3168/jds.2015-10535.

Laven, R. A. and H. Hunt. 2002. Evaluation of copper sulphate, formalin and peracetic acid in footbaths for the treatment of digital dermatitis in cattle. Vet. Rec. 151:144–46. DOI:10.1136/vr.151.5.144.

Leso, L., M. Barbari, M. A. Lopes, F. A. Damasceno, P. Galama, J. L. Taraba, and A. Kuipers. 2020. Invited review: Compost-bedded pack barns for dairy cows. J. Dairy Sci. 103:1072-1099. https://doi.org/10.3168/jds.2019-16864.

Lobeck, K. M., M. I. Endres, E. M. Shane, S. M. Godden, and J. Fetrow. 2011. Animal welfare in cross-ventilated, compost-bedded pack, and naturally ventilated dairy barns in the upper Midwest. J Dairy Sci.94:5469-5479. https://doi.org/10.3168/jds.2011-4363.

Mardis, E. R. 2008. Next-generation DNA sequencing methods. Annu. Rev. Genomics Hum. Genet. 9:387-402. DOI:10.1146/annurev.genom.9.081307.164359.

Morris, C. A. 2007. A review of genetic resistance to disease in Bos taurus cattle. The Veterinary Journal. 174:481-491.https://doi.org/10.1016/j.tvjl.2006.09.006.

Naderi, S., M. Bohlouli, T. Yin and S. König. 2018. Genomic breeding values, SNP effects and gene identification for disease traits in cow training sets. Animal genetics. 49:178-192.

Nicholas, F. W. 2005. Animal breeding and disease. Philosophical Transactions of the Royal Society B: Biological Sciences. 360:1529-1536. DOI:10.1098/rstb.2005.1674.

Nielsen, M. W., M. L. Strube, A. Isbrand, W. D. H. M. Al-Medrasi, M. Boye, T. K. Jensen, K. Nuss, and K. A. Steiner. 2004. Spezielle Diagnostik und Therapie. In: Fiedler A, Maierl J, Nuss K, Hrsg. Erkrankungen der Klauen und Zehen des Rindes: Mit 12 Tabellen. Stuttgart: Schattauer. p. 77–129.

Ofner-Schröck, E., M. Zähner, G. Huber, K. Guldimann, T. Guggenberger, and J. Gasteiner. 2015. Compost Barns for Dairy Cows—Aspects of Animal Welfare. OJAS 05(02):124–131. https://doi.org/10.4236/ojas.2015.52015.

Onyiro, O. M., L. J. Andrews and S. Brotherstone. 2008. Genetic parameters for digital dermatitis and correlations with locomotion, production, fertility traits and longevity in Holstein-Friesian dairy cows. J. Dairy Sci. 91:4037–4046.

Peñagaricano, F., B. D. Valente, J. P. Steibel, R. O. Bates, C. W. Ernst, H. Khatib and G. J. M. Rosa. 2015. Searching for causal networks involving latent variables in complex traits: Application to growth, carcass, and meat quality traits in pigs. Journal of Animal Science, 93:4617-4623.

Perry, B., and D. Grace. 2009. The impacts of livestock diseases and their control on growth and development processes that are pro-poor. Philosophical Transactions of the Royal Society B: Biological Sciences. 364:2643-2655. DOI:10.1098/rstb.2009.0097.

Petersen, S. O. 2018. Greenhouse gas emissions from liquid dairy manure: Prediction and mitigation. J. Dairy Sci. 101:6642-6654. https://doi.org/10.3168/jds.2017-13301.

Polsky, L. and M. A. von Keyserlingk. 2017. Invited review: Effects of heat stress on dairy cattle welfare. Journal of dairy science, 100:8645-8657.

Rasmussen, M., N. Capion, K. Klitgaard, T. Rogdo, T. Fjeldaas, M. Boye, and T. K. Jensen. 2012. Bovine digital dermatitis: possible pathogenic consortium consisting of Dichelobacter nodosus and multiple Treponema species. Vet. Microbiol. 160:151–61. DOI:10.1016/j.vetmic.2012.05.018.

Read, D. H., and R. L. Walker. 1994. Papillomatous digital dermatitis of dairy cattle: Pathologic findings. Pages 156–158 in Proc. 8th Int. Sym. Dis. of Rum. Digit. Banff, Canada.

Read, D.H. and R. L. Walker. 1998. Papillomatous digital dermatitis (footwarts) in California dairy cattle: clinical and gross pathologic findings. J. Vet. Diagn. Invest. 10:67–76. DOI:10.1177/104063879801000112.

Rehbein, P., K. Brügemann, T. Yin, U. U von Borstel, X. L. Wu and S. König. 2013. Inferring relationships between clinical mastitis, productivity and fertility: A recursive model

application including genetics, farm associated herd management, and cow-specific antibiotic treatments. Preventive Veterinary Medicine. 112:58-67.

Rodriguez-Lainz, A., D. W. Hird, R. L. Walker, and D. H. Read. 1996. Papillomatous digital dermatitis in 458 dairies. J. Am. Vet. Med. Assoc. 209:1464–67.

Rodriguez-Lainz, A., P. Melendez-Retamal, D. W. Hird, D. H. Read, and R. L. Walker. 1999. Farm- and host-level risk factors for papillomatous digital dermatitis in Chilean dairy cattle. Prev. Vet. Med. 42:87–97.

Rodrigues, C. A., M. C. R. Luvizotto, A. L. G. Alves, P. H. M. Teodoro, and E. A. Gregorio. 2010. Digital dermatitis of the accessory digits of dairy cows. Pesqui. Vet. Bras. 30:246–48.

Schöpke, K., S. Weidling, R. Pijl, and H.H. Swalve. 2013. Relationships between bovine hoof disorders, body condition traits, and test-day yields. J. Dairy Sci. 96:679-689. https://doi.org/10.3168/jds.2012-5728.

Schöpke, K., A. Gomez, K. A. Dunbar, H. H. Swalve, and D. Döpfer. 2015. Investigating the genetic background of bovine digital dermatitis using improved definitions of clinical status. J. Dairy Sci. 98:8164–8174. http://dx.doi.org/10.3168/jds.2015-9485.

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, and K. Orsel. 2016. Prevalence and distribution of foot lesions in dairy cattle in Alberta, Canada. J. Dairy Sci. 99:6828–6841. https://doi. org/10.3168/jds.2016-10941.

Solano, L., H. W. Barkema, C. Jacobs, and K. Orsel. 2017. Validation of the M-stage scoring system for digital dermatitis on dairy cows in the milking parlor. J. Dairy Sci. 100:1592–1603. https://doi.org/10.3168/jds.2016-11365.

Somers, J.G.C.J., K. Frankena, E. N. Noordhuizen-Stassen, and J.H.M. Metz. 2005. Risk factors for digital dermatitis in dairy cows kept in cubicle houses in The Netherlands. Preventive Veterinary Medicine. 71:11-21. https://doi.org/10.1016/j.prevetmed.2005.05.002.

Straubinger, R. K. 2015. Spirochäten. In: Selbitz H, Truyen U, Valentin-Weigand P, Hrsg. Tiermedizinische Mikrobiologie, Infektions- und Seuchenlehre. 10. Aufl. Stuttgart: Enke Verlag. p. 139–50.

Sullivan, L. E., S. D. Carter, R. Blowey, J. S. Duncan, D. Grove-White, and N. J. Evans. 2013. Digital dermatitis in beef cattle. Vet. Rec. 173:582–83. DOI: 10.1136/vr.101802.

Turner, M. E. and C. D. Stevens. 1959. The regression analysis of causal paths. Biometrics. 15:236-258.

Urban, D. and J. Mayerl. 2014. SEM-Grundlagen. p. 25–81. In D. Urban, and J. Mayerl (eds.). Strukturgleichungsmodellierung: Ein Ratgeber für die Praxis. Springer VS, Wiesbaden.

VanRaden, P. M., C. P. Van Tassell, G. R. Wiggans, T. S. Sonstegard, R. D. Schnabel, J. F. Taylor, and F. S. Schenkel. 2009. Invited Review: Reliability of genomic predictions for North American Holstein bulls. J. Dairy Sci. 92:16-24. DOI:10.3168/jds.2008-1514.

Van der Spek, D., J. A. M. van Arendonk and H. Bovenhuis. 2015. Genome-wide association study for claw disorders and trimming status in dairy cattle. J. Dairy Sci.98: 1286-95. DOI: 10.3168/jds.2014-8302.

Wagner, P. E. 2002. Bedded pack shelters. Lancaster Farming(47):23.

Wagner, P., K. Brügemann, T. Yin, P. Engel, and S. König. 2023. Inferring Causalities of Environmental and Genetic Factors for Differential Somatic Cell Count and Mastitis Pathogens in Dairy Cows Using Structural Equation Modelling. Genes, 14:2102.

Weiss, E. and J. P. Teifke. 2007. Haut. In: Dahme E, Weiss E, Hafner-Marx A, Hrsg. Grundriss der speziellen pathologischen Anatomie der Haustiere: 5 Tabellen. 6. Aufl. Stuttgart: Enke.

Zinicola, M., H. Higgins, S. Lima, V. Machado, C. Guard, and R. Bicalho. 2015a. Shotgun Metagenomic Sequencing Reveals Functional Genes and Microbiome Associated with Bovine Digital Dermatitis. PLoS One. 10:e0133674. DOI:10.1371/journal.pone.0133674.

Zinicola, M., F. Lima, S. Lima, V. Machado, M. Gomez, D. Döpfer, C. Guard, and R. Bicalho. 2015b. Altered microbiomes in bovine digital dermatitis lesions, and the gut as a pathogen reservoir. PLoS One. 10:e0120504. DOI:10.1371/journal.pone.0120504.

CHAPTER 2

Genomic analyses of claw disorders in Holstein cows: Genetic parameters, trait associations, and genome-wide associations considering interactions of SNP and heat stress

Niklas Sölzer[1], Katharina May[1], Tong Yin[1] and Sven König[1]

[1] Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, 35390 Gießen, Germany

Accepted: May 2022

Published October 22

Journal of Dairy Science, 105(10), 8218-8236, https://doi.org/10.3168/jds.2022-22087

al., 2017). Recently, in some countries, official national genetic evaluations for DD have been introduced, considering diagnoses from producers and claw trimmers (Rensing, 2019). The estimated heritabilities for DD were in small to moderate range from 7.3 to 14.2% from pedigree-based approaches (Koenig et al., 2005; van der Linde et al., 2010; Schöpke et al., 2015), and from 5 to 36.7% when considering genomic relationship matrices (Biemans et al., 2019; Shabalina et al., 2021). The development of claw selection indices requires genetic correlations among all diseases. Malchiodi et al. (2017) estimated positive genetic correlations throughout between DD with other claw diseases, and identified the largest correlation between DD and interdigital hyperplasia (**HYP**) with 0.57. Accordingly, Gernand et al. (2012) estimated quite large genetic correlations between DD and HYP, ranging from 0.50 to 0.61 in the course of lactation; additionally, among all claw disorders, HYP was the trait with the highest heritability (up to 0.35 at the end of lactation). Götze (1952) described a strong genetic predisposition for HYP decades ago. A third claw disorder displaying increasing incidences in German dairy cow herds is sole ulceration (**SU**; Gernand et al., 2012). van der Spek et al. (2013) and Gernand et al. (2012) estimated a heritability for SU in threshold models of 0.08 and 0.07, respectively. The genetic correlation between SU and HYP was 0.18 (van der Waaij et al., 2005). For the development of selection indices, it is also imperative to study genetic associations between DD with conformation traits, female fertility, and productivity. In this regard, opposite pedigree-based genetic correlations were estimated between DD with milk yield, such as −0.31 by Onyiro et al. (2008), but 0.24 by Koenig et al. (2005). The genetic correlations between DD and the interval from calving to first insemination was 0.01 (Buch et al., 2011).

Regarding genomic analyses, GWAS for DD with ongoing gene annotations suggested potential candidate genes on chromosomes 3, 6, 9, 11, 12, 19, and 26 (Naderi et al., 2018; Sánchez-Molano et al., 2019; Kopke et al., 2020; Shabalina et al., 2020). For HYP, Zhang et al. (2019) identified a missense mutation in the gene *ROR2* on BTA 8, which was associated with distal limb ossification and brachydactyly in humans. For SU, van der Spek et al. (2015), Sánchez-Molano et al. (2019), and Butty et al. (2021) reported QTL or potential candidate genes on BTA 8, 10, 11, 12, 18, 22, and 29. In summary, some genomic studies for DD, HYP, and SU have been conducted, but ignored possible environmental effects on genetic mechanisms. Recently in middle Europe, periods of heat stress (**HS**) have increased gradually. Brügemann et al. (2013) and Gernand et al. (2019) especially identified effects of HS directly after calving on genetic parameter estimates for low heritability functional traits, and hypothesized HS effects on genetic mechanisms. In such context, consideration of high-throughput genomic marker data combined with innovative statistical modeling approaches allows deeper insights, with possibilities to infer SNP marker × HS interactions (Halli et al., 2021).

Consequently, the aim of the present study was to conduct comprehensive genomic analyses for DD, HYP, and SU including (1) the estimation of heritabilities based on genomic relationship matrices, (2) the estimation of SNP-marker effects for DD, HYP, and SU and the annotation of potential candidate genes from models considering or ignoring possible HS interactions, and (3) the estimation of genetic correlations based on genomic relationship matrices between DD, HYP, and SU with breeding goal traits reflecting health, female fertility, and productivity, and of respective correlations based on SNP effects with focus on the identified candidate gene segments.

MATERIALS AND METHODS

Cow Traits

The genotypes and phenotypes were from databases for national official genetic evaluations. Hence, no additional animal care statement was necessary. We considered 17,264 first-lactation Holstein Friesian cows from 50 large-scale co-operator herds located in the German federal states of Brandenburg and Mecklenburg-West Pomerania. The calving years covered the period from 2010 to 2016. The age at first calving ranged from 20 to 40 mo. All cows were diagnosed (1 = diseased; 0 = healthy) for the claw disorders DD, HYP, and SU, for endometritis (**EM**), and for clinical mastitis (**CM**) by veterinarians and herd managers based on the "central diagnosis key for health data recording" according to the official ICAR nomenclature (Stock et al., 2013). A cow was classified as diseased if at least one diagnosis was recorded until lactation d 365. All cows without any diagnosis until lactation d 365 were defined as healthy for the corresponding disease. Repeated entries for the same diagnosis were ignored. The average prevalence was 15.96% for DD, 8.20% for SU, 2.36% for HYP, 25.12% for EM, and 25.77% for CM.

Conformation traits included linear scores from official type trait recording for rear leg side view (**SV**), foot angle (**FA**), and rear leg rear view (**RV**) for a subset of 14,648 cows. For the conformation trait SV with an intermediate optimum (score = 5), deviations in both directions were treated equally; that is, scores 1 and 9 were allocated to class 1, scores 2 and 7 to class

Table 1. Descriptive statistics for conformation, female fertility, health, and 305-d lactation traits (respective units in brackets)

Trait	Cow (n)	Mean	SD	Minimum	Maximum
Rear leg side view (points)	14,648	5.32	1.23	1	9
Foot angle (points)	14,648	4.87	1.23	1	9
Rear leg rear view (points)	14,648	5.04	1.46	1	9
Nonreturn, d 90 (frequency)	15,452	0.44	0.50	0	1
Days from calving to first insemination (d)	15,452	71.46	21.42	21	149
Calving interval (d)	13,732	388.95	52.30	295	549
Dermatitis digitalis (frequency)	17,264	0.16	0.37	0	1
Sole ulcer (frequency)	17,264	0.08	0.27	0	1
Interdigital hyperplasia (frequency)	17,264	0.02	0.15	0	1
Endometritis (frequency)	17,264	0.25	0.43	0	1
Mastitis (frequency)	17,264	0.26	0.44	0	1
Milk yield (kg)	16,147	9,058.27	1,524.01	1,378	16,156
Fat yield (kg)	16,147	343.87	52.78	57	571
Protein yield (kg)	16,147	301.88	45.81	48	528

2, scores 3 and 6 to class 4, and the score 5 represented the best value in class 5. For RV and FA on a scale from 1 to 9, 9 was the best value.

Records for the female fertility traits nonreturn at d 90 (**NR90**) and for the interval from calving to first insemination (**CFI**) were available for a subset of 15,452 cows. For calving interval (**CIN**) between the calving dates in lactation 1 and 2, we considered records from 13,732 cows. Production data for 305-d lactation milk yield (**MY**), fat yield (**FY**), and protein yield (**PY**) were available from 16,147 cows. Table 1 shows the descriptive statistics for all traits.

Genotypes

The cows were genotyped with the Illumina BovineSNP50 v2 BeadChip (Illumina Inc.; 4,589 cows) or with the Illumina Bovine Eurogenomics 10k chip (12,675 cows). Cows with low-density 10k genotypes were imputed to the 50k panel by the project partner VIT (Vereinigte Informationssysteme Tierhaltung w.V., Verden, Germany) using the algorithm by Segelke et al. (2012). The SNP data set included 44,474 SNPs from 17,264 genotyped cows with phenotypic records. Quality control of the genotype data was performed with the software package PLINK (Purcell et al., 2007). Single nucleotide polymorphisms with a minor allele frequency <1% and a deviation from the Hardy-Weinberg equilibrium (P-value $< 1 \times 10^{-8}$) were discarded. Finally, 44,046 SNPs from 17,264 cows remained for the genomic studies.

Meteorological Data

For the identification of the nearest weather station for each herd, distances (km) between each herd and all public weather stations were calculated using the R-package GEOSPHERE (Hijmans et al., 2019), considering the respective longitudes and latitudes of the herds and the weather stations. Finally, we allocated 30 weather stations to 50 herds. The maximum distance between a herd and a weather station was 27.88 km, the minimum distance was 0.74 km, and the average distance was 13.96 km. We computed the hourly temperature-humidity index (**THI**) as follows (NRC, 1971):

$$\text{THI} = (1.8 \times \text{T} + 32) - (0.55 - 0.0055 \times \text{RH}) \times (1.8 \times \text{T} - 26),$$

where T = hourly temperature and RH = relative humidity. Afterward, we calculated the average daily THI for all days within the period from −1 d before to 21 d after calving. We focused on this interval because HS, especially during the early lactation period, contributed to an increase of claw disorder incidences (Gernand et al., 2019). The range of the daily THI within the defined period was from 32.11 to 76.95. In a next step, we defined a HS threshold with THI 68 as identified by Brügemann et al. (2012) for functional traits in different production systems in Germany. A genotyped cow underwent HS after first calving if at least one of the daily THI within the defined period exceeded 68. Otherwise, the cow was allocated to the cow group without HS. The number of cows allocated to THI >68 was 3,866, implying 22.39% of the cows from all 50 herds were exposed to HS.

Statistical Models

Estimation of Heritabilities for Claw Disorders. Single nucleotide polymorphism-based heritabilities for DD, HYP, and SU were estimated via both linear (on the observed scale) and threshold (on the

underlying liability scale) single-trait animal models using the GREML function as implemented in the GCTA software package (Yang et al., 2011). The single-trait model 1 was

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zg} + \mathbf{e}, \quad [1]$$

where $\mathbf{y}$ = vector of observations for DD, HYP, and SU; $\boldsymbol{\beta}$ = a vector of fixed effects including herd (50 herds), calving year (2010–2016), calving season (4 quarters: Jan–Mar, Apr–Jun, Jul–Sep, Oct–Dec), and first calving age (linear regression); $\mathbf{g}$ = a vector of additive genetic effects following $N\left(\mathbf{0}, \mathbf{G}\sigma_g^2\right)$, where $\mathbf{G}$ = the genomic relationship matrix constructed according to Yang et al. (2010) and σ_g^2 = the genomic variance; $\mathbf{e}$ = a vector of random residuals following $N\left(\mathbf{0}, \mathbf{I}\sigma_e^2\right)$, where $\mathbf{I}$ = an identity matrix and σ_e^2 = residual variance; and $\mathbf{X}$ and $\mathbf{Z}$ = incidence matrices for fixed and random genetic effects, respectively. In the threshold model, variances from the observed scale were transformed to the underlying liability scale according to Lee et al. (2011).

Estimation of Genetic Correlations Among Claw Disorders, and Between Claw Disorders with Female Fertility, Conformation, Health, and Production Traits. Genetic correlations among DD, HYP, and SU and between DD, HYP, and SU with conformation, female fertility, health, and 305-d lactation traits, were estimated via bivariate linear animal models using the GREML algorithm as implemented in GCTA (Yang et al., 2011). The bivariate model 2 was

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1\boldsymbol{\beta}_1 + \mathbf{Zg}_1 + \mathbf{e}_1\\ \mathbf{X}_2\boldsymbol{\beta}_2 + \mathbf{Zg}_2 + \mathbf{e}_2\end{bmatrix}, \quad [2]$$

where the subscripts 1 and 2 represent the first and the second trait, respectively. The effects were the same as described in model 1. The bivariate model considered the following (co)variance structure for random effects:

$$\text{var}\begin{bmatrix}\mathbf{u}_1\\ \mathbf{u}_2\\ \mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix} = \begin{bmatrix}\mathbf{G}\sigma_{g_1}^2 & \mathbf{G}\sigma_{g_{12}} & 0 & 0\\ \mathbf{G}\sigma_{g_{12}} & \mathbf{G}\sigma_{g_2}^2 & 0 & 0\\ 0 & 0 & \mathbf{I}\sigma_{e_1}^2 & \mathbf{I}\sigma_{e_{12}}\\ 0 & 0 & \mathbf{I}\sigma_{e_{12}} & \mathbf{I}\sigma_{e_2}^2\end{bmatrix},$$

where $\mathbf{G}$ = genomic relationship matrix; $\sigma_{g_1}^2$, $\sigma_{g_2}^2$, and $\sigma_{g_{12}}$ = genomic variances for the first and the second trait, and genomic covariance between both traits, respectively; $\mathbf{I}$ = identity matrix for the observations; $\sigma_{e_1}^2$, $\sigma_{e_2}^2$, and $\sigma_{e_{12}}$ = residual variances for the first and second trait and residual covariance between both traits, respectively.

Genome-Wide Associations and Gene Annotations. We performed a GWAS for DD, HYP, and SU in a case-control design by applying a single SNP linear mixed model and using the mlma-loco "leaving one chromosome out" option as implemented in the software GCTA (Yang et al., 2011). Statistical model 3 was defined as follows:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{x_{snp}}\boldsymbol{u_{snp}} + \mathbf{Zg} + \mathbf{e}, \quad [3]$$

where $\mathbf{x_{snp}}$ = SNP genotypes and $\boldsymbol{u_{snp}}$ = SNP effect. The remaining effects were the same as described in model 1.

The number of false positives associations was indicated by QQ plots and inflation factors (λ).

The genome-wide significance level according to Bonferroni ($\mathbf{pBF} = 0.05/\mathrm{N_{SNP}} = 44{,}046$) was 1.14×10^{-6}. Furthermore, following Kurz et al. (2018) and Klein et al. (2020), a less conservative normative significance threshold was used to identify candidate SNPs defined as $\mathbf{pCD} = 1 \times 10^{-4}$. Potential candidate genes from the Ensembl database based on the *Bos taurus* ARS-UCD1.2 genome assembly (Yates et al., 2019) were identified, and assigned to the corresponding significant or candidate SNPs. In this regard, a gene was considered as a candidate gene if at least one significantly associated SNP was located in the gene or within a window size of 200 kb up- and downstream. In the last step, physiological functions of potential candidate genes were inferred based on information from the Ensembl (Yates et al., 2019) and Genecard (Stelzer et al., 2016) databases.

Model 3 was also applied to estimate SNP effects for conformation traits, female fertility traits, EM, CM, and 305-d lactation traits. For conformation and 305-d lactation traits, we applied linear mixed model association analyses (option mlma instead of mlma-loco) as a significantly greater population stratification was observed in the GWAS for these traits ($\lambda > 2$).

Calculation of SNP Effect Correlations. We calculated correlations for the estimated SNP effects from model 3 between DD, HYP, and SU with conformation traits (SV, FA, and RV), female fertility traits (NR90, CFI, and CIN), health traits (EM and CM), and 305-d lactation traits (MY, FY, and PY). In this regard, we considered the SNPs located 200 kb up- and downstream of the respective identified potential candidate gene for DD, HYP, and SU. The total number of variants within the defined chromosome segments of all identified potential candidate genes for the 3 claw

disorders DD, HYP, and SU ranged from 5 to 24, with an average number of 10.84 variants per interval.

Genome-Wide Associations for Claw Disorders with SNP by Heat Stress Interactions. We estimated main and interaction SNP effects for DD, HYP, and SU via generalized least squares equations according to the algorithm as introduced by Yang et al. (2014). Statistical model 4 to estimate main and interaction SNP effects was

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{x_{snpi}}u_{snpi} + \mathbf{x_{interi}}u_{interi} + \mathbf{Zg} + \mathbf{Wg_{hs}} + \mathbf{e}, \quad [4]$$

where $\mathbf{y}$ = a vector of observations for DD, HYP, and SU; $\mathbf{x_{snpi}}$ = SNP genotypes; u_{snpi} = SNP main effect; $\mathbf{x_{interi}}$ = a vector of genotypes for cows undergoing HS (i.e., THI >68) after first calving; u_{interi} = SNP by HS interaction effect; $\mathbf{g_{hs}}$ = a vector of genotype by HS interaction effects for cows with HS following $N\left(\mathbf{0},\ \mathbf{G_{hs}}\sigma^2_{g_{hs}}\right)$, where $\mathbf{G_{hs}}$ = the genomic relationship matrix for the cows with HS and $\sigma^2_{g_{hs}}$ = the variance of genotype by HS interactions (estimated using the gxe option in GCTA); and $\mathbf{W}$ = a design matrix allocating phenotypic records to $\mathbf{g_{hs}}$. Hence, the term u_{interi} with the respective design matrix $\mathbf{x_{interi}}$ was used to indicate whether the genotyped cow was exposed to HS. The regression coefficients of genotypes were nested within the 2 THI classes THI ≤68 or THI >68. The remaining effects were the same as described in model 1. The significance values of main and interaction effects for each SNP were estimated using our own R-package "GWAInter.R" (Halli et al., 2021). Hence, each SNP has 2 *P*-values, specifically one for the significance of the main effect and another for the significance of the interaction effect. In the next step, potential candidate genes for interaction effects were annotated as described above for the SNPs from model 3.

RESULTS AND DISCUSSION

Heritabilities for Claw Disorders

The SNP based heritability for DD from the linear model on the observed scale was 0.04 (SE 0.005), reflecting pedigree-based estimates from linear models by Onyiro et al. (2008) and Johansson et al. (2011) (Table 2). As expected from theory (Fahrmeir and Tutz, 2001; Mrode 2005), the heritability on the underlying liability scale from the threshold model for DD was slightly larger with 0.08 (SE 0.01). Dermatitis digitalis heritabilities in Holstein populations from threshold models were 0.10 (van der Waaij et al., 2005), 0.07 (Koenig et al., 2005), and 0.09 (Gernand et al., 2012). The differences in heritability estimates from linear and threshold models for HYP and SU were obvious, but expected due to their low prevalence (Table 2). The incidence of a disease strongly determines the value of the transformed heritability when applying the transformation equation as developed by Dempster and Lerner (1954), explaining the large heritability differences on the observed and on the underlying liability scale for the diseases with low incidences. Accordingly, the linear estimated heritability for HYP was 0.03 (SE 0.006), but 0.23 (SE 0.04) from the threshold model. The threshold model heritabilities for HYP agree with estimates in Holstein populations conducted by Gernand et al. (2012) with 0.22 (SE 0.04) and by Burmester (2005) with 0.28. Similarly, for SU, the heritability was 0.10 (SE 0.02) from the threshold model, and 0.03 (SE 0.005) from the linear model. Similar heritabilities for SU were estimated by van der Spek et al. (2013), such as a heritability of 0.03 from the linear model, and 0.11 from the threshold model.

Table 2. Additive genetic variances (V_g), residual variances (V_e), and corresponding heritabilities for claw disorders from the linear model (h^2-lin) and from the model with the logit link function (h^2-logit)

	Genetic parameter[1]			
Claw disorder	V_g	V_e	h^2-lin	h^2-logit
Dermatitis digitalis	0.004 (0.001)	0.113 (0.001)	0.04 (0.005)	0.08 (0.01)
Interdigital hyperplasia	0.001 (0.0001)	0.022 (0.0002)	0.03 (0.005)	0.23 (0.04)
Sole ulcer	0.002 (0.0003)	0.067 (0.0007)	0.03 (0.005)	0.10 (0.02)

[1]Standard errors are shown in parentheses.

Genome-Wide Associations and Potential Candidate Genes for Claw Disorders

The Manhattan plot for the DD GWAS from model 3 is presented in Figure 1a. The corresponding inflation factor λ was 1.20. We identified 13 significant SNPs associated with 14 potential candidate genes (Table 3). One of the SNPs located on BTA 10 exceeded pBF. The other 12 significant SNP markers according to pCD are located on BTA 9, 10, 16, 19, and 24. The largest number of significantly associated SNPs was detected on BTA 10. Table 3 shows the significant SNPs, as well as the annotated potential candidate genes for DD. The potential candidate gene *NEO1* on BTA 10 encodes a cell surface protein that belongs to the immunoglobulin superfamily (Vielmetter et al., 1997). The involvement in cell growth and differentiation might explain the significant effect on DD. Accordingly, in the context

of cell growth, Cole et al. (2018) discussed associations between genotypes of the *NEO1* gene with fertility and embryonic size in humans and mice. Another identified potential candidate gene for DD is *DAPK2* on BTA 10. Britschgi et al. (2008) described functions of *DAPK2* on granulocytic differentiations. Granulocytes represent a subgroup of leukocytes, explaining the effects of *DAPK2* on DD, because leucocytes defend infections caused by bacteria (e.g., treponemes), fungi, or parasites. The annotated potential candidate gene *USP3* on BTA 10 is involved in protein ubiquitination (Das et al., 2020). This process, in turn, is directly or indirectly involved in numerous cellular processes, explaining the entrance of treponemes due to damaged skin cells. Keogh et al. (2021) addressed similar cell repairing mechanisms regulated by *USP3* in the context of calving difficulties and recovering after calving in dairy cows and beef heifers. The potential candidate gene *CA12* on BTA 10 was related to feed intake in Hereford and Angus cattle (Seabury et al., 2017) and with uterine pH in Holstein Friesian cattle (Kiser et al., 2019), but not directly with disease resistances. The potential candidate genes *KRT33A* and *KRT33B* on BTA 19 are involved in keratin formation (McKenzie et al., 2010). Keratin is a major component of the claw horn (Litzke, 2019), implying the penetration of treponemes in case of insufficient keratin production.

The Manhattan plot for the GWAS of HYP is presented in Figure 1b. For HYP, we identified 27 significant SNPs associated with 12 potential candidate genes. The corresponding inflation factor λ was 1.16. One of the SNPs located on BTA 13 exceeded pBF. The largest number of significant SNPs according to pCD is located on BTA 8. Table 4 includes the significant SNPs, as well as the associated candidate genes for HYP. The potential candidate gene *UBQLN1* on BTA 8 influences protein degradation in vivo, and proteins interacted with diseases such as the foot and mouth disease virus (Gladue et al., 2014). The potential candidate gene *WDPCP* on BTA 11 plays a crucial role in collective cell movement and cilia formation, explaining the involvement in disease resistance mechanisms (e.g., de las Heras-Saldana et al., 2019; Afonso et al., 2020). The potential candidate gene *ITPR1* on BTA 22 encodes an intracellular receptor for inositol-1, 4, 5-triphosphate, and was active in the presence of external stressors (Cheruiyot et al., 2021).

The Manhattan plot for the GWAS of SU is presented in Figure 1c. For SU, we identified 14 significant SNPs associated with 6 potential candidate genes. The corresponding inflation factor λ was 1.16. None of the SNPs exceeded pBF. The significant SNP markers according to pCD are located on BTA 4, 5, 6, 7, 9, 12, 13, and 27. The largest number of significant SNPs was found on BTA 13. Table 5 shows the significant SNPs for SU and the annotated potential candidate genes. However, direct relationships between the identified genes and diseases were only reported for *C20orf202* on BTA 13, due to the effect on Na/K/2CL cotransporter mechanisms (Shiozaki et al., 2014). The other potential candidate genes for SU were associated with body size (*JAZF1*; Zhao et al., 2015 and *RSP04*; Duan et al., 2021), MY (*CCDC159*; Du et al., 2019), female fertility (*MAN1A1*; Tarekegn et al., 2019), and male fertility (*CTCFL*; Han and Peñagaricano, 2016; Chen et al., 2021) in cattle.

Visual inspections of the Manhattan plots for the 3 claw disorders DD, HYP, and SU in Figures 1a–c indicate regions containing potential candidate genes on BTA 9. Especially for DD and HYP, a large number of potential candidate genes are located on BTA 8, 9, 10, and 11. Furthermore, both diseases have several potential candidate genes on BTA 19 between base pair positions 34,000,000 and 55,000,000, again indicating similar genetic mechanisms and supporting the quite large genetic correlation. From a pathological perspective and in the context of shared diseases pathways, HYP often occurs after a DD infection due to external stressors (de Jesús Argáez-Rodríguez et al., 1997). Nevertheless, we also identified a large number of variants, which did not overlap across the different claw disorders, indicating that the genetic correlation is mostly due to polygenetic effects.

Genetic Correlations and SNP Effect Correlations Among Claw Disorders, and Between Claw Disorders and Remaining Traits

Dermatitis Digitalis. Genetic and SNP effect correlations between DD with all other traits are presented in Figure 2. The genetic correlation between DD and the conformation traits SV, FA, and RV was negative throughout, but close to zero and in a narrow range from 0.05 to −0.14. From a breeding perspective, a negative correlation is favorable, implying that bulls with a fewer number of diseased daughters have the desired scores for linear type traits from the feet and leg composite. Onyiro et al. (2008) and van der Linde et al. (2010) estimated stronger genetic correlations between DD with feet and leg type traits of −0.31 and −0.67, respectively, for locomotion, and of −0.27 and −0.63, respectively, for the overall fundament score. Genetically, Kopke et al. (2020) associated favorable DD breeding values with steeper and parallel legs, supporting the results from the present study. The SNP effect correlations between DD and FA considering the SNPs from the potential candidate genes were highly variable, and ranged from −0.87 (*SHC4*) to 0.75

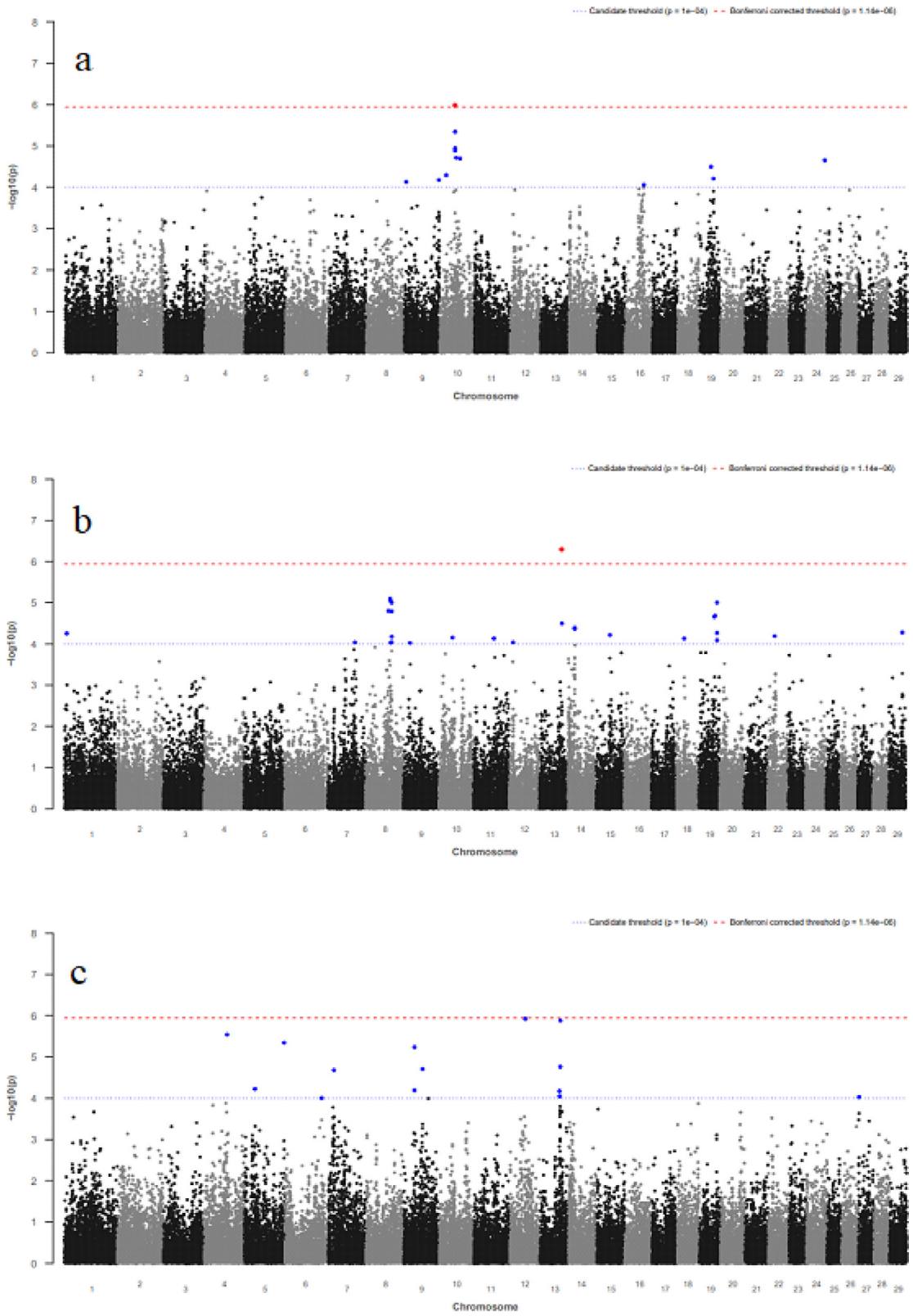

Figure 1. (a) Manhattan plot for −log(10) *P*-values of SNP effects for dermatitis digitalis; (b) Manhattan plot for −log(10) *P*-values of SNP effects for interdigital hyperplasia; (c) Manhattan plot for −log(10) *P*-values of SNP effects for sole ulcer. Genome-wide significance threshold (pBF = 1.14×10^{-6}); suggestive candidate threshold (pCD = 1×10^{-4}). pBF = genome-wide significance level according to Bonferroni; pCD = normative significance threshold.

Table 3. Significantly associated SNP markers and annotated potential candidate genes for dermatitis digitalis

BTA	SNP-ID	SNP position (bp)	*P*-value	Gene[1]	Gene position (bp)[1]
9	rs109476338	5,558,499	7.38×10^{-5}	—	—
	rs109910772	101,846,818	6.67×10^{-5}	*FGFR1OP*	101,818,057–101,845,437
	rs109910772	101,846,818	6.67×10^{-5}	*ENSBTAG00000054087*	101,855,408–101,856,823
10	rs110975142	19,887,506	5.11×10^{-5}	*NEO1*	19,888,399–20,126,828
	rs42584950	45,998,237	0.10×10^{-5}	*DAPK2*	45,948,472–46,088,593
	rs109741931	46,490,485	1.13×10^{-5}	*USP3*	46,413,154–46,513,245
	rs41256789	46,254,922	1.31×10^{-5}	*HERC1*	46,185,094–46,254,294
	rs42838073	46,609,304	0.45×10^{-5}	*CA12*	46,649,404–46,851,817
	rs41649796	49,690,693	1.93×10^{-5}	*RORA*	48,892,525–49,702,163
	rs111011739	61,347,219	2.01×10^{-5}	*SHC4*	61,284,100–61,419,065
16	rs41809605	54,144,431	8.72×10^{-5}	*LRRC38*	54,111,470–54,145,850
19	rs110240780	34,064,148	3.18×10^{-5}	*RNF112*	34,063,776–34,069,380
	rs43729493	41,559,996	6.16×10^{-5}	*KRT33A*	41,552,389–41,557,718
	rs43729493	41,559,996	6.16×10^{-5}	*KRT33B*	41,575,278–41,581,410
24	rs41647307	54,822,569	2.23×10^{-5}	*TCF4*	54,594,983–54,976,864

[1]No gene name or no gene position: SNP was not located in the gene or within a window size of 200 kb up- and downstream.

(*NEO1*). The correlation was positive (0.75) and significantly ($P < 0.05$) different from zero for SNPs from the *NEO1* gene segment, as well as for SNPs surrounding the *LRRC38* gene segment (0.85). There was a highly significant positive correlation (0.85) between DD and SV for SNPs from the *HERC1* segment. With regard to RV, the strongest SNP correlation (0.96) with DD was estimated based on SNPs from the segments surrounding the genes *KRT33A* and *KRT33B*. However, most of the SNP effect correlations for specific chromosome segments between DD and conformation traits were negative, supporting the genetic correlation estimates.

Genetic correlations between DD and female fertility traits were −0.16 for NR90, 0.12 for CFI, and 0.28 for CIN. The positive genetic correlations between DD and fertility interval traits indicate longer periods from calving to first estrus for diseased or lame cows. Accordingly, Buch et al. (2011) estimated a positive, but very small genetic correlation of 0.01 between DD with CFI. The negative genetic correlation between DD and

Table 4. Significantly associated SNP markers and annotated potential candidate genes for interdigital hyperplasia

BTA	SNP-ID	SNP position (bp)	*P*-value	Gene[1]	Gene position (bp)[1]
1	rs41639125	7,484,829	5.54×10^{-5}	—	—
7	rs41607741	78,689,485	9.16×10^{-5}	—	—
8	rs29010321	68,193,101	1.59×10^{-5}	—	—
	rs41587016	73,406,436	7.98×10^{-5}	—	—
	rs110594250	73,731,612	9.02×10^{-6}	—	—
	rs110430166	74,463,486	9.34×10^{-5}	—	—
	rs43010550	76,873,029	8.99×10^{-5}	*UBQLN1*	76,869,555–76,925,625
	rs110311453	76,927,444	9.99×10^{-6}	*UBQLN1*	76,869,555–76,925,625
	rs42295642	77,126,858	1.62×10^{-5}	*KIF27*	77,083,416–77,176,341
	rs42295713	77,243,628	6.67×10^{-5}	*RMI1*	77,241,944–77,244,793
9	rs29012613	19,914,423	9.45×10^{-5}	*BCKDHB*	19,804,029–20,074,759
10	rs43625750	41,725,845	6.98×10^{-5}	—	—
11	rs109261711	61,853,060	7.34×10^{-5}	*WDPCP*	61,723,956–62,023,241
12	rs109743542	11,839,129	9.12×10^{-5}	*VWA8*	11,675,264–12,071,822
13	rs110732787	65,832,716	5.06×10^{-5}	—	—
	rs41702929	66,361,962	3.17×10^{-5}	—	—
14	rs110259526	19,840,969	4.33×10^{-5}	—	—
	rs109185030	19,923,818	4.02×10^{-5}	—	—
15	rs109584130	41,778,626	6.05×10^{-5}	—	—
18	rs110840644	23,178,646	7.37×10^{-5}	—	—
19	rs109954470	46,980,971	2.20×10^{-5}	*TLK2*	46,911,925–47,034,030
	rs41918393	49,207,326	2.06×10^{-5}	—	—
	rs41919813	55,023,618	5.39×10^{-5}	*MFSD11*	55,014,709–55,043,048
	rs41921756	55,099,255	9.93×10^{-5}	*MXRA7*	55,081,443–55,096,598
	rs109693837	55,137,609	8.14×10^{-5}	*ST6GALNAC1*	55,121,131–55,140,468
22	rs41640905	21,522,835	6.47×10^{-5}	*ITPR1*	21,523,823–21,876,681
29	rs109416157	40,631,214	5.25×10^{-5}	*INCENP*	40,599,706–40,634,288

[1]No gene name or no gene position: SNP was not located in the gene or within a window size of 200 kb up- and downstream.

Table 5. Significantly associated SNP markers and annotated potential candidate genes for sole ulcer

BTA	SNP-ID	SNP position (bp)	*P*-value	Gene[1]	Gene position (bp)[1]
4	rs41622258	68,127,101	2.86×10^{-6}	*JAZF1*	67,992,971–68,321,145
5	rs109158459	31,569,741	5.93×10^{-5}	*C5H12orf54*	31,543,719–31,568,121
	rs109997913	117,596,182	4.52×10^{-6}	—	—
6	rs29012203	105,234,399	9.93×10^{-5}	—	—
7	rs109444369	15,754,379	2.08×10^{-5}	*CCDC159*	15,752,075–15,759,370
9	rs42785019	31,725,734	6.44×10^{-5}	*MAN1A1*	31,675,672–31,849,229
	rs42786248	31,733,303	5.75×10^{-6}	*MAN1A1*	31,675,672–31,849,229
	rs42827834	55,390,187	1.97×10^{-5}	—	—
12	rs110161003	46,739,136	1.19×10^{-6}	—	—
13	rs41256263	57,339,787	6.72×10^{-5}	—	—
	rs42555087	58,659,653	8.93×10^{-5}	*CTCFL*	58,657,726–58,682,600
	rs110791636	59,930,801	1.72×10^{-5}	*C20orf202*	59,934,485–59,939,133
	rs41703753	60,152,518	1.31×10^{-6}	*RSPO4*	60,118,678–60,153,362
27	rs42237076	796,128	9.35×10^{-5}	—	—

[1]No gene name or no gene position: SNP was not located in the gene or within a window size of 200 kb up- and downstream.

NR90 indicates improved conception for healthy cows. With focus on some specific gene segments, SNP effect correlations differed from the genetic correlations. In this regard, the SNP effect correlation between DD and NR90 was −0.89 for the *DAPK2* gene segment, and −0.91 for the *LRRC38* gene segment. For the fertility interval traits, the sign of SNP effect correlations mostly differed when compared with the genetic correlation. The genetic correlations between DD and interval traits were positive and favorable from a breeding perspective, but negative for most of the relevant chromosome segments.

The genetic correlation between DD with SU was 0.20, and 0.64 with HYP. Fiedler (2014) explained positive phenotypic correlations among DD, HYP, and SU through causalities in infection pathways. Single nucleotide polymorphism effect correlations between DD and SU considering the gene segments varied widely in the range from −0.63 (*ENSBTAG00000054087*) to 0.42 (*HERC1*). We identified significant positive SNP effect correlations between DD and HYP for 11 of the 14 genes, with largest correlations considering the SNPs from the segments *KRT33A* (0.93), *KRT33B* (0.93), *HERC1* (0.90), and *DAPK2* (0.84). The weak to moderate negative genetic correlations between DD with EM (−0.22) and with CM (−0.07) indicate different pathways for bacterial infections of different disease categories and a broad variety of bacteria species that can trigger the respective disease. Accordingly, Gernand et al. (2012) and Buch et al. (2011) found genetic correlations close to zero between DD with diseases from other health categories. For SNP effect correlations for EM, the range varied from −0.94 (*HERC1*) to 0.79 (*ENSBTAG000054087*). Most of the SNP effect correlations between DD and EM, and between DD and CM were negative.

With regard to 305-d lactation traits, genetic correlations between DD with MY (0.13), FK (0.03), and PK (0.10) indicate slight genetic antagonistic relationships between bacterial claw infections and productivity as previously shown by Koenig et al. (2005). However, with focus on the most important genes or chromosome segments for DD, SNP effect correlations with 305-d lactation MY ranged from −0.58 (*ENSBTAG00000054087*) to 0.79 (*LRRC38*), with 10 of the 14 correlations being positive. Regarding FY, SNP effect correlations with DD varied in the range from −0.91 (*RNF112*) to 0.73 (*LRRC38*), and for PY from −0.75 (*RNF112*) to 0.82 (*LRRC38*). With regard to the *LRRC38* segment, the SNP effect correlations between DD and the production traits MY, FY, and PY were significant and positive. *LRRC38* is actively involved in K transport and is associated with postpartum Ca concentration in blood and in milk of Holstein cows (Cavani et al., 2022). Calcium in turn plays an important role in milk production (Breves et al., 2016), as well as in keratinization and mature horn cell formation of the claw (Langova et al., 2020). Rodríguez et al. (2017) associated increased blood calcium losses at the beginning of lactation and hypocalcemia of especially high yielding cows with several diseases, which may explain the positive correlations between DD and the production traits based on the SNPs of the *LRRC38* segment.

Interdigital Hyperplasia. Genetic and SNP effect correlations for HYP with all other traits are presented in Figure 3. The genetic correlations between HYP and the conformation traits SV, FA, and RV were 0.03, −0.18, and −0.21, respectively. Hence, genetic improvements of FA and RV imply indirect favorable selection response in the HYP health status. Similarly, in pedigree-based approaches, van der Waaij et al. (2005) estimated negative genetic correlations between HYP

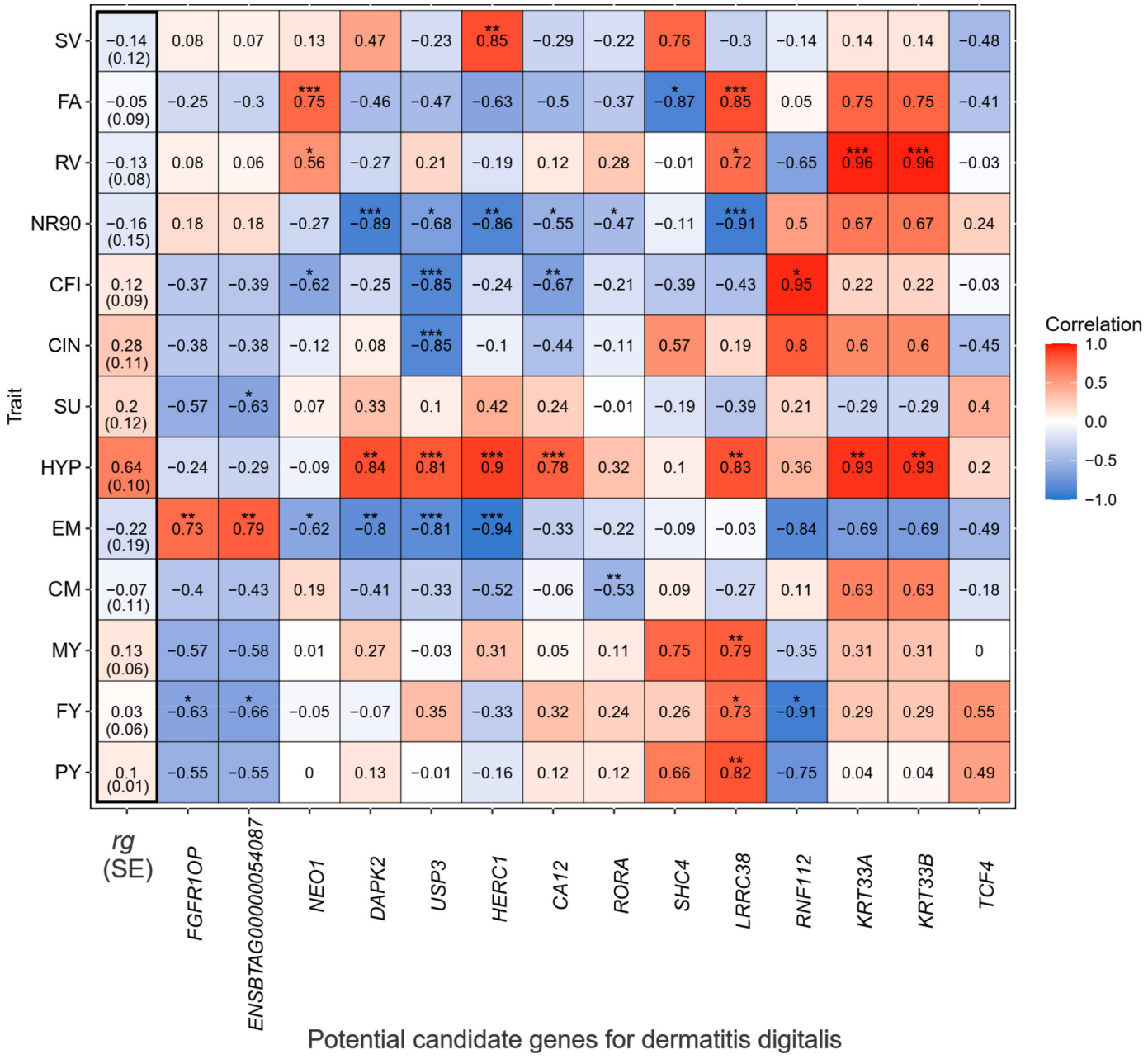

Figure 2. Genetic correlations and SNP effect correlations considering SNPs surrounding potential candidate genes for dermatitis digitalis (DD) between DD and remaining traits. Significance of the Pearson correlation: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; SV = rear leg side view; FA = foot angle; RV = rear leg rear view; NR90 = nonreturn rate at d 90; CFI = days from calving to first insemination; CIN = calving interval; SU = sole ulcer; HYP = interdigital hyperplasia; EM = endometritis; CM = clinical mastitis; MY = milk yield; FY = fat yield; PY = protein yield; rg = genetic correlation between dermatitis digitalis and the listed traits.

with FA (−0.15) and with RV (−0.35), but a weak positive genetic correlation with SV (0.04). Again, the SNP effect correlations with SV based on the identified candidate genes for HYP display a heterogeneous pattern from −0.89 (*KIF27*) to 0.80 (*ITPR1*). Regarding FA, SNP effect correlations with HYP ranged from −0.46 (*KIF27*) to 0.68 (*ITPR1*).

The genetic correlations between HYP with NR90 (−0.06), CFI (0.17), and CIN (0.11) reflect the estimates between female fertility traits with DD. Hence, increased susceptibility to HYP is genetically related to a delayed estrus activity after calving and with impaired conception. Regarding the identified important chromosome segments for HYP, 7 genes contributed to

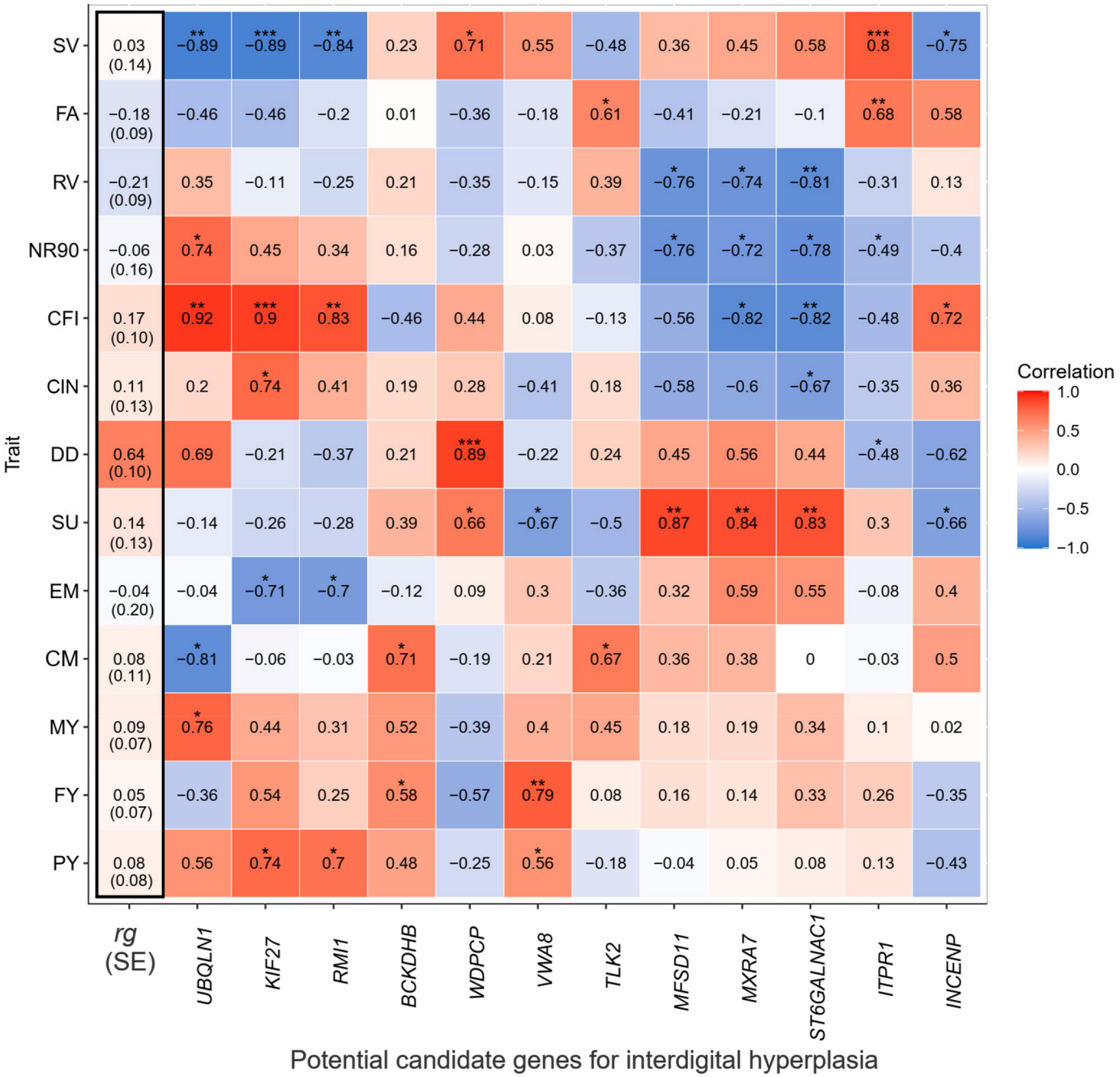

Figure 3. Genetic correlations and SNP effect correlations considering SNPs surrounding potential candidate genes for interdigital hyperplasia (HYP) between HYP and remaining traits. Significance of the Pearson correlation: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; SV = rear leg side view; FA = foot angle; RV = rear leg rear view; NR90 = nonreturn rate at d 90; CFI = days from calving to first insemination; CIN = calving interval; DD = dermatitis digitalis; SU = sole ulcer; EM = endometritis; CM = clinical mastitis; MY = milk yield; FY = fat yield; PY = protein yield; rg = genetic correlation between interdigital hyperplasia and the listed traits.

negative correlations with NR90, and 5 genes to positive correlations with NR90. Such a "balance" of gene segments contributing to either favorable or unfavorable SNP effect correlations with HYP also was identified for the fertility interval traits CFI and CIN. Highly significant correlations were found between HYP and CFI (0.90), as well as between HYP and CIN (0.74) for SNPs surrounding the *KIF27* gene. With regard to the potential candidate gene *KIF27* on BTA 8, Parker Gaddis et al. (2016) confirmed the effect on female fertility. The genetic correlation between HYP and SU was 0.14 (0.13), reflecting estimates by van der Waaij

et al. (2005) based on pedigree data. Accordingly, Koenig et al. (2005) estimated positive genetic correlations among DD, SU, and HYP, and they indicated that claw disorders "genetically appear to occur in clusters." This means that a cow with a DD infection has an increased genetic risk to show symptoms for HYP or SU, and vice versa. Phenotypically, 580 cows had more than one claw disorder; specifically, 155 cows with a diagnosis for DD and HYP, 336 cows with a diagnosis for DD and SU, 25 cows with a diagnosis for DD, HYP, and SU, and 64 cows with a diagnosis for HYP and SU. Very strong positive correlations between SNP effects for HYP and SU were calculated based on the SNPs surrounding the genes *MFSD11* (0.87), *MXRA7* (0.84), and *ST6GALNAC1* (0.83), supporting the favorable genetic correlation between HYP and SU. With regard to *WDPCP*, the correlation between DD and HYP was 0.89. *WDPCP* was associated with inflammatory response mechanisms to infections (de las Heras-Saldana et al., 2019; Afonso et al., 2020), which might play a role for both claw disorders DD and HYP. The genetic correlation between HYP and CM was 0.08, and −0.04 between HYP and EM. Similarly, Gernand et al. (2012) estimated genetic correlations of 0.03 between HYP with EM, and of 0.00 between HYP and CM. For production traits, genetic correlations with HYP were close to zero, but always positive; in particular, correlations were 0.09 with MY, 0.05 with FY, and 0.08 with PY. Accordingly, SNP effects correlations between HYP and MY were positive for 11 gene segments, and only negative based on the SNPs surrounding *WDPCP*.

Sole Ulceration. Figure 4 displays the genetic and SNP effect correlations between SU and the selected traits. Genetic correlations between SU and the conformation traits SV, FA, and RV were throughout negative with −0.21, −0.07, and −0.19, respectively. SNP effect correlations between SU and SV ranged from −0.90 to −0.06. Pronounced negative correlations were calculated between SU with SV (−0.81), with FA (−0.66), and with RV (−0.58) for the SNPs located in the *MAN1A1* segment. With regard to SU and female fertility associations, genetic correlations were 0.05 with NR90, 0.31 with CFI, and 0.23 with CIN. Accordingly, Buch et al. (2011) estimated a genetic correlation of 0.33 between SU and CFI based on pedigree data. The SNP effect correlations with focus on the SU candidate genes and female fertility traits showed a heterogeneous pattern. Again, strong correlations were found for the *MAN1A1* gene segment, such as 0.92 between the SNP effects for SU and for CIN. *MAN1A1* was suggested as a candidate gene for human health and fertility disorders, such as in the context of mesenchymal tumor development (Alonso-Garcia et al., 2020).

With regard to direct health trait diagnoses, the genetic correlation between SU and EM was 0.65, and 0.22 between SU and CM. The respective SNP effect correlations between SU and EM ranged from −0.90 (*CTCFL*) to 0.34 (*CCDC159*). Genetic correlations between SU and 305-d lactation traits were throughout antagonistic; specifically, they were 0.25 with MY, 0.26 with FY, and 0.27 with PY, reflecting estimates by Koenig et al. (2005) and Oliveira Junior et al. (2021) when using pedigree data.

Genome-Wide Associations for SNP by Heat Stress Interactions

The Manhattan plot for the SNP interaction effects with HS for DD is presented in Figure 5. A significant interaction means that the respective SNP is relevant for DD susceptibility under HS, but not under thermoneutral conditions, or vice versa. The corresponding inflation factor λ was 1.07. Two SNPs exceeded the candidate threshold. The annotated potential candidate genes for interactions effects are listed in Table 6. One significant SNP was located in close distance to the gene *THBD* on BTA 13. This protein-coding gene is involved in blood clotting and associated with tuberculosis disease in mice (Weijer et al., 2005). With regard to the main effects from model 4, the Manhattan plot (not shown) was similar with the pattern from model 3 as presented in Figure 1a. Again, most of the significant SNPs are located on BTA 10 and on BTA 16 (see Figure 1a).

The Manhattan plot for the SNP interaction effects for HYP is presented in Figure 6. The corresponding inflation factor λ was 1.01. Two of the SNPs exceeded the candidate threshold (see Table 6). For the HYP × HS interaction, a significant SNP was found in the segment of *ADGRV1* on BTA 7. *ADGRV1* was associated with metabolic BW in Holstein Friesian cows (Hardie et al., 2017). With regard to the main effects, the Manhattan plot from the interaction model 4 (not shown) was similar with the pattern from model 3 as presented in Figure 1b. Again, most of the significant SNPs are located on BTA 19 (see Figure 1b).

The Manhattan plot for the SNP interaction effects for SU with HS is presented in Figure 7. The corresponding inflation factor λ was 1.15. Fifteen of the SNPs exceeds the candidate threshold (see Table 6). For the interaction term, a significant SNP was found in the *CLCN1* segment on BTA 4. *CLCN1* is involved in the electrical excitability of skeletal muscle cells, explaining its role in the occurrence of myotonia hereditaria in Australian cattle dogs (Finnigan et al., 2007). Another significant SNP is located in close distance to the segment of *TRH*

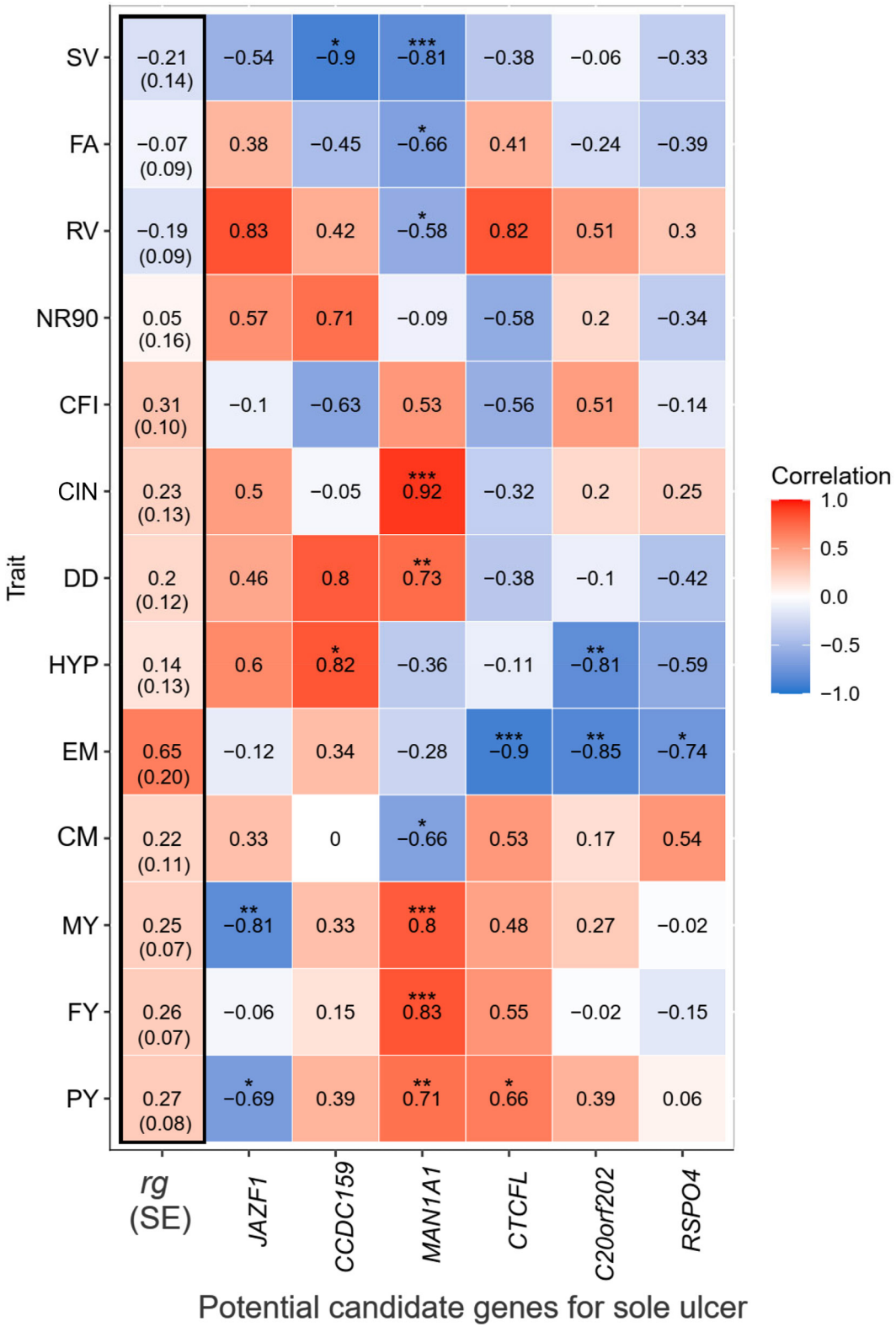

Figure 4. Genetic correlations and SNP effect correlations considering SNPs surrounding potential candidate genes for sole ulcer (SU) between SU and remaining traits. Significance of the Pearson correlation: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; SV = rear leg side view; FA = foot angle; RV = rear leg rear view; NR90 = nonreturn rate at d 90; CFI = days from calving to first insemination; CIN = calving interval; DD = dermatitis digitalis; HYP = interdigital hyperplasia; EM = endometritis; CM = clinical mastitis; MY = milk yield; FY = fat yield; PY = protein yield; rg = genetic correlation between sole ulcer and the listed traits.

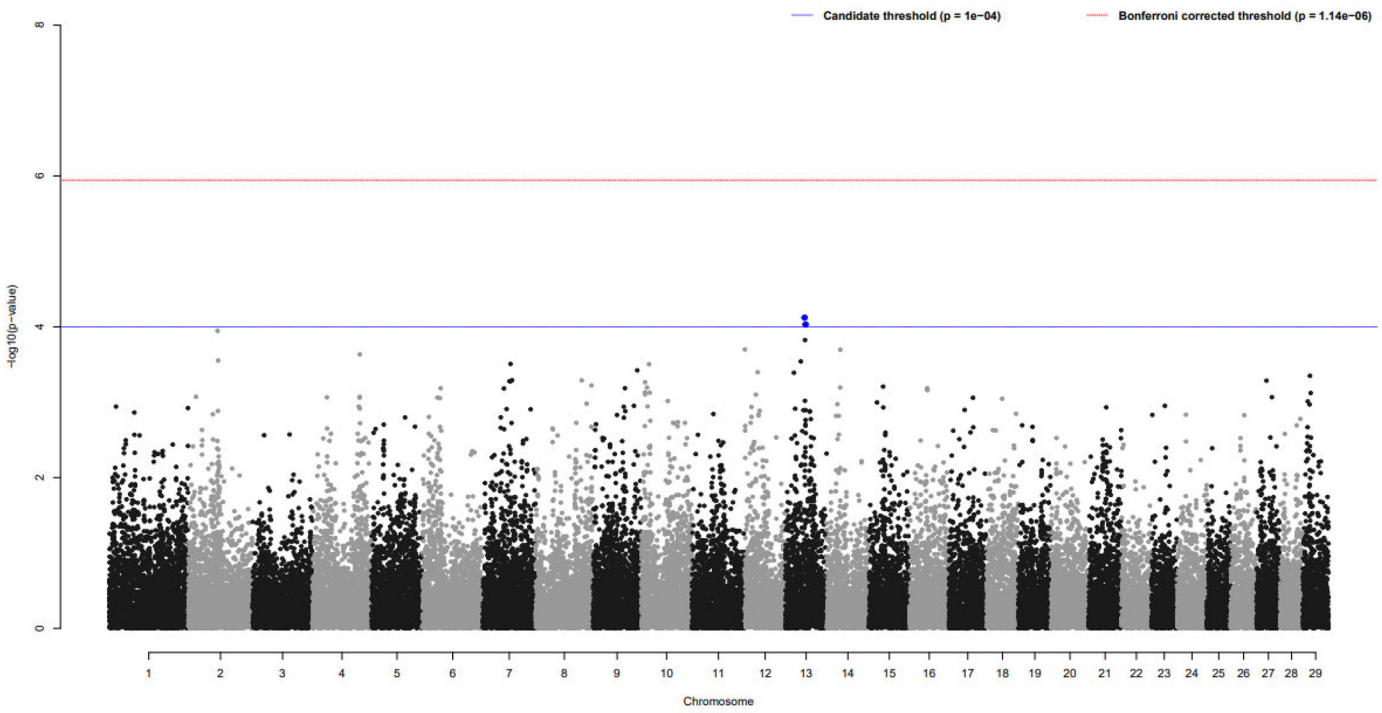

Figure 5. Manhattan plot for −log(10) *P*-values of SNP × heat stress interaction effects for dermatitis digitalis. Genome-wide significance threshold (pBF = 1.14×10^{-6}); suggestive candidate threshold (pCD = 1×10^{-4}). pBF = genome-wide significance level according to Bonferroni; pCD = normative significance threshold.

on BTA 22, which is related to growth hormone and prolactin secretion (Kaiser et al., 1994). One significant SNP is located within the segment of *RHOBTB1* on BTA 28. This protein-coding gene is involved in actin filament assembly and affected meat quality in cattle (Silva et al., 2020).

Studies addressing genotype x environment interactions for claw disorders are quite rare. Applying the classical multiple-trait approach, Shabalina et al. (2021) proved genotype × environment interactions for DD based on genetic correlations smaller than 0.80 between DD recorded in conventional or in organic farms.

Table 6. Significantly associated SNP markers and annotated potential candidate genes for SNP × heat stress interaction effects

Claw disorder	BTA	SNP-ID	SNP position (bp)	*P*-value	Gene[1]	Gene position (bp)[1]
Dermatitis digitalis	13	rs41689217	41,935,549	7.55×10^{-5}	*THBD*	41,849,505–41,854,998
Dermatitis digitalis	13	rs29024306	44,156,728	9.30×10^{-5}	—	—
Interdigital hyperplasia	2	rs110893390	10,542,376	7.23×10^{-5}	*FSIP2*	10,562,943–10,705,287
Interdigital hyperplasia	7	rs29011503	90,389,523	7.11×10^{-5}	*ADGRV1*	90,154,320–90,667,809
Sole ulcer	2	rs41597882	12,931,458	9.96×10^{-5}	*ENSBTAG00000052131*	12,940,014–12,941,224
	4	rs109245784	106,723,818	9.47×10^{-5}	*CLCN1*	106,700,966–106,738,430
	5	rs42485609	7,930,463	2.21×10^{-5}	—	—
	5	rs29017368	57,677,072	3.59×10^{-5}	*OR6C8*	57,673,709–57,674,653
	7	rs41569775	33,733,352	8.43×10^{-5}	—	—
	7	rs41618235	71,209,958	4.55×10^{-5}	*ENSBTAG00000050222*	71,358,400–71,362,800
	7	rs42668574	105,414,564	5.49×10^{-5}	—	—
	8	rs109909107	105,987,019	7.94×10^{-5}	*ASTN2*	105,642,638–106,693,624
	9	rs110131550	23,016,695	7.42×10^{-5}	*DOP1A*	22,963,808–23,089,404
	17	rs41584487	22,998,749	7.95×10^{-5}	—	—
	22	rs29013532	55,883,157	4.63×10^{-5}	*TRH*	55,886,672–55,889,873
	25	rs110927692	12,436,815	5.16×10^{-5}	—	—
	25	rs109782763	12,512,507	4.98×10^{-5}	—	—
	25	rs110912511	12,724,331	8.00×10^{-5}	*ERCC4*	12,833,435–12,874,714
	28	rs110136757	16,691,439	4.49×10^{-5}	*RHOBTB1*	16,637,438–16,777,483

[1]No gene name or no gene position: SNP was not located in the gene or within a window size of 200 kb up- and downstream.

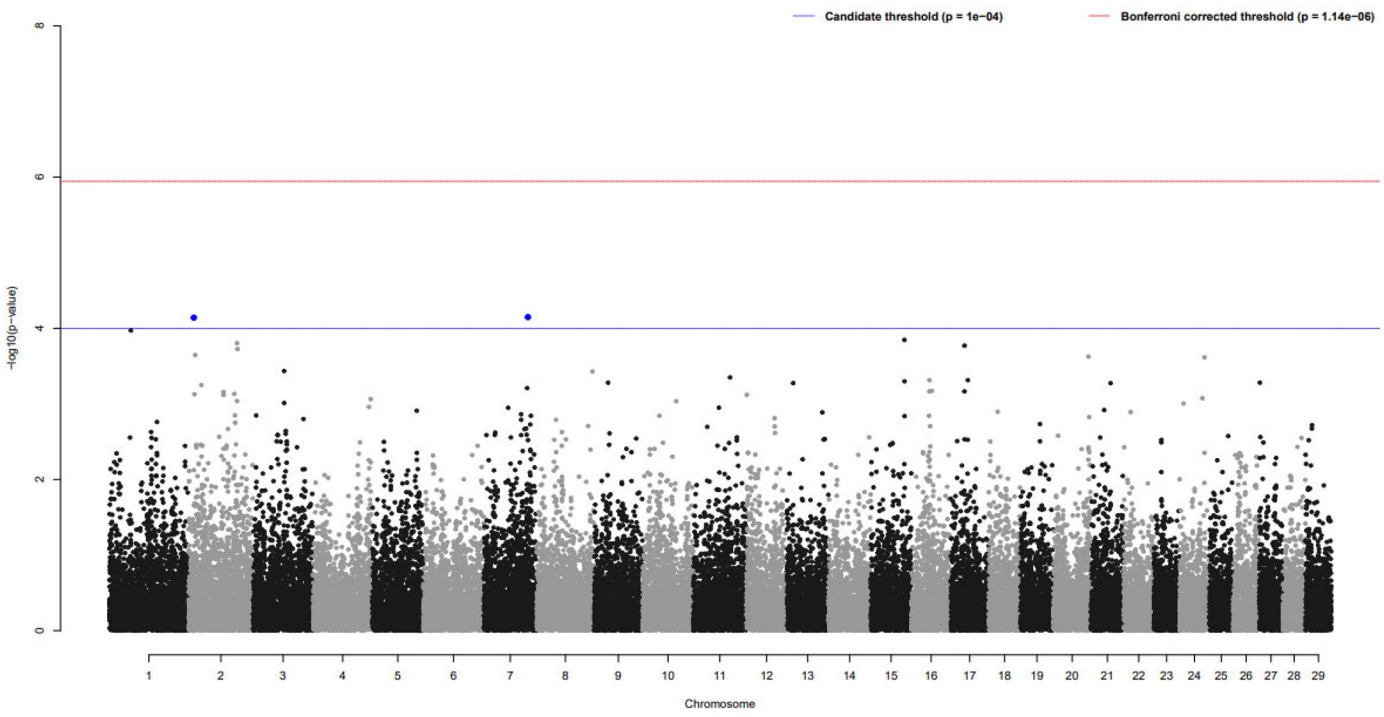

Figure 6. Manhattan plot for −log(10) *P*-values of SNP × heat stress interaction effects for interdigital hyperplasia. Genome-wide significance threshold (pBF = 1.14×10^{-6}); suggestive candidate threshold (pCD = 1×10^{-4}). pBF = genome-wide significance level according to Bonferroni; pCD = normative significance threshold.

Accordingly, for the claw disorder sole hemorrhage, Swalve et al. (2014) indicated that the etiology of a disease is highly dependent on the particular environment. In their study, they found strong associations of genotypes for *IQGAP1* on sole hemorrhage. However, in a previous study (Urao et al., 2010), effects of *IQGAP1* on blood circulation in mice feet depended on the environmental stress conditions. Brenig et al. (2003) indicated that the *SLC26a2* Gen (bovine diastrophic sulfate transporter) is involved in mechanisms regulating cell sulfate intake in the cattle claw. However, in associated analyses conducted in bulls kept on station, the same authors reported stable surface particularities on *SLC26a2* expressions.

CONCLUSIONS

Single nucleotide polymorphism-based heritabilities were 0.04 and 0.03 for DD, 0.08 and 0.10 for SU, and 0.03 and 0.23 for HYP from linear and threshold models, respectively. The heritability differences from linear and threshold models reflect theoretical expectations when considering the respective disease prevalence. The genetic and the SNP based correlations between DD, HYP, and SU with conformation traits from the feet and leg composite were throughout favorable from a breeding point of view. Genetic correlations between DD, SU, and HYP with other breeding goal traits indicated impaired female fertility, an impaired udder health status, and a productivity decline of diseased cows. Genetic correlations among DD, SU, and HYP were moderate to large, indicating that different claw disorders have similar genetic mechanisms. Nevertheless, we identified disease specific potential candidate genes, and genetic associations based on the surrounding SNPs partly differed from the genetic relationships. Especially for candidate genes affecting 2 traits simultaneously, SNP effect correlations were close to 1 or to −1. With regard to SNP × HS interactions, we identified significant SNPs on several chromosomes, and annotated the potential candidate genes *FSIP2*, *CLCN1*, *ADGRV1*, *DOP1A*, *THBD*, and *RHOBTB1*. These results indicate gene specific mechanisms of claw disorders only in specific environments.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support provided by the German National Science Foundation, DFG (Bonn, Germany), through grant number KO 3520/8-1. We thank the H. Wilhelm Schaumann Foundation (Hamburg, Germany) for providing a scholar-

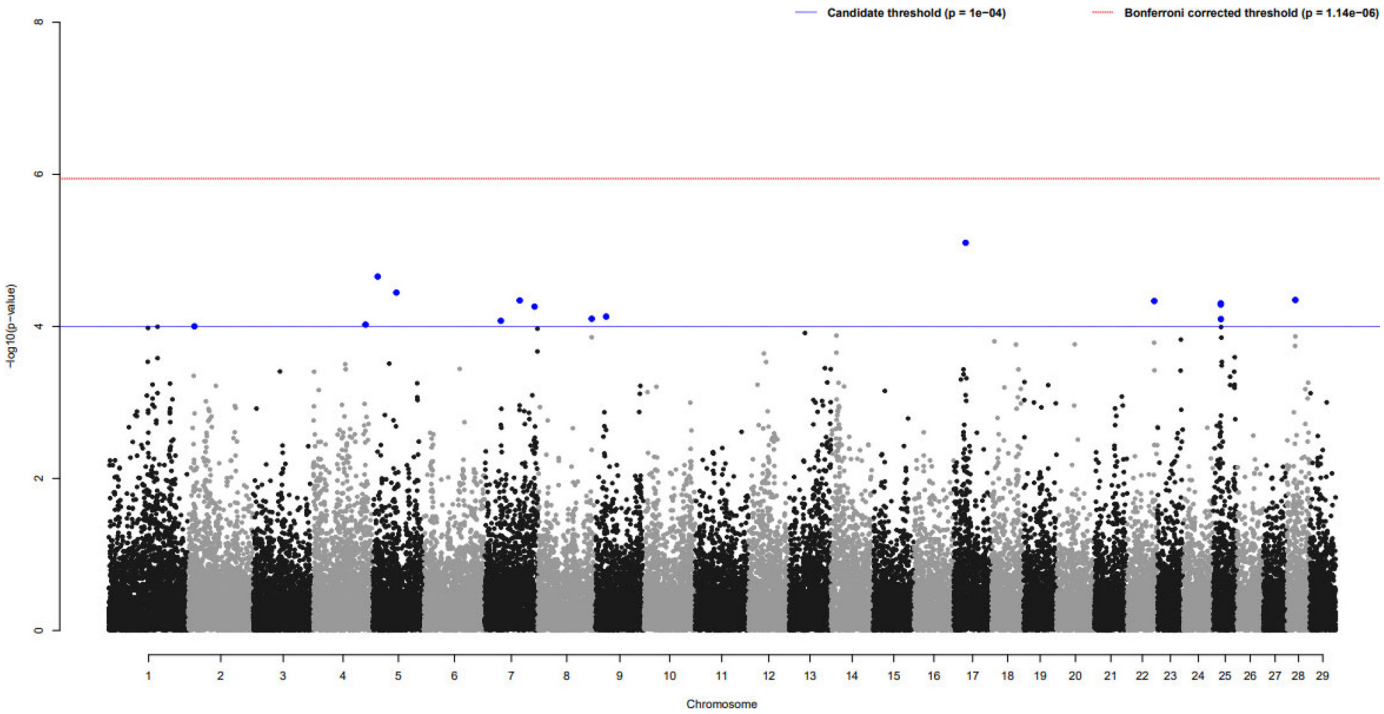

Figure 7. Manhattan plot for −log(10) *P*-values of SNP × heat stress interaction effects for sole ulcer. Genome-wide significance threshold (pBF = 1.14×10^{-6}); suggestive candidate threshold (pCD = 1×10^{-4}). pBF = genome-wide significance level according to Bonferroni; pCD = normative significance threshold.

ship to Niklas Sölzer. The authors have not stated any conflicts of interest.

REFERENCES

Afonso, J., M. R. S. Fortes, A. Reverter, W. J. S. Diniz, A. S. M. Cesar, A. O. Lima, J. Petrini, M. M. de Souza, L. L. Coutinho, G. B. Mourão, A. Zerlotini, C. F. Gromboni, A. R. A. Nogueira, and L. C. A. Regitano. 2020. Genetic regulators of mineral amount in Nelore cattle muscle predicted by a new co-expression and regulatory impact factor approach. Sci. Rep. 10:8436. https://doi.org/10.1038/s41598-020-65454-7.

Alonso-Garcia, V., C. Chaboya, Q. Li, B. Le, T. J. Congleton, J. Florez, V. Tran, G.-Y. Liu, W. Yao, C. B. Lebrilla, and F. A. Fierro. 2020. High mannose N-Glycans promote migration of bone-marrow-derived mesenchymal stromal cells. Int. J. Mol. Sci. 21:7194. https://doi.org/10.3390/ijms21197194.

Biemans, F., M. C. M. de Jong, and P. Bijma. 2019. A genome-wide association study for susceptibility and infectivity of Holstein Friesian dairy cattle to digital dermatitis. J. Dairy Sci. 102:6248–6262. https://doi.org/10.3168/jds.2018-15876.

Blowey, R. W., and M. W. Sharp. 1988. Digital dermatitis in dairy cattle. Vet. Rec. 122:505–508. https://doi.org/10.1136/vr.122.21.505.

Brenig, B., B. G. Baumgartner, B. Kriegesmann, F. Habermann, R. Fries, and H. H. Swalve. 2003. Molecular cloning, mapping, and functional analysis of the bovine sulfate transporter SLC26a2 gene. Gene 319:161–166. https://doi.org/10.1016/S0378-1119(03)00806-0.

Breves, G., K. Elfers, and B. Schröder. 2016. Laktation, Milchbildung, Nährstoffflüsse und Regulation. 26. Hülsenberger Gespräche 07. – 08.06.2016.

Britschgi, A., E. Trinh, M. Rizzi, M. Jenal, A. Ress, A. Tobler, M. F. Fey, K. Helin, and M. P. Tschan. 2008. DAPK2 is a novel E2F1/KLF6 target gene involved in their proapoptotic function. Oncogene 27:5706–5716. https://doi.org/10.1038/onc.2008.179.

Brügemann, K., E. Gernand, U. König von Borstel, and S. König. 2012. Defining and evaluating heat stress thresholds in different dairy cow production systems. Arch. Tierzucht 55:13–24. https://doi.org/10.5194/aab-55-13-2012.

Brügemann, K., E. Gernand, U. U. von Borstel, and S. König. 2013. Application of random regression models to infer the genetic background and phenotypic trajectory of binary conception rate by alterations of temperature × humidity indices. Livest. Sci. 157:389–396. https://doi.org/10.1016/j.livsci.2013.08.009.

Bruijnis, M. R. N., H. Hogeveen, and E. N. Stassen. 2010. Assessing economic consequences of foot disorders in dairy cattle using a dynamic stochastic simulation model. J. Dairy Sci. 93:2419–2432. https://doi.org/10.3168/jds.2009-2721.

Buch, L. H., A. C. Sorensen, J. Lassen, P. Berg, J. Eriksson, J. H. Jakobsen, and M. K. Sorensen. 2011. Hygiene-related and feed-related hoof diseases show different patterns of genetic correlations to clinical mastitis and female fertility. J. Dairy Sci. 94:1540–1551. https://doi.org/10.3168/jds.2010-3137.

Burmester, J. 2005. Analyse von Daten aus dem Managementprogramm für Klauenpfleger mittels Schwellenwertmodellen. MS Thesis. University of Göttingen, Göttingen, Germany.

Butty, A. M., T. C. S. Chud, D. F. Cardoso, L. S. F. Lopes, F. Miglior, F. S. Schenkel, A. Cánovas, I. M. Häfliger, C. Drögemüller, P. Stothard, F. Malchiodi, and C. F. Baes. 2021. Genome-wide association study between copy number variants and hoof health traits in Holstein dairy cattle. J. Dairy Sci. 104:8050–8061. https://doi.org/10.3168/jds.2020-19879.

Cavani, L., M. B. Poindexter, C. D. Nelson, J. E. P. Santos, and F. Peñagaricano. 2022. Gene mapping, gene-set analysis, and genomic prediction of postpartum blood calcium in Holstein cows. J. Dairy Sci. 105:525–534. https://doi.org/10.3168/jds.2021-20872.

Chen, Z., L. F. Brito, H. Luo, R. Shi, Y. Chang, L. Liu, G. Guo, and Y. Wang. 2021. Genetic and genomic analyses of service sire ef-

fect on female reproductive traits in Holstein cattle. Front. Genet. 12:713575. https://doi.org/10.3389/fgene.2021.713575..

Cheruiyot, E. K., M. Haile-Mariam, B. G. Cocks, I. M. MacLeod, R. Xiang, and J. E. Pryce. 2021. New loci and neuronal pathways for resilience to heat stress in cattle. Sci. Rep. 11:16619. https://doi.org/10.1038/s41598-021-95816-8.

Cole, J. B., K. L. Parker Gaddis, D. J. Null, C. Maltecca, and J. S. Clay. 2018. Genome-wide association study and gene network analysis of fertility, retained placenta, and metritis in US Holstein cattle. World Congress of Genetics Applied in Livestock Production. Vol. Biol. Dis. Resist. 1:171.

Das, S., A. P. Chandrasekaran, B. Suresh, S. Haq, J. H. Kang, S. J. Lee, J. Kim, J. Kim, S. Lee, H. H. Kim, K. S. Kim, and S. Ramakrishna. 2020. Genome-scale screening of deubiquitinase subfamily identifies USP3 as a stabilizer of Cdc25A regulating cell cycle in cancer. Cell Death Differ. 27:3004–3020. https://doi.org/10.1038/s41418-020-0557-5.

de las Heras-Saldana, S., S. A. Clark, N. Duijvesteijn, C. Gondro, J. H. J. van der Werf, and Y. Chen. 2019. Combining information from genome-wide association and multi-tissue gene expression studies to elucidate factors underlying genetic variation for residual feed intake in Australian Angus cattle. BMC Genomics 20:939. https://doi.org/10.1186/s12864-019-6270-4.

de Jesús Argáez-Rodríguez, F., D. W. Hird, J. H. de Anda, D. H. Read, and A. Rodríguez-Lainz. 1997. Papillomatous digital dermatitis on a commercial dairy farm in Mexicali, Mexico: Incidence and effect on reproduction and milk production. Prev. Vet. Med. 32:275–286. https://doi.org/10.1016/S0167-5877(97)00031-7.

Dempster, E. R., and I. M. Lerner. 1954. Population size and effectiveness of selection. Evolution 8:291. https://doi.org/10.1111/j.1558-5646.1954.tb01455.x.

Döpfer, D., K. Anklam, D. Mikheil, and P. Ladell. 2012. Growth curves and morphology of three *Treponema* subtypes isolated from digital dermatitis in cattle. Vet. J. 193:685–693. https://doi.org/10.1016/j.tvjl.2012.06.054.

Du, C., T. Deng, Y. Zhou, T. Ye, Z. Zhou, S. Zhang, B. Shao, P. Wei, H. Sun, F. A. Khan, L. Yang, and G. Hua. 2019. Systematic analyses for candidate genes of milk production traits in water buffalo (*Bubalus bubalis*). Anim. Genet. 50:207–216. https://doi.org/10.1111/age.12739.

Duan, X., B. An, L. Du, T. Chang, M. Liang, B. Yang, L. Xu, L. Zhang, J. Li, G. E, and H. Gao. 2021. Genome-wide association analysis of growth curve parameters in Chinese Simmental beef cattle. Animals (Basel) 11:192. https://doi.org/10.3390/ani11010192.

Fahrmeir, L., and G. Tutz. 2001. Multivariate Statistical Modelling Based on Generalized Linear Models. Springer Series in Statistics. Springer.

Fiedler, A. 2014. Rusterholz's sole ulcers are home-made. Accessed Dec. 14, 2021. https://www.milchpraxis.com/rusterholzsches-sohlengeschwuer/.

Finnigan, D. F., W. J. B. Hanna, R. Poma, and A. J. Bendall. 2007. A novel mutation of the CLCN1 gene associated with Myotonia hereditaria in an Australian cattle dog. J. Vet. Intern. Med. 21:458–463. https://doi.org/10.1111/j.1939-1676.2007.tb02990.x.

Gernand, E., S. König, and C. Kipp. 2019. Influence of on-farm measurements for heat stress indicators on dairy cow productivity, female fertility, and health. J. Dairy Sci. 102:6660–6671. https://doi.org/10.3168/jds.2018-16011.

Gernand, E., P. Rehbein, U. U. von Borstel, and S. König. 2012. Incidences of and genetic parameters for mastitis, claw disorders, and common health traits recorded in dairy cattle contract herds. J. Dairy Sci. 95:2144–2156. https://doi.org/10.3168/jds.2011-4812.

Gladue, D. P., V. O'Donnell, R. Baker-Bransetter, J. M. Pacheco, L. G. Holinka, J. Arzt, S. Pauszek, I. Fernandez-Sainz, P. Fletcher, E. Brocchi, Z. Lu, L. L. Rodriguez, and M. V. Borca. 2014. Interaction of foot-and-mouth disease virus nonstructural protein 3A with host protein DCTN3 is important for viral virulence in cattle. J. Virol. 88:2737–2747. https://doi.org/10.1128/JVI.03059-13.

Götze, R. 1952. Praktische Hinweise zur Erkennung der Erbgesundheit und Erbfruchtbarkeit aus dem Erscheinungsbild des Zuchtbullen. Tierarztl. Umsch. 7:466–474.

Halli, K., S. F. Vanvanhossou, M. Bohlouli, S. König, and T. Yin. 2021. Identification of candidate genes on the basis of SNP by time-lagged heat stress interactions for milk production traits in German Holstein cattle. PLoS One 16:e0258216. https://doi.org/10.1371/journal.pone.0258216.

Han, Y., and F. Peñagaricano. 2016. Unravelling the genomic architecture of bull fertility in Holstein cattle. BMC Genet. 17:143. https://doi.org/10.1186/s12863-016-0454-6.

Hardie, L. C., M. J. VandeHaar, R. J. Tempelman, K. A. Weigel, L. E. Armentano, G. R. Wiggans, R. F. Veerkamp, Y. de Haas, M. P. Coffey, E. E. Connor, M. D. Hanigan, C. Staples, Z. Wang, J. C. M. Dekkers, and D. M. Spurlock. 2017. The genetic and biological basis of feed efficiency in mid-lactation Holstein dairy cows. J. Dairy Sci. 100:9061–9075. https://doi.org/10.3168/jds.2017-12604.

Hijmans, R. J., E. Williams, and C. Vennes. 2019. Package 'Geosphere'—Spherical trigonometry. version 1.5–10, 25.05.2019. https://cran.r-project.org/web/packages/geosphere/geosphere.pdf.

Johansson, K., J. Eriksen, U. S. Nielsen, J. Pösö, and G. P. Aamand. 2011. Genetic Evaluation of Claw Health in Denmark, Finland and Sweden. Proceedings of the 2011 Interbull Meeting: Interbull Bulletin No. 44.

Kaiser, U. B., R. A. Katzenellenbogen, P. M. Conn, and W. W. Chin. 1994. Evidence that signalling pathways by which thyrotropin-releasing hormone and gonadotropin-releasing hormone act are both common and distinct. Mol. Endocrinol. 8:1038–1048. https://doi.org/10.1210/mend.8.8.7527898.

Keogh, K., T. R. Carthy, M. C. McClure, S. M. Waters, and D. A. Kenny. 2021. Genome-wide association study of economically important traits in Charolais and Limousin beef cows. Animal 15:100011. https://doi.org/10.1016/j.animal.2020.100011.

Kiser, J. N., E. Clancey, J. G. N. Moraes, J. Dalton, G. W. Burns, T. E. Spencer, and H. L. Neibergs. 2019. Identification of loci associated with conception rate in primiparous Holstein cows. BMC Genomics 20:840. https://doi.org/10.1186/s12864-019-6203-2.

Klein, S. L., C. Scheper, K. May, and S. König. 2020. Genetic and non-genetic profiling of milk β-hydroxybutyrate and acetone and their associations with ketosis in Holstein cows. J. Dairy Sci. 103:10332–10346. https://doi.org/10.3168/jds.2020-18339.

Klitgaard, K., M. W. Nielsen, H. C. Ingerslev, M. Boye, and T. K. Jensen. 2014. Discovery of bovine digital dermatitis-associated *Treponema* spp. in the dairy herd environment by a targeted deep-sequencing approach. Appl. Environ. Microbiol. 80:4427–4432. https://doi.org/10.1128/AEM.00873-14.

Koenig, S., A. R. Sharifi, H. Wentrot, D. Landmann, M. Eise, and H. Simianer. 2005. Genetic parameters of claw and foot disorders estimated with logistic models. J. Dairy Sci. 88:3316–3325. https://doi.org/10.3168/jds.S0022-0302(05)73015-0.

Kopke, G., K. Anklam, M. Kulow, L. Baker, H. H. Swalve, F. B. Lopes, G. J. M. Rosa, and D. Döpfer. 2020. The identification of gene ontologies and candidate genes for digital dermatitis in beef cattle from a genome-wide association study. Int. J. Vet. Sci. Res. 6:027–037.

Kurz, J. P., Z. Yang, R. B. Weiss, D. J. Wilson, K. A. Rood, G. E. Liu, and Z. Wang. 2018. A genome-wide association study for mastitis resistance in phenotypically well-characterized Holstein dairy cattle using a selective genotyping approach. Immunogenetics 71:35–47. https://doi.org/10.1007/s00251-018-1088-9.

Langova, L., I. Novotna, P. Nemcova, M. Machacek, Z. Havlicek, M. Zemanova, and V. Chrast. 2020. Impact of nutrients on the hoof health in cattle. Animals (Basel) 10:1824. https://doi.org/10.3390/ani10101824.

Lee, S. H., N. R. Wray, M. E. Goddard, and P. M. Visscher. 2011. Estimating missing heritability for disease from genome-wide association studies. Am. J. Hum. Genet. 88:294–305. https://doi.org/10.1016/j.ajhg.2011.02.002.

Litzke, L. 2019. The Hoof. Hrsg. 7. Thieme. https://doi.org/10.1055/b-006-161628.

Malchiodi, F., A. Koeck, S. Mason, A. M. Christen, D. F. Kelton, F. S. Schenkel, and F. Miglior. 2017. Genetic parameters for hoof health traits estimated with linear and threshold models using alternative

cohorts. J. Dairy Sci. 100:2828–2836. https://doi.org/10.3168/jds.2016-11558.
McKenzie, G. W., J. Abbott, H. Zhou, Q. Fang, N. Merrick, R. H. Forrest, J. R. Sedcole, and J. G. Hickford. 2010. Genetic diversity of selected genes that are potentially economically important in feral sheep of New Zealand. Genet. Sel. Evol. 42:43. https://doi.org/10.1186/1297-9686-42-43.
Mrode, R. A. 2005. Linear Models for the Prediction of Animal Breeding Values. 2. CABI Publ.
Mülling, C., and J. Hagen. 2012. Significance of claw diseases and functional anatomy of the claw. Prakt. Tierarzt 93:4–10.
Naderi, S., M. Bohlouli, T. Yin, and S. König. 2018. Genomic breeding values, SNP effects and gene identification for disease traits in cow training sets. Anim. Genet. 49:178–192. https://doi.org/10.1111/age.12661.
NRC. 1971. A Guide to Environmental Research on Animals. Natl. Acad. Sci.
Oliveira Junior, G. A., F. S. Schenkel, L. Alcantara, K. Houlahan, C. Lynch, and C. F. Baes. 2021. Estimated genetic parameters for all genetically evaluated traits in Canadian Holsteins. J. Dairy Sci. 104:9002–9015. https://doi.org/10.3168/jds.2021-20227.
Onyiro, O. M., L. J. Andrews, and S. Brotherstone. 2008. Genetic parameters for digital dermatitis and correlations with locomotion, production, fertility traits and longevity in Holstein-Friesian dairy cows. J. Dairy Sci. 91:4037–4046. https://doi.org/10.3168/jds.2008-1190.
Parker Gaddis, K. L., D. J. Null, and J. B. Cole. 2016. Explorations in genome-wide association studies and network analyses with dairy cattle fertility traits. J. Dairy Sci. 99:6420–6435. https://doi.org/10.3168/jds.2015-10444.
Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. W. de Bakker, M. J. Daly, and P. C. Sham. 2007. PLINK: A tool set for whole-genome association and population-based linkage analysis. Am. J. Hum. Genet. 81:559–575. https://doi.org/10.1086/519795.
Read, D. H., and R. L. Walker. 1998. Papillomatous digital dermatitis (footwarts) in California dairy cattle: Clinical and gross pathologic findings. J. Vet. Diagn. Invest. 10:67–76. https://doi.org/10.1177/104063879801000112.
Rensing, S. 2019. Breading health with precision. Milchrind. 1:4–7.
Rodríguez, E. M., A. Arís, and A. Bach. 2017. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. J. Dairy Sci. 100:7427–7434. https://doi.org/10.3168/jds.2016-12210.
Sánchez-Molano, E., V. Bay, R. F. Smith, G. Oikonomou, and G. Banos. 2019. Quantitative trait loci mapping for lameness associated phenotypes in Holstein-Friesian dairy cattle. Front. Genet. 10:926. https://doi.org/10.3389/fgene.2019.00926.
Schöpke, K., A. Gomez, K. A. Dunbar, H. H. Swalve, and D. Döpfer. 2015. Investigating the genetic background of bovine digital dermatitis using improved definitions of clinical status. J. Dairy Sci. 98:8164–8174. https://doi.org/10.3168/jds.2015-9485.
Seabury, C. M., D. L. Oldeschulte, M. Saatchi, J. E. Beever, J. E. Decker, Y. A. Halley, E. K. Bhattarai, M. Molaei, H. C. Freetly, S. L. Hansen, H. Yampara-Iquise, K. A. Johnson, M. S. Kerley, J. Kim, D. D. Loy, E. Marques, H. L. Neibergs, R. D. Schnabel, D. W. Shike, M. L. Spangler, R. L. Weaber, D. J. Garrick, and J. F. Taylor. 2017. Genome-wide association study for feed efficiency and growth traits in U.S. beef cattle. BMC Genomics 18:386. https://doi.org/10.1186/s12864-017-3754-y.
Segelke, D., J. Chen, Z. Liu, F. Reinhardt, G. Thaller, and R. Reents. 2012. Reliability of genomic prediction for German Holsteins using imputed genotypes from low-density chips. J. Dairy Sci. 95:5403–5411. https://doi.org/10.3168/jds.2012-5466.
Shabalina, T., T. Yin, and S. König. 2020. Influence of common health disorders on the length of productive life and stayability in German Holstein cows. J. Dairy Sci. 103:583–596. https://doi.org/10.3168/jds.2019-16985.
Shabalina, T., T. Yin, K. May, and S. König. 2021. Proofs for genotype by environment interactions considering pedigree and genomic data from organic and conventional cow reference populations. J. Dairy Sci. 104:4452–4466. https://doi.org/10.3168/jds.2020-19384.
Shiozaki, A., Y. Nako, D. Ichikawa, H. Konishi, S. Komatsu, T. Kubota, H. Fujiwara, K. Okamoto, M. Kishimoto, Y. Marunaka, and E. Otsuji. 2014. Role of the $Na^+/K^+/2Cl^-$ cotransporter NKCC1 in cell cycle progression in human esophageal squamous cell carcinoma. World J. Gastroenterol. 20:6844–6859. https://doi.org/10.3748/wjg.v20.i22.6844.
Silva, D. B. S., L. F. S. Fonseca, D. G. Pinheiro, A. F. B. Magalhães, M. M. M. Muniz, J. A. Ferro, F. Baldi, L. A. L. Chardulo, R. D. Schnabel, J. F. Taylor, and L. G. Albuquerque. 2020. Spliced genes in muscle from Nelore cattle and their association with carcass and meat quality. Sci. Rep. 10:14701. https://doi.org/10.1038/s41598-020-71783-4.
Solano, L., H. W. Barkema, C. Jacobs, and K. Orsel. 2017. Validation of the M-stage scoring system for digital dermatitis on dairy cows in the milking parlor. J. Dairy Sci. 100:1592–1603. https://doi.org/10.3168/jds.2016-11365.
Solano, L., H. W. Barkema, S. Mason, E. A. Pajor, S. J. LeBlanc, and K. Orsel. 2016. Prevalence and distribution of foot lesions in dairy cattle in Alberta, Canada. J. Dairy Sci. 99:6828–6841. https://doi.org/10.3168/jds.2016-10941.
Stelzer, G., N. Rosen, I. Plaschkes, S. Zimmerman, M. Twik, S. Fishilevich, T. I. Stein, R. Nudel, I. Lieder, Y. Mazor, S. Kaplan, D. Dahary, D. Warshawsky, Y. Guan-Golan, A. Kohn, N. Rappaport, M. Safran, and D. Lancet. 2016. The GeneCards suite: From gene data mining to disease genome sequence analyses. Curr. Protoc. Bioinformatics 54:1.30.1–1.30.33. https://doi.org/10.1002/cpbi.5.
Stock, K. F., J. Cole, J. Pryce, N. Gengler, A. Bradley, L. Andrews, B. Heringstad, and C. Egger-Danner. 2013. Standardization of health data. ICAR guidelines including health key. ICAR Tech. Ser. 17:75–81. http://hdl.handle.net/2268/216992.
Swalve, H. H., C. Floren, M. Wensch-Dorendorf, K. Schöpke, R. Pijl, K. Wimmers, and B. Brenig. 2014. A study based on records taken at time of hoof trimming reveals a strong association between the IQ motif-containing GTPase-activating protein 1 (*IQGAP1*) gene and sole hemorrhage in Holstein cattle. J. Dairy Sci. 97:507–519. https://doi.org/10.3168/jds.2013-6997.
Tarekegn, G. M., P. Gullstrand, E. Strandberg, R. Båge, E. Rius-Vilarrasa, J. M. Christensen, and B. Berglund. 2019. Genetic parameters of endocrine fertility traits based on in-line milk progesterone profiles in Swedish Red and Holstein dairy cows. J. Dairy Sci. 102:11207–11216. https://doi.org/10.3168/jds.2019-16691.
Urao, N., M. Razvi, J. Oshikawa, R. D. McKinney, R. Chavda, W. F. Bahou, T. Fukai, and M. Ushio-Fukai. 2010. IQGAP1 is involved in post-ischemic neovascularization by regulating angiogenesis and macrophage infiltration. PLoS One 5:e13440. https://doi.org/10.1371/journal.pone.0013440.
van der Linde, C., G. de Jong, E. P. C. Koenen, and H. Eding. 2010. Claw health index for Dutch dairy cattle based on claw trimming and conformation data. J. Dairy Sci. 93:4883–4891. https://doi.org/10.3168/jds.2010-3183.
van der Spek, D., J. A. M. van Arendonk, and H. Bovenhuis. 2015. Genome-wide association study for claw disorders and trimming status in dairy cattle. J. Dairy Sci. 98:1286–1295. https://doi.org/10.3168/jds.2014-8302.
van der Spek, D., J. A. M. van Arendonk, A. A. A. Vallée, and H. Bovenhuis. 2013. Genetic parameters for claw disorders and the effect of preselecting cows for trimming. J. Dairy Sci. 96:6070–6078. https://doi.org/10.3168/jds.2013-6833.
van der Waaij, E. H., M. Holzhauer, E. Ellen, C. Kamphuis, and G. de Jong. 2005. Genetic parameters for claw disorders in Dutch dairy cattle and correlations with conformation traits. J. Dairy Sci. 88:3672–3678. https://doi.org/10.3168/jds.S0022-0302(05)73053-8.
Vielmetter, J., X. N. Chen, F. Miskevich, R. P. Lane, K. Yamakawa, J. R. Korenberg, and W. J. Dreyer. 1997. Molecular characterization of human neogenin, a DCC-related protein, and the mapping of its gene (NEO1) to chromosomal position 15q22.3–q23. Genomics 41:414–421. https://doi.org/10.1006/geno.1997.4688.

Weijer, S., C. W. Wieland, S. Florquin, and T. van der Poll. 2005. A thrombomodulin mutation that impairs activated protein C generation results in uncontrolled lung inflammation during murine tuberculosis. Blood 106:2761–2768. https://doi.org/10.1182/blood-2004-12-4623.

Yang, J., B. Benyamin, B. P. McEvoy, S. Gordon, A. K. Henders, D. R. Nyholt, P. A. Madden, A. C. Heath, N. G. Martin, G. W. Montgomery, M. E. Goddard, and P. M. Visscher. 2010. Common SNPs explain a large proportion of the heritability for human height. Nat. Genet. 42:565–569. https://doi.org/10.1038/ng.608.

Yang, J., S. H. Lee, M. E. Goddard, and P. M. Visscher. 2011. GCTA: A tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88:76–82. https://doi.org/10.1016/j.ajhg.2010.11.011.

Yang, J., N. A. Zaitlen, M. E. Goddard, P. M. Visscher, and A. L. Price. 2014. Advantages and pitfalls in the application of mixed-model association methods. Nat. Genet. 46:100–106. https://doi.org/10.1038/ng.2876.

Yates, A. D., P. Achuthan, W. Akanni, J. Allen, J. Allen, J. Alvarez-Jarreta, M. R. Amode, I. M. Armean, A. G. Azov, R. Bennett, J. Bhai, K. Billis, S. Boddu, J. C. Marugán, C. Cummins, C. Davidson, K. Dodiya, R. Fatima, A. Gall, C. G. Giron, L. Gil, T. Grego, L. Haggerty, E. Haskell, T. Hourlier, O. G. Izuogu, S. H. Janacek, T. Juettemann, M. Kay, I. Lavidas, T. Le, D. Lemos, J. G. Martinez, T. Maurel, M. McDowall, A. McMahon, S. Mohanan, B. Moore, M. Nuhn, D. N. Oheh, A. Parker, A. Parton, M. Patricio, M. P. Sakthivel, A. I. Abdul Salam, B. M. Schmitt, H. Schuilenburg, D. Sheppard, M. Sycheva, M. Szuba, K. Taylor, A. Thormann, G. Threadgold, A. Vullo, B. Walts, A. Winterbottom, A. Zadissa, M. Chakiachvili, B. Flint, A. Frankish, S. E. Hunt, G. IIsley, M. Kostadima, N. Langridge, J. E. Loveland, F. J. Martin, J. Morales, J. M. Mudge, M. Muffato, E. Perry, M. Ruffier, S. J. Trevanion, F. Cunningham, K. L. Howe, D. R. Zerbino, and P. Flicek. 2019. Ensembl 2020. Nucleic Acids Res. 48:D682–D688. https://doi.org/10.1093/nar/gkz966.

Zhang, X., H. H. Swalve, R. Pijl, F. Rosner, M. Wensch-Dorendorf, and B. Brenig. 2019. Interdigital hyperplasia in Holstein cattle is associated with a missense mutation in the signal peptide region of the tyrosine-protein kinase transmembrane receptor gene. Front. Genet. 10:1157. https://doi.org/10.3389/fgene.2019.01157.

Zhao, F., S. McParland, F. Kearney, L. Du, and D. P. Berry. 2015. Detection of selection signatures in dairy and beef cattle using high-density genomic information. Genet. Sel. Evol. 47:49. https://doi.org/10.1186/s12711-015-0127-3.

ORCIDS

Niklas Sölzer https://orcid.org/0000-0003-3167-5177
Katharina May https://orcid.org/0000-0001-7809-1385
Tong Yin https://orcid.org/0000-0001-6065-731X
Sven König https://orcid.org/0000-0002-1716-2511

CHAPTER 3

Genetic evaluations and genome-wide association studies for specific digital dermatitis diagnoses in dairy cows considering genotype x housing system interactions

Niklas Sölzer [1], Kerstin Brügemann[1], Tong Yin[1] and Sven König[1]

[1] Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, 35390 Giessen, Germany

Accepted: December 2023

Published: June 2024

Journal of Dairy Science, 107(6), 3724-3737, https://doi.org/10.3168/jds.2023-24207

J. Dairy Sci. 107:3724–3737
https://doi.org/10.3168/jds.2023-24207

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Niklas Sölzer, Kerstin Brügemann, Tong Yin, and Sven König*
Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, 35390 Gießen, Germany

ABSTRACT

The present study aimed to use detailed phenotyping for the claw disorder digital dermatitis (DD) considering specific DD stages in 2 housing systems (conventional cubicle barns [CON] and compost-bedded pack barns [CBPB]) to infer possible genotype × housing system interactions. The DD stages included 2,980 observations for the 3 traits DD-sick, DD-acute, and DD-chronic from 1,311 Holstein-Friesian and 399 Fleckvieh-Simmental cows. Selection of the 5 CBPB and 5 CON herds was based on a specific protocol to achieve a high level of herd similarity with regard to climate, feeding, milking system, and location, but with pronounced housing-system differences. Five other farms had a "mixed system" with 2 subherds, one representing CBPB and the other one CON. The CBPB system was represented by 899 cows (1,530 observations), and 811 cows (1,450 observations) represented the CON system. The average disease prevalence was 20.47% for DD-sick, 13.88% for DD-acute, and 5.34% for DD-chronic, with a higher prevalence in CON than in CBPB. After quality control of 50K genotypes, 38,495 SNPs from 926 cows remained for the ongoing genomic analyses. Genetic parameters for DD-sick, DD-acute, and DD-chronic were estimated by applying single-step approaches for single-trait repeatability animal models considering the whole dataset, and separately for the CON and CBPB subsets. Genetic correlations between same DD traits from different housing systems, and between DD-sick, DD-chronic, and DD-acute, were estimated via bivariate animal models. Heritabilities based on the whole dataset were 0.16 for DD-sick, 0.14 for DD-acute, and 0.11 for DD-chronic. A slight increase of heritabilities and genetic variances was observed in CON compared with the "well-being" CBPB system, indicating a stronger genetic differentiation of diseases in a more challenging environment. Genetic correlations between same DD traits recorded in CON or CBPB were close to 0.80, disproving obvious genotype × housing system interactions. Genetic correlations among DD-sick, DD-acute and DD-chronic ranged from 0.58 to 0.81. SNP main effects and SNP × housing system interaction effects were estimated simultaneously via GWAS, considering only the phenotypes from genotyped cows. Ongoing annotations of potential candidate genes focused on chromosomal segments 100 kb upstream and downstream from the significantly associated candidate SNP. GWAS for main effects indicated heterogeneous Manhattan plots especially for DD-acute and DD-chronic, indicating particularities in disease pathogenesis. Nevertheless, a few shared annotated potential candidate genes, that is, *METTL25*, *AFF3*, *PRKG1*, and *TENM4* for DD-sick and DD-acute, were identified. These genes have direct or indirect effects on disease resistance or immunology. For the SNP × housing system interaction, the annotated genes *ASXL1* and *NOL4L* on BTA 13 were relevant for DD-sick and DD-acute. Overall, the very similar genetic parameters for the same traits in different environments and negligible genotype × housing system interactions indicate only minor effects on genetic evaluations for DD due to housing-system particularities.
Key words: claw health, genetic parameters, genome-wide associations, genotype × housing system interactions

INTRODUCTION

One of the most important claw disorders in dairy cattle with the highest incidence and pronounced effects on farm economy is digital dermatitis (**DD**; Klitgaard et al., 2014; Solano et al., 2016). Digital dermatitis is a multifactorial claw infection (Blowey and Sharp, 1988; Read and Walker, 1994). In addition to the predominant bacterial effects caused by the genus *Treponema* spp., housing conditions, feeding characteristics, and genetics of the host play an important role (Döpfer et al., 2012; Solano et al., 2017). In some countries, official genetic evaluations for DD have been established, and these are mostly based on binary disease diagnoses from producers and

Received September 25, 2023.
Accepted December 6, 2023.
*Corresponding author: sven.koenig@agrar.uni-giessen.de

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

claw trimmers (Rensing, 2019). The pedigree-based binary DD heritabilities were in a small-to-moderate range from 7.3% to 14.2% (König et al., 2005; van der Linde et al., 2010; Schöpke et al., 2015), and from 5% to 36.7% when considering genomic relationship matrixes (Biemans et al., 2019; Shabalina et al., 2020). Enlarging the data pool for genomic predictions and genetic-parameter estimations is possible in so-called single-step approaches, simultaneously considering genomic and pedigree relationships (e.g., Lourenco et al., 2020; Misztal et al., 2020). With regard to functional traits, single-step evaluations have been carried out for milkability (Guarini et al., 2019a), for female fertility (Matilainen et al., 2018; Guarini et al., 2019b), and for diseases (Vukasinovic et al., 2017). Shabalina et al. (2020) compared genetic-parameter estimates for clinical mastitis considering the different matrixes **A**, **G**, and **H**, but results from pure genomic and single-step approaches were quite similar.

In genomic evaluations for claw disorders, mostly producer-recorded disease diagnoses or data from routine claw trimming are used. The advantage of producer-recorded diseases is the use of simplified diagnosis schemes as mostly implemented in herd-management software programs, which contributes to large datasets for genetic evaluations (Zwald et al., 2004). However, for complex diseases and especially for DD with specific acute and chronic stages, disease pathogenesis is insufficiently depicted through simple binary diagnoses (Döpfer et al., 1997). Schöpke et al. (2015) introduced a recording system for different DD stages for quantitative genetic analyses. Such comprehensive DD scoring is associated with additional efforts regarding time and labor and requires veterinarian expertise, but heritabilities were larger compared with a binary DD definition based on producer records (Schöpke et al., 2015). Consequently, in genomic analyses, stronger association signals for molecular markers and annotated potential candidate genes are expected when considering a detailed phenotyping strategy reflecting disease pathogenesis. In GWAS for simple binary DD with subsequent gene annotations, potential candidate genes were detected on chromosomes 3, 6, 9, 11, 12, 19, and 26 (Naderi et al., 2018; Kopke, 2019; Sánchez- Molano et al., 2019; Shabalina et al., 2021). However, most of the significantly associated SNPs only surpassed the less conservative suggestive threshold, but were not significant according to the strict Bonferroni criterion.

In addition to precise phenotyping, detailed modeling of environmental effects might contribute to a deeper understanding of genomic mechanisms. For disease-indicator traits, obvious genotype × environment interactions were detected when modeling the environment through herd gradients reflecting herd size or the hygiene status (Yin and König, 2018). Environmental alterations might contribute to differences in gene expressions, as experimentally shown in heat-stressed mice (Cammack et al., 2009). In dairy cattle genomic studies, Shabalina et al. (2021) identified different significant SNPs for DD in GWAS conducted either in organic or in conventional populations. Sölzer et al. (2022) focused on genotype by climate interactions and annotated different potential candidate genes for DD under heat stress and thermoneutral conditions. In addition to population or climatic effects, specific housing characteristics might contribute to the claw health status and possible genotype × housing interactions for DD. In the context of DD improvements, Lobeck et al. (2011) promoted compost-bedded pack barns (**CBPB**) as a favorable housing alternative. In conventional freestall herds (**CON**), the phenotypic expression of lameness is mostly induced through the concrete floor (Somers et al., 2005; Kester et al., 2014). However, different breeds responded differently in CBPB and CON, pointing to possible genotype × housing system interactions (Leso et al., 2020). In this regard, Wagner et al. (2021) detected specific significant SNPs for udder health due to housing particularities. The annotated potential candidate genes were related to the regulation of the immune system under environmental stress.

Consequently, the aim of the present study was to conduct comprehensive genetic and genomic analyses for novel traits derived from DD scoring including (1) the estimation of genetic parameters in a single-step approach separately for the overall, CON, and CBPB datasets, and (2) GWAS and the annotation of potential candidate genes for main and interaction effects with the discrete housing system considering the phenotypic records from genotyped cows.

MATERIALS AND METHODS

DD Recording

Phenotyping for DD traits considered 2,980 observations of 1,311 lactating Holstein-Friesian (**HF**) and 399 Fleckvieh-Simmental cows. The DD scoring in the years 2021 and 2022 was accomplished according to a validated diagnosis scheme (Döpfer et al., 1997), considering the DD stages M.0 to M.4.1 of the claws from the hind legs. For DD stage diagnosing, the cows were scored by one trained veterinarian. In this regard, the veterinarian saw all cows from the herd at the same recording date. The interval of consecutive herd visits was 3 mo. A specific mirror was used to diagnose the different DD stages as accurately as possible. The single DD stages were grouped into 3 traits: DD-sick (including the stages M.1–M.4.1), DD-acute (including the stages M.1, M.2, or M.3) and DD-chronic (including the stages M.4 or M.4.1). Stage M.0 represents healthy cows without any

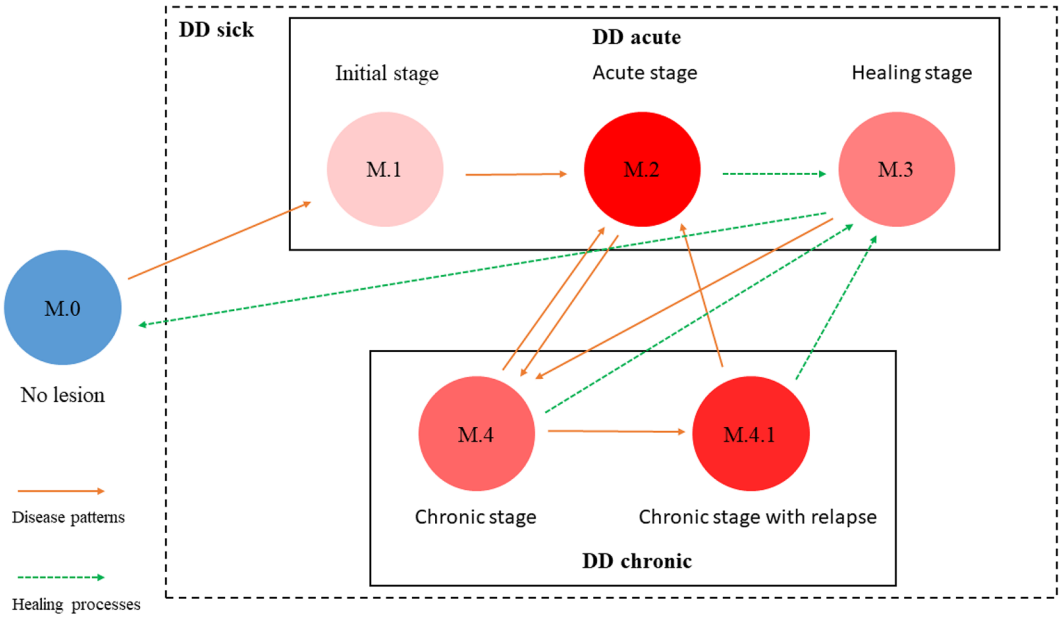


Figure 1. Digital dermatitis (DD) stages according to the diagnosis scheme by Döpfer et al. (1997) and classification into the DD trait categories DD-sick, DD-acute, and DD-chronic Briefly, the DD scoring was accomplished considering the DD stages M.0 to M.4.1 of the claws from the hind legs. For DD stage diagnosing, the cows were scored by one trained veterinarian. A specific mirror was used to diagnose the different DD stages as accurately as possible. The single DD stages were grouped into 3 traits: DD-sick (including the stages M.1–M.4.1), DD-acute (including the stages M.1, M.2, or M.3) and DD-chronic (including the stages M.4 or M.4.1). Stage M.0 represents healthy cows without any lesions.

lesions. Figure 1 illustrates the different DD stages according to Döpfer et al. (1997) and the stage transformations into the aforementioned DD traits. For different observed DD stages at different cow legs, the respective cow was considered to be sick = 1 for, for example, a DD-chronic entry at the right hind leg, and as sick = 1 for, for example, a DD-acute entry at the left hind leg. Hence, all DD stages were analyzed separately considering the binary data structure (no entry for the respective DD stage implied a score = 0 for healthy), and using the cow as the observational unit. The average prevalence (always calculated in relation to the healthy control unit from the same herd visit) was 20.47% for DD-sick, 13.88% for DD-acute, and 5.34% for DD-chronic.

Housing-System Characteristics

The cows were kept in 15 farms located in the German federal states of Bavaria, Hesse, and Rhineland-Palatinate, reflecting the housing system CON or CBPB. The CON farms were typical German cubicle farms with a concrete floor. The herd size per farm ranged from 25 to 840 cows. Lactation numbers of cows ranged from 1 to 14. Five farms had only the CON system, 5 other farms had only the CBPB system, and the remaining 5 farms had both CON and CBPB systems. The farms with both systems had 2 farm buildings, that is, one for the CBPB cows and the other one for the CON cows, which were considered as to 2 separate herds in the ongoing analyses. The CON herds were chosen as control herds for a respective CBPB herd with regard to geographic coordinates, climate, feed ration and feeding system, and herd size according to the guidelines for selecting case (i.e., compost) and control (i.e., cubicle) herds as defined by an expert group from the European Freewalk Consortium (Blanco-Penedo et al., 2020). The prevalences were 10.65% and 26.39% for DD-sick, 7.45% and 17.11% for DD-acute, and 1.49% and 9.03% for DD-chronic, for cows kept in CBPB and CON, respectively.

Genotypes

Genotyping of cows was organized by the breeding organizations in the routine process of national genetic evaluations, and the farmers actively participate in this routine based on specific contracts with the breeding organizations. Hence, no additional animal care approval was needed for this study. A subset of 935 cows was genotyped with the Illumina BovineSNP50 v2 BeadChip (Illumina Inc.). Quality control of the genotype data was performed using the software package PLINK (Purcell et al., 2007). SNPs with a minor allele frequency <1% and a significant deviation from the Hardy-Weinberg equilibrium ($P < 1 \times 10^{-8}$) were discarded. Finally, 38,495 SNPs from 926 cows remained for the genomic studies. The average coefficient for relationships based on SNP data

Table 1. Number of observations, number of cows, and prevalence for the 3 DD traits (sick, acute, and chronic) in 2 housing systems (CBPB and CON)

	All cows		Genotyped cows	
Item	CBPB	CON	CBPB	CON
No. of observations	1,530	1,450	859	883
No. of cows	899	811	441	485
DD-sick	10.65	26.93	11.97	30.79
DD-acute	7.45	17.11	8.55	20.00
DD-chronic	1.43	7.15	1.71	11.97

[1]Trait definitions as explained in Materials and Methods and in Figure 1.

between the HF and Fleckvieh-Simmental cows from Bavaria was 4.24%, indicating the use of Red Holstein bulls in the Simmental population in past decades. Numbers of cows and observations with phenotypes and genotypes in both CON and CBPB production systems are given in Table 1.

Statistical Models

Single-Step Genetic-Parameter Estimations. For the estimation of genetic (co)variance components and breeding values for DD-sick, DD-chronic, and DD-acute via ssGBLUP, the genetic-statistical single-trait repeatability model 1 was defined in matrix notation as follows:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{Wpe} + \mathbf{e}, \quad (1)$$

where $\mathbf{y}$ was a vector that included observations for DD-sick, DD-chronic, or DD-acute; $\boldsymbol{\beta}$ was a vector of fixed effects, including herd, year of diagnosis, season of diagnosis (4 seasons: January to March; April to June; July to September; October to December), parity (5 classes: 1, 2, 3, 4, and ≥5) and breed; $\mathbf{a}$ was a vector of random additive genetic effects, with $\mathbf{a} \sim N\left(\mathbf{0}, \mathbf{H}\sigma_a^2\right)$, where σ_a^2 was the additive genetic variance; $\mathbf{pe}$ was the vector of random permanent environmental effects for repeated measurements across lactations, with $\mathbf{pe} \sim N\left(\mathbf{0}, \mathbf{I}\sigma_{pe}^2\right)$, where σ_{pe}^2 was the permanent environmental variance; $\mathbf{e}$ was the vector of random residual effects with $\mathbf{e} \sim N\left(\mathbf{0}, \mathbf{I}\sigma_e^2\right)$, where σ_e^2 was the residual variance; and $\mathbf{X}$, $\mathbf{Z}$, and $\mathbf{W}$ were the incidence matrixes for fixed, additive genetic, and permanent environmental effects, respectively. The combined inverse of the $\mathbf{H}$ matrix was computed according to Legarra et al. (2009) by considering $\mathbf{G_w} = (0.95 \times \mathbf{G} + 0.05 \times \mathbf{A_{22}})$, where $\mathbf{A_{22}}$ was the submatrix of the pedigree-based relationship matrix for genotyped animals and $\mathbf{G}$ was the genomic relationship matrix (VanRaden, 2008). The pedigree-relationship matrix considered ancestors back to at least 3 generations and comprised 11,385 animals with genetic relationships to the cows with phenotypes. The oldest ancestor in the pedigree dataset was born in 1921. Model 1 was applied to the whole dataset, as well as for the subdatasets CON and CBPB.

Genetic correlations between DD-sick$_{CBPB}$ with DD-sick$_{CON}$, between DD-chronic$_{CBPB}$ with DD-chronic$_{CON}$, and between DD-acute$_{CBPB}$ with DD-acute$_{CON}$ were estimated via bivariate animal models and considering the same DD trait as 2 different traits. The effects were the same as defined for model 1. However, a cow was kept in the same housing system during the recording period, implying not estimable residual covariances and residual correlations. Genetic correlation estimates were compared with breeding value correlations (breeding values from the single-trait analyses) considering phenotyped cows and sires with DD daughter records.

Genetic correlations between DD-sick, DD-chronic, and DD-acute for the whole dataset considering cows from both housing systems were estimated in bivariate repeatability models, considering the same fixed and random effects as described above for model 1. Nevertheless, due to the DD trait definitions, DD-chronic and DD-acute represent a subsample of DD-sick, implying some kind of autocorrelations due to trait dependencies.

All (co)variance components were estimated using the AI-REML algorithm as implemented in the BLUPF90 software package (Misztal et al., 2018).

Genome-Wide Associations for DD Stages with Housing System Interactions. The GWAS considering only the phenotypic records from genotyped cows (see Table 1) were applied to estimate SNP main effects and SNP × housing system-interaction effects for DD-sick, DD-acute and DD-chronic. For the interaction analyses, we considered only one observation per cow and DD trait, that is, the DD record from the most recent lactation. Calculations were performed using our own software package GWAInter.R (Halli et al., 2021) and applying generalized least squares methodology and the algorithm as introduced by Yang et al. (2014). The respective statistical model 2 used to estimate main and interaction SNP effects was:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{x}_{snpi}\,\mathbf{u}_{snpi} + \mathbf{x}_{interi}\mathbf{u}_{interi} + \mathbf{Za} + \mathbf{e}, \quad (2)$$

where $\mathbf{y}$ = a vector of observations for DD-sick, DD-chronic, or DD-acute; $\mathbf{x}_{\mathbf{snpi}}$ = a vector for SNP genotypes; $\mathbf{u}_{\mathbf{snpi}}$ = a vector including SNP main effect; $\mathbf{x}_{\mathbf{interi}}$ = a vector of genotypes for cows in the CBPB system; $\mathbf{u}_{\mathbf{interi}}$ = a vector for the difference in regression coefficients, that is, SNP effects in the CBPB system compared with SNP effects in the CON system (SNP × housing system interaction effect). The notations for fixed and random effects are described in model 1. The modeling approach implied 2 *P*-values for each SNP: one for the significance of the main effect and another one for the significance of the interaction effect. The genome-wide significance level according to Bonferroni (**pBF**) was defined with pBF = 0.05, no. of SNP = 1.3e−06. A normative significance threshold (**pCD**) was used to identify potential candidate SNPs, considering pCD = 1e−04 (Kurz et al., 2019). Annotated potential candidate genes located in 100 kb upstream and downstream from the significantly associated candidate SNP were detected using Ensembl, release 102 (Zerbino et al., 2018).

RESULTS AND DISCUSSION

Genetic Parameters for DD Traits

The estimates for single-step variance components and heritabilities are given in Table 2. The heritabilities for the 3 DD traits were in a narrow range with 0.16 for DD-sick, 0.14 for DD-acute, and 0.11 for DD-chronic. The more precise phenotyping of DD based on veterinarian expertise contributed to larger genetic variances and heritabilities compared with producer health diagnoses as outlined by Schöpke et al. (2015) for pedigree-based genetic relationships. This is in agreement with results for other health or functional traits. For udder health trait genetic analyses, Wagner et al. (2021) emphasized the more precise genetic evaluations when using laboratory measurements including specific mastitis pathogens compared with analyses based on producer-recorded clinical mastitis. With regard to female fertility and classical trait definitions as used in genetic evaluations, Berry et al. (2014) reported small heritabilities ranging from 0.01 to 0.18. Heritabilities were substantially larger, up to 0.33, with genetic studies on the underlying endocrine profiles such as measurements for progesterone (Häggman et al., 2019). However, a more accurate phenotyping strategy is always associated with additional efforts regarding logistics, time, and labor, which hamper the creation of very large datasets.

In addition to the phenotyping strategy or the trait definition, environmental conditions might contribute to genetic-parameter differences between studies. In the present study, heritabilities and variance components for same DD traits were quite similar for the overall dataset, CBPB and CON (Table 2). Nevertheless, additive genetic, permanent environmental, and residual variances for all DD traits were larger in the CON environment. With regard to the comparison of same-variance components between the CON and CBPB systems for the same DD trait, the larger differences for additive genetic variances than for the permanent environmental and residual components contributed to the smallest DD heritabilities in the CBPB data subset. The CBPB production system reflects a modern "well-being environment," with strong focus on cow welfare (Leso et al., 2020). Schierenbeck at al. (2011) reported larger standard deviations for estimated breeding values and daughter yield deviations, indicating a more pronounced genetic differentiation, in "challenging environments" for low heritability health traits. That finding was supported by Schafberg et al. (2006) for detailed recorded mastitis traits including specific major pathogens. In such a context, the lower prevalence of diseases in superior environments, that is, a higher fraction of healthy cows in the CBPB system than in the more challenging CON system, might hamper the identification of genetic differences. Effects of incidences on genetic variations and heritability estimates have been theoretically derived for threshold model applications and human diseases by Dahlqwist et al. (2019). Consequently, using a health dataset from cooperator

Table 2. Heritabilities (h^2) with corresponding SE in brackets, additive genetic variances $\left(\sigma_a^2\right)$, permanent environmental variances $\left(\sigma_{pe}^2\right)$ and residual variances $\left(\sigma_e^2\right)$ for DD-sick, DD-acute, and DD-chronic cows

Trait[1]	Dataset	h^2 (SE)	σ_a^2	σ_{pe}^2	σ_e^2
DD-sick	All	0.16 (0.03)	2.46	0.88	11.76
	CBPB	0.13 (0.06)	1.83	0.70	11.07
	CON	0.18 (0.05)	2.97	0.94	12.02
DD-acute	All	0.14 (0.03)	1.97	0.04	12.07
	CBPB	0.11 (0.06)	1.55	0.03	12.01
	CON	0.15 (0.04)	2.36	0.04	13.12
DD-chronic	All	0.11 (0.01)	0.66	0.01	5.36
	CBPB	0.09 (0.04)	0.52	0.01	5.11
	CON	0.16 (0.04)	1.16	0.04	5.98

[1]Trait definition as explained in Materials and Methods and in Figure 1.

Table 3. Genetic correlations and breeding value correlations (from the cows with DD records) between same DD traits recorded in different environments

Trait combination[1]	Genetic correlation (SE)	Breeding value correlation
DD-sick$_{CBPB}$ with DD-sick$_{CON}$	0.85 (0.15)	0.83
DD-acute$_{CBPB}$ with DD-acute$_{CON}$	0.73 (0.19)	0.68
DD-chronic$_{CBPB}$ with DD-chronic$_{CON}$	0.82 (0.21)	0.77

[1]Trait definition as explained in Materials and Methods and in Figure 1. Subscripts indicate housing environment.

herds reflecting a superior environment, Gernand et al. (2013) focused on only major claw disorders displaying lactation incidences larger than 10%, due to failure in convergence or extremely small genetic variances for rare diseases.

The genetic correlations for the same DD traits from different housing systems were 0.85 between DD-sick$_{CBPB}$ with DD-sick$_{CON}$, 0.73 between DD-acute$_{CBPB}$ with DD-acute$_{CON}$, and 0.82 between DD-chronic$_{CBPB}$ with DD-chronic$_{CON}$ (Table 3). The genetic correlation estimates had quite large standard errors, but the respective breeding value correlations (based on the breeding values from the single-trait analyses) were very similar. The breeding value correlations (Pearson correlation coefficient) between the same DD traits from the different datasets (whole dataset, CBPB, CON) for cows with phenotypic records as well as for 61 sires with more than 5 phenotyped daughters in both systems were in the range from 0.68 (DD-acute CON with DD-acute CBPB for cows) to 0.83 (DD-sick CON with DD-sick whole dataset for sires). Only in the case of a breeding value accuracy of 1 was a correlation between breeding values identical with a genetic correlation (Calo et al., 1973). According to the theoretical derivations made by Robertson (1959), correlations smaller than 0.80 indicate significant genotype × housing interactions. The genetic correlations and breeding value correlations close to 0.80 (see Table 3) indicate only slight rerankings of animals in the different housing systems. Substantially smaller genetic correlations for health traits were reported by Shabalina et al. (2021) when creating datasets according to organic or conventional criteria. Such classification simultaneously includes feeding, housing, and management aspects. The CON–CBPB classification strongly focused on the housing separation, while keeping feeding, milking, and management effects as identical as possible. For different datasets of large-scale CON herds reflecting different feeding systems, Yin and König (2018) estimated very small genetic correlations between same health and health indicator traits.

Considering the overall dataset including cows from both housing systems, the genetic correlations between the different DD traits were 0.81 (DD-sick with DD-acute), 0.93 (DD-sick with DD-chronic) and 0.58 (DD-acute with DD-chronic; Table 4). Hence, consideration of specific DD stages and disease pathogenesis according to veterinary expertise (Döpfer et al., 1997) reflects a differing genetic background for DD-acute and DD-chronic. The genetic correlations between DD-sick with DD-chronic, and between DD-sick with DD-acute, might be biased because of the existing autocorrelations and trait dependencies (DD-acute and DD-chronic are subsets of DD-sick). The phenotypic correlations among the DD traits were slightly smaller than the respective genetic correlations (Table 4).

Genome-Wide Associations and Potential Candidate Genes for DD Traits

The Manhattan plot from the GWAS for SNP main effects of the 3 DD traits is shown in Figure 2, with DD-sick in Figure 2a. The corresponding inflation factor λ was 1.32. We identified 23 significant SNPs for DD-sick associated with 24 potential candidate genes as summarized in Supplemental Table S1. However, only one of these SNPs (on BTA 29) exceeded pBF. This SNP is located in the exon of the gene *TENM4*. Generally, the largest number of significant SNPs according to the more relaxed normative threshold was detected on BTA 29. The annotated potential candidate gene *UMODL1* on BTA 1 is related to immune response and regulation of neutrophil granulocyte migration (Fortin et al., 2019). Neutrophil granulocytes are the category of defense cells responding first to bacterial infections, such as in the case of DD pathogens. In humans, *UMODL1* regulates immune and reproductive mechanisms (Wang et al., 2012) and recently has been identified as a candidate gene for female fertility in Brown Swiss cattle (Manca et al., 2021). The annotated potential candidate gene *NUGGC*

Table 4. Genetic (above diagonal) and phenotypic correlations (below diagonal) among the different DD traits[1]

Trait	DD-sick	DD-acute	DD-chronic
DD-sick		0.81 (0.09)	0.93 (0.08)
DD-acute	0.73 (0.01)		0.58 (0.13)
DD-chronic	0.67 (0.02)	0.55 (0.02)	

[1]Analyses were based on the overall dataset including cows from both housing systems (compost-bedded pack barn or conventional cubicle barn). Trait definitions as explained in Materials and Methods and in Figure 1.

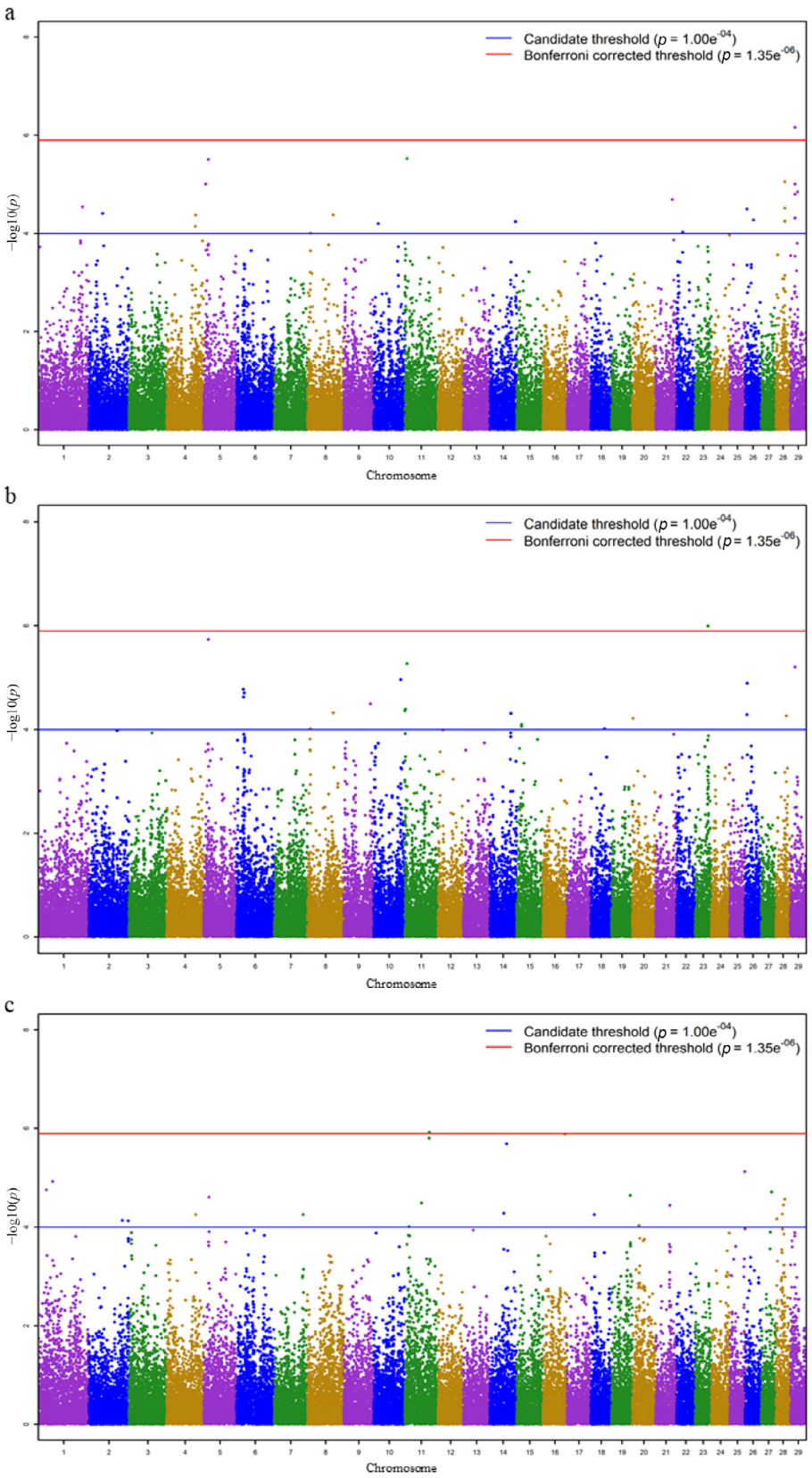


Figure 2. Manhattan plot for −log(10) *P*-values of SNP main effects for DD. (a) DD-sick, (b) DD-acute, and (c) DD-chronic. The DD traits are explained in Materials and Methods and in Figure 1.

on BTA 8 is involved in the cellular response to alterations of lipopolysaccharide levels (e.g., in the formation of the outer cell membrane of gram-negative bacteria; Raetz and Whitfield, 2002). Linking *NUGGC* to DD, gram-negative treponemes represent the main bacterial milieu of a DD infection (Döpfer et al., 2012). Furthermore, *NUGGC* was intensively discussed in the context of foot-and-mouth disease infections, with overexpression during humoral immune response (Eschbaumer et al., 2016). The 2 potential candidate genes *CCDC88C* and *PPP4R3A* on BTA 21 were associated with metabolic cow diseases in early lactation (Klein et al., 2021). Metabolic diseases contribute to stress and impair the immune defense mechanisms, in causality implying an increased susceptibility to claw infections (Schöpke et al., 2015).

The Manhattan plot from the GWAS for DD-acute GWAS is presented in Figure 2b. The corresponding inflation factor λ was 1.31. We identified 21 significant SNPs associated with 24 potential candidate genes as summarized in Supplemental Table S1. One of these SNPs on BTA 23 exceeded pBF. The largest number of significant SNPs according to the normative threshold was detected on BTA 6 and BAT 11. The potential candidate gene *NRXN3* on BTA 10 affected neuronal receptors, specifically the differentiation of synapses (Reissner et al., 2013). Functions of *NRXN3* mainly addressed behavioral disorders and temperament abnormalities in mice (Brown et al., 2011) and in Brahman cattle (Paredes-Sánchez et al., 2020). In dairy cattle genomic health studies, Klein et al. (2020) and Hayirli (2006) associated *NRXN3* with metabolic disorders. The potential candidate gene *UBR5* on BTA 14 is involved in the ubiquitination of proteins, but was deregulated in case of severe disease stressors, especially due to some cancers (Shearer et al., 2015). van der Spek et al. (2015) related genotypes of *UBR5* to the claw disease interdigital hyperplasia in Holstein cows. Strong genetic correlations between interdigital hyperplasia and DD were estimated in pedigree-based analyses (König et al., 2005; Gernand et al., 2012), supporting the results from genomic studies and the identified overlapping genomic mechanisms (Sölzer et al., 2022). The annotated potential candidate gene *DOCK2* on BTA 20 for DD-acute is directly involved in immune defense mechanisms. A mutation in this gene implied immunodeficiency in humans, due to impaired functions of T cells and defects of B and NK cells (Dobbs et al., 2015).

The Manhattan plot for the SNP main effects of DD-chronic GWAS is presented in Figure 2c. The corresponding inflation factor λ was 1.39. We identified 25 significant SNPs associated with 33 potential candidate genes as summarized in Supplemental Table S1. Two of these SNPs exceeded pBF, one located on BTA 11 and the other one located on BTA 16. The largest number of significant SNPs according to pCD was detected on BTA 11 and 28. The significant SNP ARS-BRGL-NGS-23211 on BTA 11 is located in a segment of the gene *WDR54*, as well as in close chromosomal proximity to 16 further potential candidate genes. All of these genes, that is, *AUP1*, *TLX2*, *PCGF1*, *LBX2*, *CCDC142*, *MRPL53*, *WBP1*, *RTKN*, *WDR54*, *C11H2orf81*, *MGC152281*, *ENSBTAG000050345*, and *DCTN1*, were associated with multifocal paratuberculosis in cattle (Canive et al., 2021). Paratuberculosis is caused by *Mycobacterium avium* ssp. *paratuberculosis*, and very similar to DD, the phenotypic expression of this multifactorial disease is strongly related to immune system deficiencies (Canive et al., 2021). The annotated potential candidate gene *TACR2* on BTA 28 is involved in inflammatory processes and immune defense mechanisms, and was described in the context of substance P release in pigs (Jakimiuk et al., 2017). Substance P causes vasodilation and an increase in vascular permeability and is responsible for the chemotaxis of leukocytes (Harrison and Geppetti, 2001).

Some overlapping association signals were found when comparing the Manhattan plots for DD-sick and DD-acute, and for DD-sick and DD-chronic. For DD-sick and DD-acute, the annotated potential candidate gene *METTL25* on BTA 5 is involved in DNA methylation (de Greef et al., 2023). Another shared potential candidate gene, *AFF3* on BTA 11, was discussed in the context of expressions in B cells and oncogenesis (Shi et al., 2018), and associated with abortion in Holstein heifers (Oliver et al., 2019). The shared potential candidate gene *PRKG1* on BTA 26 contributes to the cyclic guanosine monophosphate signaling pathway (Hofmann et al., 2009), and was associated with milk fatty acid contents in Chinese Holstein populations (Shi et al., 2019). A further shared potential candidate gene for DD-sick and DD-acute is *TENM4* on BTA 29. *TENM4* is a marker for myoblast quiescence (Pietrosemoli et al., 2017) and was discussed in the context of zilpaterol application in cattle (Reith et al., 2022). Zilpaterol is a β agonist with effects on muscle mass accumulation in farm animals. However, zilpaterol application under heat-stress conditions contributed to a downregulation of *TENM4* expression (Reith et al., 2022), indicating an environmental effect on gene activities.

A shared potential candidate gene for DD-sick and DD-chronic is *MCU* on BTA 28. The protein encoded by *MCU* interacts with mitochondrial calcium uptake (Tarasov et al., 2012). Calcium in turn plays an important role in milk production (Breves et al., 2016), as well as in keratinization and mature horn cell formation of the claw (Langova et al., 2020). Rodríguez et al. (2017) associ-

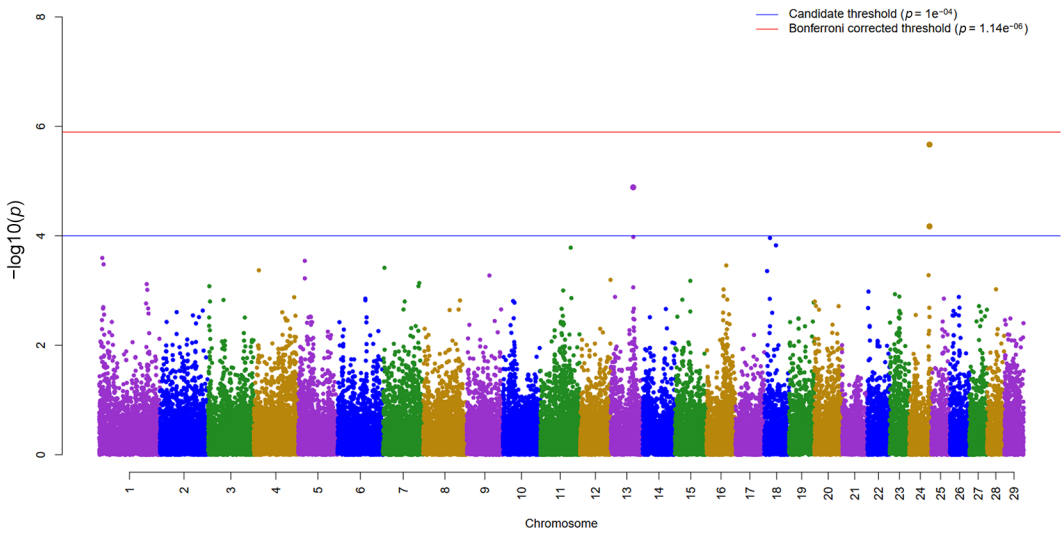


Figure 3. Manhattan plot for −log(10) *P*-values of SNP interaction effects with the housing system for DD-sick. The DD traits are explained in Materials and Methods and in Figure 1. Briefly, the DD scoring was accomplished according to a validated diagnosis scheme (Döpfer et al., 1997), considering the DD stages M.0 to M.4.1 of the claws from the hind legs. For DD stage diagnosing, the cows were scored by one trained veterinarian. A specific mirror was used to diagnose the different DD stages as accurately as possible. The single DD stages were grouped into 3 traits: DD-sick (including the stages M.1–M.4.1), DD-acute (including the stages M.1, M.2, or M.3) and DD-chronic (including the stages M.4 or M.4.1). Stage M.0 represents healthy cows without any lesions.

ated increased blood calcium losses at the beginning of lactation and hypocalcemia of high-yielding cows with several diseases, including claw disorders. The potential candidate gene *ITGA11* for DD-sick and DD-chronic on BTA 10 is involved in integrin-mediated cell adhesion and cell migration (Zhang et al., 2002) and was associated with DD in beef cattle (Kopke et al., 2020).

Genome-Wide Associations for SNP Interaction Effects with Housing-System Effects

The Manhattan plots for the SNP interaction effects with the housing system are displayed in Figure 3 (DD-sick), Figure 4 (DD-acute), and Figure 5 (DD-chronic). As indicated above, a significant interaction means that the

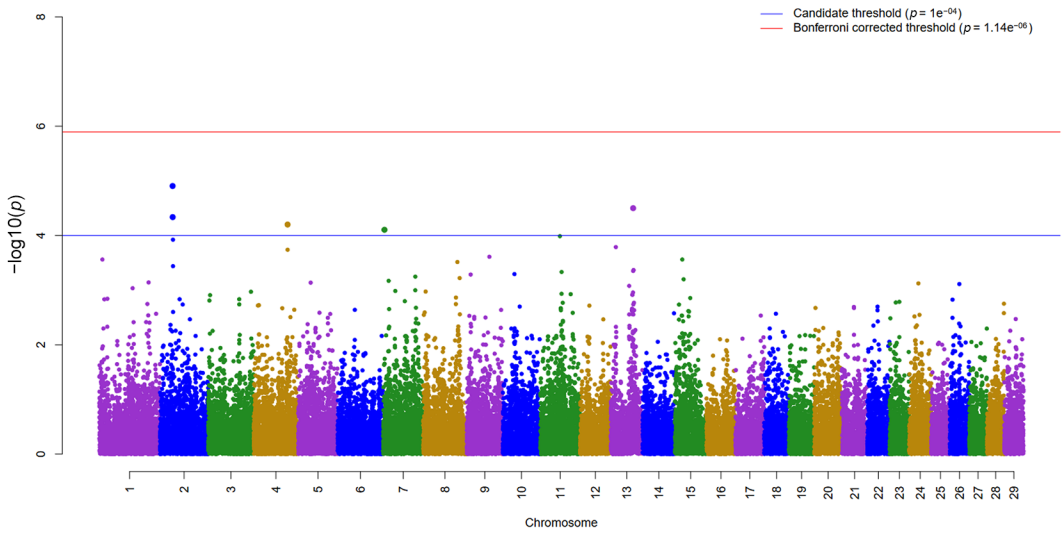


Figure 4. Manhattan plot for −log(10) *P*-values of SNP interaction effects with the housing system for DD-acute. The DD traits are explained in Materials and Methods and in Figure 1. Briefly, the DD scoring was accomplished according to a validated diagnosis scheme (Döpfer et al., 1997), considering the DD stages M.0 to M.4.1 of the claws from the hind legs. For DD stage diagnosing, the cows were scored by one trained veterinarian. A specific mirror was used to diagnose the different DD stages as accurately as possible. The single DD stages were grouped into 3 traits: DD-sick (including the stages M.1–M.4.1), DD-acute (including the stages M.1, M.2, or M.3) and DD-chronic (including the stages M.4 or M.4.1). Stage M.0 represents healthy cows without any lesions.

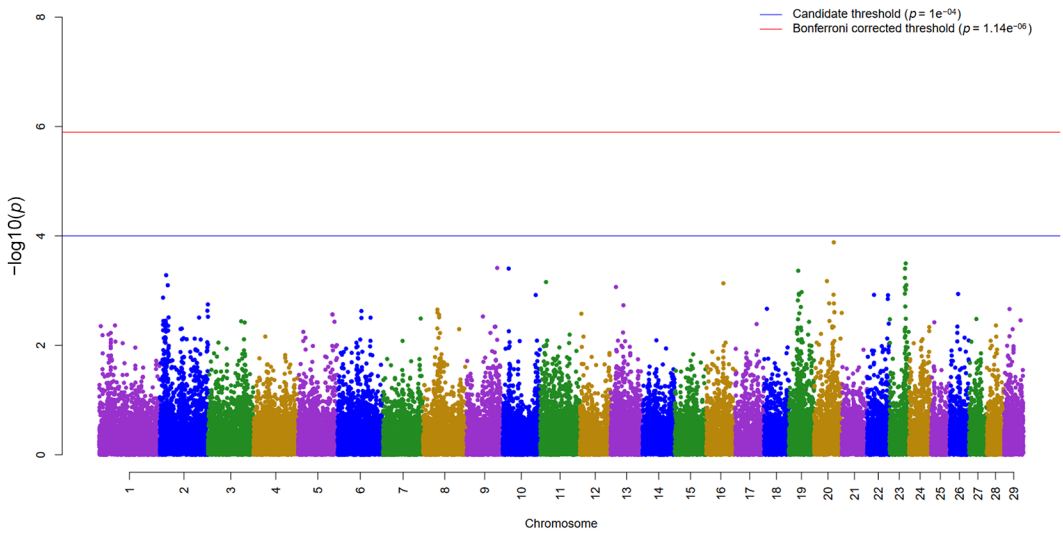


Figure 5. Manhattan plot for −log(10) *P*-values of SNP interaction effects with the housing system for DD-chronic. The DD traits are explained in Materials and Methods and in Figure 1. Briefly, the DD scoring was accomplished according to a validated diagnosis scheme (Döpfer et al., 1997), considering the DD stages M.0 to M.4.1 of the claws from the hind legs. For DD stage diagnosing, the cows were scored by one trained veterinarian. A specific mirror was used to diagnose the different DD stages as accurately as possible. The single DD stages were grouped into 3 traits: DD-sick (including the stages M.1–M.4.1), DD-acute (including the stages M.1, M.2, or M.3) and DD-chronic (including the stages M.4 or M.4.1). Stage M.0 represents healthy cows without any lesions.

respective SNP is relevant for a housing system CBPB, but not for CON, or vice versa. The corresponding inflation factor λ was 1.17 for DD-sick, 1.2 for DD-acute, and 1.47 for DD-chronic. The annotated potential candidate genes for interaction effects are listed in Table 5. For DD-sick and DD-acute, 3 and 5 SNPs, respectively, exceeded the candidate threshold, but no SNP was significantly associated with DD-chronic. For DD-sick and DD-acute, the same SNP on BTA 13 was significant for the interaction component. This SNP is located close to the genes *ASXL1* and *NOL4L*. Both genes were associated with the occurrence of cancer (Stein et al., 2011; Lin et al., 2021). Naderi et al. (2020) reported significant effects of *ASXL1* and *NOL4L* on binary DD when evaluating high-yielding HF

Table 5. Significantly associated SNP markers and annotated potential candidate genes for the interaction effect with the housing system

Trait[1]	BTA	SNP-ID	SNP position (bp)	*P*-value	Gene[2]	Gene position (bp)[1]
DD-sick	13	rs110282784	61,954,306	1.29×10^{-5}	*ASXL1*	61,807,148–61,871,197
	13	rs110282784	61,954,306	1.29×10^{-5}	*NOL4L*	61,874,277–61,955,381
	24	rs110415388	57,151,899	2.15×10^{-6}	—	—
	24	rs41643954	57,185,546	6.70×10^{-5}	—	—
DD-acute	2	rs110334343	30,980,589	1.24×10^{-5}	*SCN2A*	30,880,623–31,019,443
	2	rs43707331	31,096,836	4.61×10^{-5}	*SCN2A*	30,880,623–31,019,443
	2	rs43707331	31,096,836	4.61×10^{-5}	*ENSBTAG00000050020*	31,096,287–31,096,586
	2	rs43707331	31,096,836	4.61×10^{-5}	*SCN3A*	31,103,231–31,183,473
	4	rs109248092	92,474,426	6.30×10^{-5}	*LEP*	92,436,922–92,453,653
	4	rs109248092	92,474,426	6.30×10^{-5}	*RBM28*	92,499,373–92,531,680
	4	rs109248092	92,474,426	6.30×10^{-5}	*PRRT4*	92,537,251–92,545,269
	4	rs109248092	92,474,426	6.30×10^{-5}	*ENSBTAG00000031866*	92,565,558–92,569,356
	4	rs109248092	92,474,426	6.30×10^{-5}	*IMPDH1*	92,569,423–92,586,808
	7	rs43496217	3,079,094	7.87×10^{-5}	*GJC2*	2,972,742–2,982,335
	7	rs43496217	3,079,094	7.87×10^{-5}	*GUK1*	2,983,133–2,993,613
	7	rs43496217	3,079,094	7.87×10^{-5}	*MRPL55*	2,993,262–2,997,182
	7	rs43496217	3,079,094	7.87×10^{-5}	*C7H1orf35*	2,996,902–3,000,276
	7	rs43496217	3,079,094	7.87×10^{-5}	*ARF1*	3,001,505–3,019,569
	7	rs43496217	3,079,094	7.87×10^{-5}	*WNT3A*	3,035,810–3,087,361
	7	rs43496217	3,079,094	7.87×10^{-5}	*WNT9A*	3,155,441–3,163,239
	13	rs110282784	61,954,306	3.16×10^{-5}	*ASXL1*	61,807,148–61,871,197
	13	rs110282784	61,954,306	3.16×10^{-5}	*NOL4L*	61,874,277–61,955,381

[1]Trait definitions as explained in Materials and Methods and in Figure 1.

[2]No gene name or no gene position: SNP was not located in the gene or within a window size of 100 kb up- and downstream.

cows from CON systems. However, they could not confirm their findings in a subsample of black and white dual-purpose cattle (founder breed of modern HF), which were mostly kept in grassland systems. Hence, effects of the breed or of the system, or both, contributed to SNP significances. Additionally, *NOL4L* was suggested as potential candidate gene for udder health (Wolf et al., 2021). More than 95% of all somatic cells are leucocytes, and levels of leucocytes have been suggested as indicator for bacterial infections of the udder (Bradley and Green, 2005). Consequently, Gernand et al. (2012) postulated a close genetic relationship between SCS and bacterial claw infections, including DD, but the genetic correlation between DD and SCS was close to zero. For DD-acute, 2 significant SNPs for the interaction component are located on BTA 2 close to the genes *SCN2A* and *SCN3A*. Both genes encode proteins that are involved in the construction of voltage-gated sodium channels, playing an important role in the transmission of action potentials in neurons. Mutations in the *SCN2A* and *SCN3A* genes were associated with epilepsy in humans (Holland et al., 2008). Another significant SNP for the interaction effect of DD-acute on BTA 4 is located in close chromosomal distance to the genes *LEP*, *RBM24*, and *IMPDH1*. The *LEP* gene is involved in many cellular signal pathways in the organism, including the regulation of the innate and adaptive immune system. For example, *LEP* promotes the activation of neutrophil granulocytes (Francisco et al., 2018). Neutrophil granulocytes, in turn, are involved in initial defense mechanisms against bacteria through phagocytosis and exocytosis (Guo and Ward, 2005), explaining the effects on DD. In dairy cattle, Shabalina et al. (2020) reported significant effects of *LEP* and of *RBM28* on the length of productive life for cows kept in conventional dairy farms, but they could not verify their results in organic environments (Shabalina et al., 2021). The annotated potential candidate gene *IMPDH1* was involved in immunological signal pathways (Jonsson and Carlsten, 2002; Malvisi et al., 2016). In heat-stressed Angus cattle, the expression of *IMPDH1* was downregulated under simultaneous zilpaterol application, as previously indicated for *TENM4* (Reith et al., 2022). The significant SNP for the interaction component of DD-acute is located on BTA 7. One annotated potential candidate gene is *MRPL55*, which is involved in protein biosynthesis in the mitochondrion and encodes a protein of the 39 S-subunit (Mai et al., 2017). *MRPL55* was associated with early abortions in mice (Cheong et al., 2020). In Brown Swiss cattle, *MRPL55* was suggested as a potential candidate gene for female fertility, with the largest effects on the interval between first and last insemination (Häfliger et al., 2021). The identified potential candidate gene for the DD-acute interaction effect is located on BTA 7, and was associated with milk yield traits in Chinese Holstein cows (Lu et al., 2022).

CONCLUSIONS

Heritabilities and additive genetic variances were slightly larger for same DD traits in the CON than in the "well-being" CBPB system, indicating a stronger genetic differentiation of diseases in a more challenging conventional environment. However, genetic correlations between the same DD traits in the different systems (CON and CBPB) were close to 0.80, disproving possible genotype × housing interactions. The genetic correlation between DD-acute and DD-chronic was 0.58, indicating a partly different genetic background for acute and chronic diseases. The GWAS for main SNP effects indicated heterogeneous Manhattan plots especially for DD-acute and DD-chronic, supporting the differences or particularities in disease pathogenesis. Nevertheless, we identified a few shared annotated potential candidate genes, *METTL25*, *AFF3*, *PRKG1*, and *TENM4* for DD-sick and DD-acute, which mostly have been reported in the context of immunology. For SNP × housing system interactions, only a few significant SNPs were detected.

NOTES

The authors acknowledge funding of this study by the German Ministry for Agriculture and Food (Bonn, Germany) in the framework of the EU-Eranet project "Climate Care Cattle Farming Systems" (CCCfarming), grant no. 2819ERA09A. We thank the H. Wilhelm Schaumann Foundation (Hamburg, Germany) for providing a scholarship to Niklas Sölzer. Supplemental material for this article is available at http://dx.doi.org/10.22029/jlupub-18177. Genotyping of cows was organized by the breeding organizations in the routine process of national genetic evaluations, and the farmers actively participate in this routine based on specific contracts with the breeding organizations. Hence, no additional animal care approval was needed for this study. The authors have not stated any conflicts of interest.

Abbreviations used: CBPB = compost-bedded pack barns; CON = conventional cubicle barns; DD = digital dermatitis; HF = Holstein-Friesian; pBF = significance level according to Bonferroni; pCD = significance threshold.

REFERENCES

Berry, D. P., E. Wall, and J. E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8:105–121. https://doi.org/10.1017/S1751731114000743.

Biemans, F., M. C. M. de Jong, and P. Bijma. 2019. A genome-wide association study for susceptibility and infectivity of Holstein Friesian dairy cattle to digital dermatitis. J. Dairy Sci. 102:6248–6262. https://doi.org/10.3168/jds.2018-15876.

Blanco-Penedo, I., W. Ouweltjes, E. Ofner-Schrök, K. Brügemann, and U. Emanuelson. 2020. Animal welfare in free walk systems in Europe. J. Dairy Sci. 103:5773–5782. https://doi.org/10.3168/jds.2019-17315.

Blowey, R. W., and M. W. Sharp. 1988. Digital dermatitis in dairy cattle. Vet. Rec. 122:505–508. https://doi.org/10.1136/vr.122.21.505.

Bradley, A., and M. Green. 2005. Use and interpretation of somatic cell count data in dairy cows. In Pract. 27:310–315. https://doi.org/10.1136/inpract.27.6.310.

Breves, G., K. Elfers, and B. Schröder. 2016. Laktation, Milchbildung, Nährstoffflüsse und Regulation. 26. Hülsenberger Gespräche, Hamburg, Germany.

Brown, S. M., S. J. Clapcote, J. K. Millar, H. S. Torrance, S. M. Anderson, R. Walker, A. Rampino, J. C. Roder, P. A. Thomson, D. J. Porteous, and K. L. Evans. 2011. Synaptic modulators *Nrxn1* and *Nrxn3* are disregulated in a Disc1 mouse model of schizophrenia. Mol. Psychiatry 16:585–587. https://doi.org/10.1038/mp.2010.134.

Calo, L. L., R. E. McDowell, L. D. VanVleck, and P. D. Miller. 1973. Genetic aspects of beef production among Holstein-Friesians pedigree selected for milk production. J. Anim. Sci. 37:676–682. https://doi.org/10.2527/jas1973.373676x.

Cammack, K. M., E. Antoniou, L. Hearne, and W. R. Lamberson. 2009. Testicular gene expression in male mice divergent for fertility after heat stress. Theriogenology 71:651–661. https://doi.org/10.1016/j.theriogenology.2008.09.029.

Canive, M., O. González-Recio, A. Fernández, P. Vázquez, G. Badia-Bringué, J. L. Lavin, J. M. Garrido, R. A. Juste, and M. Alonso-Hearn. 2021. Identification of loci associated with susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* infection in Holstein cattle using combinations of diagnostic tests and imputed whole-genome sequence data. PLoS One 16:e0256091. https://doi.org/10.1371/journal.pone.0256091.

Cheong, A., R. Lingutla, and J. Mager. 2020. Expression analysis of mammalian mitochondrial ribosomal protein genes. Gene Expr. Patterns 38:119147. https://doi.org/10.1016/j.gep.2020.119147.

Dahlqwist, E., P. K. E. Magnusson, Y. Pawitan, and A. Sjölander. 2019. On the relationship between the heritability and the attributable fraction. Hum. Genet. 138:425–435. https://doi.org/10.1007/s00439-019-02006-8.

de Greef, E., A. Suh, M. J. Thorstensen, K. E. Delmore, and K. C. Fraser. 2023. Genomic architecture of migration timing in a long-distance migratory songbird. Sci. Rep. 13:2437. https://doi.org/10.1038/s41598-023-29470-7.

Dobbs, K., C. Dominguez Conde, S. Zhang, S. Parolini, M. Audry, J. Chou, E. Haapaniemi, S. Keles, I. Bilic, S. Okada, M. J. Massaad, S. Rounioja, A. M. Alwahadneh, N. K. Serwas, K. Capuder, E. Çiftçi, K. Felgentreff, T. K. Ohsumi, V. Pedergnana, B. Boisson, Ş. Haskoloğlu, A. Ensari, M. Schuster, A. Moretta, Y. Itan, O. Patrizi, F. Rozenberg, P. Lebon, J. Saarela, M. Knip, S. Petrovski, D. B. Goldstein, R. E. Parrott, B. Savas, A. Schambach, G. Tabellini, C. Bock, T. A. Chatila, A. M. Comeau, R. S. Geha, L. Abel, R. H. Buckley, A. İkincioğulları, W. Al-Herz, M. Helminen, F. Doğu, J.-L. Casanova, K. Boztuğ, and L. D. Notarangelo. 2015. Inherited DOCK2 deficiency in patients with early-onset invasive infections. N. Engl. J. Med. 372:2409–2422. https://doi.org/10.1056/NEJMoa1413462.

Döpfer, D., K. Anklam, D. Mikheil, and P. Ladell. 2012. Growth curves and morphology of three *Treponema* subtypes isolated from digital dermatitis in cattle. Vet. J. 193:685–693. https://doi.org/10.1016/j.tvjl.2012.06.054.

Döpfer, D., A. Koopmanns, F. A. Meijer, I. Szakáll, Y. H. Schukken, W. Klee, R. B. Bosma, J. L. Cornelisse, A. J. van Asten, and A. A. ter Huurne. 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and *Campylobacter faecalis*. Vet. Rec. 140:620–623. https://doi.org/10.1136/vr.140.24.620.

Eschbaumer, M., C. Stenfeldt, G. R. Smoliga, J. M. Pacheco, L. L. Rodriguez, R. W. Li, J. Zhu, and J. Arzt. 2016. Transcriptomic analysis of persistent infection with foot-and-mouth disease virus in cattle suggests impairment of apoptosis and cell-mediated immunity in the nasopharynx. PLoS One 11:e0162750. https://doi.org/10.1371/journal.pone.0162750.

Fortin, C., A. Leader, N. Mahutte, S. Hamilton, M. C. Leveille, M. Villeneuve, and M. A. Sirard. 2019. Gene expression analysis of follicular cells revealed inflammation as a potential IVF failure cause. J. Assist. Reprod. Genet. 36:1195–1210. https://doi.org/10.1007/s10815-019-01447-4.

Francisco, V., J. Pino, V. Campos-Cabaleiro, C. Ruiz-Fernández, A. Mera, M. A. Gonzalez-Gay, R. Gómez, and O. Gualillo. 2018. Obesity, fat mass and immune system: Role for leptin. Front. Physiol. 9:640. https://doi.org/10.3389/fphys.2018.00640.

Gernand, E., D. A. Döhne, and S. König. 2013. Genetic background of claw disorders in the course of lactation and their relationships with type traits. J. Anim. Breed. Genet. 130:435–444. https://doi.org/10.1111/jbg.12046.

Gernand, E., P. Rehbein, U. U. von Borstel, and S. König. 2012. Incidences of and genetic parameters for mastitis, claw disorders, and common health traits recorded in dairy cattle contract herds. J. Dairy Sci. 95:2144–2156. https://doi.org/10.3168/jds.2011-4812.

Guarini, A. R., D. A. L. Lourenco, L. F. Brito, M. Sargolzaei, C. F. Baes, F. Miglior, I. Misztal, and F. S. Schenkel. 2019b. Genetics and genomics of reproductive disorders in Canadian Holstein cattle. J. Dairy Sci. 102:1341–1353. https://doi.org/10.3168/jds.2018-15038.

Guarini, A. R., D. A. L. Lourenco, L. F. Brito, M. Sargolzaei, C. F. Baes, F. Miglior, S. Tsuruta, I. Misztal, and F. S. Schenkel. 2019a. Use of a single-step approach for integrating foreign information into national genomic evaluation in Holstein cattle. J. Dairy Sci. 102:8175–8183. https://doi.org/10.3168/jds.2018-15819.

Guo, R. F., and P. A. Ward. 2005. Role of C5A in inflammatory responses. Annu. Rev. Immunol. 23:821–852. https://doi.org/10.1146/annurev.immunol.23.021704.115835.

Häfliger, I. M., F. R. Seefried, M. Spengeler, and C. Drögemüller. 2021. Mining massive genomic data of two Swiss Braunvieh cattle populations reveals six novel candidate variants that impair reproductive success. Genet. Sel. Evol. 53:95. https://doi.org/10.1186/s12711-021-00686-3.

Häggman, J., J. Christensen, E. Mäntysaari, and J. Juga. 2019. Genetic parameters for endocrine and traditional fertility traits, hyperketonemia and milk yield in dairy cattle. Animal 13:248–255. https://doi.org/10.1017/S1751731118001386.

Halli, K., S. F. Vanvanhossou, M. Bohlouli, S. König, and T. Yin. 2021. Identification of candidate genes on the basis of SNP by time-lagged heat stress interactions for milk production traits in German Holstein cattle. PLoS One 16:e0258216. https://doi.org/10.1371/journal.pone.0258216.

Harrison, S., and P. Geppetti. 2001. Substance P. Int. J. Biochem. Cell Biol. 33:555–576. https://doi.org/10.1016/S1357-2725(01)00031-0.

Hayirli, A. 2006. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Vet. Res. Commun. 30:749–774. https://doi.org/10.1007/s11259-006-3320-6.

Hofmann, F., D. Bernhard, R. Lukowski, and P. Weinmeister. 2009. cGMP regulated protein kinases (cGK). Handb. Exp. Pharmacol. 191:137–162. https://doi.org/10.1007/978-3-540-68964-5_8.

Holland, K. D., J. A. Kearney, T. A. Glauser, G. Buck, M. Keddache, J. R. Blankston, I. W. Glaaser, R. S. Kass, and M. H. Meisler. 2008. Mutation of sodium channel SCN3A in a patient with cryptogenic pediatric partial epilepsy. Neurosci. Lett. 433:65–70. https://doi.org/10.1016/j.neulet.2007.12.064.

Jakimiuk, A., P. Podlasz, M. Chmielewska-Krzesinska, and K. Wasowicz. 2017. Characterisation, localisation and expression of porcine *TACR1*, *TACR2* and *TACR3* genes. Vet. Med. (Praha) 62:443–455. https://doi.org/10.17221/23/2017-VETMED.

Jonsson, C. A., and H. Carlsten. 2002. Mycophenolic acid inhibits inosine 50-monophosphate dehydrogenase and suppresses production of pro-inflammatory cytokines, nitric oxide, and LDH in macrophages. Cell. Immunol. 216:93–101. https://doi.org/10.1016/S0008-8749(02)00502-6.

Kester, E., M. Holzhauer, and K. Frankena. 2014. A descriptive review of the prevalence and risk factors of hock lesions in dairy cows. Vet. J. 202:222–228. https://doi.org/10.1016/j.tvjl.2014.07.004.

Klein, S., C. Scheper, K. May, and S. König. 2020. Genetic and non-genetic profiling of milk β-hydroxybutyrate and acetone and their

associations with ketosis in Holstein cows. J. Dairy Sci. 103:10332–10346. https://doi.org/10.3168/jds.2020-18339.
Klein, S.-L., T. Yin, H. H. Swalve, and S. König. 2021. Single-step genomic best linear unbiased predictor genetic parameter estimations and genome-wide associations for milk fatty acid profiles, interval from calving to first insemination, and ketosis in Holstein cattle. J. Dairy Sci. 104:10921–10933. https://doi.org/10.3168/jds.2021-20416.
Klitgaard, K., M. W. Nielsen, H. C. Ingerslev, M. Boye, and T. K. Jensen. 2014. Discovery of bovine digital dermatitis-associated *Treponema* spp. in the dairy herd environment by a targeted deep-sequencing approach. Appl. Environ. Microbiol. 80:4427–4432. https://doi.org/10.1128/AEM.00873-14.
König, S., A. R. Sharifi, H. Wentrot, D. Landmann, M. Eise, and H. Simianer. 2005. Genetic parameters of claw and foot disorders estimated with logistic models. J. Dairy Sci. 88:3316–3325. https://doi.org/10.3168/jds.S0022-0302(05)73015-0.
Kopke, G. 2019. Genomic and genetic-statistical analysis on susceptibility to digital dermatitis in Holstein cattle. DMV thesis. Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany. https://d-nb.info/1250342848/34.
Kopke, G., K. Anklam, M. Kulow, L. Baker, H. H. Swalve, F. B. Lopes, G. J. M. Rosa, and D. Döpfer. 2020. The identification of gene ontologies and candidate genes for digital dermatitis in beef cattle from a genome-wide association study. Int. J. Vet. Sci. Res. 6:027–037 https://doi.org/10.17352/ijvsr.000050.
Kurz, J. P., Z. Yang, R. B. Weiss, D. J. Wilson, K. A. Rood, G. E. Liu, and Z. Wang. 2019. A genome-wide association study for mastitis resistance in phenotypically well-characterized Holstein dairy cattle using a selective genotyping approach. Immunogenetics 71:35–47. https://doi.org/10.1007/s00251-018-1088-9.
Langova, L., I. Novotna, P. Nemcova, M. Machacek, Z. Havlicek, M. Zemanova, and V. Chrast. 2020. Impact of nutrients on the hoof health in cattle. Animals (Basel) 10:1824. https://doi.org/10.3390/ani10101824.
Legarra, A., I. Aguilar, and I. Misztal. 2009. A relationship matrix including full pedigree and genomic information. J. Dairy Sci. 92:4656–4663. https://doi.org/10.3168/jds.2009-2061.
Leso, L., M. Barbari, M. A. Lopes, F. A. Damasceno, P. Galama, J. L. Taraba, and A. Kuipers. 2020. Invited review: Compost-bedded pack barns for dairy cows. J. Dairy Sci. 103:1072–1099. https://doi.org/10.3168/jds.2019-16864.
Lin, F., J. Zhou, X. Li, and X. Wang. 2021. NOL4L, a novel nuclear protein, promotes cell proliferation and metastasis by enhancing the PI3K/AKT pathway in ovarian cancer. Biochem. Biophys. Res. Commun. 559:121–128. https://doi.org/10.1016/j.bbrc.2021.04.055.
Lobeck, K. M., M. I. Endres, E. M. Shane, S. M. Godden, and J. Fetrow. 2011. Animal welfare in cross-ventilated, compost-bedded pack, and naturally ventilated dairy barns in the upper Midwest. J. Dairy Sci. 94:5469–5479. https://doi.org/10.3168/jds.2011-4363.
Lourenco, D., A. Legarra, S. Tsuruta, Y. Masuda, I. Aguilar, and I. Misztal. 2020. Single-step genomic evaluations from theory to practice: using SNP chips and sequence data in BLUPF90. Genes (Basel) 11:790. https://doi.org/10.3390/genes11070790.
Lu, X., A. A. I. Arbab, I. M. Abdalla, D. Liu, Z. Zhang, T. Xu, G. Su, and Z. Yang. 2022. Genetic parameter estimation and genome-wide association study-based loci identification of milk-related traits in Chinese Holstein. Front. Genet. 12:799664. https://doi.org/10.3389/fgene.2021.799664.
Mai, N., Z. M. A. Chrzanowska-Lightowlers, and R. N. Lightowlers. 2017. The process of mammalian mitochondrial protein synthesis. Cell Tissue Res. 367:5–20. https://doi.org/10.1007/s00441-016-2456-0.
Malvisi, M., F. Palazzo, N. Morandi, B. Lazzari, J. L. Williams, G. Pagnacco, and G. Minozzi. 2016. Responses of bovine innate immunity to *Mycobacterium avium* subsp. *paratuberculosis* infection revealed by changes in gene expression and levels of microRNA. PLoS One 11:e0164461. https://doi.org/10.1371/journal.pone.0164461.
Manca, E., A. Cesarani, L. Falchi, A. S. Atzori, G. Gaspa, A. Rossoni, N. P. P. Macciotta, and C. Dimauro. 2021. Genome-wide association study for residual concentrate intake using different approaches in Italian Brown Swiss. Ital. J. Anim. Sci. 20:1957–1967. https://doi.org/10.1080/1828051X.2021.1963864.
Matilainen, K., I. Strandén, G. P. Aamand, and E. A. Mäntysaari. 2018. Single step genomic evaluation for female fertility in Nordic Red dairy cattle. J. Anim. Breed. Genet. 135:337–348. https://doi.org/10.1111/jbg.12353.
Misztal, I., D. Lourenco, and A. Legarra. 2020. Current status of genomic evaluation. J. Anim. Sci. 98:skaa101. https://doi.org/10.1093/jas/skaa101.
Misztal, I., S. Tsuruta, D. A. L. Lourenco, Y. Masuda, I. Aguilar, A. Legarra, and Z. Vitezica. 2018. Manual for BLUPF90 family programs. Accessed Feb. 22, 2021. University of Georgia, Athens, GA. http://nce.ads.uga.edu/wiki/doku.php?id=documentation.
Naderi, S., M. Bohlouli, T. Yin, and S. König. 2018. Genomic breeding values, SNP effects and gene identification for disease traits in cow training sets. Anim. Genet. 49:178–192. https://doi.org/10.1111/age.12661.
Naderi, S., M. H. Moradi, M. Farhadian, T. Yin, M. Jaeger, C. Scheper, P. Korkuc, G. A. Brockmann, S. König, and K. May. 2020. Assessing selection signatures within and between selected lines of dual-purpose black and white and German Holstein cattle. Anim. Genet. 51:391–408. https://doi.org/10.1111/age.12925.
Oliver, K. F., A. M. Wahl, M. Dick, J. A. Toenges, J. N. Kiser, J. M. Galliou, J. G. N. Moraes, G. W. Burns, J. Dalton, T. E. Spencer, and H. L. Neibergs. 2019. Genomic analysis of spontaneous abortion in Holstein heifers and primiparous cows. Genes (Basel) 10:954. https://doi.org/10.3390/genes10120954.
Paredes-Sánchez, F. A., A. M. Sifuentes-Rincón, E. Casas, W. Arellano-Vera, G. M. Parra-Bracamonte, D. G. Riley, T. H. Welsh, and R. D. Randel. 2020. Novel genes involved in the genetic architecture of temperament in Brahman cattle. PLoS One 15:e0237825. https://doi.org/10.1371/journal.pone.0237825.
Pietrosemoli, N., S. Mella, S. Yennek, M. B. Baghdadi, H. Sakai, R. Sambasivan, F. Pala, D. Di Girolamo, and S. Tajbakhsh. 2017. Comparison of multiple transcriptomes exposes unified and divergent features of quiescent and activated skeletal muscle stem cells. Skelet. Muscle 7:28. https://doi.org/10.1186/s13395-017-0144-8.
Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. W. de Bakker, M. J. Daly, and P. C. Sham. 2007. PLINK: A tool set for whole-genome association and population-based linkage analysis. Am. J. Hum. Genet. 81:559–575. https://doi.org/10.1086/519795.
Raetz, C. R. H., and C. Whitfield. 2002. Lipopolysaccharide endotoxins. Annu. Rev. Biochem. 71:635–700. https://doi.org/10.1146/annurev.biochem.71.110601.135414.
Read, D. H., and R. L. Walker. 1994. Papillomatous digital dermatitis of dairy cattle: Pathologic findings. Pages 156–158 in Proc. 8th Int. Sym. Dis. of Rum. Digit. Banff, Canada.
Reissner, C., F. Runkel, and M. Missler. 2013. Neurexins. Genome Biol. 14:213. https://doi.org/10.1186/gb-2013-14-9-213.
Reith, R. R., R. L. Sieck, P. C. Grijalva, R. M. Swanson, A. M. Fuller, D. E. Diaz, T. B. Schmidt, D. T. Yates, and J. L. Petersen. 2022. Transcriptome analyses indicate that heat stress-induced inflammation in white adipose tissue and oxidative stress in skeletal muscle is partially moderated by zilpaterol supplementation in beef cattle. J. Anim. Sci. 100:skac019. https://doi.org/10.1093/jas/skac019.
Rensing, S. 2019. Breeding health with precision. Milchrind. 1:4–7.
Robertson, A. 1959. The sampling variance of the genetic correlation coefficient. Biometrics 15:469–485. https://doi.org/10.2307/2527750.
Rodríguez, E. M., A. Arís, and A. Bach. 2017. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. J. Dairy Sci. 100:7427–7434. https://doi.org/10.3168/jds.2016-12210.
Sánchez-Molano, E., V. Bay, R. F. Smith, G. Oikonomou, and G. Banos. 2019. Quantitative trait loci mapping for lameness associated phenotypes in Holstein-Friesian dairy cattle. Front. Genet. 10:926. https://doi.org/10.3389/fgene.2019.00926.
Schafberg, R., F. Rosner, and H. H. Swalve. 2006. Examinations on intramammary infections in dairy cows based on pathogen-specific data. Article 15-13 in 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, MG, Brazil.

Schierenbeck, S., F. Reinhardt, R. Reents, H. Simianer, and S. König. 2011. Identification of informative cooperator herds for progeny testing based on yield deviations. J. Dairy Sci. 94:2071–2082. https://doi.org/10.3168/jds.2010-3466.

Schierenbeck, S., F. Reinhardt, R. Reents, H. Simianer, and S. König. 2011. Identification of informative cooperator herds for progeny testing based on yield deviations. J. Dairy Sci. 94:6143–6152. https://doi.org/10.3168/jds.2011-4574.

Schöpke, K., A. Gomez, K. A. Dunbar, H. H. Swalve, and D. Döpfer. 2015. Investigating the genetic background of bovine digital dermatitis using improved definitions of clinical status. J. Dairy Sci. 98:8164–8174. https://doi.org/10.3168/jds.2015-9485.

Shabalina, T., T. Yin, and S. König. 2020. Influence of common health disorders on the length of productive life and stayability in German Holstein cows. J. Dairy Sci. 103:583–596. https://doi.org/10.3168/jds.2019-16985.

Shabalina, T., T. Yin, K. May, and S. König. 2021. Proofs for genotype by environment interactions considering pedigree and genomic data from organic and conventional cow reference populations. J. Dairy Sci. 104:4452–4466. https://doi.org/10.3168/jds.2020-19384.

Shearer, R. F., M. Iconomou, C. K. W. Watts, and D. N. Saunders. 2015. Functional roles of the E3 ubiquitin ligase UBR5 in cancer. Mol. Cancer Res. 13:1523–1532. https://doi.org/10.1158/1541-7786.MCR-15-0383.

Shi, L., X. Lv, L. Liu, Y. Yang, Z. Ma, B. Han, and D. Sun. 2019. A post-GWAS confirming effects of *PRKG1* gene on milk fatty acids in a Chinese Holstein dairy population. BMC Genet. 20:53. https://doi.org/10.1186/s12863-019-0755-7.

Shi, Y., Y. Zhao, Y. Zhang, N. J. AiErken, N. Shao, R. Ye, Y. Lin, and S. Wang. 2018. *AFF3* upregulation mediates tamoxifen resistance in breast cancers. J. Exp. Clin. Cancer Res. 37:254. https://doi.org/10.1186/s13046-018-0928-7.

Solano, L., H. W. Barkema, C. Jacobs, and K. Orsel. 2017. Validation of the M-stage scoring system for digital dermatitis on dairy cows in the milking parlor. J. Dairy Sci. 100:1592–1603. https://doi.org/10.3168/jds.2016-11365.

Solano, L., H. W. Barkema, S. Mason, E. A. Pajor, S. J. LeBlanc, and K. Orsel. 2016. Prevalence and distribution of foot lesions in dairy cattle in Alberta, Canada. J. Dairy Sci. 99:6828–6841. https://doi.org/10.3168/jds.2016-10941.

Sölzer, N., K. May, T. Yin, and S. König. 2022. Genomic analyses of claw disorders in Holstein cows: Genetic parameters, trait associations, and genome-wide associations considering interactions of SNP and heat stress. J. Dairy Sci. 105:8218–8236. https://doi.org/10.3168/jds.2022-22087.

Somers, J. G. C. J., K. Frankena, E. N. Noordhuizen-Stassen, and J. H. M. Metz. 2005. Risk factors for digital dermatitis in dairy cows kept in cubicle houses in The Netherlands. Prev. Vet. Med. 71:11–21. https://doi.org/10.1016/j.prevetmed.2005.05.002.

Stein, B. L., D. M. Williams, C. O'Keefe, O. Rogers, R. G. Ingersoll, J. L. Spivak, A. Verma, J. P. Maciejewski, M. A. McDevitt, and A. R. Moliterno. 2011. Disruption of the *ASXL1* gene is frequent in primary, post-essential thrombocytosis and post-polycythemia vera myelofibrosis, but not essential thrombocytosis or polycythemia vera: Analysis of molecular genetics and clinical phenotypes. Haematologica 96:1462–1469. https://doi.org/10.3324/haematol.2011.045591.

Tarasov, A. I., F. Semplici, M. A. Ravier, E. A. Bellomo, T. J. Pullen, P. Gilon, I. Sekler, R. Rizzuto, and G. A. Rutter. 2012. The mitochondrial Ca2+ uniporter MCU is essential for glucose-induced ATP increases in pancreatic β-cells. PLoS One 7:e39722. https://doi.org/10.1371/journal.pone.0039722.

van der Linde, C., G. de Jong, E. P. C. Koenen, and H. Eding. 2010. Claw health index for Dutch dairy cattle based on claw trimming and conformation data. J. Dairy Sci. 93:4883–4891. https://doi.org/10.3168/jds.2010-3183.

van der Spek, D., J. A. M. van Arendonk, and H. Bovenhuis. 2015. Genome-wide association study for claw disorders and trimming status in dairy cattle. J. Dairy Sci. 98:1286–1295. https://doi.org/10.3168/jds.2014-8302.

VanRaden, P. M. 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91:4414–4423. https://doi.org/10.3168/jds.2007-0980.

Vukasinovic, N., N. Bacciu, C. A. Przybyla, P. Boddhireddy, and S. K. DeNise. 2017. Development of genetic and genomic evaluation for wellness traits in US Holstein cows. J. Dairy Sci. 100:428–438. https://doi.org/10.3168/jds.2016-11520.

Wagner, P., T. Yin, K. Brügemann, P. Engel, C. Weimann, K. Schlez, and S. König. 2021. Genome-wide associations for microscopic differential somatic cell count and specific mastitis pathogens in Holstein cows in compost-bedded pack and cubicle farming systems. Animals (Basel) 11:1839. https://doi.org/10.3390/ani11061839.

Wang, W., Y. Tang, L. Ni, E. Kim, T. Jongwutiwes, A. Hourvitz, R. Zhang, H. Xiong, H. C. Liu, and Z. Rosenwaks. 2012. Overexpression of uromodulin-like1 accelerates follicle depletion and subsequent ovarian degeneration. Cell Death Dis. 3:e433. https://doi.org/10.1038/cddis.2012.169.

Wolf, M. J., T. Yin, G. B. Neumann, P. Korkuć, G. A. Brockmann, S. König, and K. May. 2021. Genome-wide association study using whole-genome sequence data for fertility, health indicator, and endoparasite infection traits in German black pied cattle. Genes (Basel) 12:1163. https://doi.org/10.3390/genes12081163.

Yang, J., N. A. Zaitlen, M. E. Goddard, P. M. Visscher, and A. L. Price. 2014. Advantages and pitfalls in the application of mixed-model association methods. Nat. Genet. 46:100–106. https://doi.org/10.1038/ng.2876.

Yin, T., and S. König. 2018. Heritabilities and genetic correlations in the same traits across different strata of herds created according to continuous genomic, genetic, and phenotypic descriptors. J. Dairy Sci. 101:2171–2186. https://doi.org/10.3168/jds.2017-13575.

Zerbino, D. R., P. Achuthan, W. Akanni, M. R. Amode, D. Barrell, J. Bhai, K. Billis, C. Cummins, A. Gall, C. G. Girón, L. Gil, L. Gordon, L. Haggerty, E. Haskell, T. Hourlier, O. G. Izuogu, S. H. Janacek, T. Juettemann, J. K. To, M. R. Laird, I. Lavidas, Z. Liu, J. E. Loveland, T. Maurel, W. McLaren, B. Moore, J. Mudge, D. N. Murphy, V. Newman, M. Nuhn, D. Ogeh, C. K. Ong, A. Parker, M. Patricio, H. S. Riat, H. Schuilenburg, D. Sheppard, H. Sparrow, K. Taylor, A. Thormann, A. Vullo, B. Walts, A. Zadissa, A. Frankish, S. E. Hunt, M. Kostadima, N. Langridge, F. J. Martin, M. Muffato, E. Perry, M. Ruffier, D. M. Staines, S. J. Trevanion, B. L. Aken, F. Cunningham, A. Yates, and P. Flicek. 2018. Ensembl 2018. Nucleic Acids Res. 46:D754–D761. https://doi.org/10.1093/nar/gkx1098.

Zhang, W.-M., S. N. Popova, C. Bergman, T. Velling, M. Kusch Gullberg, and D. Gullberg. 2002. Analysis of the human integrin α11 gene (ITGA11) and its promoter. Matrix Biol. 21:513–523. https://doi.org/10.1016/S0945-053X(02)00054-9.

Zwald, N. R., K. A. Weigel, Y. M. Chang, R. D. Welper, and J. S. Clay. 2004. Genetic selection for health traits using producer-recorded data. I. Incidence rates, heritability estimates, and sire breeding values. J. Dairy Sci. 87:4287–4294. https://doi.org/10.3168/jds.S0022-0302(04)73573-0.

Supplemental Table S1. Significantly associated SNP markers and annotated potential candidate genes for the main effects for DD-sick (**S1-A**), for DD-acute (**S1-B**) and for DD-chronic (**S1-C**). The DD traits are explained in the materials and methods and in Figure 1.

S1-A: Trait DD-sick

BTA	SNP-ID	SNP position (bp)	*P*-value	Gene[1]	Gene position (bp)[1]
1	rs43278831	142,486,334	2.86×10^{-5}	*UMODL1*	142,327,757 – 142,403,210
	rs43278831	142,486,334	2.86×10^{-5}	*ABCG1*	142,488,705 – 142,561,127
	rs43278831	142,486,334	2.86×10^{-5}	*TFF3*	142,565,466 – 142,569,246
2	rs41755558	42,129,770	3.90×10^{-5}	*GALNT13*	41,676,135 – 42,259,624
4	rs41654218	93,338,554	7.21×10^{-5}	*AHCYL2*	93,142,664 – 93,333,822
	rs41654218	93,338,554	7.21×10^{-5}	*STRIP2*	93,338,888 – 93,401,401
	rs41590634	94,699,823	4.26×10^{-5}	-	-
5	rs109115428	5,361,321	9.88×10^{-6}	-	-
	rs110162221	12,104,374	3.10×10^{-6}	*METTL25*	11,908,969 – 12,015,429
8	rs110174079	10,759,225	9.87×10^{-5}	*ELP3*	10,593,713 – 10,712,508
	rs110174079	10,759,225	9.87×10^{-5}	*NUGGC*	10,720,463 – 10,767,022
	rs110174079	10,759,225	9.87×10^{-5}	*SCARA5*	10,796,505 – 10,929,733
	rs41570498	82,271,544	4.20×10^{-5}	-	-
10	rs109925557	15,077,217	6.29×10^{-5}	*PIAS1*	14,905,205 – 15,030,499
	rs109925557	15,077,217	6.29×10^{-5}	*CALM4*	15,035,521 – 15,044,913
	rs109925557	15,077,217	6.29×10^{-5}	*CLN6*	15,051,437 – 15,067,688
	rs109925557	15,077,217	6.29×10^{-5}	*FEM1B*	15,105,565 – 15,119,491
	rs109925557	15,077,217	6.29×10^{-5}	*ITGA11*	15,129,322 – 15,262,276
11	-	4,996,418	2.99×10^{-6}	*AFF3*	4,665,771 – 5,289,303
14	rs110080711	78,454,550	5.79×10^{-5}	-	-
21	rs41584200	56,195,714	2.04×10^{-5}	*CCDC88C*	56,040,106 – 56,183,841
	rs41584200	56,195,714	2.04×10^{-5}	*PPP4R3A*	56,210,637 – 56,245,304

22	rs41613366	19,452,386	9.48×10^{-5}	*GRM7*	18,673,820 – 19,567,204
26	rs110166523	8,101,467	3.20×10^{-5}	*PRKG1*	6,899,619 – 8,313,722
26	rs110358694	28,763,335	5.31×10^{-5}	-	-
28	rs109412394	28,725,060	5.68×10^{-5}	*MCU*	28,709,027 – 28,902,834
	rs29011010	28,794,085	8.83×10^{-6}	*MCU*	28,709,027 – 28,902,834
	rs29011010	28,794,085	8.83×10^{-6}	*ENSBTAG00000054039*	28,879,757 – 28,880,758
	rs109467698	28,923,986	3.07×10^{-5}	*MCU*	28,709,027 – 28,902,834
	rs109467698	28,923,986	3.07×10^{-5}	*ENSBTAG00000054039*	28,879,757 – 28,880,758
	rs109467698	28,923,986	3.07×10^{-5}	*OIT3*	28,915,123 – 28,942,806
	rs109467698	28,923,986	3.07×10^{-5}	*PLA2G12B*	28,944,315 – 28,963,077
29	rs110485277	16,816,497	4.87×10^{-5}	*TENM4*	16,813,276 – 17,402,612
	rs41649476	16,837,556	1.61×10^{-5}	*TENM4*	16,813,276 – 17,402,612
	rs109486457	16,996,267	6.90×10^{-7}	*TENM4*	16,813,276 – 17,402,612
	rs111027650	17,037,783	9.89×10^{-5}	*TENM4*	16,813,276 – 17,402,612
	rs41584940	25,443,243	1.44×10^{-5}	-	-

[1]No gene name or no gene position: SNP was not located in the gene or within a window size of 100 kb up- and downstream.

S1-B: Trait DD-acute

BTA	SNP-ID	SNP position (bp)	*P*-value	Gene[1]	Gene position (bp)[1]
5	rs110162221	12,104,374	1.85×10^{-6}	*METTL25*	11,908,969 – 12,015,429
6	rs108990454	20,399,602	1.68×10^{-5}	-	-
	rs43464456	21,186,796	2.37×10^{-5}	-	-
	rs110082224	23,297,539	1.98×10^{-5}	-	-
8	rs29020862	10,109,654	9.70×10^{-5}	*EXTL3*	9,980,042 – 10,017,423
	rs29020862	10,109,654	9.70×10^{-5}	*FZD3*	10,150,817 – 10,238,564
	rs41570498	82,271,544	4.77×10^{-5}	-	-
9	rs109322240	94,740,322	3.23×10^{-5}	*SYNJ2*	94,544,473 – 94,653,549
	rs109322240	94,740,322	3.23×10^{-5}	*SERAC1*	94,662,572 – 94,714,692
	rs109322240	94,740,322	3.23×10^{-5}	*GTF2H5*	94,714,760 – 94,729,301
	rs109322240	94,740,322	3.23×10^{-5}	*TULP4*	94,820,216 – 94,960,850
10	rs41657367	90,874,981	1.10×10^{-5}	*NRXN3*	90,495,258 – 91,099,930
11	rs111007356	265,663	4.37×10^{-5}	*FBLN7*	245,530 – 303,203
	rs111007356	265,663	4.37×10^{-5}	*ZC3H6*	144,638 – 189,991
	rs111007356	265,663	4.37×10^{-5}	*ZC3H8*	203,490 – 230,821
	rs111007356	265,663	4.37×10^{-5}	*TMEM87B*	319,215 – 361,708
	rs43654724	1,861,245	4.10×10^{-5}	*NPHP1*	1,697,163 – 1,762,645
	rs43654724	1,861,245	4.10×10^{-5}	*MALL*	1,773,930 – 1,807,073
	rs43654724	1,861,245	4.10×10^{-5}	*MAL*	1,848,632 – 1,873,328
	rs43654724	1,861,245	4.10×10^{-5}	*ENSBTAG00000034657*	1,913,421 – 1,918,226
	rs43654724	1,861,245	4.10×10^{-5}	*MRPS5*	1,922,410 – 1,945,833
	-	4,996,418	5.41×10^{-6}	*AFF3*	4,665,771 – 5,289,303
14	rs41627975	61,969,914	4.92×10^{-5}	*UBR5*	61,987,835 – 62,126,918
15	rs41633845	20,745,006	8.68×10^{-5}	*ARHGAP20*	20,598,185 – 20,812,735
	rs41633846	20,775,708	8.04×10^{-5}	*ARHGAP20*	20,598,185 – 20,812,735
18	rs41886115	42,508,672	9.64×10^{-5}	*ENSBTAG00000003856*	42,597,926 – 42,599,478
20	rs110030994	1,829,447	6.09×10^{-5}	*DOCK2*	1,562,432 – 2,001,666
	rs110030994	1,829,447	6.09×10^{-5}	*INSYN2B*	1,768,224 – 1,792,301
23	rs41667526	44,517,863	1.01×10^{-6}	*ADTRP*	44,610,190 – 44,677,675

S1-C: DD-chronic

BTA	SNP-ID	SNP position (bp)	*P*-value	Gene[1]	Gene position (bp)[1]
1	rs42408102	28,434,475	1.76×10^{-5}	-	-
	rs43670957	46,322,462	1.18×10^{-5}	-	-
2	rs109136926	117,962,757	7.36×10^{-5}	*TRIP12*	117,811,397 – 117,918,447
	rs109136926	117,962,757	7.36×10^{-5}	*FBXO36*	117,962,254 – 118,069,148
	rs43314636	134,714,711	7.45×10^{-5}	-	-
4	rs43413106	94,623,036	5.55×10^{-5}	*KLF14*	94,523,445 – 94,524,428
5	rs41649678	15,293,572	2.46×10^{-5}	-	-
7	rs41658549	95,785,453	5.53×10^{-5}	*PCSK1*	95,746,303 – 95,790,112
11	rs109559885	10,211,360	9.86×10^{-5}	*AUP1*	10,110,333 – 10,114,465
	rs109559885	10,211,360	9.86×10^{-5}	*DQX1*	10,114,072 – 10,120,751
	rs109559885	10,211,360	9.86×10^{-5}	*TLX2*	10,122,252 – 10,124,335
	rs109559885	10,211,360	9.86×10^{-5}	*PCGF1*	10,130,551 – 10,132,842
	rs109559885	10,211,360	9.86×10^{-5}	*LBX2*	10,138,281 – 10,140,220
	rs109559885	10,211,360	9.86×10^{-5}	*CCDC142*	10,151,984 – 10,158,175
	rs109559885	10,211,360	9.86×10^{-5}	*MRPL53*	10,159,213 – 10,162,298
	rs109559885	10,211,360	9.86×10^{-5}	*MOGS*	10,164,061 – 10,167,845
	rs109559885	10,211,360	9.86×10^{-5}	*WBP1*	10,168,029 – 10,170,419
	rs109559885	10,211,360	9.86×10^{-5}	*INO80B*	10,170,214 – 10,173,993
	rs109559885	10,211,360	9.86×10^{-5}	*RTKN*	10,187,533 – 10,202,365
	rs109559885	10,211,360	9.86×10^{-5}	*WDR54*	10,201,853 – 10,205,764
	rs109559885	10,211,360	9.86×10^{-5}	*C11H2orf81*	10,205,805 – 10,211,683
	rs109559885	10,211,360	9.86×10^{-5}	*MGC152281*	10,211,644 – 10,218,786
	rs109559885	10,211,360	9.86×10^{-5}	*ENSBTAG00000050345*	10,219,762 – 10,221,111
	rs109559885	10,211,360	9.86×10^{-5}	*DCTN1*	10,238,630 – 10,269,100
	rs109559885	10,211,360	9.86×10^{-5}	*ENSBTAG00000010072*	10,306,886 – 10,307,580
	rs110074079	48,555,061	3.26×10^{-5}	*REEP1*	48,518,871 – 48,654,804
	rs110074079	48,555,061	3.26×10^{-5}	*ENSBTAG00000054778*	48,639,480 – 48,676,952
	rs29023108	76,425,215	1.58×10^{-6}	-	-
	rs41567966	77,091,873	1.19×10^{-6}	-	-

14	rs42509128	39,032,005	5.23×10^{-5}	-	-
	rs43040593	49,344,976	$2.04 \times 10^{-.6}$	-	-
16	rs108956332	74,432,920	1.30×10^{-6}	-	-
18	rs109262661	10,201,072	5.56×10^{-5}	*CDH13*	9,350,020 – 10,154,230
	rs109262661	10,201,072	5.56×10^{-5}	*HSBP1*	10,162,416 – 10,166,868
	rs109262661	10,201,072	5.56×10^{-5}	*MLYCD*	10,240,324 – 10,254,080
	rs109262661	10,201,072	5.56×10^{-5}	*OSGIN1*	10,285,040 – 10,339,375
19	rs41927485	57,412,632	2.29×10^{-5}	-	-
20	-	18,571,733	9.29×10^{-5}	*ELOVL7*	18,454,411 – 18,531,445
	-	18,571,733	9.29×10^{-5}	*ENSBTAG00000049955*	18,482,424 – 18,482,765
	-	18,571,733	9.29×10^{-5}	*DEPDC1B*	18,560,198 – 18,649,542
	rs41613300	18,626,250	9.29×10^{-5}	*ELOVL7*	18,454,411 – 18,531,445
	rs41613300	18,626,250	9.29×10^{-5}	*DEPDC1B*	18,560,198 – 18,649,542
21	rs41643781	48,482,014	3.61×10^{-5}	*CLEC14A*	48,394,033 – 48,395,926
25	rs381524820	41,563,799	7.47×10^{-6}	*MICALL2*	41,461,263 – 41,480,286
	rs381524820	41,563,799	7.47×10^{-6}	*UNCX*	41,614,415 – 41,615,906
27	rs110888316	32,828,175	1.95×10^{-5}	-	-
28	rs3423094174	3,452,115	6.88×10^{-5}	-	-
	rs43734734	20,968,839	5.43×10^{-5}	-	-
	rs42143510	25,781,265	3.55×10^{-5}	*HK1*	25,694,146 – 25,769,790
	rs42143510	25,781,265	3.55×10^{-5}	*TACR2*	25,768,939 – 25,781,767
	rs42143510	25,781,265	3.55×10^{-5}	*TSPAN15*	25,803,071 – 25,855,714
	rs29011010	28,794,085	2.70×10^{-5}	*MCU*	28,709,027 – 28,902,834
	rs29011010	28,794,085	2.70×10^{-5}	*ENSBTAG00000054039*	28,879,757 – 28,880,758

[1]No gene name or no gene position: SNP was not located in the gene or within a window size of 100 kb up- and downstream.

CHAPTER 4

Inferring effects of barn emissions, housing conditions and genetics on specific dermatitis digitalis diagnoses in dairy cows

Niklas Sölzer[1], Kerstin Brügemann[1], Tong Yin[1], Petra Engel[1] and Sven König[1]

[1] Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, 35390 Giessen, Germany

Accepted: January 2025

Published: March 2025

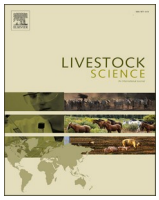
Livestock Science, 293, 105650, https://doi.org/10.1016/j.livsci.2025.105650

Livestock Science 293 (2025) 105650

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci



Inferring effects of barn emissions, housing conditions and genetics on specific dermatitis digitalis diagnoses in dairy cows

Niklas Sölzer, Kerstin Brügemann, Petra Engel, Sven König *

Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, 35390, Gießen, Germany

HIGHLIGHTS OF THIS STUDY

- Specific dermatitis digitalis disease stages indicate a differing genetic background with different significant SNPs and annotated candidate genes for acute and chronic stages.
- The most relevant housing effects on dermatitis digitalis were the bedding material, the air volume in the barn, temperature, humidity and wind speed in the barn, and the ammonia concentration.
- Considering all effects in structural equation models indicated the pre-dominance of housing effects on acute dermatitis digitalis, but stronger genetic effects on the chronic stage.

ARTICLE INFO

Keywords:
Dermatitis digitalis stages
Compost bedded pack barn
Housing effects
Genome-wide associations
Structural equation models

ABSTRACT

The aim of the present study was to infer effects of housing systems, cow phenotypes and genomics on specific stages of the claw disorder dermatitis digitalis (**DD**) of dairy cows kept in compost bedded pack barns (**CBPB**) and conventional cubicle barns (**CCB**) applying structural equation models (**SEM**). Housing system characterisations, herd hygiene status determination and greenhouse gas emission recordings considered 11 farms, whereas 6 farms represented the CBPB system, 2 farms represented the CCB system, and 3 farms "mixed farming" system with CBPB for sub-herd A and CCB for sub-herd B. In these 11 farms, 1,047 Holstein-Friesian and Fleckvieh-Simmental cows (1,611 observations) were phenotyped for the DD stages DD sick, DD acute and DD chronic. Cows from 4 further farms without housing and greenhouse gas emission data were considered for DD phenotyping and SNP genotyping, implying the availability of 2,980 DD observations from 1,710 cows for genomic studies of DD traits. In a first step, generalized linear mixed models were applied to identify the most relevant housing characteristics on DD sick, DD acute and DD chronic. Least-squares-means for infection probabilities were generally smaller in CBPB than in CCB. With regard to compost, barn air and barn emission characteristics in CBPB, a bedding temperature in the range < 28°C, a C:N ratio in the bedding material > 21, a pH-value in the bedding material > 8.8, small NH_3 concentrations (< 0.55) in the barn air, as well as small as moderate air humidity, were associated with the highest DD health status. The single-step GWAS indicated similar Manhattan plots for DD sick and DD acute, and respective shared potential candidate genes based on gene annotations from the *Bos taurus* ARS1.2 genome assembly. Three same SNPs were significantly associated (according to normative significance threshold) with DD acute and DD sick, but no overlaps in this regard were identified for other DD stages. Strong association signals in the Manhattan plots according to strict pBF were identified for DD chronic including three further SNPs, and for DD acute including the SNP *Hapmap47993-BTA-56668* (HAP) located on BTA 23. These SNPs together with latent variables for the cow DD individuality (**DD indiv**, including phenotypes and estimated breeding values for DD stages), cow productivity before and after a DD diagnosis, and the relevant barn and housing characteristics (as identified via mixed model applications), were simultaneously considered in SEM for DD sick, DD acute and DD chronic. Housing and barn characteristics played a predominant role with regard to infection risks for DD sick and DD acute. In contrast for DD chronic, path coefficients on DD indiv were quite large for DD chronic EBVs, as well as for single SNP effects.

* Corresponding author at: Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, Ludwigstraße 21b, 35390, Gießen, Germany.
E-mail address: sven.koenig@agrar.uni-giessen.de (S. König).

https://doi.org/10.1016/j.livsci.2025.105650
Received 18 November 2024; Received in revised form 16 January 2025; Accepted 20 January 2025
Available online 21 January 2025

1. Introduction

Structural as well as demographical changes plus increasing demands raised by the society imply respective challenges in future dairy cattle farming strategies (Readts et al., 2017). On the one hand, due to the growing global population size, there is an increasing demand for high-quantity and high-quality edible food resources with moderate prices. On the other hand, strict standards and restrictions plus legal guidelines with regard to animal welfare, animal housing and climate and resource protection, affect the costs of dairy cattle farming (Beaver et al., 2020; Galama et al., 2020). As a compromise combining both aspects, i.e., cow productivity and innovative welfare-friendly cattle housing, compost bedded pack barns (**CBPB**), have been suggested (Leso et al., 2020). A high level of animal welfare and corresponding productivity can be achieved in the free lying area, which is littered with compostable material (e.g., wood chips, sawdust or shavings) (Janni et al., 2007). Major characteristics of the bedding material are the bedding temperature, and dry matter and the carbon-to-nitrogen ratio (**C:N-ratio**) in the bedding material (Bewley et al., 2013; Eckelkamp et al., 2016). Furthermore, the composting process in the lying area affects the compost fertiliser quality criteria (Black et al., 2013; Bewley et al., 2017). However, the effects of such housing and composting characteristics on cow health traits are unclear. Climatic effects and barn-specific emissions including air humidity, air temperature and wind speed have direct effects on cow traits (e.g. Halli et al., 2023), but imply also indirect effects via compost alterations (Giambra et al., 2021). Vice versa, the housing system might affect greenhouse gas emissions, e.g., through the storage technique of slurry, compost or manure inside the cow barn. First effects of greenhouse gas emissions directly recorded inside the barn including ammonia and carbon dioxide on cow health traits and possible genotype-by-emission interactions, were outlined by König et al. (2022).

One of the most important cow diseases worldwide with quite large incidences across housing systems is the claw disorder dermatitis digitalis (**DD**) (Klitgaard et al., 2014; Solano et al., 2016). Dermatitis digitalis is a multi-factorial claw infection (Blowey and Sharp, 1988; Read and Walker, 1994). In addition to the predominant bacterial environment (bacteria of the *Treponema spp. Genus*), the housing conditions, feeding and genetics of the hosts play a decisive role (Döpfer et al., 2012; Solano et al., 2017). For in-depth DD studies, Döpfer et al. (1997) developed a specific scoring system, with focus on a detailed scoring of specific DD disease stages. Previous genetic studies utilising the specific DD stages focused on the estimation of genetic parameters (Schöpke et al., 2015), on the identification of functional genetic variants (Oelschlaegel et al., 2022) and on proofs for possible genotype-by-climate interactions (Sölzer et al., 2022). In the housing context, Sölzer et al. (2024) explicitly estimated heritabilities for DD stages in CBPB, which were in a range from 0.09 to 0.18 (SE in the range from 0.02 to 0.05). Furthermore, based on breeding value correlations, Sölzer et al. (2024) indicated genotype-by-housing interactions when considering the breeding values from CBPB and from cows kept in conventional farming systems.

Inferring the complex interplay among genetics, housing conditions, climate parameters, cow emissions and individual cow characteristics (production level, overall health status) via standard mixed model applications might be a challenge. Possible confounding effects as well as mutual recursive or causal relationships among response variables and between traits and effects hamper applications of standard mixed model theory (Rehbein et al., 2013). For taking into account mutual trait relationships and to assess effects of parameters which are not directly measurable, structural equation models (**SEM**) have been suggested (Gana and Broc, 2019). SEM are standard analytical tools in social sciences and psychology, because they are able to measure causal relationships and to represent complex causal processes via measurable (manifest) variables and non-measurable (latent) variables (Bielby and Hauser 1977; Hair et al., 2014). With regard to animal health analyses, Detilleux et al. (2013) applied SEM to infer risk factors and tolerance mechanisms for bovine mastitis infections. Wagner et al. (2023) used an SEM approach to detangle causal relationships among environmental and genetic factors on udder health.

The objective of this study was to infer the effects of different risk factors including individual, genomic, herd and housing characteristics on claw health. In this regard, the present study aimed on a detailed recording for different DD stages, on a detailed characterisation of the housing environment including measurements of greenhouse gases inside the barn in CBPB and in conventional cubicle barns (**CCB**), and on a detailed genomic characterisation using dense SNP marker data. In a first analysis step, we applied generalized linear mixed models to especially infer the effects of compost characteristics and gas emissions on DD traits. Secondly, the genetics part focussed on genome-wide association studies (**GWAS**) and the estimation of genomic breeding values. Finally, the comprehensive data sources and results from specific analyses were simultaneously considered in integrative SEM analyses.

2. Materials and methods

2.1. Cow trait recording

The complete dataset with regard to housing characteristics and cow traits considered 1,611 observations for different DD stages from 1,047 cows during the recording years 2021 and 2022. The breed of the cows was Holstein-Friesian (**HF**, 1050 observations) and Fleckvieh-Simmental (**FS**, 561 observations). The cows were kept on 11 farms in the German federal states of Bavaria, Hesse and Rhineland-Palatinate. Six farms represented the CBPB system, 2 farms represented the CCB system, and 3 farms represented a "mixed farming" system with CBPB for sub-herd A and CCB for sub-herd B. The herd size ranged between 25 and 800 milking cows. Cows were from parities 1 to 14. 62.5% of all cows had observations from at least 2 DD recording dates.

The detailed DD scoring was done according to a validated scheme considering the DD stages M.0 to M.4.1 of the claws from all four legs (Döpfer et al., 1997). For ongoing analyses, specific DD stages were grouped into three DD traits according to disease pathogenesis. The trait DD sick included the stages M.1 - M.4.1, DD acute included the stages M.1, M.2 or M.3, and DD chronic included the stages M.4 or M.4.1. The different DD stages and the respective trait grouping is illustrated in Fig. 1. The average prevalence was 24.64 for DD sick, 16.88 for DD acute and 7.08 for DD chronic. Production data of theses cows considered records for milk yield, fat percentage, protein percentage and somatic cell count from the nearest test-day before DD scoring (1390 observations) and from the nearest test-day after DD scoring (1487 observations). Furthermore, the cleanliness of the cows, as a parameter reflecting the herd hygiene status, was determined at the date of DD scoring using the monitoring scheme by Cook (2002). In this regard, the lower leg, upper leg and flank were scored on a scale from 1 to 4. The score = 1 indicated a very clean cow, score = 2 was assigned for minor splashing, score 3 implied distinct plaques of manure, and score = 4 was used for a very dirty cow with confluent plaque of manure.

This dataset including the 1,047 cows with DD records could be associated with the herd hygiene status, housing characterizations (see chapter 2.2.1) and greenhouse gas emission recording (see chapter 2.2.2) at the same time. Genomic analyses (see chapter 2.3.1) to identify the most interesting potential candidate genes based on a larger dataset including 2,980 observations from 1,710 cows from 15 herds. However, from these additional 4 herds, we had no hygiene scores, housing and barn characteristics and greenhouse gas emissions, implying to exclude these herds from the fixed effect analyses (see chapter 2.2.3) and the integrative structural equation modelling (see chapter 2.4.1).

2.2. Barn and housing characteristics recording and respective association analyses with DD traits

2.2.1. Analysis of the bedding material in compost bedded pack barns

At each farm visit for DD scoring, the bedding temperature was measured at eight sampling points spread across the whole bedding area at a litter depth of 20 cm (Testo 435-2, Testo SE & Co. KGaA, Titisee-Neustadt, Germany), and compost samples were taken. The compost samples were taken at three depths per sampling point. The first sample was taken from the surface, another sample at a depth of 5 cm to 10 cm, and the third sample at a depth of 20 cm. The samples from the same sampling point were mixed, placed in a plastic bag and stored in a cool container. Samples were used for dry mass determination in the laboratory at the same day, while the remaining content was frozen for further analyses. For dry mass determination, the compost samples were dried in the drying oven at 105°C for 24 hours. For the ongoing analyses, the frozen samples were slowly melted in the refrigerator. The pH-value was measured in the laboratory according to the HBU 3.5.1 method, DIN 19684-1. For the determination of carbon and nitrogen in the compost, the melted sample was dried at 60°C, and afterwards milled. The airtight sealed sample were stored at room temperature and contents for carbon, nitrogen, nitrate and ammonium were determined in the laboratory of the department for "Organic Farming with focus on sustainable soil use" at University of Giessen.

2.2.2. Measuring of greenhouse gases

In cooperation with the company MSA (MSA Industries, Pennsylvania, United States), we developed a mobile gas measuring system, which was used to measure the gas concentrations for carbon dioxide (**CO_2**) (in the percentage range; infrared measurement; ULTIMA® XIR gas detector from MSA, Pennsylvania, United States), methane (**CH_4**) (in the ppm range; electro-chemical measurement; Monicon S500L Gas Monitor from Monicon Technology Ltd, Galway, Ireland) and ammonia (**NH_3**) (in the ppm range; electro-chemical measurement; PrimaX® P Gas Transmitter from MSA, Pennsylvania, United States). Gas measurements considered all herds from both housing systems CBPB and CCB. Previous to the gas recordings, 8 to 10 measuring points inside and outside the barn were marked. At these measuring points, the gas measuring trolley was installed at a height from 40 to 80 cm for periods of 5 minutes. In parallel, air temperature (**T**), relative humidity (**RH**) and wind speed (**WS**) were recorded at each measuring point using a mobile weather station (Testo 435-2, Testo SE & Co. KGaA, Titisee-Neustadt, Germany).

2.2.3. Statistical models to infer effects of farm characteristics on DD traits

For studying the effects of compost characteristics, climate and greenhouse gas emissions directly recorded inside the cow barn on the three binary DD traits (DD sick, DD acute, DD chronic), generalized linear mixed models with a logit-link function as implemented in the SAS GLIMMIX procedure (SAS, 2022), have been applied. The statistical model 1 was defined as follows:

$$y_{ijklmn} = \mu + Herd_i + Season_i + Parity_j + Breed_l + DIM_m + Herd_l[BTc; CNc; pHc; NH_3c; Tc; RHc]_n + cow_o + e_{ijklmno} \quad (1)$$

where μ was the overall mean effect, $Herd_i$ was the fixed effect for the *i-th* herd, $Season_j$ was the fixed effect for the *jt-th* season (4 seasons: Mar-May, Jun-Aug, Sep-Nov, Dec-Feb) of DD scoring, $Parity_k$ was the *k-th* lactation number of the cow (1, 2, 3, >3), $Breed_l$ was the fixed effect for the *l-th* breed, DIM_m was the linear regression on days in milk for the lactation stage reflecting the days after calving at DD recording (range: 11 to 336 days), $Herd_l[BTc; CNc; pHc; NH_3c; Tc; RHc]_m$ was the nesting of the herd effect within the different housing characteristics in consecutive runs. The housing characteristics were the fixed effect classes for the bedding temperature (***BTc***) in the compost at a depth of 20 cm class (< 28°C, 28°C–36°C, > 36°C, control), for the C:N ratio (***CNc***) in the bedding material (< 21, 21-29, > 29, control), for the pH-value of the bedding material (***pHc***) (< 8.5, 8.5-8.9, >8.9, control), for the NH_3 concentration (***NH_3c***) in the barn air (< 0.55 ppm, 0.55 ppm-1.15 ppm,

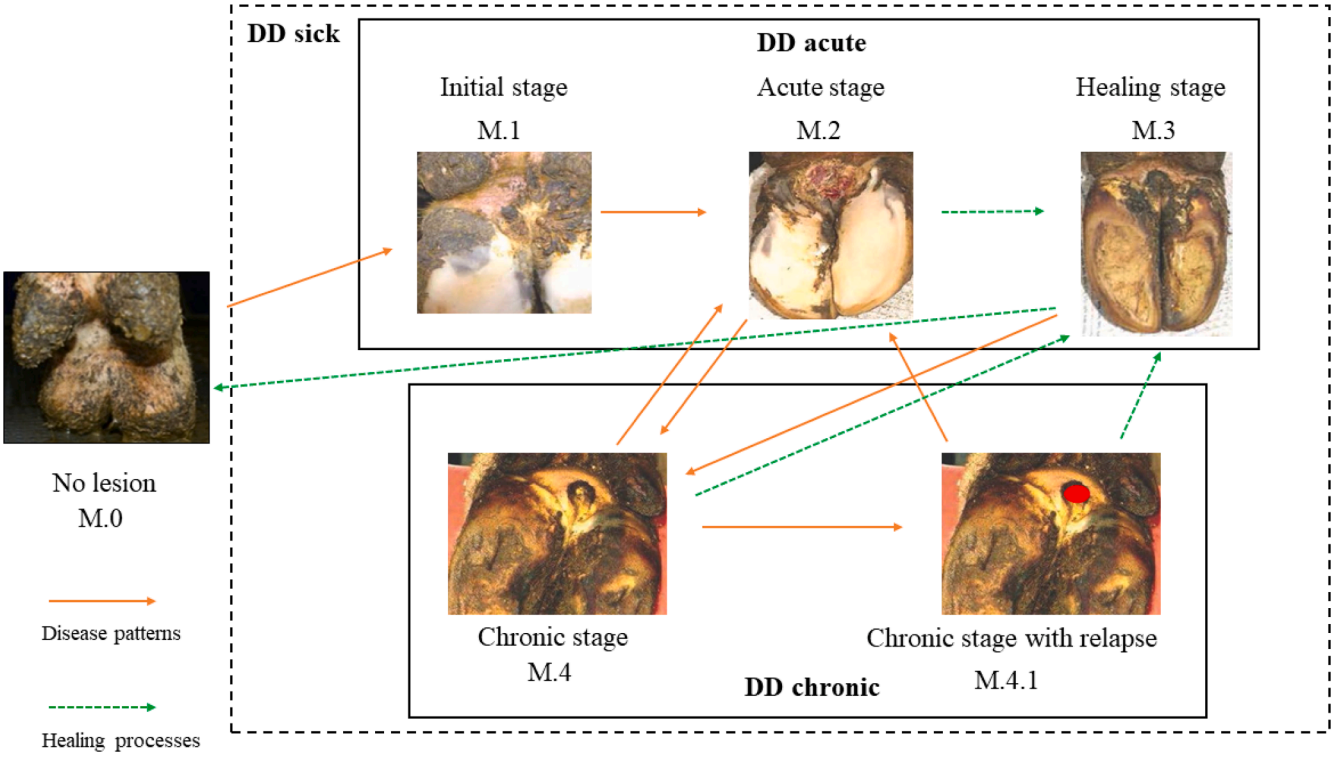


Fig. 1. Overview for the different dermatitis digitalis (DD) stages (according to Döpfer et al., 1997) and respective classifications into the DD traits DD sick, DD acute and DD chronic. The arrows indicate the change of the different DD stages, with green-dotted arrows representing the healing process, and red-solid arrows representing the changes from healthy towards DD stages and possible changes among DD stages.

> 1.15 ppm, control), for the barn air temperature (***Tc***) (< 7.5°C, 7.5°C-12.5°C, > 12.5°C, control) and for the barn humidity (***RHc***) (<65, 65-85, >85, control). The repeated measurements for the *o-th* cow were considered through the random cow effect (cow_0), and $e_{ijklmno}$ *was the random* residual effect. Least-squares-means **(LSmeans**) for DD disease probabilities (**Pr**) were calculated for the observed scale applying the following transformation: $Pr = \frac{e^{estimate}}{1+e^{estimate}}$ with estimate being the LSmeans on the logit scale.

2.3. Genomic analyses

2.3.1. Genotyping and quality control

Cows from 4 further farms without housing and greenhouse gas emission data were considered for DD phenotyping and SNP genotyping, implying the availability of 2,980 DD observations from 1,710 cows for genetic studies of DD traits. A subset of 935 cows was genotyped with the *Illumina BovineSNP50 v2 BeadChip* (Illumina Inc.). All herds involved in this study participate at the German national health monitoring and genotyping program to implement genomic evaluations for health traits since 2021. In consequence, all female cattle from these herds are routinely genotyped, and the genotypes stored at the national genetic evaluation centres were used for this study. The smaller number of genotyped cows compared to the number of phenotyped cows is due the delayed start of calf genotyping in most herds later than in the year 2019.

Quality control of the genotype data was performed using the software package PLINK (Purcell et al., 2007). Quality control criteria considered a call rate > 95%, a minor allele frequency (**MAF**) > 0.01, and SNPs not significantly deviating from Hardy-Weinberg equilibrium ($P > 0.001$). We considered only SNPs located on *Bos taurus* autosomes, and we excluded cows with more than 95% identical genotypes. After quality control, 38,495 SNPs from 926 cows with DD phenotypes were available for the genomic studies. With regard to the complete dataset (i.e., the cows from herds with housing characteristics and hygiene status), 454 cows (776 observations) were genotyped.

2.3.2. Estimation of genetic parameters and genomic breeding values

For the estimation of variance components, genetic parameters and genomic breeding values for DD-sick, DD-acute and DD-chronic, we used the DD dataset including the 2,980 DD observations from the 1,710 cows (926 cows with genotypes). In this regard, single-step analyses (**ssGBLUP**) was applied, using the AI-REML algorithm as implemented in the AIREMLF90 program (Misztal et al., 2018). The respective genetic-statistical single trait animal model 2 was defined in matrix notation as follows:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Za} + \mathbf{Wp} + \mathbf{e} \quad (2)$$

where **y** was a vector including observations for the three DD traits; **β** was a vector of fixed effects including herd-year season of diagnosis (4 seasons: Jan – Mar.; Apr.- Jun.; Jul.- Sep.; Oct.- Dec.), breed, parity (1 (26.9% of all observations), 2 (27.3% of all observations), 3 (18.4% of all observations), 4 (13.8% of all observations), and ≥ 5 (13.6% of all observations)), and a linear regression on days in milk (**DIM**) reflecting the days after calving at DD recording (range: 11 to 336 days); **a** was a vector of random additive-genetic effects; **p** was a vector of random permanent environmental effects, **e** was a vector of random residual effects; **X, Z** and **W** were the incidence matrices for **β, a**, and **p**, respectively. It was assumed that $\mathbf{a} \sim N(0, \boldsymbol{H}\sigma_a^2)$, $\mathbf{p} \sim N(0, \mathbf{I}\sigma_p^2)$ and $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$ where σ_a^2, σ_p^2 and σ_e^2 are the additive-genetic, permanent environmental, and residual variances, respectively. The combined inverse of the **H** matrix was computed using the PREGSF90 program by blending the pedigree relationship matrix ($\mathbf{G_w}$; Legarra et al., 2009). $\mathbf{G_w}$ was calculated as follows $\mathbf{G_w} = (0.95 \times \mathbf{G} + 0.05 \times \mathbf{A_{22}})$, where $\mathbf{A_{22}}$ was the submatrix of the pedigree-based relationship matrix for genotyped animals and **G** was the genomic relationship matrix (VanRaden, 2008). The pedigree relationship matrix considered at least a depth of 3 generations backwards, with oldest founder animals born in 1906, contributing to a pedigree dataset of 11,385 animals. The 1,710 cows with DD records originated from 416 different sires, and from 1,299 different dams. Genomic relationships between HF and FS were due the introgression of Red Holstein genes into the FS population at the beginning of the 1980s.

2.3.3. Genome-wide association study

The single-step GWAS for the estimations of SNP marker effects based on the genomic breeding values as obtained from ssGBLUP analyses. The SNP effects and *p*-values were estimated with the "OPTION snp_p_value" as implemented in POSTGSF90 (Aguilar et al., 2019). The genome-wide significance level according to Bonferroni was defined (**pBF** = 0.05/ N_{SNP} = 1.3e-06). Additionally, a less stringent normative significance threshold (**pCD**) was considered, with pCD = 1e-04 (Kurz et al., 2019).

2.3.4. Annotation of potential candidate genes

For gaining deeper insights into possible physiological mechanisms, we annotated potential candidate genes located in a window 100 kb upstream or downstream from the significantly associated candidate SNP by utilizing the Ensembl database, release 102, on the basis of the *Bos taurus* ARS1.2 genome assembly (Zerbino et al., 2018). SNP were mapped to corresponding genes from the *Bos taurus* annotation from the Ensembl database (http://www.ensembl.org/biomart/martview), applying the R package biomaRt (Durinck et al., 2009). The SNP markers related to the potential candidate genes with a significance according to pBF, or with a significance according to pCD for one of the DD traits (DD-sick or DD-acute or DD-chronic), were integrated into ongoing SEM modelling approaches (see the next chapter 2.4.1 and Table 1). The description of functions of the annotated potential candidate genes based on reports given in the literature.

2.4. Structural equation modelling for integrative analyses considering of housing characteristics and genomic information simultaneously

2.4.1. Latent and indicator variables

We set up a SEM separately for DD-sick, DD-acute and DD-chronic by applying the *lavaan* package (Rosseel, 2012) as implemented in R, version 4.4.2 for Windows (R Core Team, 2020). In this regard, we considered 5 latent variables in the SEM, often defined as the 'measurement part of the SEM' (Detilleux et al., 2013). Each latent variable covered observed variables, i.e., so called indicator variables. These are either directly related to the latent variables (indicators) or are present as free variables (free indicators) in the SEM (Moshagen, 2012). The latent variables as well as the descriptive indicator and free indicator variables are listed in Table 1. In this regard, the first latent variable was related to the individuality of a cow (**DD indiv**) considering their phenotypes for the DD traits, the respective estimated genomic breeding values (**ebv**) for the DD traits, and the phenotypes for the hygiene score (**hyg**) and somatic cell score (**scs**) as related disease indicator traits. The second latent variable was the genomics (**Gen**) component, considering the most significant SNPs from the previous GWAS as explained above. The influence of cow productivity on DD traits as well as possible recursive effects were depicted through the latent variables for test-day production before (**Prb**) and after the date (**Pra**) for DD scoring. Considered indicator variables were the respective test-day records for milk yield (**myb, mya**), fat content (**fcb, fca**) and protein content (**pcb, pca**), as well as the respective lactation number (**lnr**) and lactation stage (**lsb, lsa**) considering the days in milk after calving. The latent variable for the barn characteristics (**barn**) included the overall housing system information (**sys**), the air volume in the barn (**aiv**), the barn temperature (**T**), the relative humidity in the barn (**RH**), wind speed in the barn (**wsp**), and the ammonia concentration in the barn air (**NH3**).

Table 1
Latent variables and indicator variables as considered in the structural equation model (abbreviations of the variables (in bold) are used in Figs. 3, 4 and 5).

Latent variables in the SEM	Indicator variables in the SEM
DD phenotypes and estimated breeding values of the cow reflecting the DD individuality **(DD indiv)**	Phenotypic scores for **DD sick, DD acute** and **DD acute**
	Hygiene score according to Cook (2002) **(hyg)**
	Somatic cell score before DD scoring **(scs)**
	Estimated genomic breeding value for DD sick **(ebv DD sick)**, for DD acute **(ebv DD acute)** and DD chronic **(ebv DD chronic)**
Genomics **(Gen)**	Significant SNPs for a DD diagnosis[1]
	ARS-BFGL-NGS-29426 **(AR6)** on BTA 5
	ARS-BFGL-NGS-39422 **(AR2)** on BTA 29
	ARS-BFGL-NGS-75315 **(AR5)** on BTA 16
	BTB-0118497 **(BTB)** on BTA 8
	Hapmap47993-BTA-56668 **(HAP)** on BTA 23
	Hapmap40478-BTA-106311 **(HP1)** on BTA 11
	Hapmap58551-rs29023108 **(HP8)** on BTA 11
Yields for test-day production traits before **(Prb)** and after **(Pra)** a DD diagnosis	Milk yield before DD scoring **(myb)**
	Fat content before DD scoring **(fcb)**
	Protein content before DD scoring **(pcb)**
	Milk yield after DD scoring **(mya)**
	Fat content after DD scoring **(fca)**
	Protein content after DD scoring **(pca)**
	Lactation number **(lnr)**
	Lactation stage before DD scoring **(lsb)**
	Lactation stage after DD scoring **(lsa)**
Barn characteristic **(barn)**	Housing system (compost or cubicles) **(sys)**
	Air volume in the barn **(aiv)**
	Temperature in the barn **(T)**
	Relative humidity in the barn **(RH)**
	Wind speed in the barn **(wsp)**
	Ammonia concentration in the barn **(NH3)**

[1] Signficant SNPs for at least 2 DD traits according to pCD, or at least for one DD trait according to pBF.

2.4.2. Structural equation model definition

In matrix notation, the SEM was defined as follows:

$$\mathbf{y} = \boldsymbol{\Lambda}\eta + \boldsymbol{\upsilon}$$

$$\boldsymbol{\eta} = \mathrm{B}\eta + \boldsymbol{\zeta}$$

where $\mathbf{y}$ was the vector of observed variables, $\boldsymbol{\eta}$ was the corresponding vector for latent variables, and $\boldsymbol{\upsilon}$ and $\boldsymbol{\zeta}$ were the corresponding vectors of error terms. It was assumed that $\mathrm{E}(\boldsymbol{\upsilon}) = \mathbf{0}$, $\mathrm{var}(\boldsymbol{\upsilon}) = \boldsymbol{\Theta}$, $\mathrm{E}(\boldsymbol{\zeta}) = \mathbf{0}$, and $\mathrm{var}(\boldsymbol{\zeta}) = \boldsymbol{\Psi}$. Elements ($\lambda$) of $\boldsymbol{\Lambda}$ were partial regression coefficients relating latent variables to the observed variables, while elements (β) of $\mathbf{B}$ connected latent variables among them (direct and indirect effects). We decided to apply the following model quality criteria: For the comparative fit index (**CFI**) a cut-off value close to 0.95, for the standardised root mean square residual (**SRMR**) a cut-off value close to 0.08, and for the root mean square error of approximation (**RMSEA**) a cut-off value close to 0.06 (Hu and Bentler, 1999).

Effects of the latent constructs were assessed by studying the path coefficients (λn). Path coefficients can range between -1 and +1, with a value ≥ 0.20 or ≤ -0.20 indicating a significant correlation (Chin, 1998). Path coefficients outside the theoretical parameter range (< -1 or > 1) are due to the standard errors.

3. Results and discussion

3.1. Effects of compost and housing characteristics on DD traits

This chapter addresses the results from the fixed effect model (model 1) as defined in chapter 2.2.3. The overall *F*-Test displayed significant effects ($P < 0.01$) of the compost and housing characteristics BTc, CNc, pHc, NH3c, Tc and RHc on DD sick, DD acute and DD chronic. The LSmeans for disease probabilities of the three DD traits for the different levels of these compost and housing characteristics are given in the Supplementary Figures S1 (BTc), S2 (CNc), S3 (pHc), S4 (NH3c), S5 (Tc) and S6 (RHc). The generally better health status for cows kept in the CBPB when compared to the control group (CCB) was observed for all sub-models with respective LSmeans as displayed in the Supplementary Figures S1-S6. With regard to cows kept in the CBPB, bedding temperatures $< 35°C$ at a depth of 20 cm were associated with smallest LSmeans for infection probabilities for DD sick, DD acute and DD chronic. Optimal composting processes in the CBPP were reported for bedding temperatures in the range from 43.3°C to 65°C at a depth of 15 cm to 31 cm (Janni et al., 2007; Bewley et al., 2013). However, such quite high bedding temperatures imply optimal and durable composting processes as being the case in the experienced CBPB farms in the US. In the CBPB in Germany as considered in the present study, the farms characterized by low bedding temperatures have large resources of bedding material and focus on a bedding management with frequent re-spreading, aiming on a generally dry and clean lying surface. For CBPB farms, 2 possible optimal bedding management practices have been reported. First, to initiate a real and sustainable composting process (argument for high bedding temperatures), or, on the other hand, the focus on the clean lying area and the high hygiene status, realized through the narrow re-spreading intervals (argument for low bedding temperatures). In such management context, the focus on the dry and clean lying areas was the most efficient strategy to prevent any DD infections (Alvergnas et al., 2019).

With regard to CNc, the smallest C:N range (CNc < 21) in the bedding material was associated with highest LSmeans for the incidences of DD sick, DD acute and DD chronic. Rosen et al. (2000) and Galama (2014) indicated a quite broad optimal C:N-ratio from 15:1 to 25:1. In the present study, LSmeans for DD disease probabilities did not differ significantly ($P > 0.05$) between CNc > 28 and CNc 21-28, but a narrow ratio with CNc < 21 was associated with a significant increase for the risk of an infection for DD sick and DD acute.

With regard to the effects of pH-values in the bedding material, significant differences ($P < 0.01$) were only observed when comparing infection probabilities for DD chronic in pHc 8.5-8.8 and in pHc >8.8. The pairwise difference of LSmeans was 15.8%. This finding, i.e., a better DD health status with increasing pH-values, is in agreement with survival pattern of bovine digital dermatitis treponemes (Bell et al., 2023).

LSmeans for the probability of occurrence of the DD traits in dependency of NH3c indicate highest infections risks for the highest level of NH3 concentrations (> 1.14 ppm) in the barn air. Ammonia is generated in the conversion process of urea through urease enzymes, mainly in faeces, in manure of the bedding material and in urine (Bristow et al., 1992). The risk of a DD infection increased under wet and unhygienic conditions, which contributed to a higher level of ammonia concentrations (Somers et al., 2005).

An increase of barn temperatures was associated with significantly increased probabilities for an infection for all three DD traits, i.e., highest LSmeans for DD disease probabilities at Tc > 12.4. Favorable effects of low barn temperatures on a broad pattern of claw disorders were shown by Gernand et al. (2019) for other housing systems than CBPB. The effect of barn humidity indicated significant differences between LSmeans ($P < 0.01$) for DD sick and DD chronic, displaying highest infection probabilities for the highest humidity class (RHc > 84). The stronger effect of air temperatures than of air humidities on cow

health and wellbeing was recently indicated by König et al. (2025), because the applied temperature-humidity formulas considered temperature with stronger weighing factors than humidity.

3.2. SNPs and potential candidate genes with significant effects on DD traits

Shared significant SNPs for at least two DD traits (according to pCD), or significant SNPs for one DD trait (according to strict pBF) integrated into the SEM (see Table 1 in chapter 2.4.1) based on the SNP effects from the GWAS as depicted in Fig. 2. This Fig. was also part in the interpretations by Sölzer et al. (2024) as a side-product in GWAS with additional SNP interaction effects. The respective Manhattan plot for DD sick (inflation factor λ was 1.32) is given in Fig. 2a, for DD acute in Fig. 2b (inflation factor λ was 1.31), and for DD (inflation factor λ was 1.39) in Fig. 2c.

Significant SNPs on two DD traits comprised *ARS-BFGL-NGS-29426* **(AR6)** located on BTA 5 (for DD sick and DD acute), *ARS-BFGL-NGS-39422* **(AR2)** located on BTA 29 (for DD sick and DD acute), and *BTB-0118497* **(BTB)** located on BTA 8 (for DD sick and DD acute). Hence, same SNPs with significant effects on two DD traits always were identified for DD acute and DD sick, but not for other DD stage combinations including DD chronic. This is in agreement with the DD disease pathology, indicating different biologic functions with obvious different phenotypic characteristics for chronic and acute disease stages (e.g., Döpfer et al., 1997; Schöpke et al., 2015). In this regard, different genetic mechanisms related to specific immune responses can be assumed (Canive et al., 2021). Consequently, the pattern in den Manhattan plots obviously differed between DD acute and DD chronic, but some similarities were identified for DD acute and DD sick. Strong association signals in the Manhattan plots according to strict pBF were identified for DD chronic including the SNP *ARS-BFGL-NGS-75315* (**AR5**) located on BTA 16, the SNP *Hapmap40478-BTA-106311* (**HP1**) located on BTA 11, and the SNP *Hapmap58551-rs29023108* (**HP8**) located on BTA 11. Furthermore, the SNP *Hapmap47993-BTA-56668* (**HAP**) located on BTA 23 was significant according to strict pBF for DD acute. These ssignificant SNPs according to pCD for at least two DD traits or with strong association signals for one DD trait according to pBF, were considered in the ongoing SEM and are listed in Table 1.

Because of the overlapping significant SNPs for at least two DD traits, we were interested in the annotation of potential candidate genes, and the respective functions of these genes as described in the literature. However, only the significant SNPs were considered in the ongoing SEM, but not the annotated genes. Due to the similar Manhattan plot pattern for DD sick and DD acute, we expected to identify shared potential candidate genes for both DD traits. In consequence, for DD sick and DD acute, we annotated the shared potential candidate gene *METTL25* on BTA 5. The detection of this shared potential candidate gene is the consequence of the significant *SNP ARS-BFGL-NGS-29426* (AR6) for both DD traits located in close chromosomal distance. Several studies (e.g., de Greef et al., 2023) highlighted *METTL25* in the context of DNA methylation. According to the "Gene Cards Human Database" (https://www.genecards.org/), *METTL25* is defined as a protein coding gene. In this database, immunodeficiency is indicated as a disease being strongly associated with *METTL25*. König and May (2018) identified several cow diseases due to immunodeficiency, especially major claw disorders including DD. The potential candidate gene *TENM4* is located on BTAT 29 in the window harbouring the SNP *ARS-BFGL-NGS-39422* (AR2), also displaying significant effects on DD sick and DD acute. According to the "Gene Cards Human Database" (https://www.genecards.org/) *TENM4* (Teneurin Transmembrane Protein 4) is a protein coding gene, and associated listed diseases in this database were very specific neurological disorders. Pietrosemoli et al. (2017) reported the involvement of *TENM4* in regulative processes of myoblast quiescence and respective interfaces with neurological functions. In farm animals (beef cattle), Reith et al. (2022) identified differentiated *TENM4* gene expressions in relation to environmental stress and to feeding particularities (zilpaterol supplementation). The aspect of stress in this context might be very interesting, supporting findings by Sölzer et al. (2022) in genome-wide associations with heat stress interactions.

For the SNP *BTB-0118497* (BTB) displaying significant effects on both DD traits DD acute and DD sick, no potential candidate gene was annotated. An explanation might be the narrow chromosomal segment of ±100 kb as used for gene annotations, because in other studies also windows comprising 250 kb (e.g., Klein et al., 2021) or even 400 kb (e.g., Bohlouli et al. 2022) were defined, contributing to an inflating number of annotated potential candidate genes. However, for the ongoing integrated structural equation modelling, we intended to limit the genomics input data, which explains the strict procedure in all genomic analyses. Nevertheless, albeit the non-significant SNPs according to pCD or pBF, we annotated *AFF3* on BTA 11 as a further potential candidate gene for both stages DD sick and DD acute. *AFF3* was already highlighted in the DD study by Sölzer et al. (2024). *AFF3* is expressed in B cells, and its expression was related with different types of cancer (Shi et al., 2019). So far, there is no direct link connecting *AFF3* with claw disorders, but effects on pregnancy in dairy cows were reported (Oliver et al., 2019). A further annotated potential candidate gene for DD sick and DD acute was *PRKG1* BTA 26, but different significant SNPs in this chromosomal segment were identified for both DD traits.

3.3. Integrative analysis: path coefficients from the structural equation modelling

3.3.1. Overall remarks

In this chapter, we address the results from the SEM simultaneously considering the previously identified major compost and housing characteristics, and the previously identified significant SNPs. Regarding the importance of path coefficients, we focused in the interpretations of the absolute values, and we did not differentiate between negative or positive signs for the same coefficient. Especially in the case of SNP genotypes, the same homozygous genotype could be assigned with a code "0" in a run for trait A, but with a code "2" in a run for trait B. Such automatic assignments might affect the signs of other path coefficients.

3.3.2. Structural equation models for DD sick and DD acute

Fig. 3 shows completely standardized estimates for the path coefficients of the SEM for DD sick, and Fig. 4 for DD acute. Overlapping pattern for the Manhattan plots (Fig. 2) with shared potential candidate genes (Table 1) were identified for both DD traits, and in consequence, effects and respective path coefficients in the SEM indicated close similarities. The latent variable for the barn characteristics with path coefficients for barn of 0.38 on DD indiv (for DD sick) and of 0.37 on DD indiv (for DD chronic) indicate quite strong effects on both DD disease stages, mainly due to the major effects of the barn characteristics on the indicator variables representing the barn climate, especially T (0.85 in the DD sick SEM, 0.86 in the DD acute SEM) and RH (-0.86 in the DD sick SEM, -0.85 in the DD acute SEM). It is well know that barn characteristics (type of the farm building, etc.) strongly determine the barn climate, with causal effects on cow claw disorders including DD (Gernand et al., 2019) and on health trait indicators (Lambertz et al., 2013). Nevertheless, the effects of barn climate on DD are very complex, especially in the context of proven genotype x barn climate interactions (Sölzer et al., 2022). The ammonia gas concentrations also were closely related with the barn characteristics (0.82 in the SEM for DD sick, and 0.82 in the SEM for DD acute), indicating the importance of the latent variable NH3 on DD infections. Ammonia strongly determines air quality, with causal effects on health traits. In this regard, van Leenen et al. (2020) described the detrimental effect of increased ammonia exposure on the bovine respiratory disease complex in beef and dairy cattle calves and heifers, with time-lagged effects on cow disease susceptibility including claw disorders. Phenotypic as well as genetic

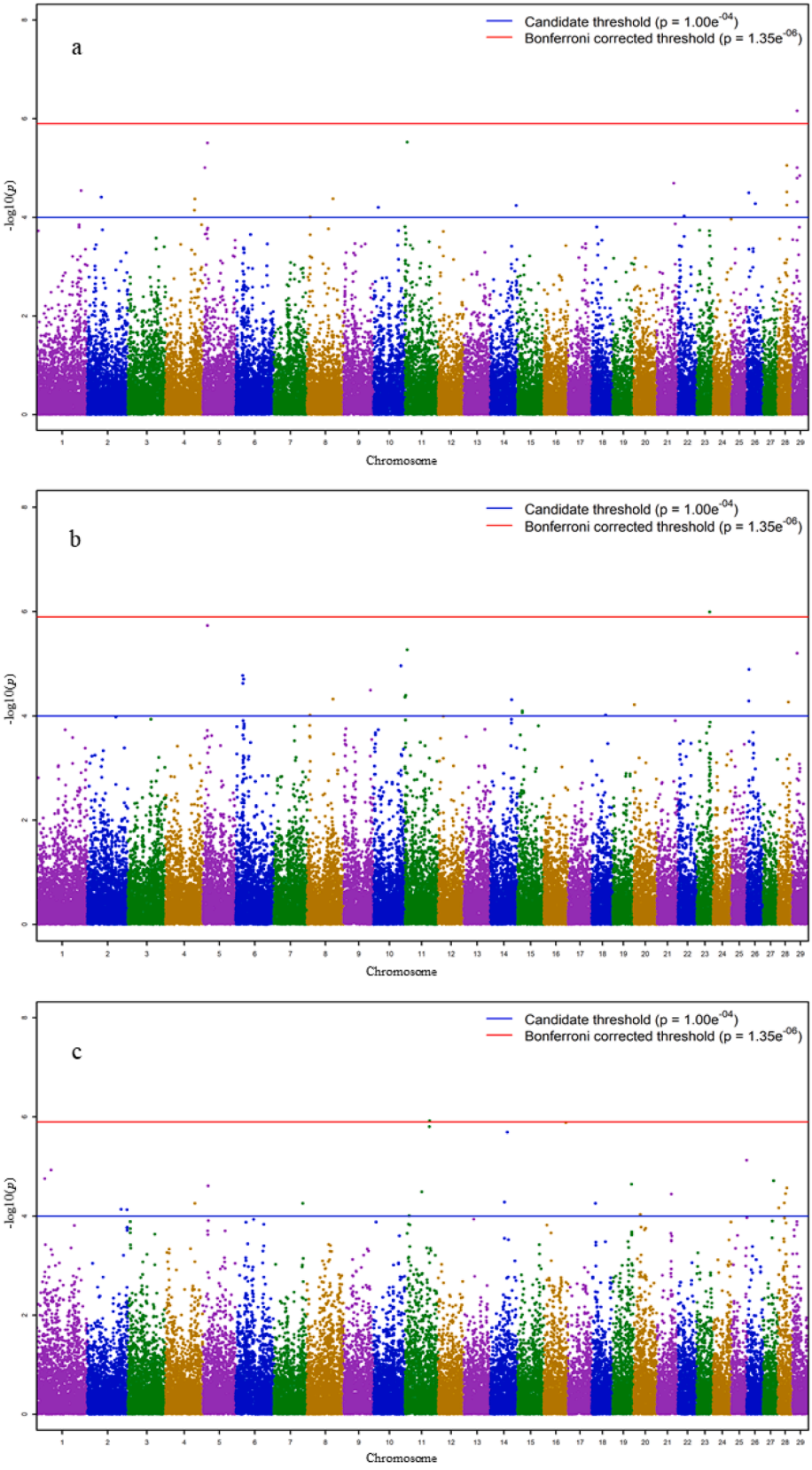


Fig. 2. Manhattan plots for the genome-wide associations for the specific dermatitis digitalis stages DD sick (**a**), DD acute (**b**) and DD chronic (**c**).

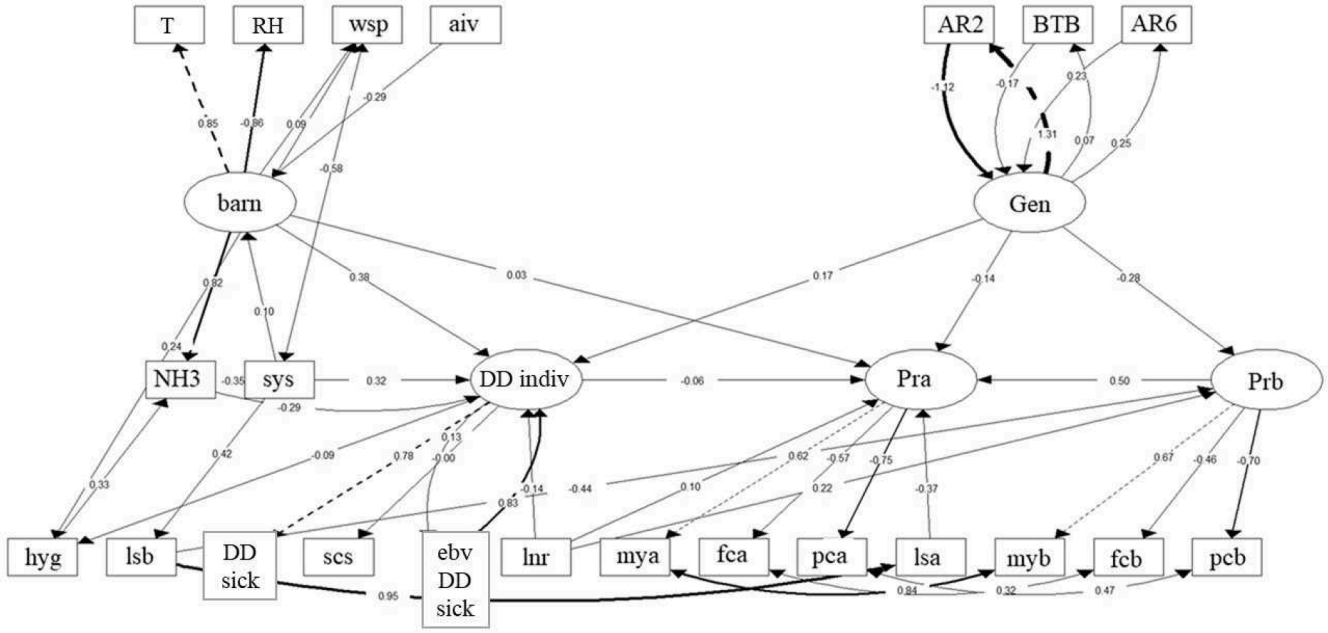


Fig. 3. Structural equation model for DD sick with respective path coefficients for latent and observed variables (hyg = hygiene score, scs = somatic cell score, ebv = estimated breeding value, AR6 = SNP *ARS-BFGL-NGS-29426*, AR2 = SNP *ARS-BFGL-NGS-39422*, AR5 = SNP *ARS-BFGL-NGS-75315,* BTB = SNP *BTB-0118497*, HAP = SNP *Hapmap47993-BTA-56668*, HP1 = SNP *Hapmap40478-BTA-106311*, HP8 = SNP *Hapmap58551-rs29023108*, myb = milk yield before DD scoring, fcb = fat yield before DD scoring, pcb = protein yield before DD scoring, mya = milk yield after DD scoring, fca = fat yield after DD scoring, pca = protein yield after DD scoring, lnr = lactation number, lsb = lactation stage before DD scoring, lsa = lactation stage after DD scoring, sys = housing system, aiv = air volume in the barn, T = temperature in the barn, RH = relative humidity in the barn, wsp = wind speed in the barn, NH3 = ammonia concentration in the barn, DD indiv = latent variable for the cow individuality, Gen = latent variable for gemomics, barn = latent variable for barn characteristics).

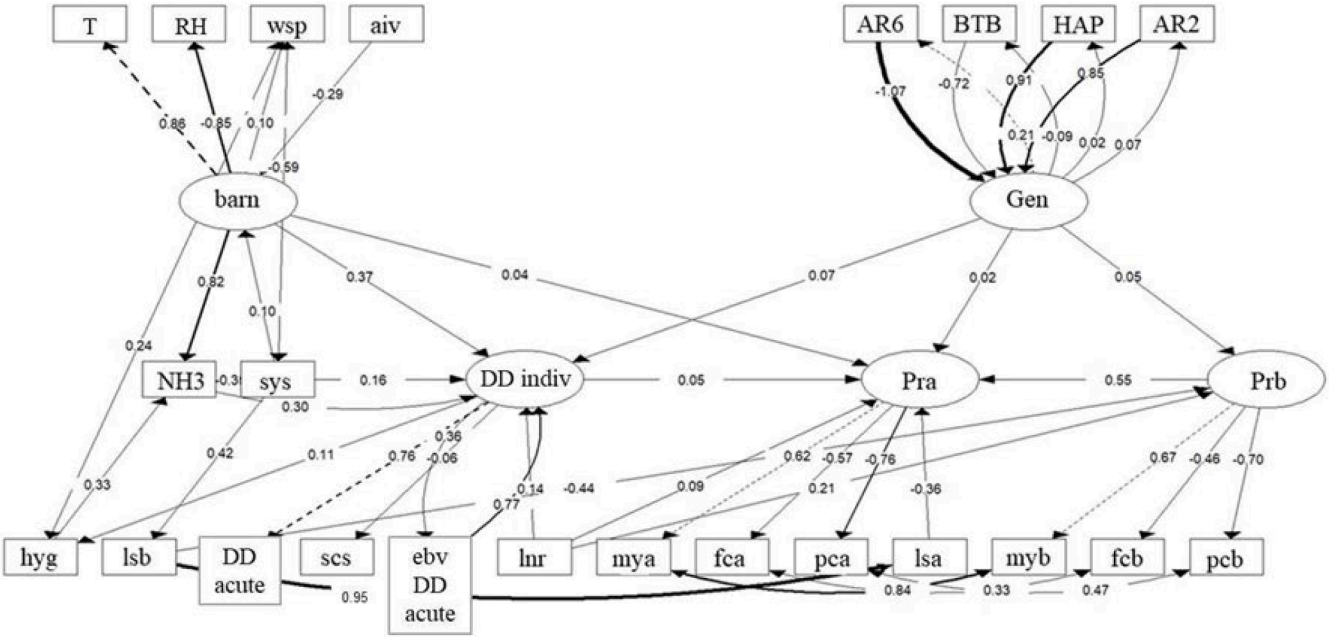


Fig. 4. Structural equation model for DD acute with respective path coefficients for latent and observed variables ((hyg = hygiene score, scs = somatic cell score, ebv = estimated breeding value, AR6 = SNP *ARS-BFGL-NGS-29426*, AR2 = SNP *ARS-BFGL-NGS-39422*, AR5 = SNP *ARS-BFGL-NGS-75315,* BTB = SNP *BTB-0118497*, HAP = SNP *Hapmap47993-BTA-56668*, HP1 = SNP *Hapmap40478-BTA-106311*, HP8 = SNP *Hapmap58551-rs29023108*, myb = milk yield before DD scoring, fcb = fat yield before DD scoring, pcb = protein yield before DD scoring, mya = milk yield after DD scoring, fca = fat yield after DD scoring, pca = protein yield after DD scoring, lnr = lactation number, lsb = lactation stage before DD scoring, lsa = lactation stage after DD scoring, sys = housing system, aiv = air volume in the barn, T = temperature in the barn, RH = relative humidity in the barn, wsp = wind speed in the barn, NH3 = ammonia concentration in the barn, DD indiv = latent variable for the cow individuality, Gen = latent variable for gemomics, barn = latent variable for barn characteristics).

associations among respiratory diseases and later cow productivity and claw health were also inferred by Mahmoud et al. (2017).

Quite strong associations were identified between the general effect of the housing system (CBPB or CCB) and barn wind speed with path coefficients between sys and wsp of -0.58 in the SEM for DD sick, and of -0.59 in the SEM for DD acute. The pronounced path coefficient between sys and wsp might be due to the fact that the CBPB are constructed as open buildings allowing more wind-permeability than CCB. Especially in CBPB, wps is an important factor influencing hygiene and cow health. Specifically, moisture and heat can only be released from the compost bedding material if the lying area is adequately ventilated. Leso et al. (2020) emphasized the importance of technical mechanisms to increase wsp in CBPB to make the lying area more attractive and more hygienic for the milking cows. Accordingly, in other free stall or open housing systems, favorable effects of wind speed on cow hygiene, and in causality on cow health, were reported (Pinto et al., 2020).

Moderate causal relationships were identified between ammonia emissions and cow hygiene, i.e., path coefficients of 0.33 between hyg and NH3 for the DD sick as well as for the DD acute SEM approach. The causalities can be interpreted as follows: increased contamination of cows is a strong indication of wet and unclean lying areas and alleys. In turn, an increase of manure in the barn implies an associated increase of ammonia formation (Edouard et al., 2019).

The cow-related variables play a comparatively minor role in the SEM for DD sick and DD acute, especially with regard to effects of DD indiv on ongoing productivity in terms of milk yield. The respective path coefficients were -0.06 (in the SEM for DD sick) and 0.05 (in the SEM for DD acute). The path coefficients very close to zero reflect previous phenotypic and genetic correlations between production traits and claw disorders, indicating neutral relationships or only slight genetic antagonisms (König et al., 2005; Gernand et al., 2013; Sölzer et al., 2022).

The overall genomics effect through individual significant SNPs on DD indiv was comparably small with path coefficients of 0.17 between Gen and DD indiv in the SEM for DD sick, and with 0.07 in the SEM for DD acute. Hence, the small path coefficients support the assumption for an infinitesimal model of inheritance for DD, indicating only small additional gain when focusing on only a few specific SNPs or annotated potential candidate genes. The SNPs considered in the SEM explained less than 1% of the genetic variance, supporting the results by Pimentel et al. (2011) who estimated variance components for chromosomal segments. In contrast, the path coefficient was moderate (0.36) between DD indiv and the respective EBV for DD acute. Such moderate associations support the application of the so-called "genomic herd management", i.e., early intra-herd selections based on genomic breeding values to improve the phenotypic cow health status (e.g., McNeel et al., 2017). Nevertheless, according to selection index theory (Dekkers, 2007), the trait heritability strongly determines associations between breeding values and phenotypes, supporting the larger path coefficients for barn characteristics than for cow genetic factors on phenotypic variations for low heritability DD traits. The single-step heritability from model 2 was small with 0.16 (SE = 0.03) for DD sick. Accordingly, the path coefficient was quite small (0.13) between the EBV for DD sick and DD indiv. With regard to the genomics component, the SNP *ARS-BFGL-NGS-29426* (AR6) indicated moderate effects on the latent genomics variable (Gen) in the SEM for DD sick (0.22) as well as in the SEM for DD acute (-1.07). The same SNP was significant for the interaction term in genome-wide associations with climate interactions for the trait DD (Sölzer et al., 2022). The SNP *ARS-BFGL-NGS-29426* is located in a QTL segment directly affecting the susceptibility to DD infections (Croué et al., 2019). The SNP AR2 displayed a strong effect (-1.12) on the latent variable for Gen in the SEM for DD sick, and the pathway coefficients were -0.72 for BTB, and 0.91 for HAP in the SEM for DD acute. All these SNPs were annotated with potential candidate genes which were involved in fundamental biological and immunological processes (Wong and Eirin-Lopez, 2021).

As indicated above, path coefficients show high similarity in the SEM for DD sick and in the SEM for DD acute. A larger difference was only observed for the path reflecting the housing system, i.e. of sys on DD indiv. The respective path coefficient was 0.32 in the DD sick SEM, but 0.16 in the DD acute SEM. Despite the identified overlap of genomic mechanisms for DD sick and DD acute with shared potential candidate genes, differences in disease pathogenesis for different DD stages have been reported (Döpfer et al., 1997). The correlation between genomic breeding values for DD sick and DD acute in the present study for cows with phenotypes was 0.79, indicating a similar genetic background, but also some DD stage genomic particularities. Such particularities were identified by Sölzer et al. (2024) with regard to specific housing system analyses, i.e. genetic analyses in CBPB or in CCB. Furthermore, the disease prevalence for binary traits might affect genetic parameters and path coefficients in SEM. The disease prevalence in CBPB was 10.65% for DD sick and 7.45% for DD acute. Even stronger prevalence differences were observed in CCB, with 26.93% for DD sick and 17.11% for DD acute. Effects of the disease prevalence on causal phenotypic and genetic trait relationships were proven from a theoretical perspective (Freund and Walpole, 1980), as well as based on real claw disorder data (König et al., 2008).

3.3.3. Structural equation model for DD chronic

Completely standardized estimates for the path coefficients in the SEM for DD chronic are shown in Fig. 5. Generally, the magnitude of effects among latent variables, and among latent and indicator variables, reflect relationships as outlined for DD sick and DD acute. The path coefficient from the latent variable "barn" on latent DD indiv was smaller in the SEM for DD chronic (0.28) than in the SEM for DD sick (0.38) or DD acute (0.37). Again, with regard to the barn characteristics, strongest effects were observed for the barn temperature (0.87) and humidity (0.85), which were strongly significant (according to Chin et al., 1998). The path coefficients of "barn" on NH3 (0.81) and of "hyg" on NH3 (0.33) indicate strong similarities with results for the DD sick SEM and DD acute SEM, again explaining the moderate interplay among housing characteristics, cow hygiene and ammonia emissions (Pinto et al., 2020). Moderate path coefficients were found between "wsp" and sys (-0.59) and between NH3 and "sys" (0.33), indicating the causalities of the overall housing system (CBPB or CCB) on barn air parameters and ammonia emissions. The open construction of the farm buildings in the CBPB system enables improved air-permeability compared to standard closed farm buildings in the CCB system. Such CBPB and CCB characterizations were outlined by Fehmer et al. (2021). The quite strong interplay among variables for barn characteristics in the SEM for DD chronic might explain the strong significance for the path coefficient of sys on DD indiv. Again, an indicator for the differing effects of the housing system (CBPB versus CBB) on DD chronic prevalence, which was tenfold higher in compost barns compared to the control group in the study by Sölzer et al. (2024). The cow-related factors associated with productivity directly after (Pra) and before (Prb) the DD diagnosis indicated weak direct relationships with DD indiv, or only indirect associations. For example, the path coefficient of DD indiv on Pra was 0.02, and was exactly zero for the effect of lnr on DD indiv. Productivity or lactation stage effects before the DD diagnosis were indirectly associated with DD indiv through other latent variables. The non-significant effects of lactation number and lactation stage on DD confirms results from previous studies. In contrast to other claw disorders, also König et al. (2005) found that the susceptibility to a DD infection was slightly higher in first parity than in adult cows, maybe due to evolved resistances. Furthermore, an effect of selection was postulated, because especially in the large-scale cow herds in Germany, intra-herd selection strategies strongly focused of early replacements of DD susceptible cows (Swalve et al., 2018). Very interesting is the strong association between DD indiv and the EBV for DD chronic (path coefficient of 1.85), and also of the latent genomics variable (Gen) on DD indiv (path coefficient of 1.14). In consequence, genetic selection suggests stronger favorable effects on DD chronic than on DD sick or on DD acute, again indicating

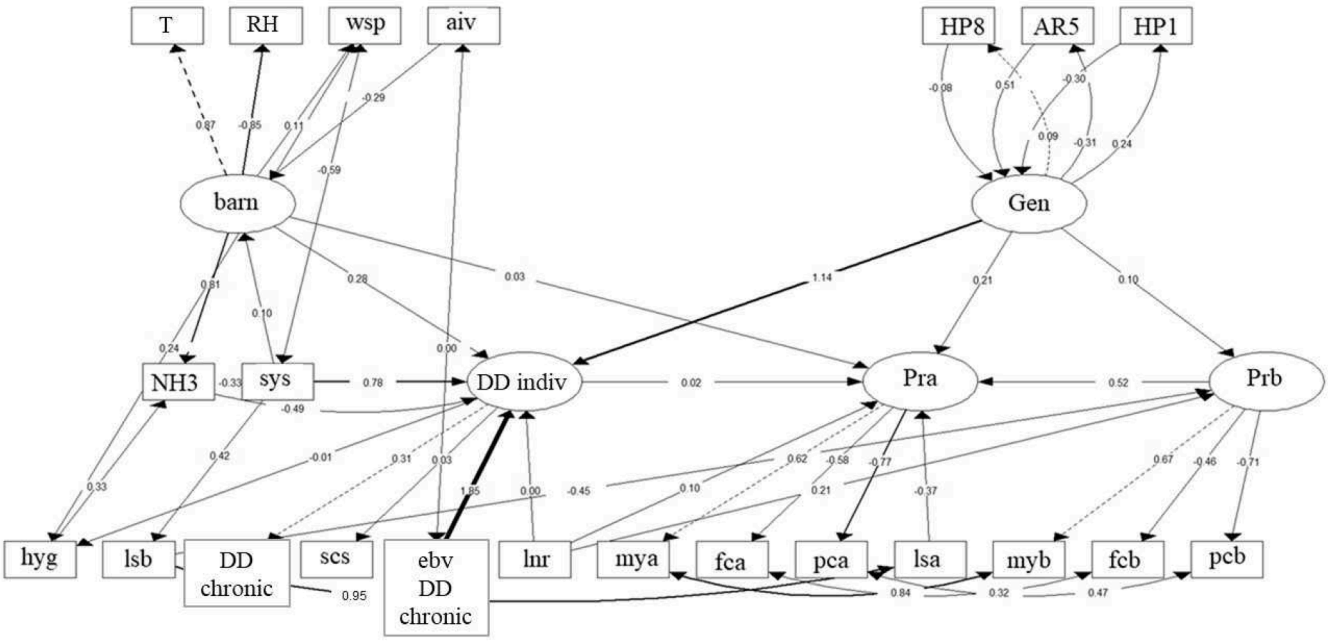


Fig. 5. Structural equation model for DD chronic with respective path coefficients for latent and observed variables ((hyg = hygiene score, scs = somatic cell score, ebv = estimated breeding value, AR6 = SNP *ARS-BFGL-NGS-29426*, AR2 = SNP *ARS-BFGL-NGS-39422*, AR5 = SNP *ARS-BFGL-NGS-75315,* BTB = SNP *BTB-0118497*, HAP = SNP *Hapmap47993-BTA-56668*, HP1 = SNP *Hapmap40478-BTA-106311*, HP8 = SNP *Hapmap58551-rs29023108*, myb = milk yield before DD scoring, fcb = fat yield before DD scoring, pcb = protein yield before DD scoring, mya = milk yield after DD scoring, fca = fat yield after DD scoring, pca = protein yield after DD scoring, lnr = lactation number, lsb = lactation stage before DD scoring, lsa = lactation stage after DD scoring, sys = housing system, aiv = air volume in the barn, T = temperature in the barn, RH = relative humidity in the barn, wsp = wind speed in the barn, NH3 = ammonia concentration in the barn, DD indiv = latent variable for the cow individuality, Gen = latent variable for gemomics, barn = latent variable for barn characteristics).

differing genetic mechanisms for the different DD stages. Major gene effects only for specific DD stages were recently reported by Oelschlaegel et al. (2022). Accordingly, in the present study, the correlations between EBVs for DD chronic with DD sick (0.58) and with DD acute (0.55) were smaller than the EBV correlation between DD sick and DD acute (0.81).

4. Conclusion

A "2-step-approach" was successfully applied to infer effects of different types of variables at different scales (climate scale, housing scale, individual cow scale, genomic scale) on the specific disease stages DD acute, DD chronic and DD sick. "2-step-approach" means the identification of most relevant housing characteristics and genomic characteristics in independent first steps, and afterwards integrating the most relevant identified factors as input parameters into the SEM in the final step 2. In step 1 in the fixed effect analyses, the climate variables T and RH, the greenhouse gas emission NH3 and specifically in the CBPB system the bedding parameters including the bedding temperature, the pH-value of the bedding material and the C:N ratio of the compost, indicated significant effects on all DD stages. In step 1 for genomics, similar pattern for Manhattan plots of SNP effects were identified for DD acute and DD sick, but differing effects with regard to the results from the GWAS and annotated potential candidate genes, were identified for DD chronic. In the integrative SEM approach, the considered specific SNPs played a minor role compared to the housing and climatic effects with path coefficients close to zero for the infection risk on DD sick and DD acute. In contrast for DD chronic, path coefficients on DD indiv were quite large for genomic breeding values for DD chronic, as well as for single SNP effects. In consequence, the success of the application of genomic tools for the reduction of DD infections strongly depends on DD stages, due to the differing genetic background in DD disease stage pathogenesis.

CRediT authorship contribution statement

Niklas Sölzer: Writing – review & editing, Methodology, Investigation, Data curation. **Kerstin Brügemann:** Validation, Investigation, Formal analysis, Conceptualization. **Petra Engel:** Writing – review & editing, Data curation, Conceptualization. **Sven König:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

We declare that we have no conflicts of interest.

Acknowledgements

The authors acknowledge funding of this study by the German Ministry for Agriculture and Food in the framework of the EU-Eranet project "Climate Care Cattle Farming Systems (CCCfarming), grant no. 2819ERA09A. We thank the H. Wilhelm Schaumann Foundation (Hamburg, Germany) for providing a scholarship to Niklas Sölzer.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2025.105650.

References

Aguilar, I., Legarra, A., Cardoso, F., Masuda, Y., Lourenco, D., Misztal, I., 2019. Frequentist p-values for large-scale-single step genome-wide association, with an application to birth weight in American Angus cattle. Genet. Sel. Evol. 51, 28. https://doi.org/10.1186/s12711-019-0469-3.

Alvergnas, M., Strabel, T., Rzewuska, K., Sell-Kubiak, E., 2019. Claw disorders in dairy cattle: effects on production, welfare and farm economics with possible prevention methods. Livestock Science 222, 54–64. https://doi.org/10.1016/j.livsci.2019.02.011.

Beaver, A., Proudfoot, K.L., von Keyserlingk, M.A.G., 2020. Symposium review: considerations for the future of dairy cattle housing: An animal welfare perspective. J. Dairy Sci. 103, 5746–5758. https://doi.org/10.3168/jds.2019-17804.

Bell, J.M., Crosby-Durrani, H.E., Blowey, R.W., Carter, S.D., Evans, N.J., 2023. Survival of bovine digital dermatitis treponemes in conditions relevant to the host and farm environment. Anaerobe 82, 102766. https://doi.org/10.1016/j.anaerobe.2023.102766.

Bewley, J.M., Taraba, J.L., McFarland, D., Garrett, P., Graves, R., Holmes, B., Kammel, D., Porter, J., Tyson, J., Weeks, S., Wright, P., 2013. Guidelines for Managing Compost Bedded-Pack Barns. The Dairy Practices Council, Ritchboro, PA.

Bewley, J.M., Robertson, L.M., Eckelkamp, E.A., 2017. A 100-year review: lactating dairy cattle housing management. J. Dairy Sci. 100, 10418–10431. https://doi.org/10.3168/jds.2017-13251.

Bielby, W.T., Hauser, R.M., 1977. Structural equation models. Annual Review of Sociology. 3, 137–161. https://doi.org/10.1146/annurev.so.03.080177.001033.

Black, R.A., Taraba, J.L., Day, G.B., Damasceno, F.A., Bewley, J.M, 2013. Compost bedded pack dairy barn management, performance, and producer satisfaction. J. Dairy Sci. 96, 8060–8074. https://doi.org/10.3168/jds.2013-6778.

Blowey, R.W., Sharp, M.W., 1988. Digital dermatitis in dairy cattle. Vet. Rec. 122, 505–508.

Bohlouli, M., Halli, K., Yin, T., Gengler, N., König, S., S, 2022. Genome-wide associations for heat stress response suggest potential candidate genes underlying milk fatty acid composition in dairy cattle. J. Dairy Sci. 105, 3323–3340. https://doi.org/10.3168/jds.2021-21152.

Bristow, A.W., Whitehead, D.C., Cockburn, J.E., 1992. Nitrogenous constituents in the urine of cattle, sheep and goats. J. Sci. Food Agric. 59, 387–394.

Canive, M, González-Recio, O., Fernández, A., Vázquez, P., Badia-Bringué, G., Lavin, J.L., Garrido, J.M., Juste, R.A., Alonso- Hearn, M., 2021. Identification of loci associated with susceptibility to Mycobacterium avium subsp. paratuberculosis infection in Holstein cattle using combinations of diagnostic tests and imputed whole-genome sequence data. Plos One 16, e0256091. https://doi.org/10.1371/journal.pone.0256091.

Chin, W.W., 1998. Commentary: issues and opinion on structural equation modeling. MIS Quarterly 22, 7–16. http://www.jstor.org/stable/249674.

Croué, I., Michenet, A., Leclerc, H., Ducrocq, V., 2019. Genomic analysis of claw lesions in Holstein cows: opportunities for genomic selection, quantitative trait locus detection, and gene identification. J. Dairy Sci. 102, 6306–6318. https://doi.org/10.3168/jds.2018-15979.

Cook, N.B., 2002. The influence of barn design on dairy cow hygiene, lameness and udderhealth. In: Proc. 35th Ann. Conv. Am. Assoc. Bov. Pract. Madison, Wisconsin, pp. 97–103.

de Greef, E., Suh, A., Thorstensen, M.J., Delmore, K.E., Fraser, K.C., 2023. Genomic architecture of migration timing in a long-distance migratory songbird. Sci Rep 13, 2437. https://doi.org/10.1038/s41598-023-29470-7.

Dekkers, J.C., 2007. Prediction of response to marker-assisted and genomic selection using selection index theory. J. Anim. Breed. Genet. 124, 331–341.

Detilleux, J., Theron, L., Duprez, J.N., Reding, E., Humblet, M.-F., Planchon, V., Delfosse, C., Bertozzi, C., Mainil, J., Hanzen, C., 2013. Structural equation models to estimate risk of infection and tolerance to bovine mastitis. Genet. Sel. Evol. 45, 6. https://doi.org/10.1186/1297-9686-45-6.

Döpfer, D., Koopmanns, A., Meijer, F.A., Szakáll, I., Schukken, Y.H., Klee, W., Bosma, R. B., Cornelisse, J.L., van Asten, A.J., ter Huurne, A.A., 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis. Vet Rec. 140, 620–623.

Döpfer, D., Anklam, K., Mikheil, D., Ladell, P., 2012. Growth curves and morphology of three Treponema subtypes isolated from digital dermatitis in cattle. Vet. J. 193, 685–693.

Durinck, S., Spellman, P.T., Birney, E., Huber, W., 2009. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. Nat. Protoc. 4, 1184–1191. https://doi.org/10.1038/nprot.2009.97.

Eckelkamp, E.A., Taraba, J.L., Akers, K.A., Harmon, R.J., Bewley, J.M., 2016. Understanding compost bedded pack barns: interactions among environmental factors, bedding characteristics, and udder health. LivestockSci. 190, 35–42. https://doi.org/10.1016/j.livsci.2016.05.017.

Edouard, N., Charpiot, A., Robin, P., Lorinquer, E., Dollé, J.-B., Faverdin, P., 2019. Influence of diet and manure management on ammonia and greenhouse gas emissions from dairy barns. Animal 12, 2903–2912. https://doi.org/10.1017/S1751731119001368.

Fehmer, L., Herold, J., Engel, P., König, S., 2021. Individual cow methane emissions in freewalk farming systems and associations with breeding traits. //. In: Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science. Wageningen Academic Publishers The Netherlands, Davos, Switzerland, p. 499. https://doi.org/10.3920/978-90-8686-918-3, 30th August –3rd September, 2021 /ISSN 1382-6077, e-ISBN: 978-90-8686-918-3.

Freund, J.E., Walpole, R.E., 1980. Mathematical statistics. Prentice Hall, Englewood cliffs, NJ.

Galama, P.J., 2014. Report. Wageningen UR Livestock Research, p. 707.

Galama, P.J., Ouweltjes, W., Endres, M.I., Sprecher, J.R., Leso, L., Kuipers, A., Klopčič, M., 2020. Symposium review: future of housing for dairy cattle. J. Dairy Sci. 103, 5759–5772. https://doi.org/10.3168/jds.2019-17214.

Gana, K., Broc, G., 2019. Structural Equation Modeling with Lavaan. John Wiley & Sons.

Gernand, E., Döhne, D.A., König, S., 2013. Genetic background of claw disorders in the course of lactation and their relationships with type traits. J. Anim. Breed. Genet. 130 (6), 435–440.

Gernand, E., König, S., Kipp, C., 2019. Influence of on-farm measurements for heat stress indicators on dairy cow productivity, female fertility, and health. J. Dairy Sci. 102, 6660–6671. https://doi.org/10.3168/jds.2018-16011.

Giambra, I.J., Jahan, Y., Yin, T., Engel, P., Weimann, C., Brügemann, K., König, S., S, 2021. Identification of thermophilic aerobic sporeformers in bedding material of compost bedded dairy cows using microbial and molecular methods. Animals 11, 2890. https://doi.org/10.3390/ani11102890.

Hair Jr., J.F., Sarstedt, M., Hopkins, L., Kuppelwieser, V.G., 2014. Partial least squares structural equation modeling (PLS-SEM): an emerging tool in business research. Eur. Bus. Rev. 26, 106–121. https://doi.org/10.1108/EBR-10-2013-0128.

Halli, K., Cohrs, I., Brügemann, K., Koch, C., König, S., 2023. Effects of temperature-humidity index on blood metabolites of German dairy cows and their female calves. J. Dairy Sci. 106, 7281–7294. https://doi.org/10.3168/jds.2022-22890.

Hu, L., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. Struct. Eq. Model.: Multidiscipl. J. 6, 1–55. https://doi.org/10.1080/10705519909540118.

Janni, K.A., Endres, M.I., Reneau, J.K., Schoper, W.W., 2007. Compost dairy barn layout and management recommendations. Appl. Eng. Agric. 23, 97–102. https://doi.org/10.13031/2013.22333.

Klein, S.L., Yin, T., Swalve, H.H., König, S., 2021. Single-step genetic parameter estimations and genome-wide associations for milk fatty acid profiles, interval from calving to first insemination and ketosis in Holstein dairy cattle. J. Dairy Sci. 104, 10921–10933.

Klitgaard, K., Nielsen, M.W., Ingerslev, H.C., Boye, M., Jensen, T.K., 2014. Discovery of bovine digital dermatitis-associated treponema spp. in the dairy herd environment by a targeted deep-sequencing approach. Appl. Enviro. Microbiol. 80, 4427–4432. https://doi.org/10.1128/AEM.00873-14.

König, S., Sharifi, A.R., Wentrot, H., Landmann, D., Eise, M., Simianer, H., 2005. Genetic parameters of claw and foot disorders estimated with logistic models. J. Dairy Sci. 88, 3316–3325. https://doi.org/10.3168/jds.S0022-0302(05)73015-0.

König, S., Wu, X., Gianola, D., Heringstad, B., Simianer, H., 2008. Exploration of relationships between claw disorders and milk yield in Holstein cows via recursive linear and threshold Models. J. Dairy Sci. 81, 395–406.

König, S., May, K., 2018. Invited review: phenotyping strategies and quantitative-genetic background of resistance, tolerance and resilience associated traits in dairy cattle. Animal 13, 897–908. https://doi.org/10.1017/S1751731118003208.

König, S., Fehmer, L., May, K., 2022. Züchterische möglichkeiten zur reduktion von methanemissionen beim rind. Züchtungskunde 94, 44–54.

König, S., Frenken, E., Giambra, I., Abdalla, E., Heise, J., Brügemann, K., 2025. Breeding concepts on heat tolerance in dairy cattle. Züchtungskunde 1, 10–25.

Kurz, J.P., Yang, Z., Weiss, R.B., Wilson, D.J., Rood, K.A., Liu, G.E., Wang, Z., 2019. A genome-wide association study for mastitis resistance in phenotypically well-characterized Holstein dairy cattle using a selective genotyping approach. Immunogenetics 71, 35–47. https://doi.org/10.1007/s00251-018-1088-9.

Lambertz, C., Sanker, C., Gauly, M., 2013. Climatic effects on milk production traits and somatic cell score in lactating Holstein-Friesian cows in different housing systems. J. Dairy Sci. 97, 319–329.

Legarra, A., Aguilar, I., Misztal, I., 2009. A relationship matrix including full pedigree and genomic information. J. Dairy Sci. 92, 4656–4663. https://doi.org/10.3168/jds.2009-2061.

Leso, L., Barbari, M., Lopes, M.A., Damasceno, F.A., Galama, P., Taraba, J.L., Kuipers, A., 2020. Invited review: compost-bedded pack barns for dairy cows. J. Dairy Sci. 103, 1072–1099. https://doi.org/10.3168/jds.2019-16864.

Mahmoud, M., Yin, T., Brügemann, K., König, S., 2017. Phenotypic, genetic, and single nucleotide polymorphism marker associations between calf diseases and subsequent performance and disease occurrences of first-lactation German Holstein cows. J. Dairy Sci. 100, 2017–2031.

McNeel, A.K., Reiter, B.C., Weigel, D., Osterstock, J., Di Croce, F.A., 2017. Validation of genomic predictions for wellness traits in US Holstein cows. J. Dairy Sci. 100, 9115–9124.

Misztal, I., Tsuruta, S., Lourenco, D.A.L., Masuda, Y., Aguilar, I., Legarra, A., Vitezica, Z., 2018. Manual for BLUPF90 Family Programs. University of Georgia. Accessed Feb. 22, 2021. http://nce.ads.uga.edu/wiki/doku.php?id=documentation.

Moshagen, M., 2012. The model size effect in SEM: inflated goodness-of-fit statistics are due to the size of the covariance matrix. Struct. Eq. Model.: Multidiscipl. J. 19, 86–98. https://doi.org/10.1080/10705511.2012.634724.

Oelschlaegel, D., Wensch-Dorendorf, M., Kopke, G., Jungnickel, R., Waurich, B., Rosner, F., Döpfer, D., Brenig, B., Swalve, H.H.H., 2022. Functional variants associated with CMPK2 and in ASB16 influence bovine Digital Dermatitis. Front. Genet. 27 (13), 859595. https://doi.org/10.3389/fgene.2022.859595.

Oliver, K.F., Wahl, A.M., Dick, M., Toenges, J.A., Kiser, J.N., Galliou, J.M., Moraes, J.G. N., Burns, G.W., Dalton, J., Spencer, T.E., 2019. Genomic analysis of spontaneous abortion in Holstein heifers and primiparous cows. Genes 10 (12), 954. https://doi.org/10.3390/genes10120954.

Pietrosemoli, N., Mella, S., Yennek, S., Baghdadi, M.B., Sakai, H., Sambasivan, R., Pala, F., Di Girolamo, D., Tajbakhsh, S., 2017. Comparison of multiple transcriptomes exposes unified and divergent features of quiescent and activated skeletal muscle stem cells. Skelet. Muscle 7, 28. https://doi.org/10.1186/s13395-017-0144-8.

Pimentel, E.C.G., Erbe, M., König, S., Simianer, H., 2011. Genome partitioning of genetic variation for milk production and composition traits in Holstein cattle. Front. Genet. 2, 19. https://doi.org/10.3389/fgene.2011.00019.

Pinto, A., Yin, T., Reichenbach, M., Bhatta, R., Schlecht, E., König, S., 2020. Phenotypic dairy cattle trait expressions in dependency of social-ecological characteristics on rural-urban gradients. Sustainability 12 (21), 9021. https://doi.org/10.3390/su12219021.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., 2007. PLINK: a tool set for whole-genome association and popution-based linkage analysis. Am. J. Hum. Genet. 81, 559–575. https://doi.org/10.1086/519795.

Raedts, P.J.M., Garcia, S.C., Chapman, D.F., Edwards, G.R., Lane, N., Rawnsley, R.P., 2017. Is systems research addressing the current and future needs of dairy farms? Animal Prod. Sci. 57, 1311–1322. https://doi.org/10.1071/AN16647.

R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

Read, D.H., Walker, R.L., 1994. Papillomatous digital dermatitis of dairy cattle: Pathologic findings. In: Proc. 8th Int. Sym. Dis. of Rum. Digit. Banff, Canada, pp. 156–158.

Reith, R.R., Sieck, R.L., Grijalva, P.C., Swanson, R.M., Fuller, A.M., Diaz, D.E., Schmidt, T.B., Yates, D.T., Petersen, J.L., 2022. Transcriptome analyses indicate that heat stress-induced inflammation in white adipose tissue and oxidative stress in skeletal muscle is partially moderated by zilpaterol supplementation in beef cattle. J. Animal Sci. 100, skac019. https://doi.org/10.1093/jas/skac019.

Rehbein, P., Brügemann, K., Yin, T., König v. Borstel, U., Wu, X.-L., König, S., 2013. Inferring relationships between clinical mastitis, productivity and fertility: a recursive model application including genetics, farm associated herd management, and cow specific antibiotic treatments. Prevent. Veterinary Med. 112, 58–67.

Rosen, C., Halback, T.R., Mugaas, R., 2000. Composting and Mulching: A Guide to Managing Organic Yard Waste. University of Minnesota Extension publication no. BU-3296-GO. University of Minnesota, St. Paul, MN.

Rosseel, Y., 2012. Lavaan: an R package for structural equation modeling and more. Version 0.5–12 (BETA). J. Stat. Softw. 48 (2), 1–36.

SAS OnDemand for Academics, 2022. GLIMMIX Procedure. SAS Institute Inc., Cary, NC, USA.

Schöpke, K., Gomez, A., Dunbar, K.A., Swalve, H.H., Döpfer, D., 2015. Investigating the genetic background of bovine digital dermatitis using improved definitions of clinical status. J. Dairy Sci. 98, 8164–8174. https://doi.org/10.3168/jds.2015-9485.

Shi, L., Lv, X., Liu, L., Yang, Y., Ma, Z., Han, B., Sun, D., 2019. A post-GWAS confirming effects of PRKG1 gene on milk fatty acids in a Chinese Holstein dairy population. BMC Genet. 20, 53. https://doi.org/10.1186/s12863-019-0755-7.

Sölzer, N., May, K., Yin, T., König, S., 2022. Genomic analyses of claw disorders in Holstein cows: Genetic parameters, trait associations, and genome-wide associations considering interactions of SNP and heat stress. J. Dairy Sci. 105, 8218–8236. https://doi.org/10.3168/jds.2022-22087.

Sölzer, N., Brügemann, K., Yin, T., König, S., 2024. Genetic evaluations and genome-wide association studies for specific digital dermatitis diagnoses in dairy cows considering genotype x housing system interactions. J. Dairy Sci. https://doi.org/10.3168/jds.2023-24207.

Solano, L., Barkema, H.W., Pajor, E.A., Mason, S., LeBlanc, S.J., Orsel, K., 2016. Prevalence and distribution of foot lesions in dairy cattle in Alberta, Canada. J. Dairy Sci. 99, 6828–6841. https://doi.org/10.3168/jds.2016-10941.

Solano, L., Barkema, H.W., Jacobs, C., Orsel, K., 2017. Validation of the M-stage scoring system for digital dermatitis on dairy cows in the milking parlor. J. Dairy Sci. 100, 1592–1603. https://doi.org/10.3168/jds.2016-11365.

Somers, J.G.C.J., Frankena, K., Noordhuizen-Stassen, E.N., Metz, J.H.M., 2005. Risk factors for digital dermatitis in dairy cows kept in cubicle houses in The Netherlands. Prevent. Veterinary Med. 71, 11–21. https://doi.org/10.1016/j.prevetmed.2005.05.002.

Swalve, H.H., Wensch-Dorendorf, M., Kopke, G., Waurich, B., Jungnickel, R., Rosner, F., Brening, B., Döpfer, D., 2018. Estimation of genomic breeding values for the susceptibility to Digital Dermatitis in Holstein dairy cattle using improved methods for phenotyping. In: Proc. 11th World Congr. Genet. Appl. Livest. Prod. Auckland, New Zealand. In: http://www.wcgalp.org/system/files/proceedings/2018/estimation-genomic-breeding-values-susceptibility-digital-dermatitis-holstein-dairy-cattle-using.pdf.

van Leenen, K., Jouret, J., Demeyer, P., Van Driessche, L., De Cremer, L., Masmeijer, C., Boyen, F., Deprez, P., Pardon, B., 2020. Associations of barn air quality parameters with ultrasonographic lung lesions, airway inflammation and infection in group-housed calves. Prevent. Veterinary Med. 181. https://doi.org/10.1016/j.prevetmed.2020.105056.

VanRaden, P.M., 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91, 4414–4423. https://doi.org/10.3168/jds.2007-0980.

Wagner, P., Brügemann, K., Yin, T., Engel, P., König, S., 2023. Inferring causalities of environmental and genetic factors for differential somatic cell count and mastitis pathogens in dairy cows using structural equation modelling. Genes 14, 2102. https://doi.org/10.3390/genes14112102.

Wong, J.M., Eirin-Lopez, J.M., 2021. Evolution of methyltransferase-like (METTL) proteins in metazoa: a complex gene family involved in epitranscriptomic regulation and other epigenetic processes. Mol. Biol. Evol. 38, 5309–5327. https://doi.org/10.1093/molbev/msab267.

Zerbino, D.R., Achuthan, P., Akanni, W., Amode, M.R., Barrell, D., Bhai, J., Billis, K., Cummins, C., Gall, A., Girón, C.G., Gil, L., Gordon, L., Haggerty, L., Haskell, E., Hourlier, T., Izuogu, O.G., Janacek, S.H., Juettemann, T., To, J.K., Laird, M.R., Lavidas, I., Liu, Z., Loveland, J.E., Maurel, T., McLaren, W., Moore, B., Mudge, J., Murphy, D.N., Newman, V., Nuhn, M., Ogeh, D., Ong, C.K., Parker, A., Patricio, M., Riat, H.S., Schuilenburg, H., Sheppard, D., Sparrow, H., Taylor, K., Thormann, A., Vullo, A., Walts, B., Zadissa, A., Frankish, A., Hunt, S.E., Kostadima, M., Langridge, N., Martin, F.J., Muffato, M., Perry, E., Ruffier, M., Staines, D.M., Trevanion, S.J., Aken, B.L., Cunningham, F., Yates, A., Flicek, P., 2018. Ensembl 2018. Nucleic Acids Res. 46 (D1), D754–D761. https://doi.org/10.1093/nar/gkx1098.

Supplementary Figures

Figure S1. LSmeans with respective standard errors for the probability of infection of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of bedding temperatures (BTc) at a depth of 20 cm. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

Figure S2. LSmeans with respective standard errors for the probability of infection of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of C:N ratios (CNc) in the bedding material. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

Figure S3. LSmeans with respective standard errors for the probability of infection of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of pH-values (pHc) in the bedding material. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

Figure S4. LSmeans with respective standard errors for the probability of infection of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of NH_3 concentrations (NH3c) in the barn air. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

Figure S5. LSmeans with respective standard errors for the probability of infection of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of air temperatures (Tc) inside the barn. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

Figure S6. LSMeans with respective standard errors for the probability of infection of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of relative humidity (RHc) in the barn air. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

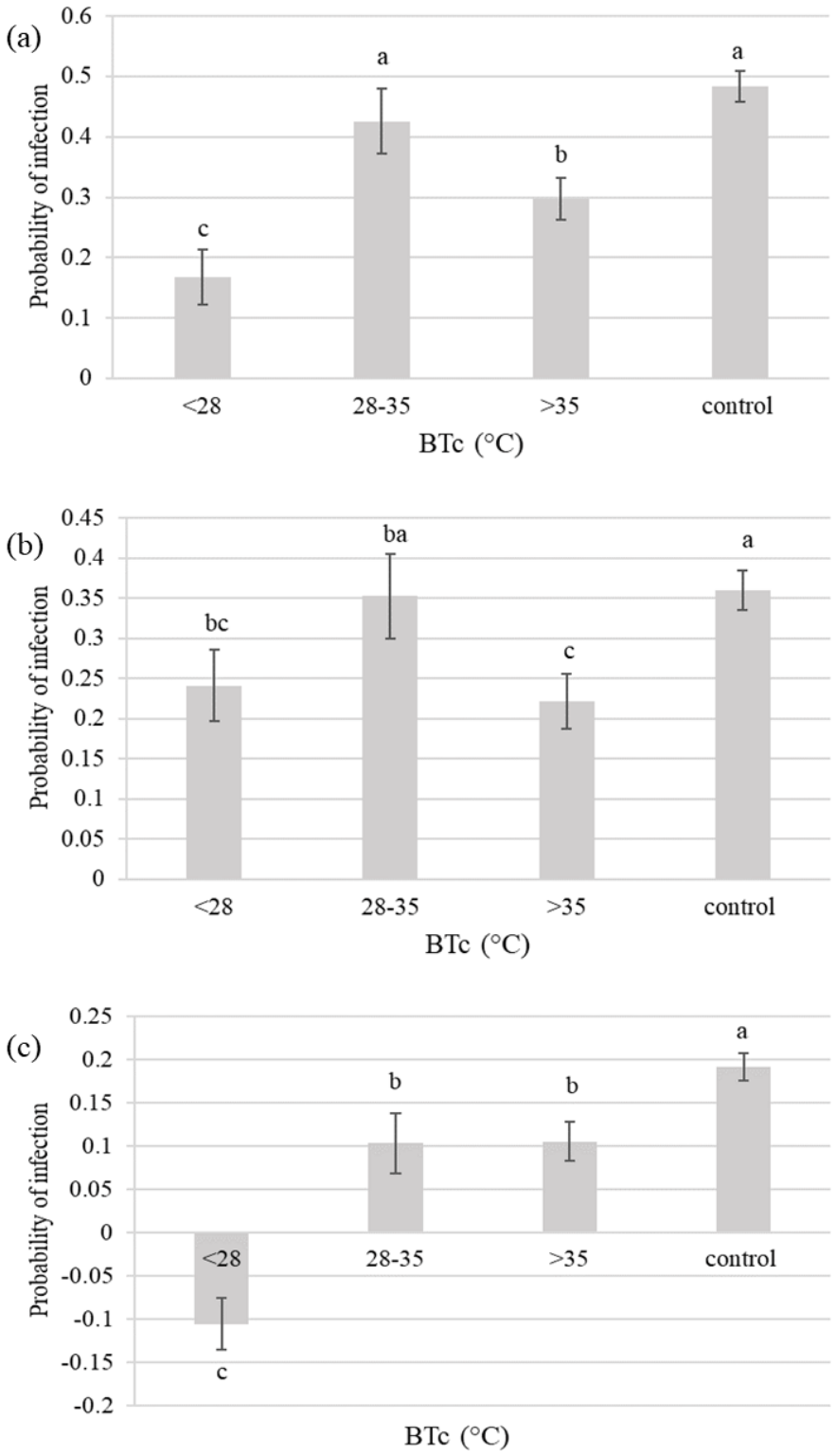


Figure S1. LSmeans with respective standard errors for the probability of occurrence of DD sick (**a**), DD acute (**b**) and DD chronic (**c**)] for different bedding temperatures (BTc) at a depth of 20 cm. Significant differences ($p < 0.05$) between LSmeans are marked with different superscripts.

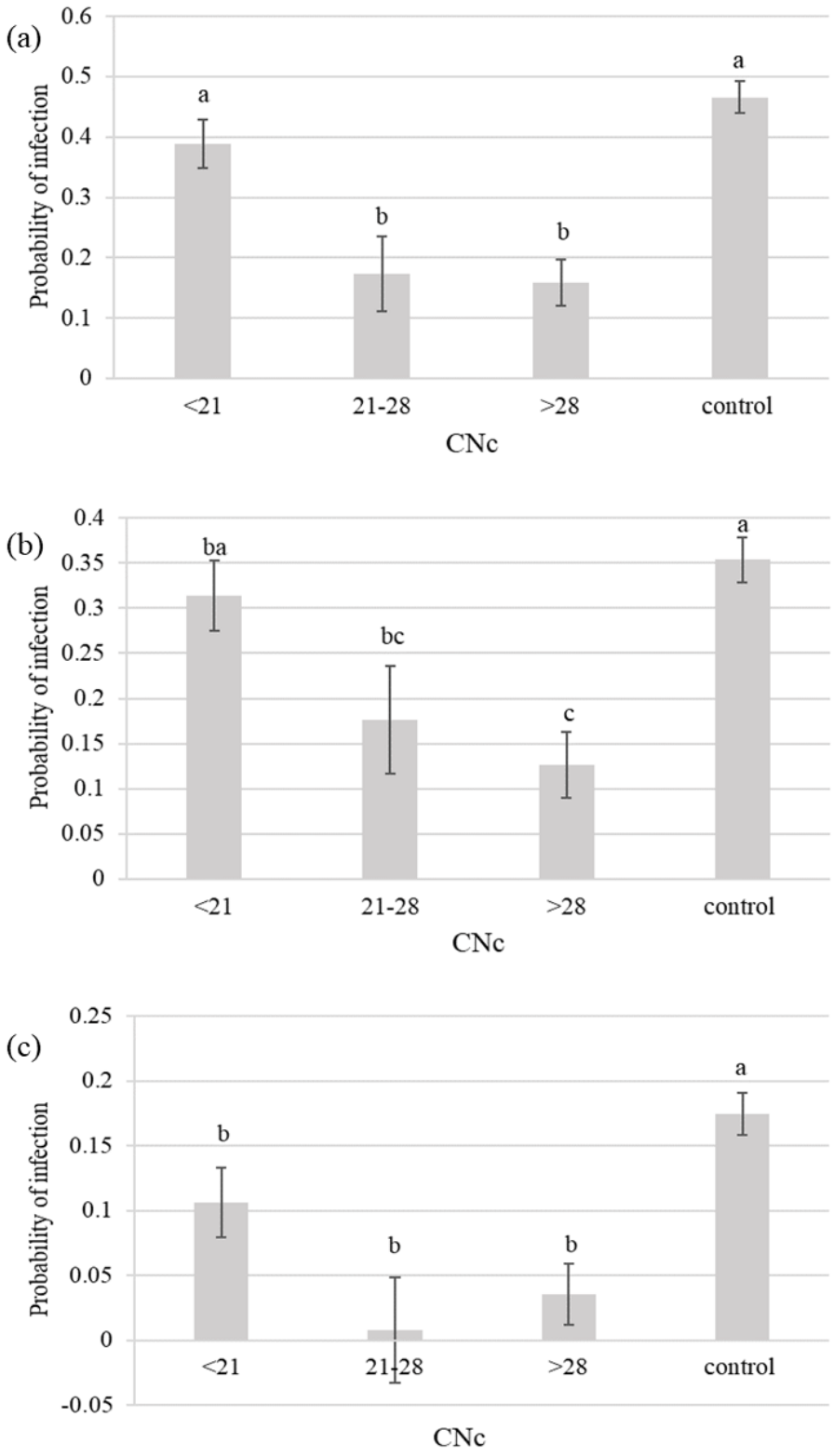
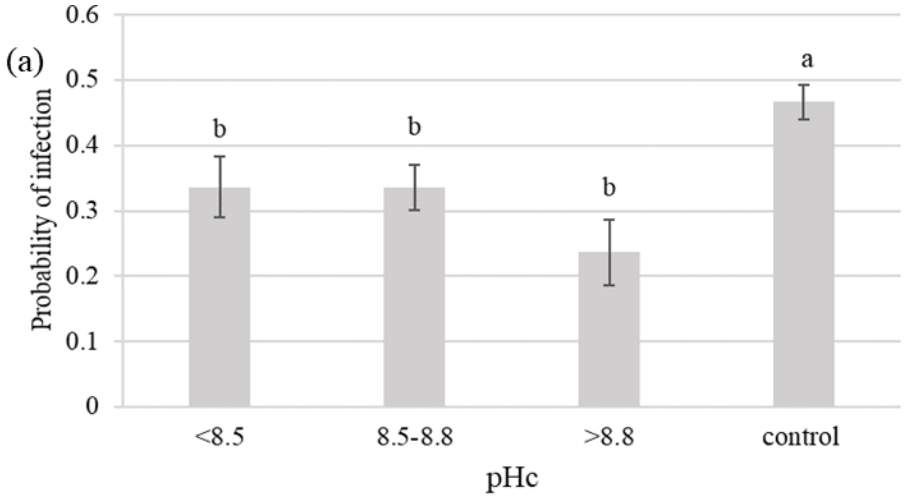
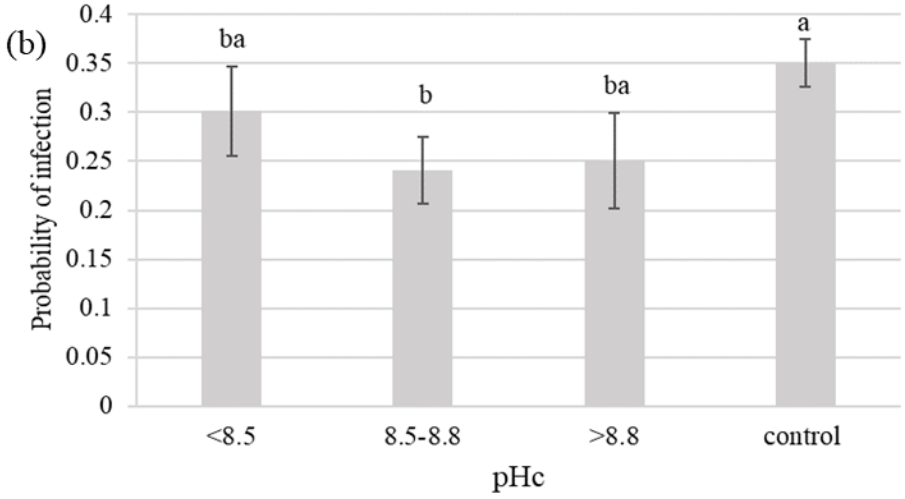


Figure S2. LSmeans with respective standard errors for the probability of occurrence of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of C:N ratios (CNc) in the bedding material. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.





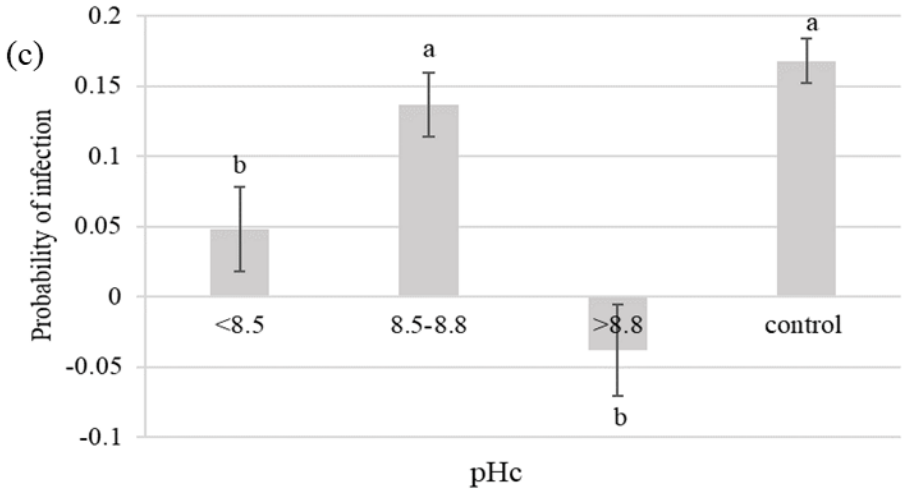


Figure S3. LSmeans with respective standard errors for the probability of occurrence of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of pH-values (pHc) in the bedding material. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

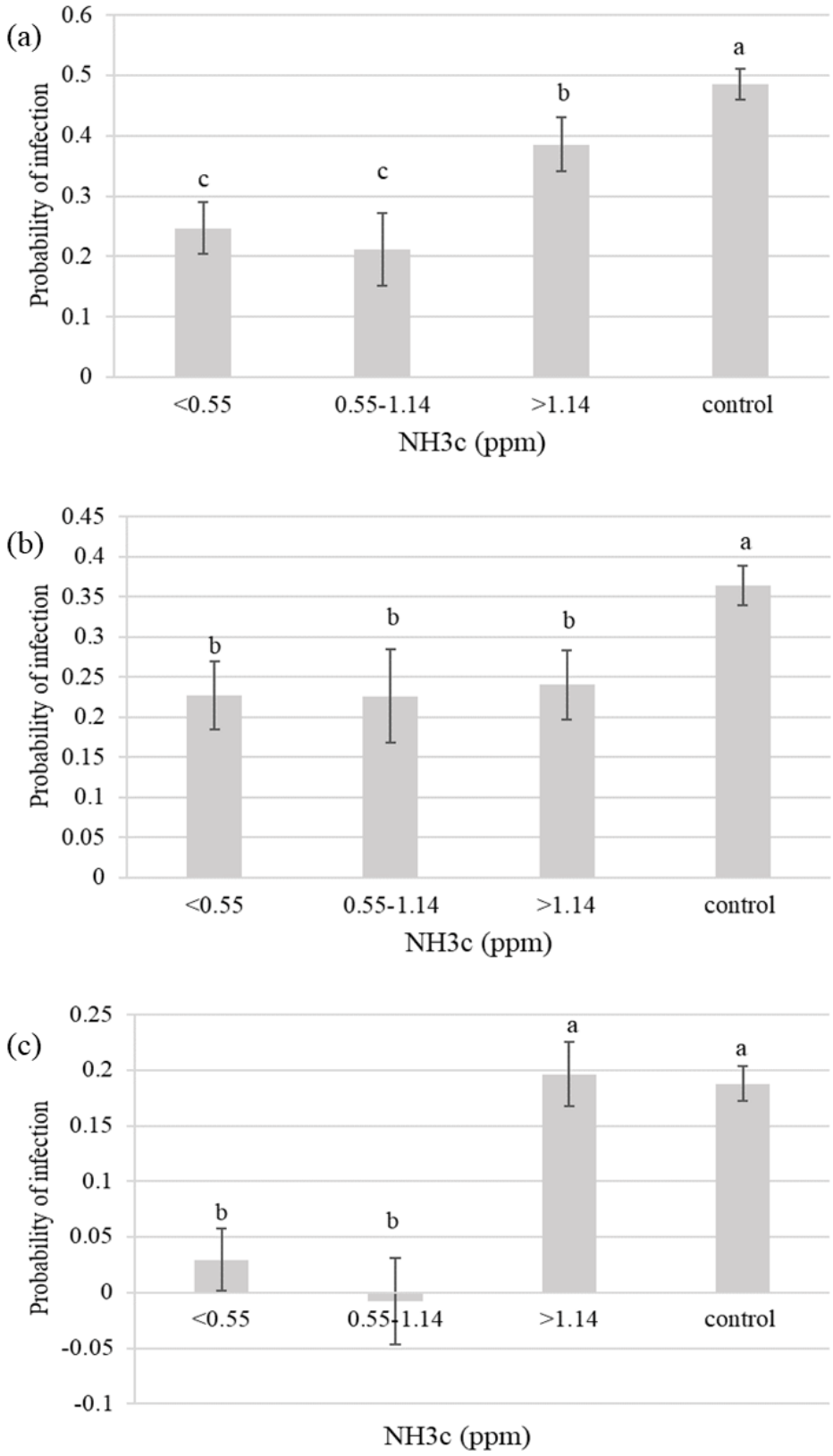


Figure S4. LSmeans with respective standard errors for the probability of occurrence of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of NH_3 concentrations (NH3c) in the barn air. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

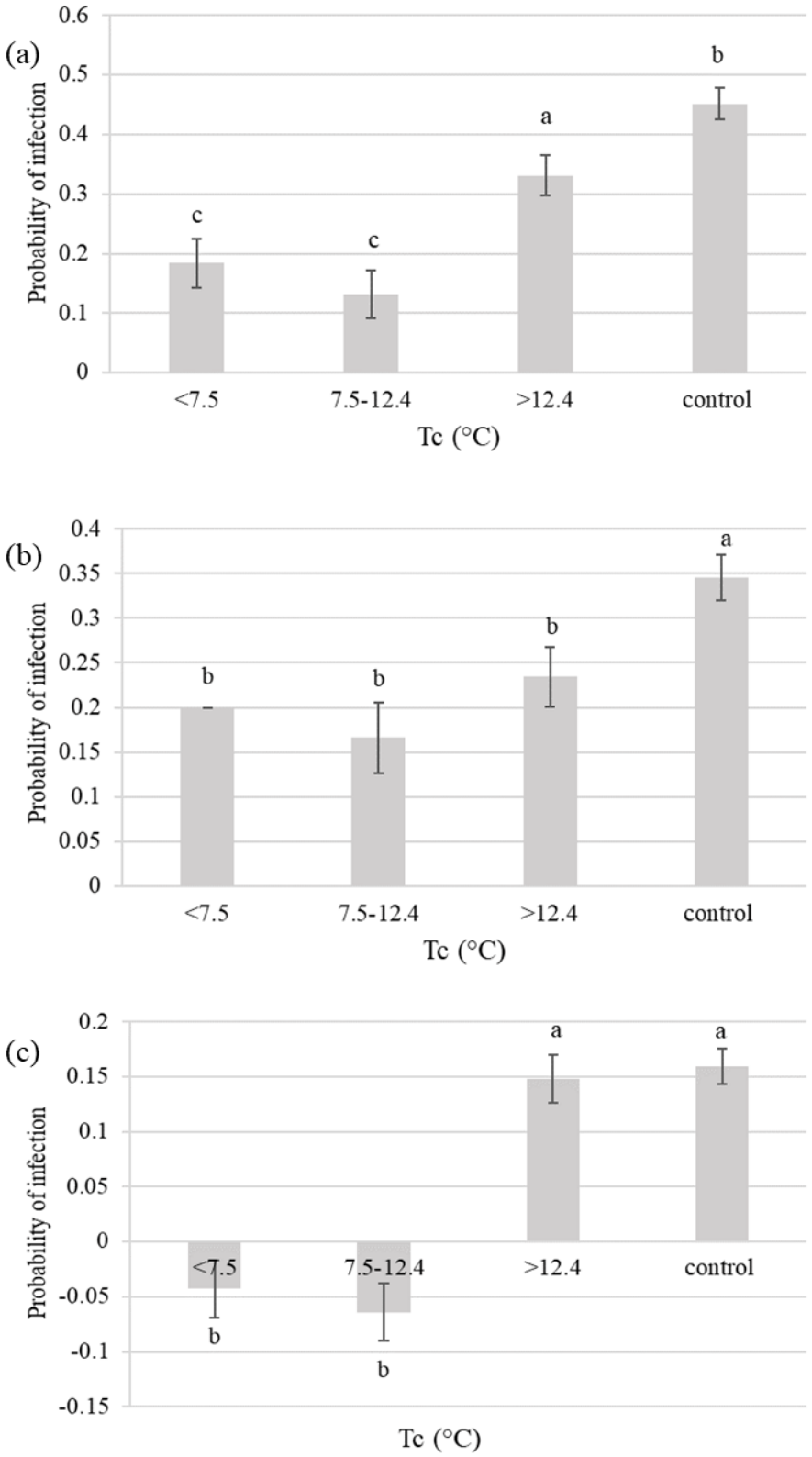


Figure S5. LSmeans with respective standard errors for the probability of occurrence of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of air temperatures (Tc) inside the barn. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

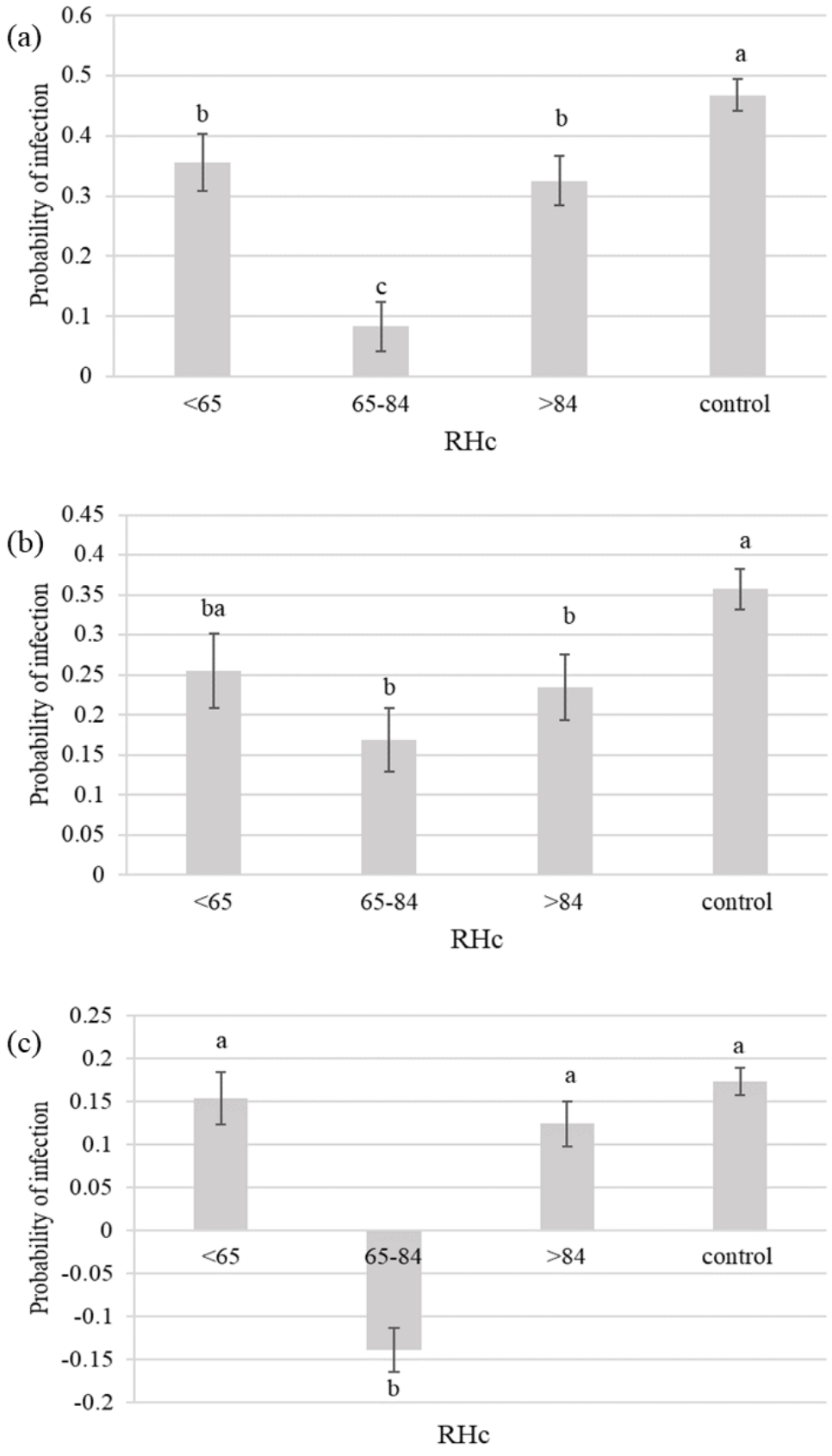


Figure S6. LSMeans with respective standard errors for the probability of occurrence of DD sick (**a**), DD acute (**b**) and DD chronic (**c**) for classes of relative humidity (RHc) in the barn air. Significant differences ($p < 0.05$) between LSmeans are marked with different letters.

5 General Discussion

The analyses in the present study dealt with the genetic background of selected claw disorders in general and with Dermatitis digitalis (**DD**) in particular. As a novel approach, interaction effects with environmental descriptors were also estimated. Furthermore, a more precise phenotyping of DD was carried out in the two housing environments of a classic cubicle barn (**CON**) and a compost bedded pack barn (**CBPB**). Furthermore, ammonia, nitrous oxide, carbon dioxide and methane emissions were measured at various points in the barns. These were analysed with other traits of the housing environment, cow phenotypes and genetic effects in structural equation models (SEM) to draw better conclusions about the parameters influencing DD. In Chapter 2, heritabilities for DD, Hyperplasia Interdigitalis (**HYP**) and Sole ulcer (**SU**) were estimated in a provided data set. Furthermore, genetic correlations between the three claw disorders and other breeding goal traits were calculated. In the further course, disease-specific candidate genes were identified and SNP effect correlations were calculated. As a final point in this chapter, SNP x heat stress (**HS**) interactions were estimated and significant SNPs were identified here as well. In chapter 3, work was now carried out on the own DD data set. Here, the occurrence in the housing systems **CON** and **CBPB** was analysed, for which the three trait categories DD sick, DD acute and DD chronic were introduced. Heritabilities and genetic correlations were estimated in relation to the three DD traits, potential candidate genes were identified and SNP x housing system interactions were calculated. In chapter 4, a two-step procedure was used. In the first step, fixed effect analyses were conducted to test the climate variables temperature (**T**) and humidity (**RH**), the ammonia emissions (**NH3c**) and the bedding characteristics: bedding temperature (**BTc**), C:N-ratio (**CNc**) and pH-value (**pHc**) for their significance with regard to the DD stages. In the second step, the parameters were linked to cow phenotypes and genetic parameters in structural equation models (**SEM**) to determine their strength of influence on a DD disease.

Further on in the chapter, the genomic evaluations are looked at, how more precise phenotyping affects disease resistance, whether alternative housing systems have an influence, and how breeding strategies can be adapted. Finally, concluding remarks and practical recommendations are given.

5.1 Genomic analyses of claw diseases

At the outset of the study design planning process, the DD scoring system was initially discussed. It was resolved that monitoring would be conducted in accordance with the M-stages proposed by Döpfer et al. (1997). Following the initial scoring on the participating farms,

it became evident that the recording of relevant phenotypes and the corresponding number of phenotypes, which are pivotal for genetic studies (Berry et al., 2011), would necessitate a considerable investment of time. It was therefore decided to analyse an existing data set, which contained a substantial amount of disease data, in order to investigate DD. This enabled the initial genetic-statistical modeling to be performed, which could subsequently be applied to the data to be collected later. During the preliminary examination of the data set, it became evident that the investigation of additional claw disorders, beyond DD, could prove beneficial. For example, Gernand et al. (2012) estimated the heritability of HYP to be 0.22 and described a closer genetic relationship to DD. In accordance with the adage that all good things come in threes, SU was selected in addition to the claw diseases already mentioned as a potential trait for investigation. The heritabilities of SU, which ranged from 0.03 to 0.11, were comparable to those of DD, depending on the estimation model employed (Onyiro et al., 2008; van der Spek et al., 2013). Moreover, Fiedler (2014) outlined positive phenotypic correlations between DD, HYP and SU, which can be attributed to causal relationships in the infection pathways. All these considerations ultimately led to the study presented in Chapter 2. In total, 17,264 first-lactating Holstein-Friesian cows were examined for the three claw disorders. The prevalences were 15.96% for DD, 8.20% for SU and 2.36% for HYP. A further advantage of the data set available was that all animals with diagnoses of the claw disorders were also genotyped and had corresponding data from the areas of conformation, female fertility, health and 305-d lactation (see Table 1 in Chapter 2). Such data quantities would not have been possible in our own data collection and of course give the study greater significance.

Finally, the study was able to show that, with regard to heritabilities, there are large differences between the estimates in the linear model (HYP 0.03; SU 0 .03) and those in the threshold model (HYP 0.23 and SU 0.10; Gernand et al., 2012; van der Spek et al., 2013). The Genome-wide association studies (**GWAS**) results for all three claw disorders identified potential candidate genes associated with cell metabolism and immune defence. For DD, two candidate genes were identified that are involved in keratin formation: *KRT33A* and *KRT33B* (McKenzie et al., 2010). In keratinocytes, possible inflammatory processes occur in the event of infection with treponema (Evans et al., 2014). The genetic correlations between DD, SU and HYP were consistently positive and amounted to 0.20 (SU) and 0.64 (HYP). Koenig et al. (2005) already suggested that there is a closer genetic relationship between the three disorders mentioned. From a pathological perspective, it can be noted that HYP often emerges after a DD infection due to external stressors (de Jesús Argáez-Rodríguez et al., 1997). It would be beneficial for future studies to investigate the assumption that the different claw disorders have a similar genetic mechanism in greater detail, in order to better identify possible genetic disease resistance.

5.2 Relationship between detailed phenotyping of Dermatitis digitalis and disease resistance

In order to successfully select animals for disease resistance, it is essential to collect accurate phenotypes (Berry et al., 2011). Schöpke et al. (2015) established a scoring system for the different DD stages, which is considerably more complex and time-consuming than the simpler binary assessment, but provides higher heritabilities (Schöpke et al., 2015). For this reason, the study design for the present work was based on the M-stages (Döpfer et al., 1997; Schöpke et al., 2015). In total, 2,980 observations from 1,311 lactating Holstein Friesian and 399 Fleckvieh-Simmental cows with corresponding scoring were collected. The heritabilities were 0.16 for DD-sick, 0.14 for DD-acute, and 0.11 for DD-chronic, which is higher than the values described in Chapter 2 for the binary DD trait. This conclusion was also reached by Wagner et al. (2021) in their study on udder health, where mastitis diagnoses by farmers (producer-recorded) were compared with cell fractions found in the milk. The genetic correlations between DD-acute and DD-chronic (0.58) suggest a partially different genetic background for acute and chronic disease progression, could not be identified by the binary approach. The GWAS of the SNP main effects also provided heterogeneous Manhattan plots, especially for DD-acute and DD-chronic, thus supporting the hypothesis of partial differences in disease pathogenesis. Nevertheless, some common candidate genes related to immunology were identified, especially for DD-sick and DD-acute. For farmers, breeding for reduced DD prevalence would be worthwhile in any case and would at least offset the potentially higher costs of more work and time required for more accurate DD scoring. On the one hand, this would result in fewer painful DD lesions, which would not only improve animal welfare but could also lead to reduced milk and performance losses. The costs of treating the animals would decrease significantly, as would the use of antibiotics (Kopke, 2019). However, it should be noted that genetic progress in one trait may usually mean a step backwards in another trait, so genetic relationships with other diseases and production traits would also need to be taken into account when breeding for DD resistance (Berry et al., 2011; Kopke, 2019). Nevertheless, a comprehensive selection would certainly lead to an improvement in claw health (Häggman and Juga 2013). Furthermore, genetically favoured animals could be characterised by a generally improved immune system, so that positive effects could also be seen in other traits (Koenig, et al., 2005; Oberbauer et al., 2013).

5.3 Influence of alternative housing systems such as the compost bedded pack barn on Dermatitis digitalis

New alternative housing systems with a free lying area such as the CBPB are designed to promote animal welfare and improve animal health on the farm (Leso et al., 2020). Lobeck et al. (2011) attribute positive effects to the CBPB, especially with regard to a possible DD disease, as the phenotypic occurrence of lameness in CON is induced by the concrete floor (Somers et al., 2005; Kester et al., 2014). This was one of the motivations for analysing the occurrence of DD in more detail in Chapters 3 and 4 in both CBPB and CON. It was already noticeable in the estimated prevalences that these were significantly lower in the CBPB farms than in the CON farms for all three DD traits. For the DD-sick characteristic in particular, the prevalences here were 10.65 in the CBPB and 26.93 in the CON. The heritabilities and variance components for the same DD traits were quite similar for the whole data set CBPB and CON. Nevertheless, additive genetic, permanent environmental, and residual variances for all DD traits were larger in the CON environment than in the CBPB. The larger differences in additive genetic variances compared to the others may be due to the lower heritability for DD traits in the CBPB data set. The genetic correlations for the same DD traits showed quite high correlations between the housing systems for DD-sick (0.85). It should be mentioned here that the genetic correlations showed quite high standard errors, but the breeding value correlations were very similar. According to the theoretical derivations of Robertson (1959), correlations of less than 0.80 indicate significant genotype x housing interactions. A significant interaction means that the respective SNP is relevant for the housing system CBPB but not for CON, or vice versa. Several significant SNPs and potential candidate genes were found, some of them associated with immunological processes or other traits of breeding interest. With regard to the LS-Means for various influencing factors from the CBPB (bedding temperature, C:N ratio) in relation to the three recorded DD traits, no direct conclusions can be reached as to whether the ‘perfect’ CBPB provides a smaller number of DD diagnoses. For example, the bedding temperature, which in the best case should be between 43.3°C - 65°C (Janni et al., 2007), shows that different temperature classes assumed the lowest values depending on the DD trait. In the SEMs, where the path coefficients allow a comment on the strength of the effect of the latent constructs. These lie in the interval between -1 and 1, whereby one can already refer to a significant correlation from a value of >= 0.2 or <= 0.2 (Chin et al., 1998). The coefficients for DD-sick (-0.32) and DD-chronic (0.78) are quite high, so that a closer relationship between the housing system and a possible DD disease can generally be mentioned here. In conclusion, the CBPB certainly has a positive effect on claw health in general and for a DD disease in particular, which is also shown by the significantly lower

prevalence of CBPB. However, the management of the lying area, which provides a dry environment for the claws, is still important (Leso et al., 2020; Bewley et al., 2017).

5.4 Effects of genotype-environment interactions

Genotype-by-environment interactions reflect how a genotype leads to different expressions of the phenotype in different environments (Hammani et al., 2009). However, the assessment of genotype-by-environment interactions for a given trait depends very much on the quality of the definition of the trait and on the number of factors influencing that trait (Barkema et al., 1998; Streit et al., 2013). Probably the most important environmental factor is the level of feeding, which is determined by the average milk yield of the herd (Hayes et al., 2003). In order to create the most consistent conditions possible for the study, and to make the environmental conditions as similar as possible for all, the study in Chapter 2 focused on large cooperating herds in northeast Germany. These had already been selected in a previously running cow reference cohort (grant number: 031A416C) and were characterised by very consistent environmental conditions. In the GWAS conducted in Chapter 2, a total of 13 significant SNPs were found, which were associated with 14 potential candidate genes. One of the SNPs, which was localised on BTA 10, exceeded pBF. The other 12 significant SNPs were located on BTA 9, 10, 16, 19 and 24. Other studies have already found potential candidate genes on BTA 3, 6 , 9, 11, 12, 19 and 26 (Naderi et al., 2018; Sánchez-Molano et al., 2019; Kopke et al., 2020). Most of the potential candidate genes found were involved in biological and immunological processes. For example, the candidate gene *DAPK2* was identified on BTA 10. *DAPK2* is involved in the differentiation of granulocytes (Britschgi et al., 2008). On BTA 19, the candidate genes *KRT33A* and *KRT33B,* which are involved in keratin formation, were found (McKenzie et al., 2010). In the GWAS for HYP, 27 significant SNPs were identified with 12 potential candidate genes. A SNP on BTA 13 exceeded pBF. In the GWAS for SU, 14 significant SNPs were identified, which were associated with 6 potential candidate genes. These were localised on BTA 4, 5, 6, 7, 9, 12, 13 and 27. In the literature, potential candidate genes had previously been described on BTA 8, 10, 11, 12, 18, 22 and 29 (van der Spek et al., 2015; Sánchez-Molano et al., 2019; Butty et al., 2021).

In the context of changing climatic conditions and an increase in extreme weather situations, SNP x HS interactions were also estimated for the three claw disorders DD, HYP and SU in Chapter 2. Since cows spend more time standing under HS conditions, this indirectly influences the increase of lameness in the herd (Polsky and von Keyserlingk, 2017). A significant interaction means that the respective SNP is relevant for DD, HYP or SU susceptibility under HS, but not under thermo neutral conditions, or vice versa. For the SNP x

HS interactions with regard to DD, two SNPs exceeded the candidate threshold. A potential candidate gene was *THBD* on BTA 13. For the SNP interaction effects for HYP, two SNPs also exceeded the candidate threshold. A potential candidate gene here was *ADGRV1* on BTA 7. This gene is associated with metabolic BW in Holstein Friesian cows (Hardie et al., 2017). For the interaction effects for SU with HS, fifteen SNPs exceeded the candidate threshold. One of the potential candidate genes, *RHOBTB1* on BTA 28, is a protein-coding gene and is involved in actin filament assembly, thus influencing beef quality in cattle (Silva et al., 2020). Studies addressing genotype x environment interactions for claw disorders are quite rare. Applying the classical multiple-trait approach, Shabalina et al. (2021) proved genotype x environment interactions for DD based on genetic correlations smaller than 0.80 between DD recorded in conventional or in organic farms. Accordingly for the claw disorder sole hemorrhage, Swalve et al. (2014) indicated that the etiology of a disease is highly depent on the particular environment. Also, in the SNP x HS interactions performed in Chapter 2, gene-specific mechanisms were only observed in specific environments.

In Chapter 3, the three DD stages recorded in the two housing environments CON and CBPB were analysed in more detail. Since the different housing systems already had a major environmental influence here, at least feeding, management and productivity should be similar. Schierenbeck et al. (2011) have already described major genetic differentiations in ‘changing environments’ for low-heritability health traits. In such context, the lower prevalence of diseases in superior environments, that is, a higher fraction of healthy cows in the CBPB system than in the more challenging CON system, might hamper the identification of genetic differences. The GWAS for DD-sick identified 23 significant SNPs with 24 associated potential candidate genes. A SNP on BTA 29 exceeded pBF. This was localised in the exon of the *TENM4* gene. The GWAS for DD-acute identified 21 significant SNPs associated with 24 potential candidate genes. One SNP on BTA 23 exceeded pBF. In the GWAS for DD-chronic, 25 significant SNPs were identified, which were associated with 33 potential candidate genes. Two of the SNPs exceeded pBF. For the interaction effects with the housing environments for DD-sick and DD-acute, 3 and 5 SNPs exceeded the candidate threshold. In this context, a significant interaction means that the respective SNP is relevant for the CBPB housing environment but not for CON, or vice versa. For DD-sick and DD-acute, the same SNP on BTA 13 was significant for the interaction component. This SNP was in close proximity to the *ASXL1* and *NOL4L* genes. Naderi et al. (2020) reported significant effects of *ASXL1* and *NOL4L* on binary DD when evaluating high-yielding HF cows from CON systems. No SNP was significantly associated with DD-chronic. The genetic correlations between the same DD traits in the different systems (CON and CBPB) were close to 0.80, disproving possible genotype x housing interactions, but only a few significant SNPs were detected. Shabalina et al. (2021) reported smaller genetic correlations for health traits, differentiating between organic and

conventional criteria. Since these include not only housing but also feeding and management, the environmental influence is of course much higher than with the CBPB-CON classification, which is only based on the housing system.

5.5 Structural equation models as a tool to enhance the understanding of Dermatitis digitalis

Since DD is a multifactorial disease with a wide range of parameters that are very difficult to model using ‘classic’ animal breeding approaches, SEMs were set up in Chapter 4. Detilleux et al. (2013) and Wagner et al. (2023) have already used SEMs to better understand the influences on udder health. Since udder health is also influenced by many direct and indirect factors, initial parallels to our project with SEM for DD could already be drawn here. Finally, three SEMs were constructed in Chapter 4 for the DD stages defined in Chapter 3. Latent variables were selected in each case for DD indiv (DD phenotypes and estimated breeding values of the cow reflecting the DD individuality), Gen (genomics), Prb (yields for test-day production trait before), Pra (yields for test-day production trait after) and barn (barn characteristic). The latent variables were in turn described by more than 20 measurable (manifest) variables. The SEMs for DD-sick and DD-acute showed similar results. Here, the latent variable barn had the greatest influence on DD indiv, followed by the housing system. Genomics and production parameters played a rather subordinate role here. Among the manifest variables related to the barn latent variable, temperature and humidity again had the greatest influence, closely followed by ammonia concentration. This once again underlines the importance of proper management in this area. Certainly, there are still some areas for optimisation to be found in the farms. It was striking that the SEM for DD-chronic showed a slightly different result. Here, genomics had a significantly greater influence on the latent variable DD indiv than in the other two SEMs, suggesting a differentiated genetic background in the pathogenesis of the individual DD stages. Furthermore, the housing system had a high influence on DD indiv, an impression that could already be observed in the phenotypic prevalences for the individual DD stages in the different housing systems. The latent variable barn had a significantly lower influence here, with the individual manifest variables having similar influence coefficients, as already reported for DD-sick and DD-acute. These results also further support the management recommendations made there. In summary, SEM is a useful addition to ‘classical’ animal breeding models. On the one hand, many variables, especially those that are difficult to measure, can be incorporated into the SEM. On the other hand, the model complexity can be reduced by using latent variables. In this way, new traits can be identified as health indicators, which can then be included in ‘classical’ modelling and breeding

value estimation (Wagner, 2024). Possible SEM for DD or claw disorders in general could be further improved by an even more precise phenotyping of the environment and other relevant parameters (Wagner, 2024). For example, the presence of microorganisms in the bedding material of the CBPB or on the walkways, in the cubicles of the CON could be analysed in more detail and incorporated into an SEM. This would allow new insights to be gained, particularly with regard to the microbial flora influencing DD. Another starting point would be a more precise analysis of the Somatic Cell Count (**SCC**) using the Differential Somatic Cell Count (**DSCC**). Since the analyses carried out in this study using the Somatic Cell Score (**SCS**) did not produce any significant results with regard to DD, Gernand et al. (2012) suspected a close genetic relationship between SCS and bacterial claw disorders. Perhaps the more precise differentiation using DSCC via an SEM could provide new insights.

5.6 Evaluation of claw disorders in the total breeding value and effects for breeding programmes

Until 1996, Holstein Friesian in Germany were bred exclusively for milk production. Due to the dairy-focused genetics, good farm and feeding management, and the increasing importance of scientific knowledge, milk production increased to a remarkable level. However, since the longevity remained at an unsatisfactory level, efforts were made to focus breeding more on improving animal health. In 1997, conformation (an indicator of claw and udder health), cell count (udder health), longevity, fertility and calving behaviour were integrated into the total breeding value on a proportional basis. The proportion of the trait milk yield decreased from 100% to 45% until 2008. This trend will continue with the introduction of the economic breeding value in 2020 and the new total breeding value in 2021. The total breeding value is now composed as follows: health (18%), longevity (18%), milk production (36%), conformation (15%), fertility (7%), calf health (3%) and calving behaviour (3%; vit, 2024). With health breeding values, claw health can be improved. Since 2019, there have been direct health traits such as Digital dermatitis. The data was provided from a large-scale project in Germany, Luxembourg and Austria and was recorded by the farms (veterinarian, hoof trimmer, farmer). The overall “RZhealth” index, which is made up of the breeding values for udder health (40%), health of the reproductive tract (15%), metabolic health (25%) and claw health (20%), provides an initial orientation for improving animal health. Since claw health was rated as the most important breeding goal in the coming years by 47% of the dairy farmers in a cattle breeding organisation in western Germany, it is worth taking a closer look at the individual traits here. The breeding value for claw health includes the following traits: dermatitis digitalis (30%), sole ulcers (15%), sole hemorrhage (15%), white line disease (15%), laminitis (15%) and

hyperplasia interdigitalis (10%). This allows direct breeding for healthier claws, although the low heritability of the traits means that the environmental influence on animal health should definitely be taken into account (vit, 2024). Since December 2023, there has been a breeding value estimation for claw health in the Simmental and Brown Swiss breeds. This includes the claw disorders (dermatitis digitalis, hyperplasia interdigitalis, white line defects, sole ulcer, heel horn erosion, laminitis and a trait that includes all other claw disorders). Depending on their economic importance, the individual traits are combined to form the claw health score (digital dermatitis 20%). The claw health score is published in the fitness block but has no influence on the total breeding value (Emmerling et al., 2023).

According to a survey of ICAR member countries, it was already clear in 2012 that claw health is a topic of great interest (Stock et al., 2012). In countries with routine breeding value estimation, hoof trimming scores have been successfully harmonised (Egger-Danner, 2015). The Scandinavian countries in particular have been pioneers in this area, with Denmark launching the 'Nordic claw recording programme' in 2010. In the meantime, claw scores have been harmonised between the four Scandinavian countries (Denmark, Sweden, Finland and Norway) and a common breeding value estimation has been developed. Since 2012, there has been a claw health index that takes into account the various relevant data sources. Genomic breeding value estimation for claw health has been available here since 2014 (NAV, 2014; Egger-Danner, 2015). The Netherlands is also among the leading nations in this regard and has been publishing breeding values for claw health since 2010 (Egger-Danner, 2015). The first breeding value estimate was based on 141,262 trimmings in 62,187 cows. The claw disorders sole hemorrhage, dermatitis digitalis, interdigital dermatitis, wall ulcer, sole ulcer, hyperplasia interdigitalis and white line disease were recorded. The traits were included in the breeding value according to economic weighting. It was found that sole hemorrhage, dermatitis digitalis and interdigital dermatitis had the highest economic values at €4 per trait. The authors of the study were thus able to calculate a cost reduction of €1.29 per cow per year when using a breeding index that includes claw health and conformation traits (van der Linde et al., 2010). For a long-term genetic improvement of claw health, a centralised collection of data at the population level is essential. Only in this way indices can be compared and used for common breeding value estimation. Therefore, an international harmonisation of claw scores is necessary. Especially with regard to the increasing genomic selection, it is becoming more and more important that the largest possible reference samples are available for new traits and that data can be considered across national borders for breeding value estimation (Egger-Danner, 2015).

One possible approach for implementing the results of this study in breeding programmes includes the following points: For the male side, there is already the breeding value DD control

for reducing DD in the herd. This promises about 25% lower ‘clinical’ DD (scored according to D.Döpfer M2 stage) in the offspring of bulls with high DD control breeding values than in the worst group of bulls for this breeding value. However, the genetic structure of the disease pathogenesis causes only minor differences between the sires in the medium breeding value range. Therefore, no relative breeding values were given, but the 10% best bulls received the DD-Premium seal and the next 15% received the DD-control seal. To achieve further success, corresponding breeding values must also be implemented on the female side. This could best be done in ongoing studies such as ‘KuhVision’, since the animals in this project are genomically examined and linearly described. Health and claw data are even already being collected. However, more precise phenotyping is needed here, especially in the area of DD, since our results showed different genetic backgrounds for the different stages of DD. This approach is associated with a significantly higher workload, but only a more precise phenotyping can also lead to a more precise genetic evaluation (Wagner et al., 2021). Only by taking these specific measures can the health of the herd be improved in the area of claw health in the wider population. Since the claw disorders DD, HYP and SU, as shown in other studies, ‘genetically occur in clusters’ (Koenig et al., 2005), targeting a claw trait in more precise phenotyping (e.g. DD), could make a corresponding contribution to improving general claw health. Another benefit for participating farms is direct information on the claw health status of the herd, enabling early selection decisions, ideally as early as the young stock herd. Such measures can quickly and specifically improve the claw health of the herd. Another approach would be to evaluate and implement the results obtained at the genomic level. This would require a larger number of animals in order to obtain a better data basis and statistical validation. Here, too, corresponding cow reference samples with simultaneous genotyping of the animals are recommended. For HYP, Zhang et al. (2019) identified a missense mutation in the receptor gene *ROR2.* Since the study provided very specific results in this area, this approach should be pursued more intensively in order to be considered in genotyping in the mid-term. Since our results confirmed polygenic effects between HYP and DD, targeted genotyping in the HYP region would also reduce susceptibility to DD. Furthermore, our results clearly show that genetic mechanisms are only important in certain environments. Here too, the SNPs found in the specific environments could be further evaluated and implemented on SNP chips to be prepared for future climatic changes and changing housing conditions and to be able to select the better adapted animals in each case.

5.7 General conclusion and recommendations

In summary, it can be said that the genomic studies have shown that DD, SU and HYP 'genetically occur in clusters'; these similar genetic mechanisms have also been described in the literature (König et al., 2005). Furthermore, it was shown that the genetic and SNP-based correlations were positive with the conformation traits and negative with fertility, udder health and productivity. From a practical point of view, this means that it makes sense to select for cows with good conformation traits, because this also results in better claw health and mitigates the negative effects on fertility, udder health and productivity. In addition, it was shown that, with regard to the SNP x HS interactions, gene-specific mechanisms only occur in specific environments. Thus, with regard to the changing climate, attention should be paid to appropriate 'heat stress' management in order to mitigate the negative effects. Further, it was demonstrated that the heritabilities and variance components for the three DD stages are very similar. However, the correlations between DD-acute and DD-chronic showed different genetic backgrounds in some cases. The Manhattan plots of the two stages also deviated considerably from each other, which supports the assumption of possible differences in the pathogenesis of the disease. The practical user can thus only be recommended to carry out a more precise phenotyping of the individual DD stages. Since a more precise phenotyping also results in a more precise genetic evaluation (Wagner et al., 2021). The workload associated with DD scoring in the milking parlour or feeding fence can also be kept manageable. This provides a good overview of the claw health of the herd and allows proper treatment and prophylactic measures to be adapted. In terms of housing environment, heritabilities and additive genetic variances were slightly higher for the three DD stages in CON than in CBPB. This is indicated by the higher genetic differentiation in the more changing conventional environment. The genetic correlations between the two housing environments, close to 0.80, indicate possible genotype x housing system interactions. The fixed effect models indicated significant influences of the bedding characteristics and climate effects on the DD stages. In general, the LSMeans for the parameters recorded in the CBPB always showed lower probabilities of DD infection than the comparison value in the CON. It was striking that there were few DD infections despite lower bedding temperatures. For practical applications, with 'lower' bedding temperatures, which should normally be at a depth of 15 cm – 31 cm, between 43.3°C – 65°C (Janni et al., 2007; Bewley et al., 2013), it is possible to keep clean animals with healthy claws. However, this requires an appropriate amount of bedding material and a re-bedding interval that is adjusted accordingly to ensure a dry and clean lying surface. The SEM for DD-sick and DD-acute attributed a major role to housing and climate effects, in contrast to genomics. On the one hand, this underlines the importance of good management in order to be able to counteract even the smallest deviations in these areas. This requires well-educated personal,

which is able to oversee the various parameters and draw the necessary conclusions quickly. Furthermore, these results once again underline the extent that dairy farming must adapt to changing climate conditions. A slightly different picture was shown in the SEM for DD-chronic, where the genomic effects were significantly more pronounced. This also highlights the importance of accurate phenotyping of the DD stages, since the success of using genomic tools to reduce DD infections depends heavily on the particular DD stage, due to the differentiated genetic background in the disease pathogenesis of each stage.

From this study, scientific and practical recommendations are the following:

- detailed disease phenotyping strategies for DD are a pre-requisite for in-depth genomic analyses
- detailed characterisations of the farm environment (housing system, bedding material, herd hygiene status, etc.) enable respective considerations of effects in statistical modelling approaches, and in consequence, to more accurate genomic predictions and should be considered in addition to accurate phenotypes and dense genotypes in large-scale cow reference samples
- the claw disorders DD, SU and HYP displayed similar genetic and genomic mechanisms, so that practical breeding on one specific disease improves the overall resistance to claw infections
- alternative welfare-friendly housing systems such as CBPB simultaneously promote claw health. For the three DD stages, the LS means for disease prevalences in CBPB were significantly lower than in CON herds. However, a proper CBPB management with strong focus on a dry and clean lying area is imperative
- SEM is particularly useful for modelling direct and indirect factors, as well as for analyses based on nested data sets. This allows the generation of new latent variables that can be considered in other models and breeding value estimates
- the developed software package for the detection of SNP x housing and SNP x climate interaction effects is freely available and can be used in ongoing genomic researches to study specified genetic mechanisms in discrete environments
- estimated climate and housing specific genomic breeding values for DD can be used for a more precise selection of sires, i.e., to select the best sire for a specific herd environment
- the analyses of genotype x emission interactions reflect scientific novelty, and the detected potential candidate genes encourage ongoing genomic studies in this regard, e.g., the analyses of gene expression pattern with environmental alterations

References

Barkema, H. W., Y. H. Schukken, T. J. Lam, M. L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. Journal of dairy science 81(2):411–419. https://doi.org/10.3168/jds.S0022-0302(98)75591-2.

Berry, D. P., M. L. Bermingham, M. Good, and S. J. More. 2011. Genetics of animal health and disease in cattle. Ir. Vet. J. 64:1-10. DOI:10.1186/2046-0481-64-5.

Bewley, J. M., L. M. Robertson, and E. A. Eckelkamp. 2017. A 100-Year Review: Lactating dairy cattle housing management. Journal of dairy science 100(12):10418–10431. https://doi.org/10.3168/jds.2017-13251.

Britschgi, A., E. Trinh, M. Rizzi, M. Jenal, A. Ress, A. Tobler, M. F. Fey, K. Helin, and M. P. Tschan. 2008. DAPK2 is a novel E2F1/ KLF6 target gene involved in their proapoptotic function. Onco gene 27:5706–5716. https://doi.org/10.1038/onc.2008.179.

Butty, A. M., T. C. S. Chud, D. F. Cardoso, L. S. F. Lopes, F. Mi glior, F. S. Schenkel, A. Cánovas, I. M. Häfliger, C. Drögemüller, P. Stothard, F. Malchiodi, and C. F. Baes. 2021. Genome-wide association study between copy number variants and hoof health traits in Holstein dairy cattle. J. Dairy Sci. 104:8050–8061. https: //doi.org/10.3168/jds.2020-19879.

Chin, W., W. 1998. Commentary: Issues and Opinion on Structural Equation Modeling. MIS Quarterly 22:7–16. http://www.jstor.org/stable/249674.

de Jesús Argáez-Rodríguez, F., D. W. Hird, J. H. de Anda, D. H. Read, and A. Rodríguez-Lainz. 1997. Papillomatous digital dermatitis on a commercial dairy farm in Mexicali, Mexico: Incidence and effect on reproduction and milk production. Prev. Vet. Med.32:275–286. https:// doi.org/10 .1016/ S0167-5877(97)00031-7.

Detilleux, J., Theron, L., Duprez, J.N., Reding, E., Humblet, M.-F., Planchon, V., Delfosse, C., Bertozzi, C., Mainil, J., Hanzen, C., 2013. Structural equation models to estimate risk of infection and tolerance to bovine mastitis. Genet. Sel. Evol. 45, 6. https://doi.org/10.1186/1297-9686-45-6.

Döpfer, D., A. Koopmanns, F. A. Meijer, I. Szakáll, Y. H. Schukken, W. Klee, R. B. Bosma, J. L. Cornelisse, A. J. van Asten, and A. A. ter Huurne. 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis. Vet Rec. 140:620–23.

Egger-Danner C. 2015. Züchterische Verbesserung der Klauengesundheit. ZAR-Seminar 2015.

Emmerling, R., R. Paul and H. Anzenberger. 2023. Lfl Tierzucht Grub.

Evans, N. J., J. M. Brown, R. Scholey, R. D. Murray, R. J. Birtles, C. A. Hart, and S. D. Carter. 2014. Differential inflammatory responses of bovine foot skin fibroblasts and keratinocytes to digital dermatitis treponemes. Vet. Immunol. Immunopathol. 161:12–20. DOI:10.1016/j.vetimm.2014.05.005.

Fiedler, A. 2014. Rusterholz's sole ulcers are home-made. https://www.milchpraxis.com/rusterholzsches-sohlengeschwuer/.

Gernand, E., P. Rehbein, U. U. von Borstel and S. König. 2012. Incidences of and genetic parameters for mastitis, claw disorders, and common health traits recorded in dairy cattle contract herds. J Dairy Sci. 95:2144–56. DOI: 10.3168/jds.2011-4812.

Hox, J., M. Moerbeek and R. van de Schoot. 2017. Multilevel analysis: Techniques and applications. Routledge.

Häggman, J. and J. Juga. 2013. Genetic parameters for hoof disorders and feet and leg conformation traits in Finnish Holstein cows. J. Dairy Sci. 96:3319-3325.https://doi.org/10.3168/jds.2012-6334.

Hammami, H., B. Rekik, and N. Gengler. 2009. Genotype by environment interaction in dairy cattle. Biotechnologie, Agronomie, Société et Environnement(13):155–164.

Hardie, L. C., M. J. VandeHaar, R. J. Tempelman, K. A. Weigel, L. E. Armentano, G. R. Wiggans, R. F. Veerkamp, Y. de Haas, M. P. Coffey, E. E. Connor, M. D. Hanigan, C. Staples, Z. Wang, J. C. M. Dekkers, and D. M. Spurlock. 2017. The genetic and biologi cal basis of feed efficiency in mid-lactation Holstein dairy cows. J. Dairy Sci. 100:9061–9075. https://doi.org/10.3168/jds.2017-12604.

Hayes, B. J., M. Carrick, P. Bowman, and M. E. Goddard. 2003. Genotype × Environment Interaction for Milk Production of Daughters of Australian Dairy Sires from Test-Day Records. Journal of dairy science 86(11):3736–3744. https://doi.org/10.3168/jds.S0022 0302(03)73980-0.

Janni, K. A., M. I. Endres, J. K. Reneau, and W. W. Schoper. 2007. Compost Dairy Barn Layout and Management Recommendations. Applied Engineering in Agriculture 23(1):97–102. https://doi.org/10.13031/2013.22333.

Kester, E., M. Holzhauer and K. Frankena. 2014. A descriptive review of the prevalence and risk factors of hock lesions in dairy cows. The Veterinary Journal. 202:222-228. https://doi.org/10.1016/j.tvjl.2014.07.004.

Kopke, G. 2019. Genomic and genetic-statistical analysis on susceptibility to dermatitis digitalis in Holstein cattle. PhD Thesis. Leipzig University, Germany.

Kopke, G., K. Anklam, M. Kulow, L. Baker, H. H. Swalve, F. B. Lopes, G. J. M. Rosa, and D. Döpfer. 2020. The identification of gene ontologies and candidate genes for digital dermatitis in beef cattle from a genome-wide association study. Int. J. Vet. Sci. Res. 6:027–037.

Koenig, S., A. R. Sharifi, H. Wentrot, D. Landmann, M. Eise, and H. Simianer. 2005. Genetic parameters of claw and foot disorders estimated with logistic models. J. Dairy Sci. 88:3316–3325. https:// doi.org/10 .3168/jds.S0022-0302(05)73015-0.

Leso, L., M. Barbari, M. A. Lopes, F. A. Damasceno, P. Galama, J. L. Taraba, and A. Kuipers. 2020. Invited review: Compost-bedded pack barns for dairy cows. Journal of dairy science 103(2):1072–1099. https://doi.org/10.3168/jds.2019-16864.

Lobeck, K. M., M. I. Endres, E. M. Shane, S. M. Godden, and J. Fetrow. 2011. Animal welfare in cross-ventilated, compost-bedded pack, and naturally ventilated dairy barns in the upper Midwest. Journal of dairy science 94(11):5469–5479. https://doi.org/10.3168/jds.2011-4363.

McKenzie, G. W., J. Abbott, H. Zhou, Q. Fang, N. Merrick, R. H. Forrest, J. R. Sedcole, and J. G. Hickford. 2010. Genetic diversity of selected genes that are potentially economically

important in feral sheep of New Zealand. Genet. Sel. Evol. 42:43. https:/ / doi .org/10.1186/ 1297 -9686-42-43.

Naderi, S., M. Bohlouli, T. Yin, and S. König. 2018. Genomic breed ing values, SNP effects and gene identification for disease traits in cow training sets. Anim. Genet. 49:178–192. https: //doi.org/10 .1111/age.12661.

Naderi, S., M. H. Moradi, M. Farhadian, T. Yin, M. Jaeger, C. Scheper, P. Korkuc, G. A. Brockmann, S. König, and K. May. 2020. Assess ing selection signatures within and between selected lines of dual purpose black and white and German Holstein cattle. Anim. Genet. 51:391–408. https://doi.org/10.1111/age.12925.

NAV Routine Evaluation (2014): Nordic cattle genetic evaluation. www.nordicebv.info.

Oberbauer, A. M., S. L. Berry, J. M. Belanger, R. M. McGoldrick, J. M. Pinos-Rodriquez and T. R. Famula. 2013. Determining the heritable component of dairy cattle foot lesions. J Dairy Sci. 96:605–13. DOI: 10.3168/jds. 2012-5485.

Onyiro, O. M., L. J. Andrews and S. Brotherstone. 2008. Genetic parameters for digital dermatitis and correlations with locomotion, production, fertility traits and longevity in Holstein-Friesian dairy cows. J. Dairy Sci. 91:4037–4046.

Polsky, L. and M. A. von Keyserlingk. 2017. Invited review: Effects of heat stress on dairy cattle welfare. Journal of dairy science, 100:8645-8657.

Reubold, U. and J. Harrington. 2018. The influence of age on estimating sound change acoustically from longitudinal data. In Suzanne Evans Wagner & Isabelle Buchstaller (eds.), *Panel studies of variation and change*, 129–151. New York: Routledge.

Robertson, A. 1959. The sampling variance of the genetic correlation coefficient. Biometrics 15:469–485. https://doi.org/10.2307/2527750.

Sánchez-Molano, E., V. Bay, R. F. Smith, G. Oikonomou, and G. Ba nos. 2019. Quantitative trait loci mapping for lameness associated phenotypes in Holstein-Friesian dairy cattle. Front. Genet. 10:926. https://doi.org/10.3389/fgene.2019.00926.

Schierenbeck, S., F. Reinhardt, R. Reents, H. Simianer, and S. König. 2011. Identification of informative cooperator herds for progeny testing based on yield deviations. J. Dairy Sci. 94:6143–6152. https: //doi.org/10.3168/jds.2011-4574.

Schöpke, K., A. Gomez, K. A. Dunbar, H. H. Swalve, and D. Döpfer. 2015. Investigating the genetic background of bovine digital dermatitis using improved definitions of clinical status. J. Dairy Sci. 98:8164–8174. http://dx.doi.org/10.3168/jds.2015-9485.

Shabalina, T., T. Yin, and S. König. 2020. Influence of common health disorders on the length of productive life and stayability in Ger man Holstein cows. J. Dairy Sci. 103:583–596. https://doi.org/10 .3168/jds.2019-16985.

Shabalina, T., T. Yin, K. May, and S. König. 2021. Proofs for genotype by environment interactions considering pedigree and genomic data from organic and conventional cow reference populations. J. Dairy Sci. 104:4452–4466. https://doi.org/10.3168/jds.2020-19384.

Silva, D. B. S., L. F. S. Fonseca, D. G. Pinheiro, A. F. B. Magalhães, M. M. M. Muniz, J. A. Ferro, F. Baldi, L. A. L. Chardulo, R. D. Schnabel, J. F. Taylor, and L. G. Albuquerque. 2020. Spliced genes in muscle from Nelore cattle and their association with carcass and meat quality. Sci. Rep. 10:14701. https://doi.org/10.1038/s41598-020-71783-4.

Somers, J.G.C.J., K. Frankena, E. N. Noordhuizen-Stassen, and J.H.M. Metz. 2005. Risk factors for digital dermatitis in dairy cows kept in cubicle houses in The Netherlands. Preventive Veterinary Medicine. 71:11-21. https://doi.org/10.1016/j.prevetmed.2005.05.002.

Stock, K. F., J. Cole, J. Pryce, N. Gengler, A. Bradley, L. Andrews and C. Egger-Danner. 2012. Survey on the recording and use of functional traits in dairy management and breeding. Proceedings ICAR Annual Meeting on 30 May 2012 in Cork, Ireland.

Streit, M., F. Reinhardt, G. Thaller, and J. Bennewitz. 2013. Genome-wide association analysis to identify genotype × environment interaction for milk protein yield and level of somatic cell

score as environmental descriptors in German Holsteins. Journal of dairy science 96(11):7318–7324. https://doi.org/10.3168/jds.2013-7133.

Swalve, H. H., C. Floren, M. Wensch-Dorendorf, K. Schöpke, R. Pijl, K. Wimmers, and B. Brenig. 2014. A study based on records taken at time of hoof trimming reveals a strong association between the IQ motif-containing GTPase-activating protein 1 (IQGAP1) gene and sole hemorrhage in Holstein cattle. J. Dairy Sci. 97:507–519. https://doi.org/10.3168/jds.2013-6997.

van der Linde, C., G. de Jong, E. P. C. Koenen and H. Eding. 2010. Claw health index for Dutch dairy cattle based on claw trimming and conformation data. J Dairy Sci. 93:4883-4891. https://doi.org/10.3168/jds.2010-3183.

van der Spek, D., J.A.M. van Arendonk, A.A.A. Vallée and H. Bovenhuis. 2013. Genetic parameters for claw disorders and the effect of preselecting cows for trimming. J Dairy Sci. 96: 6070-6078. https://doi.org/10.3168/jds.2013-6833.

van der Spek, D., J. A. M. van Arendonk, and H. Bovenhuis. 2015. Genome-wide association study for claw disorders and trimming status in dairy cattle. J. Dairy Sci. 98:1286–1295. https://doi.org/ 10.3168/jds.2014-8302.

Vereinigte Informationssysteme Tierhaltung w.V. (vit), Heideweg 1, D-27283, Verden, Germany. https:vit.de/fileadmin/DE/Zuchtwertschaetzung/Zws_Bes_deu.pdf

Wagner, P., T. Yin, K. Brügemann, P. Engel, C. Weimann, K. Schlez, and S. König. 2021. Genome-wide associations for microscopic differential somatic cell count and specific mastitis pathogens in Holstein cows in compost-bedded pack and cubicle farming systems. Animals (Basel) 11:1839. https://doi.org/10.3390/ani11061839.

Wagner, P., Brügemann, K., Yin, T., Engel, P., K¨ onig, S., 2023. Inferring causalities of environmental and genetic factors for differential somatic cell count and mastitis pathogens in dairy cows using structural equation modelling. Genes 14, 2102. https://doi.org/10.3390/genes14112102.

Wagner, P. 2024. Phenotypic and genomic analyses of udder health in compost bedded pack barns based on microscopic differential cell count and specific mastitis pathogens. PhD Thesis. Giessen University, Germany.

Wang, Z. and L. A. Goonewardene. 2004. The use of Mixed models in the analysis of animal experiments with repeated measures data. Canadian Journal of animal science. 84:1-11.

Zhang, X., H. H. Swalve, R. Pijl, F. Rosner, M. Wensch-Dorendorf and B. Brenig. 2019. Interdigital hyperplasia in holstein cattle is associated with a missense mutation in the signal peptide region of the tyrosine-protein kinase transmembrane receptor gene. Frontiers in genetics. 10:1157.

ACKNOWLEDGMENTS

In my opinion, writing a doctoral thesis is similar to sports; you can do it alone, but it works much better and easier as part of a team.

Therefore, I would like to thank Prof. König for his excellent supervision and the thought-provoking impulses during this time. Some of the experiences I was able to gain will certainly accompany me on my further path.

I would also like to thank Dr Katharina May, Dr Tong Yin and Dr Kerstin Brügemann. Thank you for always helping me when the genetic-statistical evaluations seemed to be a dead end for me. A big thanks goes to Dr Petra Engel and Lena Fehmer; together we drove a lot of kilometres and collected even more data. And it wasn't always just the professional exchange that was at the forefront. Overall, I would like to thank all my colleagues at the Institute of Animal Breeding and Genetics. The time at the institute was very pleasant thanks to all of you and characterised by many wonderful encounters. Thank you, Cordula and Jenny, for the pleasant office atmosphere.

I would also like to extend a special thanks to my family and friends. You have always encouraged me to continue and have always had my back in difficult and stressful times.

But my very special thanks go to you, Johanna. You have been there for me throughout this time, always encouraging me to carry on. Together, we will not only overcome this challenge!

Thank you so much for letting me be part of this team!

FORMAL DECLARATION

Erklärung gemäß § 10 Absatz 6 der Promotionsordnung des Fachbereichs Veterinärwesen der Justus-Liebig-Universität Gießen vom 06.11.2012

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Gießen, den 02.04.2025

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Niklas Sölzer