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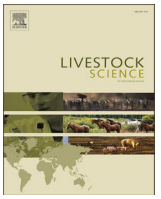

Inferring effects of barn emissions, housing conditions and genetics on specific dermatitis digitalis diagnoses in dairy cows

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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.

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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.

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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,

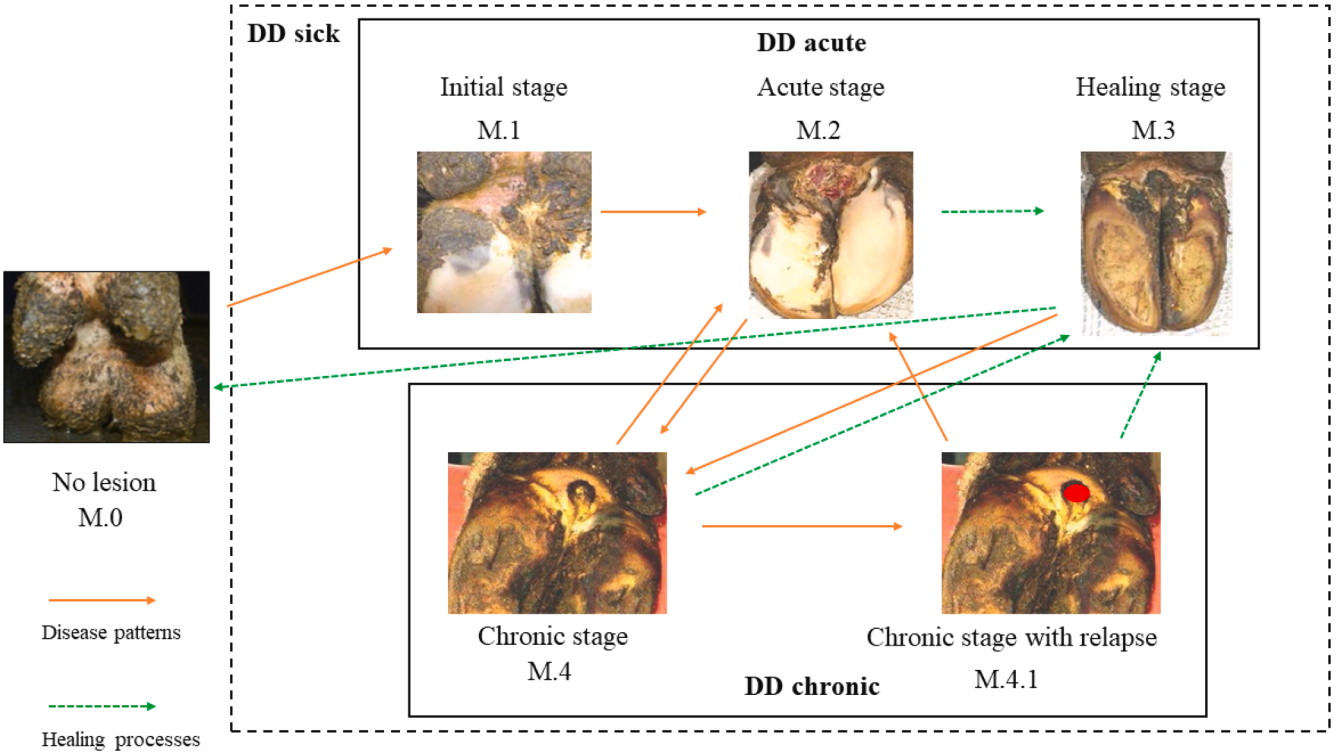

Fig. 1. Overview for the different dermatitis digitalis (DD) stages (according to Döpfer et al., 1997) and respective classifications into the DD traits DD sick, DD acute and DD chronic. The arrows indicate the change of the different DD stages, with green-dotted arrows representing the healing process, 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 **y** was the vector of observed variables, **η** was the corresponding vector for latent variables, and **υ** and **ζ** were the corresponding vectors of error terms. It was assumed that E (**υ**) = **0**, var (**υ**) = **Θ**, E (**ζ**) = **0**, and var (**ζ**) = **Ψ**. Elements (λ) of **Λ** were partial regression coefficients relating latent variables to the observed variables, while elements (β) of **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

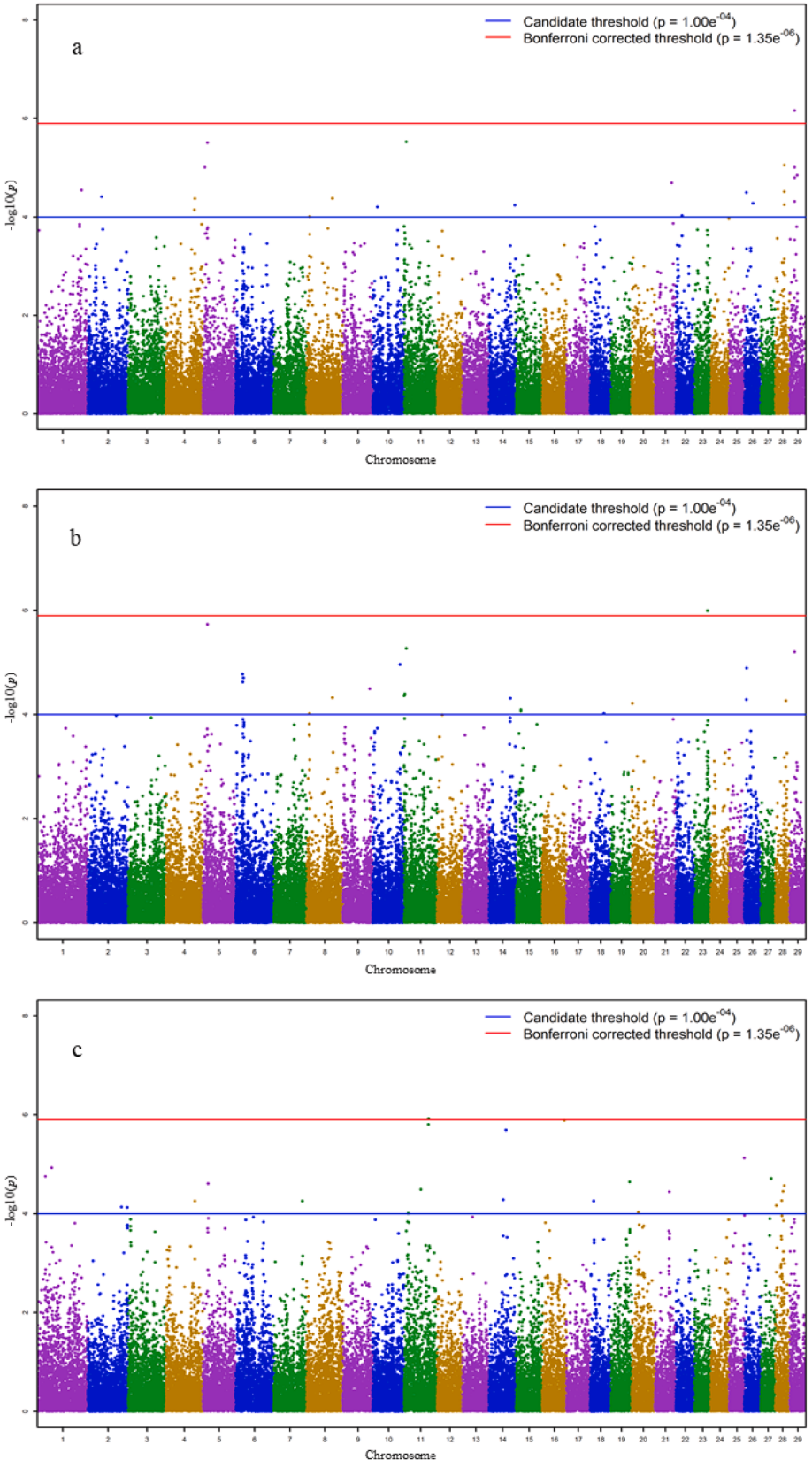

Fig. 2. Manhattan plots for the genome-wide associations for the specific dermatitis digitalis stages DD sick (**a**), DD acute (**b**) and DD chronic (**c**).

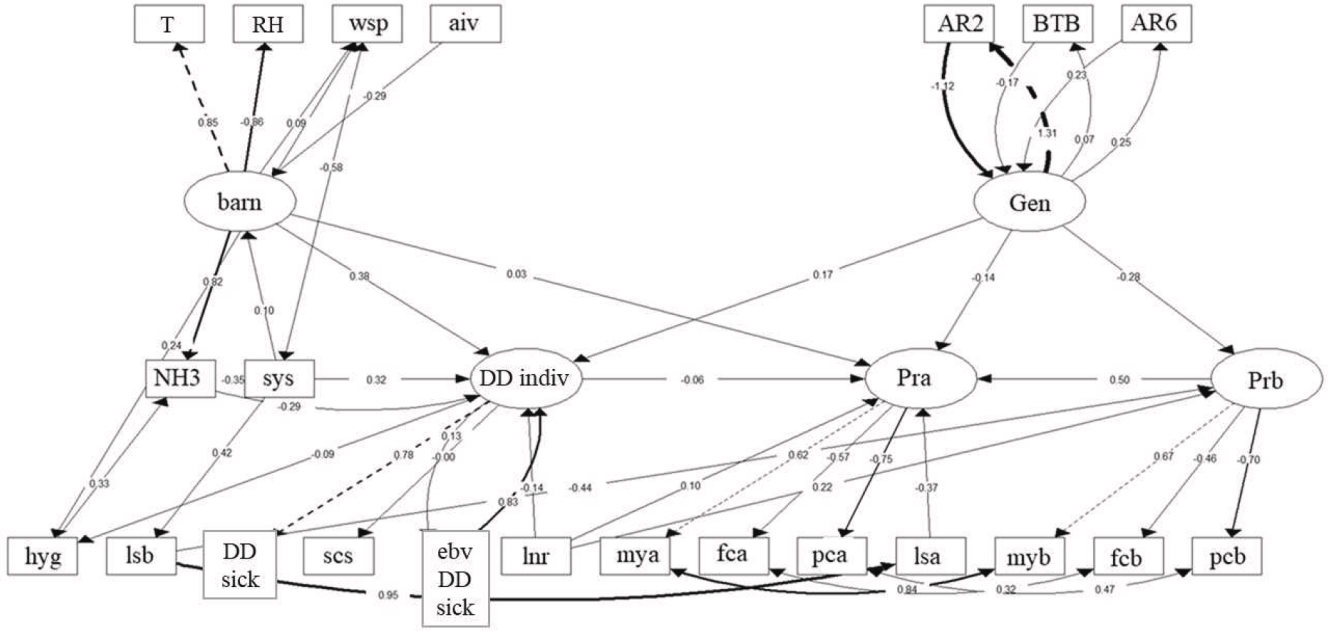

Fig. 3. Structural equation model for DD sick with respective path coefficients for latent and observed variables (hyg = hygiene score, scs = somatic cell score, ebv = estimated breeding value, AR6 = SNP *ARS-BFGL-NGS-29426*, AR2 = SNP *ARS-BFGL-NGS-39422*, AR5 = SNP *ARS-BFGL-NGS-75315,* BTB = SNP *BTB-0118497*, 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).

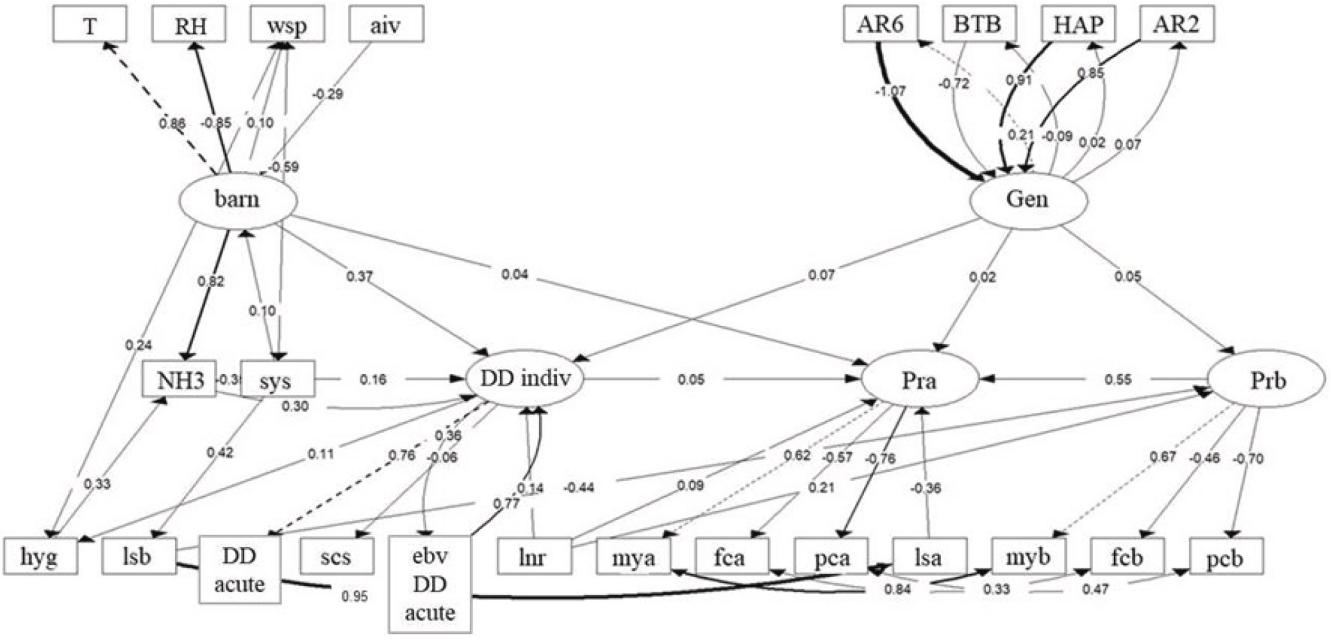

Fig. 4. Structural equation model for DD acute with respective path coefficients for latent and observed variables ((hyg = hygiene score, scs = somatic cell score, ebv = estimated breeding value, AR6 = SNP *ARS-BFGL-NGS-29426*, AR2 = SNP *ARS-BFGL-NGS-39422*, AR5 = SNP *ARS-BFGL-NGS-75315,* BTB = SNP *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

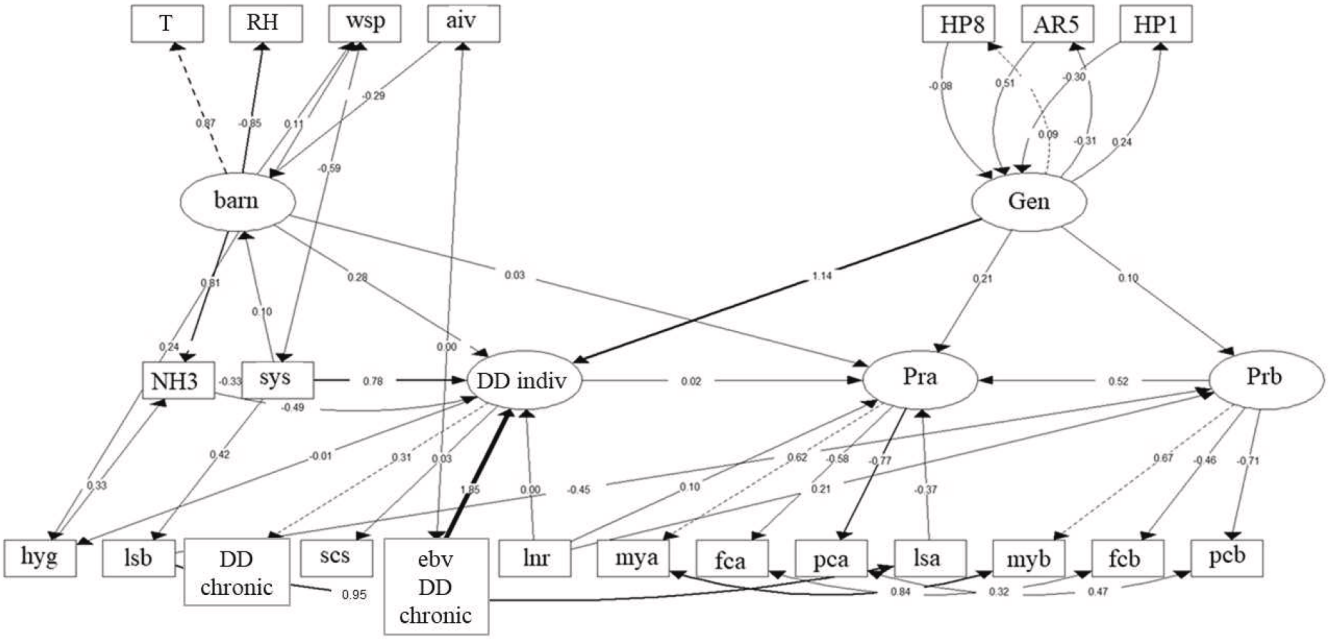

Fig. 5. Structural equation model for DD chronic with respective path coefficients for latent and observed variables ((hyg = hygiene score, scs = somatic cell score, ebv = estimated breeding value, AR6 = SNP *ARS-BFGL-NGS-29426*, AR2 = SNP *ARS-BFGL-NGS-39422*, AR5 = SNP *ARS-BFGL-NGS-75315,* BTB = SNP *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.

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Supplementary materials

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

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