

# Effect of heat stress on methane emissions of Dutch Holstein COWS

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## Abstract

Over the past decade, climate change has raised the importance of addressing heat stress in dairy cattle. The Temperature-Humidity Index (THI) is a key tool for assessing the animals' response to varying weather conditions, serving as an indicator of heat stress. Studies suggest that higher THI is linked to reduced milk production and compromised health. Despite this, little is known about the effect of temperature and humidity on methane emissions of dairy cattle. Our study aims to investigate the potential impact of temperature and humidity on methane emissions in the Dutch Holstein population. We analyzed 132,960 weekly methane concentration (CH<sub>4c</sub>) records from 7,669 cows across 72 commercial farms in the Netherlands spanning from 2019 to 2023. Each methane record was paired with weekly THI data computed from meteorological records provided by the Netherlands Meteorologisch Instituut (KNMI). Weekly THI values were calculated using the National Research Council formula, resulting in indexes ranging between 28 and 72. At the population level, a repeatability animal model included fixed effects such as herd-year-season interaction, week of lactation, and parity-age of cow at calving interaction (parities = 1, 2, 3, ≥4). Random effects included animal additive genetic effects and permanent environment effects. Methane concentrations showed a significant increase starting at a THI value of 46. At the individual level, a reaction norm model focusing on THI values higher than 46 (THI<sub>46+</sub>) was implemented. An interaction between animal additive genetic effect and THI<sub>46+</sub> level using Legendre polynomials of first order was fitted, resulting in different aggregate Estimated Breeding Values (EBVs) at different THI<sub>46+</sub> values per animal. Results demonstrated a significant THI effect ( $P < 0.001$ ) on CH<sub>4c</sub> at a population level. Estimated aggregated heritabilities at different THI<sub>46+</sub> level for CH<sub>4c</sub> ranged between 0.11 (at THI 55 and 56) and 0.50 at THI level 70 (SE=0.01). The permanent environment ratio ranged between 0.19 at THI level 70 to 0.35 at THI level 56. Based on the EBV for CH<sub>4c</sub> at the THI value lower than 46 (that is, in a thermo-neutral environment), cows were ranked into top (high emitting animals, n=50) and bottom (low emitting animals, n=50) groups. The results revealed that aggregate EBVs for low-emitting cows tended to increase as THI levels rose, whereas high-emitting cows showed decreasing EBVs at higher THI<sub>46+</sub> levels. This could potentially impact the selection of CH<sub>4</sub> emissions reduction strategies in a future affected by climate change (global warming) and/or in countries with different temperatures and humidity levels.

**Key words:** heat stress, methane emissions, temperature-humidity index.

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## Introduction

Climate change has intensified the need to address heat stress in dairy cattle over the past decade. The Temperature-Humidity Index

(THI) serves as a critical indicator of heat stress, correlating with reduced milk production and compromised health in dairy cows (Hammami et al. 2015; Herbut et al. 2018). Despite these associations, little is

understood about how temperature and humidity specifically affect methane emissions in dairy cattle. Understanding these relationships is crucial for developing effective strategies to mitigate methane emissions in dairy farming, particularly in the context of climate change. This study investigates the potential impact of temperature and humidity on methane emissions within the Dutch Holstein population.

## Materials and Methods

### Data collection

#### Methane records

The data included 7,669 Dutch Holstein cows with 132,960 records of CH<sub>4</sub> concentration (in parts per million, ppm). These records were collected in primiparous and multiparous cows during 2019 to 2023 in 72 commercial farms in the Netherlands. Parities were grouped into categories of 1, 2, 3, and 4+, and only records up to lactation week 53 were included.

#### THI records

Each CH<sub>4c</sub> record was associated with a corresponding weekly THI record computed from daily meteorological information measured in the closest meteorological station to each farm. Twenty-four meteorological stations were identified as the closest to the 72 farms. Daily meteorological records were provided by the Nederlands Meteorologisch Instituut (KNMI). Weekly THI were computed using the National Research Council formula as follows:

$$\text{THI} = (1.8 \cdot t + 32) - (0.55 - 0.0055 \cdot \text{rh}) \cdot (1.8 \cdot t - 26) \quad (1)$$

where  $t$  is daily average temperature (in degrees Celsius), and  $\text{rh}$  is the daily average relative humidity (in %). Subsequently, THI were averaged weekly to match the weekly CH<sub>4c</sub> records.

### Population-level analyses

To evaluate the effect of THI at a population level we used the following repeatability animal model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_a\mathbf{a} + \mathbf{Z}_p\mathbf{pe} + \mathbf{e} \quad (2)$$

where  $\mathbf{y}$  is the vector of phenotypes (CH<sub>4c</sub>);  $\mathbf{b}$  is the vector of fixed effects: herd, year-season interaction ( $n=312$ ), week of lactation ( $n=54$ ), age of cow at calving nested within parity ( $n=4$ ), and THI ( $n=44$ );  $\mathbf{a}$  is the vector of direct additive genetic effects;  $\mathbf{pe}$  the vector of random permanent environment effects; and  $\mathbf{e}$  is the vector of residual effects. The matrices  $\mathbf{X}$ ,  $\mathbf{Z}_a$  and  $\mathbf{Z}_p$  are the incidence matrix relating observations with the fixed effects, random genetic effects, and random permanent environment effects. Distributions of the random effects are  $\text{var}(\mathbf{a}) = \mathbf{A}\sigma^2_a$  where  $\mathbf{A}$  is the pedigree relationship matrix and  $\sigma^2_a$ , and  $\text{var}(\mathbf{pe}) = \mathbf{I}\sigma^2_{pe}$ , where  $\mathbf{I}$  is an identity matrix of an order equal to the number of observations and  $\sigma^2_{pe}$  is the residual variance, and  $\text{var}(\mathbf{e}) = \mathbf{I}\sigma^2_e$ , where  $\mathbf{I}$  is an identity matrix of an order equal to the number of observations and  $\sigma^2_e$  is the residual variance. The pedigree included 98,324 individuals, with maximum 14 generations. The estimation of the variances components and of the different effects was performed with ASReml 4.0.

### Individual level analyses

Based on the results of the previous analyses at a population level, we determined that CH<sub>4c</sub> increased from a THI value of 46. Therefore, a reaction norm model was used to evaluate the effect of THI values higher than 46 (THI<sub>46+</sub>) at an individual (cow) level. To estimate variance components and EBV for CH<sub>4c</sub> associated to heat stress the following model was used:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Q}\mathbf{Z}_a\mathbf{a}_T + \mathbf{Z}_p\mathbf{pe} + \mathbf{e} \quad (3)$$

where  $\mathbf{b}$  is the vector of fixed effects as defined for Model (2), except that THI level classes have been replaced by three THI group

classes (that is, low, mid, and high, and defined below);  $\mathbf{a}_T$  is a vector of random regression coefficients for additive genetic effects;  $\mathbf{Q}$  is the covariate matrix for first-order Legendre polynomials for THI defined below; and other vectors and matrices are the same as for Model (2).

The group “low” includes THI values between 28 and 43, the group “mid” between 43 to 58 and the group “high” between 58 to 72.

In the random terms an interaction between animal additive genetic effect and THI values ( $\geq 46$ ) was modelled using first-order Legendre polynomials as followed:

$$T = \begin{cases} 0 & \text{if THI} \leq \text{THI}_{\text{TH}} \\ \text{THI} - \text{THI}_{\text{TH}} & \text{if THI} > \text{THI}_{\text{TH}} \end{cases} \quad (4)$$

Where, THI is the THI value, and  $\text{THI}_{\text{TH}}$  is the THI (heat) threshold (46). In our case this could be represented as:

$$\text{THI}_{46+} = \text{THI}_{46+n} - 46$$

#### **Genetic parameters estimation**

Estimated (co)variance components of the random regression were used together with Legendre polynomial coefficients to calculate genetic variances and covariances for each  $\text{THI}_{46+}$  level using the methodology described in Fischer et al. (2004):

$$\mathbf{G} = \mathbf{\Phi} \mathbf{K} \mathbf{\Phi}' \quad (5)$$

where  $\mathbf{G}$  is the genetic variance-covariance matrix within trait per  $\text{THI}_{46+}$  level (matrix of size  $n \times n$ ),  $\mathbf{\Phi}$  is a matrix of order  $l \times n$ , which contains  $l$  orthogonal polynomial coefficients for each of the traits through  $n$   $\text{THI}_{46+}$  levels;  $\mathbf{K}$  is a matrix of order  $l \times l$ , which contains the estimated covariance function describing the genetic variance components for the random regression coefficients. Where  $n = 23$   $\text{THI}_{46+}$  levels (47-70) and  $l = 2$  (2 coefficients, one for the intercept and one for the lineal regression).

Phenotypic variance, heritabilities and permanent environmental ratio were estimated as follows:

$$\sigma^2_P = \mathbf{G} + \sigma^2_{pe} + \sigma^2_e \quad (6)$$

$$h^2 = \mathbf{G} / \sigma^2_P \quad (7)$$

$$pe^2 = \sigma^2_{pe} / \sigma^2_P \quad (8)$$

where  $\sigma^2_P$  is the phenotypic matrix of (co)variances,  $\mathbf{G}$  is the genetic matrix of (co)variances,  $\sigma^2_{pe}$  is the (scalar) variance for permanent environment and  $\sigma^2_e$  is (scalar) the residual variance;  $h^2$  is the heritability and  $pe^2$  is the permanent environment ratio.

#### **Estimated breeding values per THI unit**

Based on the EBV for  $\text{CH}_4c$  in a thermo-neutral conditions (that is  $\text{THI} < 46$ ), cows were ranked into top (high emitting animals,  $n=50$ ) and bottom (low emitting animals,  $n=50$ ). The purpose of this ranking was to observe how low-emitting and high-emitting animals perform under heat stress (higher THI values). Subsequently, their EBV were plotted at different  $\text{THI}_{46+}$  levels.

## **Results and Discussion**

Among the 72 herds and between 2019 and 2023, Temperature-Humidity indexes varied between 28 and 72 with an average of 50. Descriptive statistics for  $\text{CH}_4c$  (for all and per THI group) are presented in Table 1. The mean  $\text{CH}_4c$  was 572 ppm, and the mean per THI group was lower for the group with high THI compared with the group with low THI. However, the standard deviations (SD) were high. Methane concentration averages were higher than those reported by van Breukelen et al. (2022; in a subset of this population), Sypniewski et al. (2021) in Polish Holstein cows and Manzanilla-Pech et al. (2022) in Danish Holstein cows.

Table 1. Descriptive statistics for CH<sub>4</sub> concentration (ppm) for the whole population, and per group of THI value.

THI group	THI range	No. obs	No. animals	Mean	SD
Whole	28-72	132,960	7,669	572	294
Low	28-43	27,960	5,614	597	312
Mid	43-58	83,101	7,332	571	296
High	58-72	30,511	5,602	561	284

**Population level analyses**

Figure 1 shows the effects on THI level on CH<sub>4</sub>c based on the solutions of Model 2. A positive effect on CH<sub>4</sub>c is observed with increasing THI values starting at THI level of 46. Therefore, this level was used as a heat threshold (THI<sub>46+</sub>) as suggested by McWhorter et al. (2023) for the individual level analyses. However, McWhorter reported a higher heat threshold (69 THI), this could be due to geographical and climate differences.

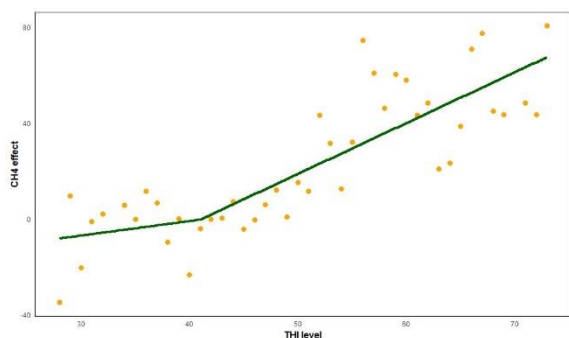


Figure 1. Effect of THI level on CH<sub>4</sub> concentrations.

**Genetic parameters for CH<sub>4</sub>c**

Estimated (co)variance components of the random regression were used together with Legendre polynomial coefficients to calculate genetic variances and covariances for each THI level after the heat threshold (THI<sub>46+</sub>). Heritabilities for CH<sub>4</sub>c at different THI<sub>46+</sub> level are presented in Figure 2. Heritabilities ranged between 0.11 (at THI 55 and 56) and 0.50 at THI level of 70. There h<sup>2</sup> agree with heritabilities for CH<sub>4</sub>c in the literature and ranging between 0.1 to 0.3 (Difford et al. 2020, Sypniewski et al. (2021) and van Breukelen et al. (2022).

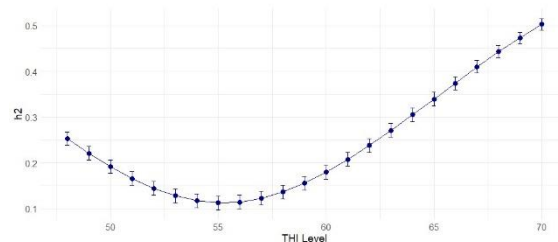


Figure 2. Heritabilities for CH<sub>4</sub> concentrations per THI level (from the heat threshold defined at a THI equal to 46). Average standard error was 0.01.

Genetic correlations for CH<sub>4</sub>c between different THI<sub>46+</sub> levels are presented in Figure 3. Genetic correlations between consecutive THI<sub>46+</sub> levels are high as expected. However, as the THI<sub>46+</sub> levels become more distant from each other, the correlations can approach zero (e.g., between THI 47 and THI 59) or show moderate negative correlations (e.g., -0.5 between THI 46 and THI 70). This negative correlation could potentially impact the selection of CH<sub>4</sub> emissions reduction strategies in a future affected by climate change (global warming) and/or in countries with different temperatures and humidity levels (e.g., Mediterranean vs. Scandinavian countries).

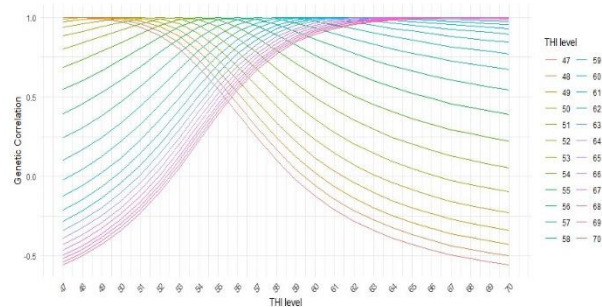


Figure 3. Genetic correlations for CH<sub>4</sub>c between different THI<sub>46+</sub> levels

**Estimated breeding values per THI unit**

When plotting aggregate EBVs of the 50 top animals and 50 bottom animals at different THI<sub>46+</sub> levels (Figure 4), the bottom animals showed an increased CH<sub>4</sub>c effect at higher THI levels, whereas the top animals reduced their CH<sub>4</sub>c effect. McWhorter et al. (2023) reported a study where 100 animals with high genetic

merit for milk production and 100 animals with high genetic merit for heat tolerance were plotted at different THI levels. The first group experienced a slight decrease in production (Holstein) and a more drastic decrease in production (Jersey) at higher THI levels. In contrast, the second group increased their production at higher THI levels. However, in this study, the 50 top and bottom animals were chosen based on their EBV at the THI<sub>46+</sub> level, which represents the intercept, and were further plotted at different THI levels.

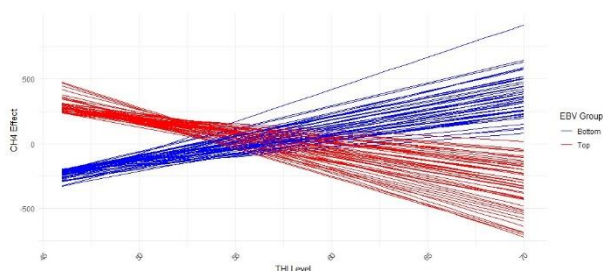


Figure 4. EBV for CH<sub>4c</sub> for top 50 (high emitting) and bottom 50 (low emitting) cows at different THI<sub>46+</sub> levels

## Conclusions

This pilot study aimed to assess the impact of THI on CH<sub>4</sub> emissions (concentration), and revealed significant findings that is the bottom animals showed an increased CH<sub>4c</sub> effect at higher THI levels, whereas the top animals reduced their CH<sub>4c</sub> effect. This knowledge could be pivotal for future selection strategies aimed at reducing CH<sub>4</sub> emissions, taking into account genotype by environment interactions. Additionally, further validation through multi-trait analysis, including milk production, is recommended. This would allow us to form the groups with animals based on their methane emissions at the same production level e.g. high and low emitting animals at a high milk production level.

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