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Genotype-by-environment interaction for methane emissions in Holstein cows under heat stress conditions

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Introduction

Livestock production is a major contributor to greenhouse gas emissions, with enteric methane from ruminants among the largest sources (Gerber *et al*., 2013). Given methane's high global warming potential, its mitigation is a priority for sustainable agriculture (Costa Jr. *et al*., 2021). Dairy cattle, due to their substantial methane output and rising global milk demand, represent a critical leverage point for reducing agricultural emissions, requiring integration of mitigation strategies into breeding and management programs (Malyugina *et al*., 2025). However, intensifying heat stress under climate change adds complexity, as it compromises productivity and may alter emission dynamics (Chen *et al*., 2024; Gauly and Ammer, 2020). Heat stress reduces feed intake, milk yield, and reproductive performance while increasing health risks (Dikmen *et al*., 2025; Nascimento *et al*., 2025).

Importantly, genotype-by-environment (G×E) interactions for methane emissions under heat stress could influence genetic evaluations, creating opportunities for breeding low-emitting animals adapted to stressful conditions. Despite growing evidence of G×E effects on production traits, research on how heat stress influences the genetic architecture of methane emissions (such as heritability estimates and sire ranking stability) remains limited. Manzanilla-Pech *et al*. (2024) provided important insights into these interactions in Dutch dairy cattle, but comparable studies in U.S. populations are lacking. Addressing this gap is critical because ignoring G×E may lead to suboptimal selection decisions, as animals ranked highly under thermoneutral conditions may not perform similarly under stress. This study investigates the genetic architecture of methane production in Holstein cows across a temperature-humidity index (THI) gradient, focusing on the impact of heat stress on heritability and sire ranking, thereby providing novel insights for climate-resilient breeding strategies.

Materials & Methods

Animals, phenotypes and weather information. Data were collected from five commercial Holstein herds across the United States using GreenFeed (C-Lock Inc., Rapid City, SD), an open-circuit gas quantification system. GreenFeed measures methane emissions from individual animals on a voluntary basis by capturing breath and eructation gases, using pelletized bait to attract cows. Phenotypic records underwent quality control by excluding observations >3.5 SD from the mean, removing the first measurement week, and applying filters for GreenFeed visit duration (≥2 min) and airflow (20–45 L/s) in accordance with C-Lock guidelines. After filtering, weekly averages of methane production (MetP, g/day) were computed for 9,923 cows. Weather data were retrieved from the NASA POWER database and used to calculate daily THI, which was aggregated into weekly averages to align with methane records.

Statistical model. A single-trait broken-stick reaction norm linear model was applied to estimate heritability across the temperature–humidity index (THI) gradient and to obtain

genomically estimated breeding values (gEBV) under heat stress (HS) conditions. A THI threshold of 70 was used to define the onset of HS, based on prior evidence showing a stable milk yield response below this value and a consistent decline thereafter (Vukasinovic *et al*., 2025). Accordingly, breeding values were assumed constant under thermoneutral conditions (THI ≤ 70), while animal-specific linear responses (slopes) were estimated for THI > 70 to capture genetic differences in HS sensitivity. Genomic evaluations were performed using single-step genomic BLUP with the algorithm for proven and young animals implemented in the BLUPF90 family of programs (Misztal *et al*., 2014). Fixed effects included lactation (1–4+) by lactation stage, age-at-calving class, and herd-year of calving, with size differences indirectly accounted for through lactation grouping. Random effects included intercept and slope terms for additive genetic and permanent environmental effects under HS, and residual variances were assumed homogeneous across the THI gradient.

Grouping bulls to assess G×E across THI gradient. Bulls with at least 100 progeny were first ranked based on their gEBV for methane emission under thermoneutral conditions (THI ≤ 70), representing baseline genetic differences in emission potential in the absence of heat stress. From this ranking, two extreme groups were defined: High emitters (n = 100) and Low emitters (n = 100). These groups were then tracked across increasing THI values to evaluate how their gEBV changed as environmental conditions worsened, with particular emphasis on severe heat stress (e.g., THI = 85). Although gEBV—and thus ranking—can be determined for each specific THI value in the reaction norm framework, defining top and bottom group based on GEBVs under thermoneutral conditions allowed for assessment of genotype-by-environment interaction, ranking stability, and differentiating sensitivity to heat stress in a manner analogous to Manzanilla-Pech *et al*. (2024).

Results & Discussion

Figure 1 shows that heritability of methane production increased steadily with THI, rising from approximately 0.22 at THI 70 to about 0.44 at THI 85, indicating that genetic differences become more pronounced under heat stress. Similar trends were reported by Manzanilla-Pech *et al*. (2024), who observed increasing heritability of methane emissions with THI, although across a narrower gradient (46–70). Comparable responses to heat stress have been reported for other traits in U.S. Holstein cattle, including higher heritabilities for feed intake traits (Nascimento *et al*., 2025) and for milk yield and conception to first service (Vukasinovic *et al*., 2025), reinforcing the role of heat stress in modifying genetic parameters across performance traits.

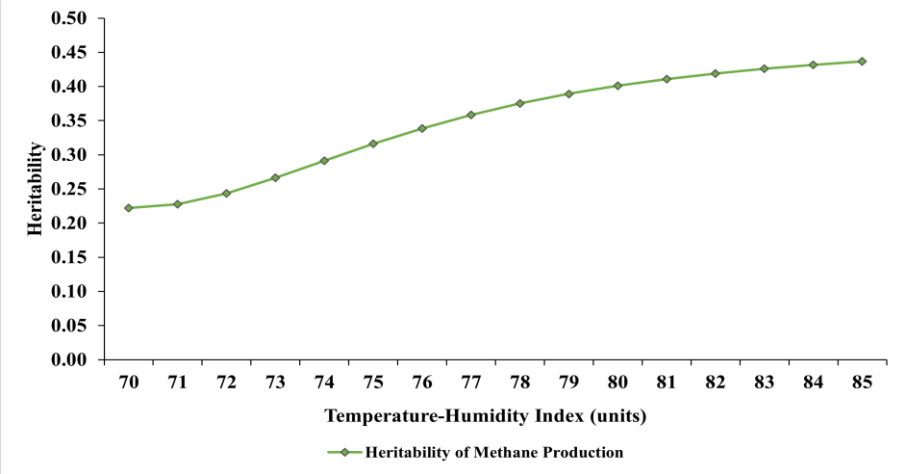

Figure 1. Heritability of methane production across the Temperature-Humidity Index gradient.

Collectively, these results confirm that heat stress introduces GxE interactions that affect ranking stability for multiple relevant traits. Such interactions have important implications for breeding programs since animals ranked highly under thermoneutral conditions may not maintain the same ranking under heat stress, and ignoring this variability could lead to suboptimal selection decisions (Tiezzi *et al*., 2017). Figure 2 illustrates this re-ranking effect, showing how bulls classified as high methane emitters under thermoneutral conditions (red lines) decrease in emission levels under heat stress, while low emitters (blue lines) tend to increase, resulting in crossover patterns across the THI gradient. These results align with those presented by Manzanilla-Pech *et al*. (2024), who observed a similar crossover pattern when plotting aggregate EBVs of the 50 top and 50 bottom animals across increasing THI levels. In their study, animals classified as high methane emitters under thermoneutral conditions showed a reduction in methane effect as THI increased, while low emitters exhibited the opposite trend—mirroring the re-ranking observed in our analysis (Figure 2). This consistency across studies reinforces the presence of genotype-by-environment interactions for methane emissions and highlights the risk of relying solely on evaluations under thermoneutral conditions.

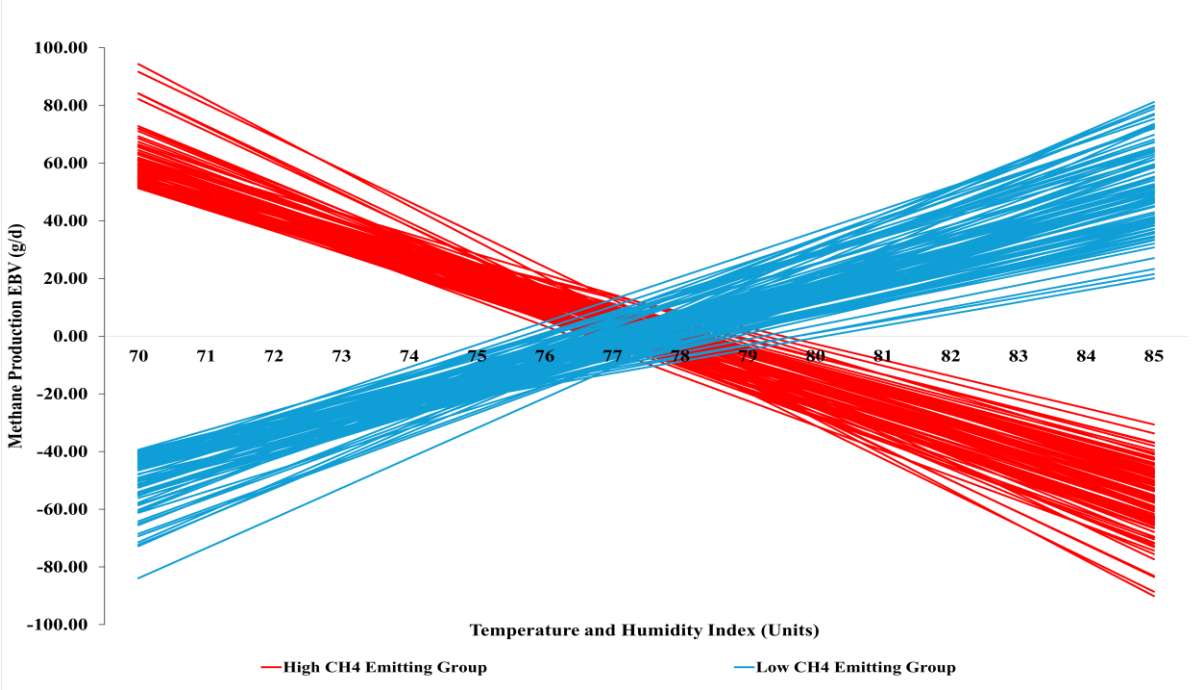

Figure 2. Sire re-ranking for methane emissions across THI values, illustrating genotype-by-environment interactions between high and low emitter groups.

Recent findings by McWhorter *et al*. (2023) provide strong evidence that G×E interactions under heat stress affect key productive traits in U.S. Holstein and Jersey cattle. Their analysis of reaction norms for milk yield, fat yield, and protein yield revealed clear re-ranking patterns across increasing THI levels. For Holsteins, bulls with high general additive genetic merit showed declines in milk yield under heat stress, while those ranked as top for heat tolerance maintained or improved performance, resulting in crossover trajectories. Similar trends were observed for Jerseys, particularly for milk yield, where heat-tolerant sires outperformed general merit sires under elevated THI. These results mirror the patterns observed in our study for methane emissions and reinforce the importance of incorporating heat stress effects into genetic evaluations. Similarly, single-step reaction norm models were employed on

Chinese Holstein cattle to evaluate reproductive traits such as age at first calving, interval from first to last service, and conception rate at the first service and significant G×E interactions were detected for all three traits with increasing values of the THI scale (Shi *et al*., 2021). The substantial extent of G×E interactions and the frequent sire re-ranking reported across numerous studies and traits underscores the importance of explicitly accounting for G×E in dairy cattle breeding programs. Incorporating this consideration will enable the selection of animals that not only exhibit superior heat tolerance but also achieve higher reproductive and productive performance, while contributing to a reduced carbon footprint through lower methane emissions under challenging climatic conditions.

Conclusions

Our findings suggested that heat stress significantly influences the genetic architecture of methane emissions in Holstein cattle. Heritability estimates increased from 0.22 under thermoneutral conditions to 0.44 at higher THI levels, indicating that genetic variance becomes more pronounced as environmental stress intensifies. This pattern reflects strong G×E interactions, which also resulted in notable sire re-ranking across the THI gradient. Bulls classified as the top high methane emitters under thermoneutral conditions showed reduced emissions under heat stress, while the top low emitters exhibited the opposite trend. These findings highlight the importance of incorporating heat stress effects into genetic evaluations and suggest that breeding programs should adopt multi-environment evaluations to address G×E and maintain selection accuracy in regions where heat stress is a major challenge. Importantly, future studies should also consider animal weight and feed intake to properly adjust methane emission analyses and improve the precision of genetic evaluations.

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