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# Effects of heat stress on the physiological responses and vaginal microbiome of Hanwoo cows

**K-H. Um[1], S-R. Cho[2], S-S. Kang[1], U-H. Kim[3], J-I. Won[1], S. Jin[1], M. Park[1], S-J. Moon[1], G-S. Jang[1], B. Shokrollahi[1], Y-C. Baek[1] & S-S. Jang[1#]**

[1]Hanwoo Research Institute, National Institute of Animal Science, RDA, Pyeongchang 25340, Korea
[2]Animal Genetic Resources Station, National Institute of Animal Science, RDA, Namwon 55717, Korea
[3]Poultry Research Institute, National Institute of Animal Science, RDA, Pyeongchang 25342, Korea

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## Abstract

Temperature increases due to climate change induce heat stress in livestock, resulting in economic losses for farmers. This study investigated the physiological responses of Hanwoo cattle to heat stress, and the effects of heat stress on the vaginal microbiome. Four cows (mean weight: 380.3 ± 37.5 kg, parity: 2.5 ± 1.0) were allocated to two groups: heat stress (temperature-humidity index (THI) 86: 33 °C, 70% humidity) and no heat stress (THI 67: 20 °C, 60% humidity). The cows were exposed to these conditions for 15 days, and the study was repeated twice with the same cohort of animals, ensuring that each cow was exposed to both thermal conditions in a crossover manner. The study revealed that feed intake was lower, while water intake, rumen temperature, rectal temperature, and respiration rate were higher in THI 86 than in THI 67 cows. The blood cortisol, glucose, and cholesterol concentrations, and the inverse Simpson index of the vaginal microbiome, were lower in THI 86 cows, while certain microorganisms (for example, *Tenericutes*) were more abundant in THI 86 than in THI 67 cows. The increase in blood oestradiol concentration during oestrus was 2.8× lower, and the duration of oestrus was shorter by 41 hours in the THI 86 than in the THI 67 cows. Blood progesterone concentrations increased after ovulation in THI 67 cows and during oestrus in THI 86 cows. In THI 86 cows, the increase in blood luteinising hormone secretion and ovulation were delayed by 10 hours and 11 hours, respectively. We concluded that heat stress raises the body temperature of Hanwoo cows, causes changes in endocrine hormones and metabolic physiology, reduces the microbial diversity in the vagina, and negatively affects the oestrus cycle.

**Keywords:** cattle, climate change, hormones, microbial diversity, reproduction, ruminants

Corresponding author: jangsc@korea.kr

## Introduction

Heat stress (HS), stemming from elevated temperatures, global warming, and heightened metabolic demands, profoundly influences the endocrine system and hormonal balance in livestock

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(Kahl *et al.*, 2015; Baek *et al.*, 2019). Susceptibility to HS, which is driven by the demand for higher production yields, results in adverse repercussions on health, reproductive capabilities, and various biological functions (Polsky & von Keyserlingk, 2017; Brivio *et al.*, 2019; Czech *et al.*, 2022). To assess the impact of HS, researchers employ the temperature-humidity index (THI) (Collier *et al.*, 2017; Khan *et al.*, 2023). According to NIAS (2014), the THI is categorised into five levels based on livestock type. For ruminants, an index below THI 72 is considered optimal, THI 72–78 indicates the need for caution (mild stress), THI 79–89 represents a warning (severe stress), THI 90–97 is classified as dangerous (serious condition), and THI 98 or higher presents a risk of death. Not only does this index measure HS severity, but it also aids in estimating the cooling needs of cows, enhancing production and management effectiveness (Zimbelman *et al.*, 2009; Kim *et al.*, 2023).

In response to HS, animals deploy intricate physiological, endocrine, and behavioural mechanisms, including reducing their feed intake, resulting in a negative energy balance and decreased milk production (Sammad *et al.*, 2020; Kim *et al.*, 2022). Chen *et al.* (2018) suggested that HS affects physiological characteristics, immune activity, circulation levels, and the microbiome, manifesting in heightened occurrences of metabolic disorders and diminished performance in growth and immune function (Kim *et al.*, 2021). In beef cattle, HS contributes to reductions in nutrient intake, body weight gain, and meat quality, potentially leading to economic losses (O'Brien *et al.*, 2010; Kim *et al.*, 2018).

At the molecular level, HS regulates numerous genes associated with heat shock proteins (HSPs), which are crucial for maintaining tissue homeostasis under stressful conditions (Min *et al.*, 2015; Baek *et al.*, 2019). Severe HS conditions are closely linked to alterations in water intake, heart rate, rectal temperature, blood parameters (cortisol, HSP70, and blood urea nitrogen), and behavioural patterns in Hanwoo steers (Kim *et al.*, 2023).

Reproductive efficiency is also affected by HS, with effects on dry matter intake, and ruminal motility and fermentation patterns (Sales *et al.*, 2021). Despite extensive research, certain aspects of fertility regulation during HS remain unclear (Dovolou *et al.*, 2023). *In vitro* studies revealed that HS effects follicular components, altering fluid composition and cell functionality (Roth, 2008; Vanselow *et al.*, 2016). Furthermore, ovarian functionality depends on an endogenous thermoregulation system preserving lower temperatures than in surrounding tissues (López-Gatius & Hunter, 2017; López-Gatius & Hunter, 2019).

Assessing the composition of the microbiota present in farm animal environments is crucial for understanding its correlation with breeding under HS conditions (Czech *et al.*, 2022). Zhao *et al.* (2019) identified bacterial species in the rumen microbiome linked to HS, revealing that while HS had no discernible effect on both alpha and beta diversity, it did influence the richness of the microbiome. Building on these findings, Sales *et al.* (2021) demonstrated that HS significantly altered the microbiota and organic acid concentrations in the rumens of beef cattle, emphasising its influence on both the ruminal and vaginal microbiomes in cattle.

In this study, we hypothesised that HS induces physiological changes in Hanwoo cows, negatively affecting hormonal levels and the balance of the vaginal microbiome. To delve deeper into these aspects, our investigation explored the changes in stress hormones and HSPs, blood metabolites, sex hormones, and the vaginal microbiome of Hanwoo cows subjected to HS.

## Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee of the Korea National Institute of Animal Science (approval no. 2019-1543).

We selected four Hanwoo cows (average body weight: 380.31 ± 37.51 kg, parity: 2.5 ± 1.0) and exposed them to two conditions: a control environment with a THI of 67 (21 °C, 60% humidity) and a HS environment with a THI of 86 (33 °C, 70% humidity), using the THI prediction model from the National Research Council (NRC, 1971):

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26.8)]$$

where: THI: temperature-humidity index, T: temperature (°C), RH: relative humidity (%).

The study was repeated twice with the same cohort of animals, ensuring that each cow was exposed to both thermal conditions in a crossover manner. Cows were individually housed in metabolic

cages with controlled temperatures and humidities for a 6-day adaptation period under THI 67 conditions, followed by the 15-day experimental period, under either THI 67 or THI 86 conditions (Figure 1). Oestrus was synchronised using a controlled internal drug release (CIDR) device (Model G1915, Zoetis Inc., USA) inserted intravaginally at the start of the 15-day experimental period and removed seven days later. On the seventh day, an intramuscular injection of 5 mL prostaglandin F2α (PGF2α, Lutalyse, Zoetis, Belgium) was administered. Daily diets included 3 kg of concentrate feed and 6 kg of rice straw, with 50 kg of water provided per day. The chemical compositions of the feeds are detailed in Table 1.

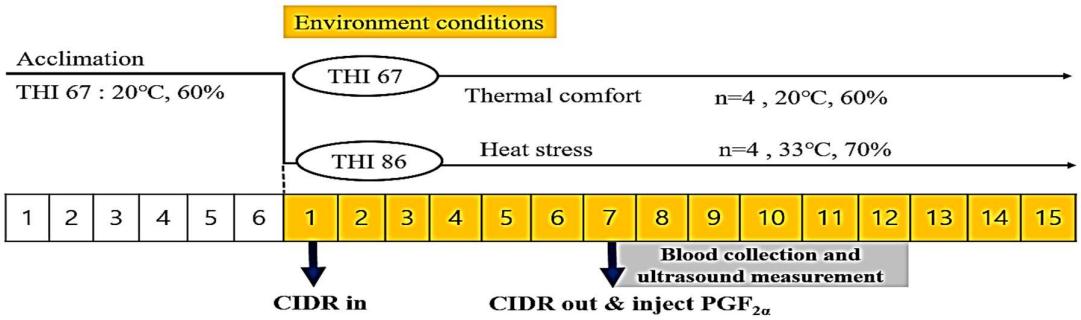


**Figure 1** Schematic of the experimental design. Four Hanwoo cows (380.31 ± 37.51 kg, parity 2.5 ± 1.0) were used. After acclimatisation to the metabolic cages under THI (temperature-humidity index) 67 conditions for six days, the cows were exposed to either THI 67 or THI 86 conditions for 15 days. On day 1 of the experimental period, a CIDR device was injected, and on day 7, the CIDR device was removed and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was injected to induce oestrus

**Table 1** Chemical composition of the used diets (as-fed basis, %)

| | Concentrate feed | Rice straw |
|---|---|---|
| **Dry matter** | 87.80 | 90.30 |
| **Crude protein** | 14.10 | 4.20 |
| **Crude fibre** | 9.00 | 31.10 |
| **Crude fat** | 3.40 | 2.50 |
| **Calcium** | 1.60 | 0.40 |
| **Phosphorus** | 0.40 | 0.10 |

The feed and water intake were calculated by measuring the amount of feed and water remaining in the respective tanks before refilling the tanks each morning. The respiratory rate was measured for one minute using a counter, and the rectal temperature was measured using a mercury thermometer (armpit glass mercury thermometer, Sanshan, Shaanxi, China). The rumen temperature was measured at two-minute intervals using gastric residence-type information and communications technology equipment (smart pill, Korea IoT corporation, Gimcheon-si, Korea), and the data were transmitted from the receiver to the server and collected at ten-minute intervals.

Blood was collected every three days for the analysis of cortisol, HSP70, and metabolite concentrations. Blood samples for the analysis of the oestradiol, luteinising hormone (LH), and progesterone concentrations were collected from 14:00 at four-hour intervals (14:00, 18:00, 22:00, and 02:00) following the removal of the CIDR device, until the last day (day 5) of ovulation. The collected blood was injected into serum vacutainer tubes (BD Vacutainer serum REF 367820, BD Biosciences, San Jose, USA), stabilised in a refrigerator at 4 °C for four hours, centrifuged at 3000 rpm for 20 minutes to separate the serum, and stored in a -80 °C ultra-low temperature freezer until analysis. The

concentrations of blood cortisol, HSP70, oestradiol, LH, and progesterone were determined using enzyme-linked immunosorbent assay (ELISA) kits [Mybiosource Co., California, USA: catalogue numbers MBS778442 (cortisol) and MBS019766 (HSP70); ABNOVA Co., Taipei, Taiwan, Republic of China: catalogue numbers KA-2276 (oestradiol), KA-2280 (LH), and KA-2281 (progesterone)], in combination with a multimode microplate reader (Ensight multimode plate reader, PerkinElmer, Waltham, MA) to measure the absorbance at a wavelength of 450 nm. An automatic biochemical analyser (Hitachi 7020 Automatic Analyzer, Hitachi, Tokyo, Japan) was used to determine the concentrations of glucose (GLU, mg/dl), total cholesterol (TCHO, mg/dl), triglyceride (TG, mg/dl), and non-esterified fatty acids (NEFA, uEq/L) in the blood.

Vaginal microbiome samples were collected at the conclusion of the experiment using the protocol outlined by Deng *et al.* (2019). DNA extraction was performed utilising the DNeasy PowerSoil Kit. The 16S rRNA gene metagenome amplicon sequencing, focusing on the V3–V4 region, was conducted on the Illumina® MiSeq® platform. Microbiome classification utilised amplicon sequence variants. Alpha and beta diversity analyses were based on the rarefaction curve, the inverse Simpson index, and UniFrac distances. Principal coordinate analysis was employed to elucidate the relationships between the samples.

All data were subjected to statistical evaluation using SAS version 9.2. Variables including feed and water intake, respiratory rate, body temperature, blood composition, and microbiome diversity under THI 67 and THI 86 conditions were compared using mean values in a t-test, with the significance threshold established at $P < 0.05$.

## Results and Discussion

Table 2 illustrates the physiological responses of the Hanwoo cows to each THI condition. Although the intake of concentrated feed did not show significant differences between treatments, the intake of roughage was notably lower under THI 86 conditions than under THI 67 conditions ($P < 0.001$). Under THI 86 conditions, water intake was 14.5 kg higher ($P < 0.05$) and the respiratory rate was 4.3× higher than under THI 67 conditions ($P < 0.001$). Both rectal and rumen temperatures in cows subjected to THI 86 were increased by ca. 0.5 °C compared to those subjected to THI 67 ($P < 0.05$).

**Table 2** Effects of temperature-humidity index (THI) level on feed and water intake, respiratory rate, rumen temperature, and rectal temperature of Hanwoo cows (mean ± standard deviation)

| | THI 67 | THI 86 | *P*-value |
|---|---|---|---|
| **Concentrate intake (kg)** | 3.00 ± 0.00 | 3.00 ± 0.00 | - |
| **Roughage intake (kg)** | $5.88^{a}$ ± 0.15 | $5.11^{b}$ ± 0.90 | <0.001 |
| **Water intake (kg)** | $24.64^{b}$ ± 3.08 | $39.12^{a}$ ± 6.40 | 0.013 |
| **Rumen temperature (°C)** | $39.34^{b}$ ± 0.13 | $39.85^{a}$ ± 0.39 | <0.001 |
| **Rectal temperature (°C)** | $38.62^{b}$ ± 0.11 | $39.01^{a}$ ± 0.25 | 0.004 |
| **Respiratory rate (breaths/min)** | $16.00^{b}$ ± 3.17 | $68.65^{a}$ ± 17.87 | <0.001 |

[a,b] Means in the same row with different superscripts differ significantly ($P < 0.05$)

These physiological changes are believed to counteract the increased body temperature and stress induced by high temperatures. Literature suggests that Hanwoo cows experience weight loss due to decreased feed intake, rumination, and nutrient absorption under HS conditions (Wang *et al.*, 2010; Soriani *et al.*, 2013; Colombo *et al.*, 2019). Furthermore, it has been reported that a THI above 82.4 can lead to fever and reduced feed intake in dairy cows (Gao *et al.*, 2017). Under conditions of HS, the body's water content becomes insufficient for normal physiological activities, necessitating an increase in water intake to compensate (Silanikove *et al.*, 2000; Schüller *et al.*, 2014). The respiratory rate, an indicator of HS in ruminants, is known to increase in high-temperature environments (Schüller *et al.*, 2014). In Hanwoo cows, the rectal temperature, a commonly used method to measure body temperature, is known to signify an increase in metabolic heat within the body (Srikandakuma *et al.*, 2004). According

to MAFRA (2018), HS in livestock can be categorised into four stages, ranging from comfort (no stress) to severe (life-threatening), each defined by specific rectal temperature thresholds. An increase in rumen temperature is associated with a decrease in rumination activity, leading to decreased feed intake and increased water intake (Conte *et al.*, 2018). Consequently, it can be concluded that the THI 86 conditions induced HS, as manifested by decreased food intake and increased water intake, respiratory rate, rectal temperature, and rumen temperature in the cows when exposed to these conditions.

Figure 2 illustrates the changes in the blood cortisol and HSP70 concentrations during the experimental period. On the sixth day of the experiment, the serum cortisol concentrations were lower in the THI 86 group than in the THI 67 group ($P$ <0.05), but no significant differences were observed during the remainder of the experimental period. No differences were found in the blood HSP70 concentrations across the experimental period.

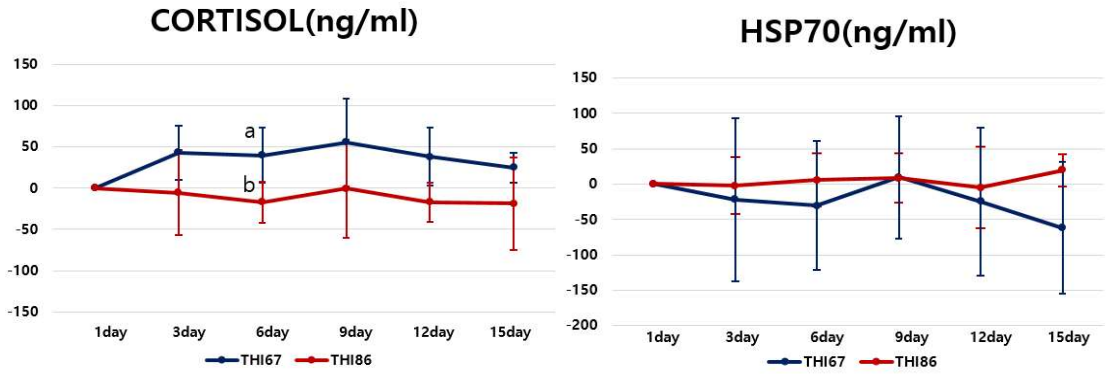


**Figure 2** Changes in blood cortisol and HSP70 concentrations (n = 4, mean ± standard deviation) in the THI 67 and THI 86 treatment groups on days 3, 6, 9, and 15, relative to study day 1. [a,b] Means with different superscripts differ significantly ($P$ <0.05). THI: temperature-humidity index

During periods of HS, the hypothalamic-pituitary-adrenal axis and the sympathetic-adrenal-medullary axis are activated to maintain homeostasis in response to stressful stimuli. Stress activates the central nervous system, which then promotes the secretion of hormones such as cortisol, regulating metabolism and reestablishing homeostasis (Carroll & Forsberg, 2007). Moreover, cortisol is known to influence the expression of HSPs, which are associated with immune responses in cows subjected to HS (Collier *et al.*, 2008). Costa *et al.* (2016) reported that cortisol impacts oocyte cells in cattle. Furthermore, research by Macfarlane *et al.* (2000) showed that stress-like levels of cortisol can suppress follicular growth and development and can block or delay the preovulatory surge of LH when cortisol is present during the late luteal and early follicular phases of the oestrous cycle. In this study, it was hypothesised that the secretion of cortisol would be lower under THI 86 conditions, to lower the metabolic heat production for body temperature control, and that this may negatively affect reproductive phenomena such as follicle development. As an increase in HSP concentrations is expected as a stress period prolongs, because of increasing damage to the cyst (Calderwood *et al.*, 2016), the absence of any significant difference in HSP70 levels in this study is believed to be because of the short duration of the test period.

Figure 3 delineates the variations in blood metabolite concentrations throughout the experimental period. A significant divergence in blood GLU levels was noted from the twelfth day, with higher concentrations in THI 67 than in THI 86 cows ($P$ <0.05). Total cholesterol levels fluctuated during the entire experimental period, increasing under THI 67 conditions and consistently decreasing under THI 86 conditions ($P$ <0.05). Triglyceride concentrations in the cows under THI 86 conditions rose from the sixth day onwards, and were higher than those in the THI 67 cows ($P$ <0.05). Non-esterified fatty acids showed a downward trend until the twelfth day in THI 86 cows, with lower levels than in the THI 67 cows, although this difference was not statistically significant.

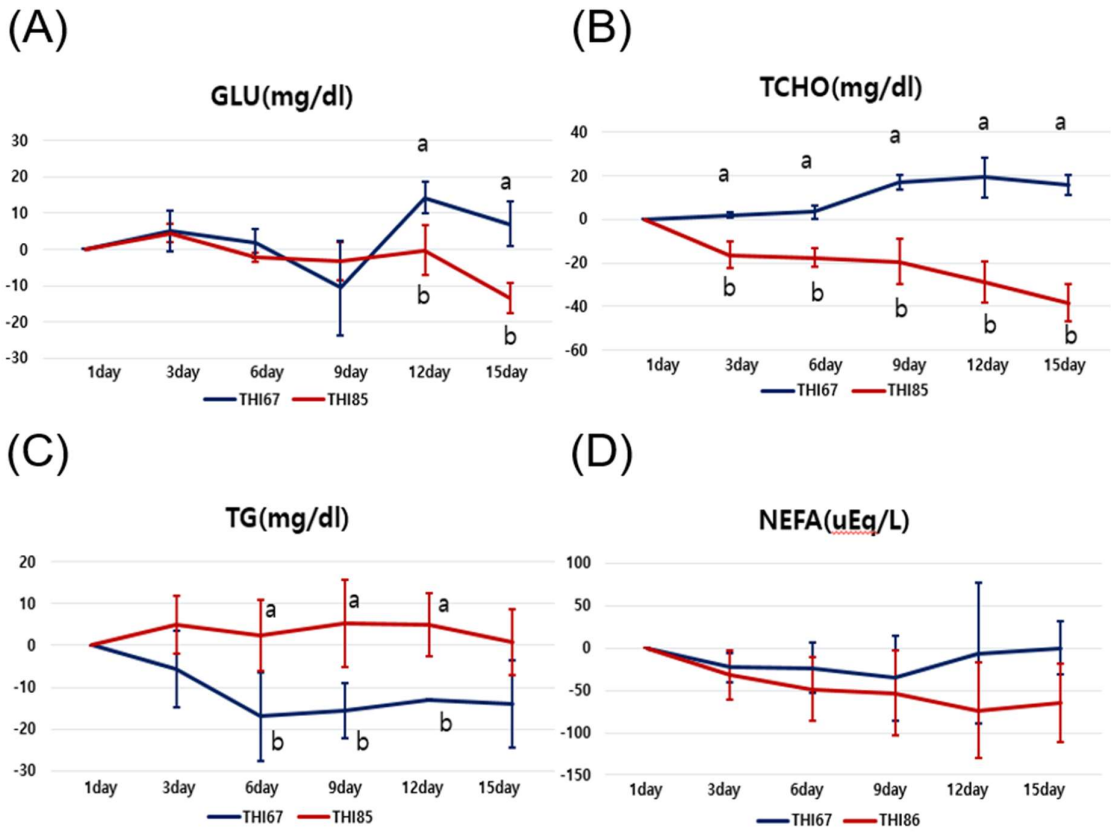


**Figure 3** Changes in glucose (GLU), total cholesterol (TCHO), triglyceride (TG), and non-esterified fatty acids (NEFA) concentrations (n = 4, mean ± standard deviation) in the THI 67 and THI 86 treatment groups on days 3, 6, 9, and 15, relative to study day 1. [a,b] Means with different superscripts differ significantly ($P$ <0.05); THI: temperature-humidity index

Blood metabolites serve as crucial indicators in nutritional physiology research, reflecting the nutritional and metabolic status of livestock (Lee *et al.*, 2013). The elevation of an animal's core body temperature beyond the norm triggers acclimation mechanisms aimed at mitigating HS effects, leading to shifts in blood metabolite and GLU levels (Rhoads *et al.*, 2010; Guo *et al.*, 2018). Blood GLU, which is primarily utilised as an energy source during HS (Febbraio, 2001), tends to decrease as the energy demands for maintaining homeostasis rise. This influences energy intake, results in the rapid consumption of blood GLU, and hinders the GLU biosynthesis process (Rhoads *et al.*, 2009; Wheelock *et al.*, 2010; Abbas *et al.*, 2020). This observation aligns with findings of Kim *et al.* (2022), where blood GLU levels diminished in cows under HS conditions. Kang *et al.* (2017) observed that elevated THI levels lead to increased fat utilisation, reducing blood TCHO concentrations. Furthermore, while some studies (Rhoads *et al.*, 2009; Kang *et al.*, 2017) noted no change in blood NEFA levels despite varying THI conditions, Abbas *et al.* (2020) reported a decrease in the NEFA concentration. In this study, although no significant change in blood NEFA concentration was detected, a decreasing trend over time was observed. This result suggests that prolonged exposure to high-temperature stress may lead to reduced NEFA concentrations in the blood. The observed decrease in blood GLU and TCHO levels and increase in TG under THI 86 conditions may interfere with lactose synthesis in cattle, causing metabolic disorders and possibly negatively affecting reproduction (Abbas *et al.*, 2020; Televičius *et al.*, 2021).

Figure 4, Figure 5, and Table 3 compare the vaginal microbiomes of the THI 67 and THI 86 cows. The number of DNA reads in the sample was 32899, and the number of amplicon sequence variants was 14076, with an average of 1134.8 ± 380.3 reads per head in the THI 67 group and 1009.8 ± 365.5 reads per head in the THI 86 group. Alpha diversity was analysed using the inverse Simpson index, and was found to be lower in the THI 86 group than in the THI 67 group ($P$ = 0.04). For the results

of the principal coordinate analysis of the beta diversity, the explanatory power was 74.17% (PC1: 60.75%, PC2: 14.46%, PC3: 9.41%) in the weighted analysis and 56.53% (PC1: 22.81%, PC2: 18.61%, PC3: 15.59%) in the unweighted analysis. Twenty-four phyla were identified in the vaginal samples, and the most abundant microorganisms were *Firmicutes* (THI 67: 56.6 ± 2.4%, THI 86: 53.4 ± 7.5%) and *Bacteroidetes* (THI 67: 19.5 ± 4.9%, THI 86: 11.8 ± 4.9%). The proportions of *Deinococcus-Thermus* and *Tenericutes* were higher in the THI 86 group than in the THI 67 group ($P$ <0.05).

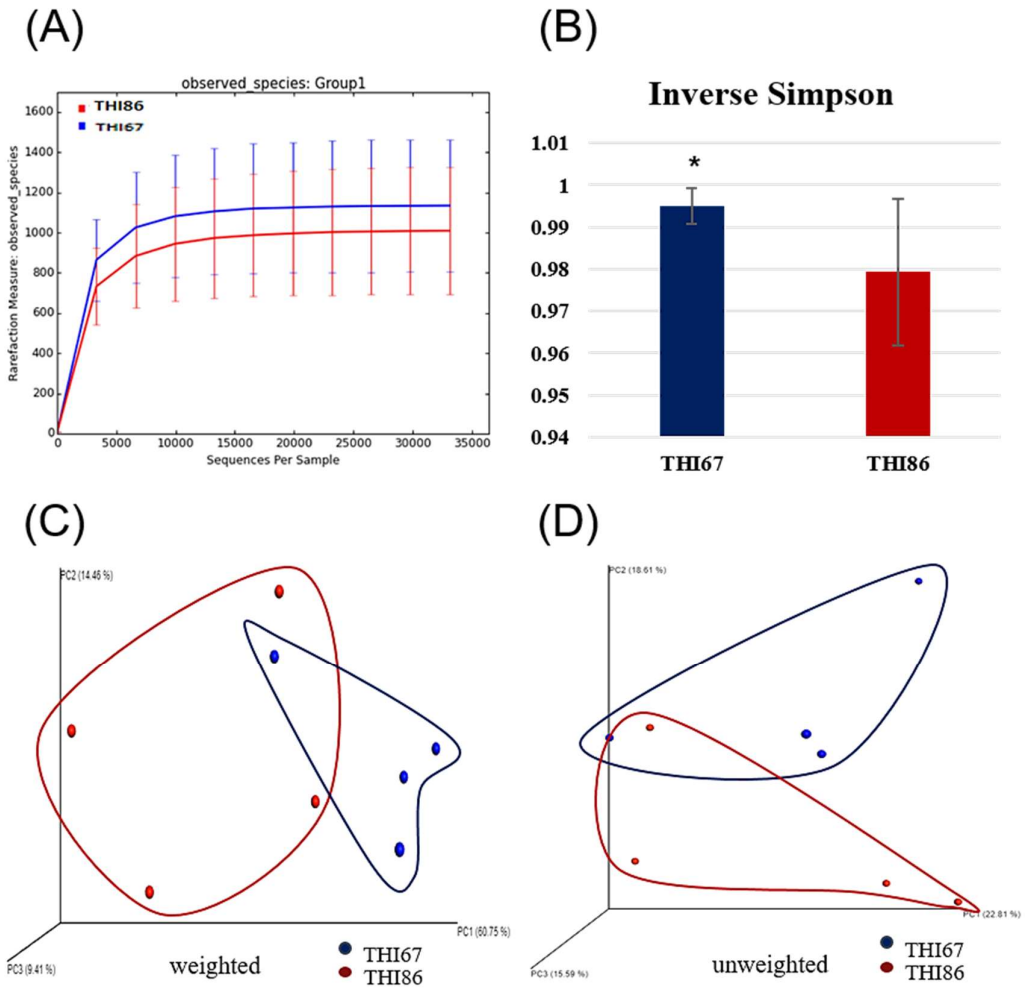


**Figure 4** Effects of heat stress on the alpha and beta diversity of the THI 67 and THI 86 groups' vaginal microbiome. (A) Rarefaction analysis for microbiota richness at 32899 reads (richness of microbiota composition: THI 67 > THI 86). (B) Alpha diversity of the faecal microbiota, presented as the inverse Simpson index (alpha diversity of microbiota: THI 67 > THI 86, $P$ = 0.04). Data shown as the mean ± standard deviation. (C) and (D), beta diversity, as shown using principal coordinate analysis plots based on community membership, and measured by the UniFrac distances. Four samples were used for the THI 67 group and four samples were used for the THI 86 group

The vaginal microenvironment plays a significant role in bovine fertility, since its resident microorganisms interact with the host mucosa and constitute the first barrier against ascending pathogens in the reproductive tract (Moreno *et al.*, 2022). *Deinococcus-Thermus* bacteria can exist anywhere, are known to be able to withstand high temperatures (Slade & Radman, 2011), and were first identified in humans (Bik *et al.*, 2006). The have also been found in the vaginal microflora of healthy women (Diop *et al.*, 2019). However, the exact role of *Deinococcus-Thermus* in humans and animals

(especially ruminants) and its exact mechanisms of action in vaginal inflammatory diseases are not yet clearly defined (Chen *et al.*, 2022).

**Table 3** Effects of heat stress on the bacterial ratio at the phylum level in the THI 67 and THI 86 treatment groups (mean ± standard deviation)

| Taxonomy | THI 67 | THI 86 | *P*-value |
|---|---|---|---|
| ***Deinococcus-Thermus* (%)** | *ND | 0.02 ± 0.02 | <0.001 |
| ***Tenericutes* (%)** | $0.28^{b}$ ± 0.04 | $0.62^{a}$ ± 0.50 | 0.002 |

THI: temperature-humidity index, *ND: not detected
[a,b] Means in the same row with different superscripts differ significantly ($P$ <0.05)

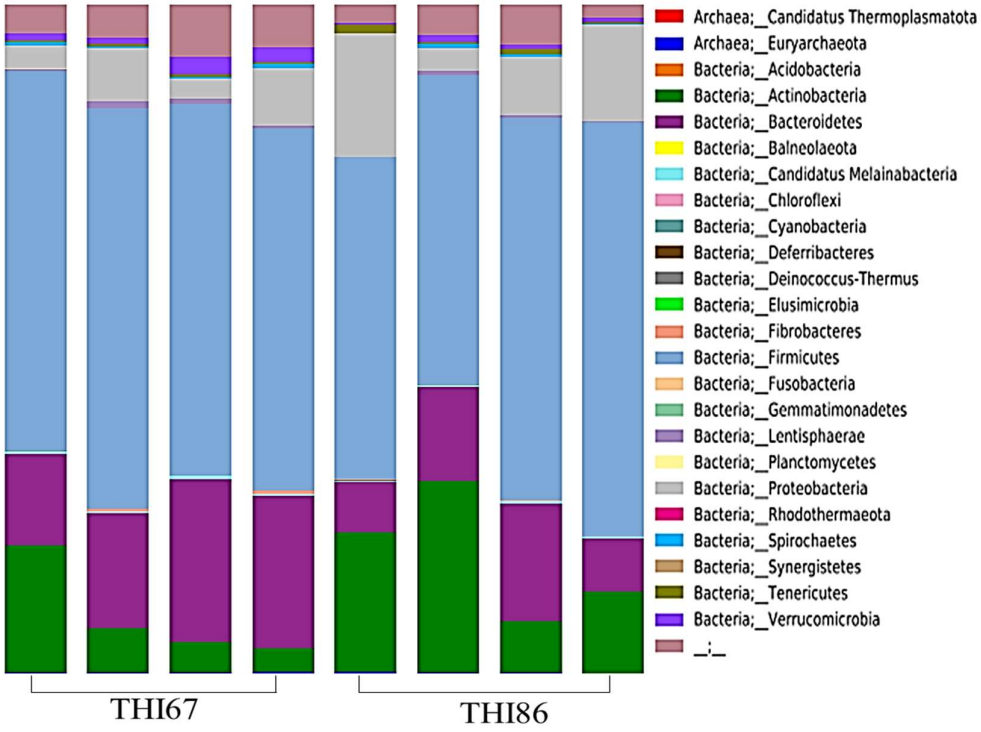


**Figure 5** Vaginal microbiome profiles at the phylum level in the THI 67 and THI 86 treatment groups. THI: temperature-humidity index

Bacteria of the *Tenericutes* phylum are found in diverse environments (Wang *et al.*, 2020) and are known to commonly appear in the reproductive organs of cattle (Ong *et al.*, 2021). In previous studies (Peng *et al.*, 2013; Jeon *et al.*, 2015; Moreno *et al.*, 2022), *Tenericutes* was detected more frequently in healthy cows than in cows with endometritis. Additionally, Yadav *et al.* (2022) reported that *Tenericutes* in the rumen increased in a high-temperature environment (THI 87–90). Based on the results of our study, the microorganism diversity in the THI 86 group was lower than that in the THI 67 group ($P$ = 0.04), but no distinct patterns were observed in either group. A number of microorganisms that are noted as surviving in high-temperature environments were more abundant in the THI 86 group than in the THI 67 group. Furthermore, *Tenericutes* was found to be more abundant in the THI 86 cows than in the THI 67 cows, and this is thought to have been caused by the high-temperature environment.

Figure 6 illustrates the changes in blood oestradiol concentrations following the removal of the CIDR device, with measurements taken every four hours. Notably, a significant increase in oestradiol was observed 40 hours post-removal across all tested animals. Specifically, under THI 67 conditions, the oestradiol concentration of one subject was 3.9× higher at 40 hours than at 36 hours after the CIDR

device removal, with this elevated level persisting for 80 hours. Conversely, under THI 86 conditions, the oestradiol concentration was 2.6× higher, and this heightened concentration continued for 40 hours. Another cow had an oestradiol concentration that was 37.8× higher at 40 hours compared to 36 hours post-removal under THI 67 conditions, and maintained this peak for 80 hours. Under THI 86 conditions, the concentration was only 3.7× higher at 40 hours post-removal, and this lasted only 36 hours. Furthermore, one cow had a 2.3-fold increase in the oestradiol concentration under THI 67 conditions from 36 hours to 40 hours post-removal, and this high concentration continued for 68 hours.

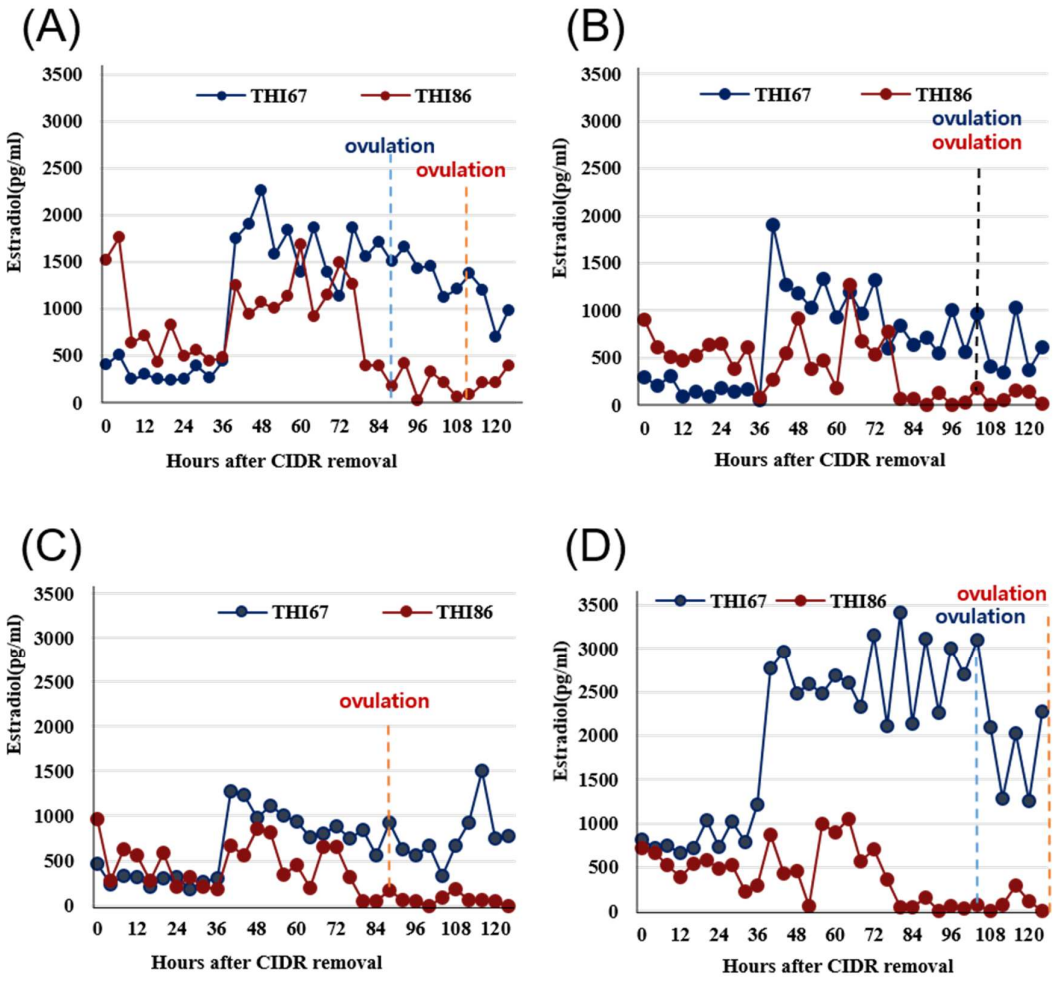


**Figure 6** Changes in blood oestradiol concentrations for each cow (A–C) at four-hour intervals after CIDR device removal. Cow (C) was not synchronised with oestrus under THI 67 conditions. THI: temperature-humidity index

These findings revealed that the average increase in oestradiol concentration during oestrus was 1424.6 ± 374.1 pg/ml under THI 67 conditions and only 507.9 ± 240.6 pg/ml under THI 86 conditions, demonstrating a 2.8-fold reduction in the oestradiol peak under THI 86 conditions compared to THI 67 conditions. Furthermore, the elevated oestradiol concentrations persisted for 76.0 ± 6.9 hours in the THI 67 cows and 35.0 ± 5.0 hours in the THI 86 cows, indicating that the duration of the increased oestradiol concentration was ca. 41 hours shorter under THI 86 conditions.

Previous studies have reported that HS adversely impacts steroid hormone synthesis by decreasing aromatase activity in ovarian granulosa cells, which are important for converting testosterone to oestradiol; this leads to a decrease in the production of oestradiol before ovulation (Wolfenson *et al.*, 2000; Ronchi *et al.*, 2001; Rozenboim *et al.*, 2007). Wolfenson *et al.* (2019) highlighted

that reduced oestradiol concentrations negatively affect oestrus duration and intensity, inhibit ovulation-related LH secretion, and disrupt ovarian cyst development, along with altering progesterone secretion and luteal function. Accordingly, these results suggest that the decreased oestradiol secretion during oestrus under the high-temperature stress of THI 86 weakened oestrus and the secretion of progesterone and LH, potentially impairing follicle and corpus luteum development.

Figure 7 shows the changes in the progesterone concentration post-CIDR removal, with samples collected at four-hour intervals. Under THI 67 conditions, an increase in progesterone concentration was noted 108 hours after CIDR device removal, whereas under THI 86 conditions, this increase occurred 40 hours post-removal and the progesterone concentration remained elevated until 112 hours post-removal. In another cow, the progesterone concentration increased 80 hours post-removal under THI 67 conditions, while under THI 86 conditions, this peak was at 72 hours post-removal, and elevated concentrations were sustained until 112 hours post-removal. In a third cow, under THI 86 conditions, progesterone levels rose from 56 hours after CIDR device removal and stayed elevated until 116 hours post-removal. Progesterone concentrations in the fourth cow increased 108 hours post-removal under THI 67 conditions and 44 hours post-removal under THI 86 conditions, with elevated concentrations lasting until 116 hours after CIDR device removal.

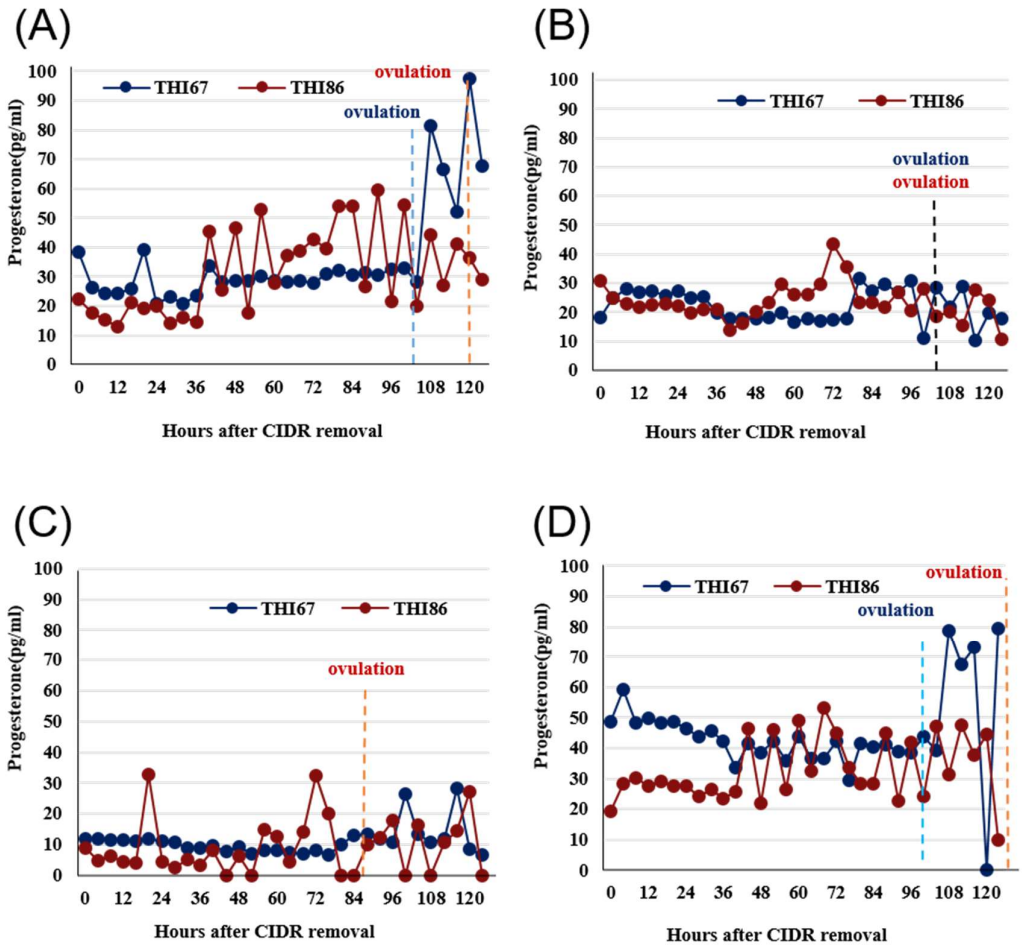


**Figure 7** Changes in blood progesterone concentrations for each cow (A–C) at four-hour intervals after CIDR device removal. Cow (C) was not synchronised with oestrus under THI 67 conditions. THI: temperature-humidity index

Blood progesterone levels may vary with the acuteness or chronicity of HS and the animal's metabolic state, influencing follicular activity and ovulation mechanisms and thus affecting oocyte and

embryo quality (De Rensis & Scaramuzzi, 2003). This study confirmed that under THI 67 conditions, progesterone concentrations increased normally post-ovulation, whereas under THI 86 conditions, progesterone concentrations increased during oestrus and before ovulation. Progesterone, which is known for its role in post-ovulation secretion and fertility (Teh *et al.*, 2016; Stevenson & Lamb, 2016), exhibited concentration changes that may have been due to HS-induced variations in lipid metabolism, TCHO, and dry matter intake (Ronchi *et al.*, 2001; De Rensis & Scaramuzzi, 2003). This could affect corpus luteum development and potentially increase the likelihood of implantation failures (Lamming & Royal, 2001).

Figure 8 shows the fluctuations in blood LH concentrations following CIDR device removal, with measurements conducted every four hours.

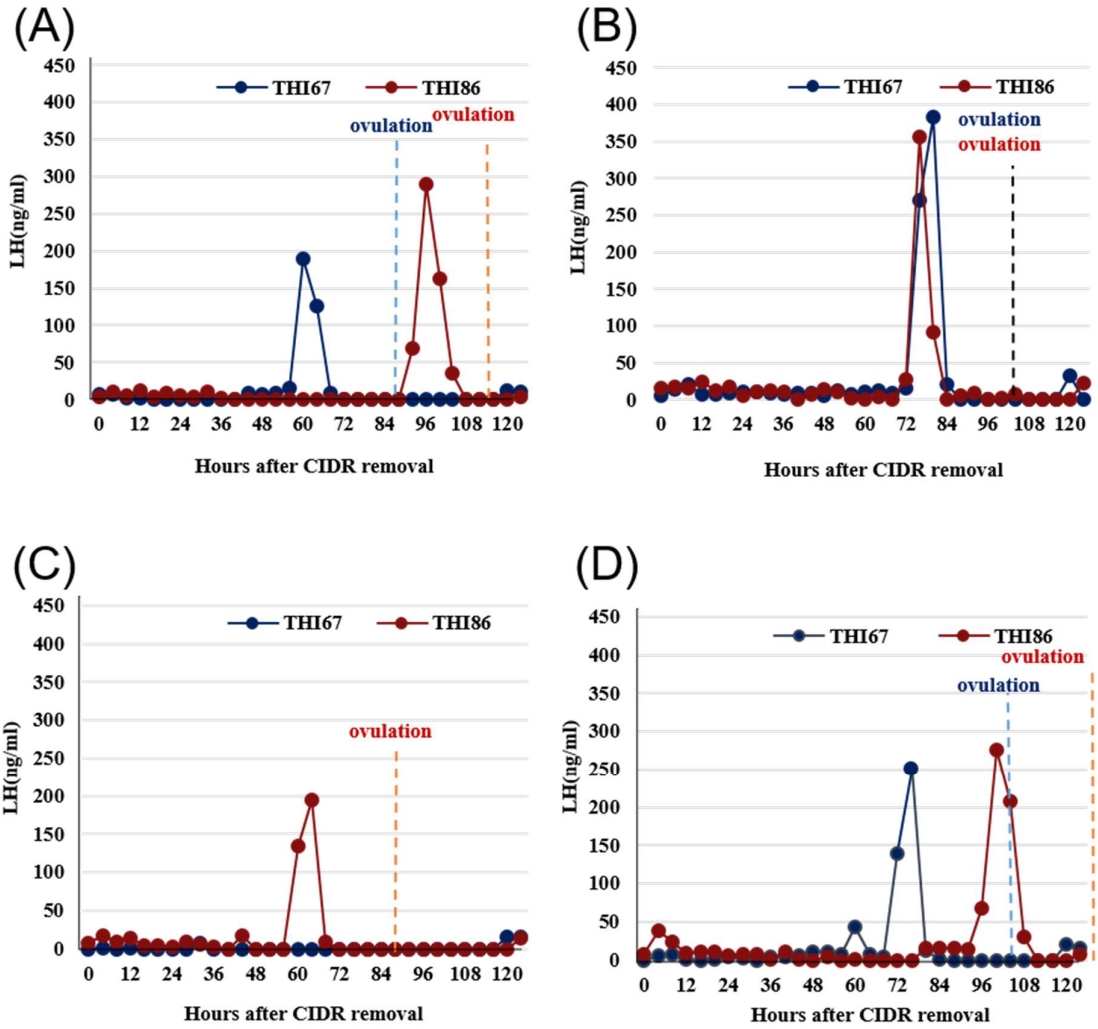


**Figure 8** Changes in blood luteinising hormone (LH) concentrations for each cow (A–C) at four-hour intervals after CIDR device removal. Cow (C) was not synchronised with oestrus under THI 67 conditions. THI: temperature-humidity index

The LH concentration for one individual rose after 60 hours and 92 hours post-removal under THI 67 and THI 86 conditions, respectively, indicating a 32-hour delay in the LH surge under THI 86 conditions. In contrast, in another cow, the LH concentration increased after 80 hours under THI 67 conditions and after 76 hours under THI 86 conditions, suggesting a four-hour acceleration of the LH surge under THI 86 conditions. A third cow, when exposed to THI 86 conditions, had an increase in the

LH concentration 64 hours post-removal. In the last cow, the LH concentration rose at 76 hours and 96 hours post-removal under THI 67 and THI 86 conditions, respectively, marking a 20-hour delay in the LH surge under THI 86 conditions.

This study found that an LH surge occurred 72 ± 10.6 hours after CIDR removal under THI 67 conditions and 82 ± 14.8 hours after CIDR removal under THI 86 conditions, indicating a 10-hour delay in the LH surge when the cows were exposed to THI 86. These results align with those of previous studies, which indicated that LH secretion patterns were altered under HS. This was interpreted as decreased and delayed LH secretion, as influenced by gonadotropin-releasing hormone, diminished LH receptor expression, reduced oestradiol secretion, elevated progesterone concentrations, and lowered GLU levels (De Rensis & Scaramuzzi, 2003; Khodaei-Motlagh *et al.*, 2011; Sammad *et al.*, 2020). Consequently, as oestradiol and progesterone concentrations rose during oestrus, the synthesis and secretion of LH in the hypothalamus and pituitary were presumably delayed.

## Conclusions

This study comprehensively demonstrated that exposure to HS (THI 86) results in significant physiological adjustments in Hanwoo cows, including elevated rumen and rectal temperatures, increased respiration rates, and heightened water intake, as well as reduced feed intake, compared to under conditions of lower thermal stress (THI 67). Notably, blood GLU and TCHO levels were lower under THI 86 conditions, whereas TG increased. Furthermore, THI 86 conditions led to a reduction in the vaginal microbial diversity at the phylum level. Crucially, HS adversely affected reproductive physiology by diminishing oestradiol synthesis and secretion during oestrus and prematurely elevating progesterone secretion, thereby delaying the LH surge and subsequent ovulation. These findings underscore the intricate interplay between HS and homeostatic mechanisms, where physiological responses aim to counterbalance elevated temperatures, albeit with repercussions on reproductive efficiency. The alteration in sex hormone dynamics and the shift towards a pro-inflammatory vaginal microbiome composition will not only disrupt the normal reproductive cycle but also have broader implications for cow productivity.

Given the projected intensification of climate change, this study highlights the urgent need for targeted research and interventions designed to mitigate HS in Hanwoo cows. Future studies should explore innovative cooling strategies, nutritional adjustments, and management practices that could alleviate the negative impacts of HS on both metabolic and reproductive physiology, ultimately safeguarding productivity. Through continued research and adaptive measures, the resilience of Hanwoo cows to climate-induced stressors can be enhanced, ensuring sustainable livestock productivity in the face of global climate challenges.

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**Authors’ contributions**

Conceptualisation: Jang, S.S. and Cho, S.R.; methodology: Jang, S.S.; validation: Kim, U.H. and Kang, S.S.; formal analysis: Won, J.I. and Jin, S.; investigation: Moon, S.J. Jang, G.S., and Park, M; data curation: Um, K.H., Park, M., and Baek, Y.C.; writing—original draft preparation: Um, K.H.; writing—review and editing: Um, K.H., Kang, S.S., and Shokrollahi, B,; supervision: Jang S.S. All authors have read and agreed to the published version of the manuscript.

**Conflict of interest declaration**

The authors declare no conflicts of interest.

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