

# Evaluation of heat stress responses in Holstein and Jersey cows by analyzing physiological characteristics and milk production in Korea

Dong-Hyun Lim<sup>1</sup>, Tae-II Kim<sup>1</sup>, Sung-Min Park<sup>1</sup>, Kwang-Seok Ki<sup>1</sup> and Younghoon Kim<sup>2\*</sup>

<sup>1</sup>Dairy Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan 31000, Korea

<sup>2</sup>Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Korea



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## \*Corresponding author

Younghoon Kim

Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Korea.  
Tel: +82-2-880-4808  
E-mail: ykeys2584@snu.ac.kr

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## ORCID

Dong-Hyun Lim

<https://orcid.org/0000-0002-8575-0198>

Tae-II Kim

<https://orcid.org/0000-0003-0532-6010>

Sung-Min Park

<https://orcid.org/0000-0001-9534-0216>

Kwang-Seok Ki

<https://orcid.org/0000-0003-0971-1389>

Younghoon Kim

<https://orcid.org/0000-0001-6769-0657>

## Abstract

We evaluated the effects of heat stress on physiological responses and milk production in Holstein and Jersey cows reared in Korea. The mean average temperature-humidity index (THI) increased significantly from May to August and then decreased until October. The mean average rectal temperature (RT) was increased in Holstein cows compared with Jersey cows, as the THI values increased from 61 to 85. The average respiratory rate (RR) was increased in Jersey cows compared with Holstein cows when the THI value increased from 61 to 85. The average surface temperature of the rumen and udder was higher in Jersey cows than in Holstein cows when the THI value increased from 61 to 85. No significant difference was noted with respect to relative serum volumes between the breeds and THI ranges, but we measured significant changes in serum pH in Holstein and Jersey cows when the THI value increased from 61 to 85. Milk production was not significantly changed in Holstein cows when the THI increased from 61 to 85, but milk production and milk protein content were significantly altered in Jersey cows when the THI increased from 61 to 85. Current study suggests that Holstein cows still have an advantage in terms of the economic returns of dairy farms in Korea. Therefore, further research is required regarding the heat tolerance of Jersey cows in Korean climatic conditions.

**Keywords:** Heat stress, Physiological response, Rectal temperature, Milk compositions, Rumen surface temperature

## INTRODUCTION

Livestock is a primary source of income for many people and provides for their everyday social and economic needs. The livestock sector employs approximately 1.3 billion people and contributes to

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**Availability of data and material**

Upon reasonable request, the datasets of this study can be available from the corresponding author.

**Authors' contributions**

Conceptualization: Lim DH, Kim TI, Park SM, Ki KS, Kim Y.

Data curation: Lim DH, Kim TI, Park SM, Ki KS, Kim Y.

Formal analysis: Lim DH, Kim TI, Park SM, Ki KS, Kim Y.

Methodology: Lim DH, Kim TI, Park SM, Ki KS.

Software: Lim DH, Kim TI, Park SM, Ki KS. Validation: Lim DH, Kim TI, Park SM, Ki KS, Kim Y.

Investigation: Lim DH, Kim TI, Park SM, Ki KS, Kim Y.

Writing - original draft: Lim DH, Kim TI, Park SM, Ki KS, Kim Y.

Writing - review & editing: Lim DH, Kim TI, Park SM, Ki KS, Kim Y.

**Ethics approval and consent to participate**

All dairy cows were maintained as stated in standard guideline, and the experimental protocol involved in this experiment was approved by the Institutional Animal Care and Use Committee (IACUC) at NIAS (study approval number: IACUC 2019-330).

approximately 40% of the world's agricultural gross domestic product (GDP) [1]. However, heat stress (HS) is one of the increasing challenges in dairy cow production. Milk production is affected by environmental and management factors. The production potential of livestock is affected by many factors; increasing environmental temperatures are a particular threat and are associated with the global warming phenomenon. HS has been found to negatively affect the quality and quantity of milk production to an increased extent in high-yield dairy cows compared to low-yield dairy cows [2]. Lactating cows are affected by HS, which may lead to altered metabolism, decreased dry matter intake, reduced milk production and reduced reproductive performance [3]. In terms of global warming, Korea suffers from extreme heat and humidity during the summer seasons from June to August. A heat wave is generally defined as a period of consecutive days or weeks of abnormally hot weather [4]. The number of heat waves (22.4 days), defined as consecutive days with maximum temperatures exceeding 33°C, in 2016 was the second highest ever recorded, following the summer of 1994.

HS occurs when any combination of environmental factors, such as air temperature, relative humidity (RH), air movement, and solar radiation, causes the effective temperature of the environment to be higher than the animal's thermoneutral zone or comfort zone [5]. The temperature humidity index (THI) is a key factor that is used as an indicator of the degree of climatic stress in animals; a THI of 72 or below is considered to be no HS, 73–77 is mild HS, 78–89 is moderate HS, and above 90 is severe HS [6]. Numerous studies have reported that dairy cows demonstrate physiological reactions to HS, as body temperatures and respiratory rates (RRs) were found to increase above a THI of 75 [7]. Collier et al. [8] determined that milk production linearly decreased with increasing THIs, rectal temperatures (RTs), and evaporative heat dissipation.

The majority of dairy cows raised in Korea are of the Holstein breed. They are relatively resistant to cold conditions but are susceptible to heat, resulting in susceptibility to HS damage. In particular, HS causes decreased milk quality and quantity, affecting the profitability of farming systems [9]. Since 2010, Korea has introduced Jersey cows, which have a smaller body size and produce less milk than Holstein dairy cows. The contents of milk fat, protein, vitamins, and minerals are also higher in Jersey cow milk than in Holstein cow milk; in Japan, the price of Jersey cow milk is higher than that of other milk varieties. Moreover, Jersey cows are popular because during times of high environmental thermal stress, with a THI above 78, their milk production and reproductive efficiency are not reduced (unlike Holstein cattle) [2]. However, there have been no scientific studies on the effects of HS responses in Holstein and Jersey cows raised in Korea. Hence, the objective of this study was to compare the degree of thermal adaptability in these two breeds (Holstein and Jersey) of dairy cows when subjected to HS conditions relevant to Korea.

## MATERIALS AND METHODS

### Animals and experimental design

The experiment was performed at the Department of Animal Resources Development, National Institute of Animal Science (Cheonan, Korea). First lactation primiparous Holstein and Jersey cows were included in an experiment carried out from May to October. At the start of the study, the Holstein cows ( $n = 7$ ) were  $31 \pm 0.8$  months of age, presented  $94 \pm 29$  days in milk (DIM), had a body weight of  $777.3 \pm 44.0$  kg, and had a milk yield of  $27.2 \pm 1.9$  kg/d; Jersey cows ( $n = 7$ ) were  $26 \pm 0.4$  months of age, presented  $82 \pm 21$  DIM, had a body weight of  $411.5 \pm 7.3$  kg and had a milk yield of  $19.1 \pm 2.5$  kg/d. The cows were housed in one section of a free-stall barn, which had metal roofs over the free stalls and feeding area but was open between the roofs. Cows were provided with supplemental cooling by fans. All dairy cows were maintained according to standard guidelines, and

the experimental protocol of this research was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Institute of Animal Science, Korea (IACUC 2019-330). Milk was collected from each cow twice daily at 06:00 and 17:00 h, and milk yields were noted at each milking. All dairy cows were fed a total mixed ration (TMR, net energy for lactation [NE<sub>L</sub>] 1.7 Mcal/kg, total digestible nutrient [TDN] 68.7%) *ad libitum*, and the TMR was formulated to meet or exceed the predicted requirements [10] for energy, protein, minerals and vitamins. The chemical composition of the TMRs was analyzed according to AOAC [11], and Van Soest et al. [12]. The nutritive value and chemical composition of the rations based on the realized TMR are presented in Table 1.

### Measurement of physiological responses

The RT, RR, and body surface temperatures of the rumen (RST) and udder (UST) were recorded once a week at 09:00, 13:00, and 15:00 ( $\pm$  30 min) between the morning and evening milking times for each cow. The RT was measured using a digital meter (model MT16C2, Microlife AG Swiss, Widnau, Switzerland). The RT was measured under two different temperature conditions by two investigators. For RT measurements, the thermometer was inserted 7 to 8 cm into the rectum. The

**Table 1.** Nutritive value and chemical composition of the TMR fed to Holstein and Jersey cows during the study period (% DM)

Items	THI	
	61–72 (May, Oct)	73–85 (Jun–Sep)
Ingredients ratio (%)		
Concentrate	38.87	33.68
Cashew nut meal	-	7.12
Beet pulp	3.72	-
Cottonseed	3.73	-
Soybean meal	1.88	3.61
Corn silage	25.02	20.83
Mixed hay	16.51	14.05
Alfalfa	5.65	10.81
Tall fescue	3.77	9.02
Bypass fat	0.21	0.20
Sodium bicarbonate	0.21	0.20
Yeast culture	0.21	0.20
Mineral mixture	0.21	0.28
Chemical composition (%)		
Moisture	53.76	54.69
Crude protein	16.31	16.57
Ether extract	5.80	5.47
Crude fiber	18.85	19.14
Crude ash	7.18	7.41
Neutral detergent fiber (NDF)	38.67	39.33
Acid detergent fiber (ADF)	22.06	21.80
Calcium	0.93	0.82
Phosphorous	0.35	0.49
TDN	74.79	75.36
NE <sub>L</sub> (Mcal/kg)	1.71	1.74

DM, dry matter; THI, temperature-humidity index; TDN, total digestible nutrient; NE<sub>L</sub>, net energy for lactation.

inserted thermometer takes approximately 1 min to contact the rectum wall and measure RT. The RT was recorded once again after a bowel movement, as inaccurate temperatures may have been obtained when the thermometer was in manure. Noncontact surface temperature measurements were obtained with an infrared thermometer (Testo 830-T1, Testo, West Chester, PA, USA). Measurements were consistently taken on cows that had been standing for longer than 5 min and from a distance of 30 cm from each measurement site. The infrared thermometer measured the RST and UST with a laser measurement spot marker and took approximately one second to measure the temperature. All cows were housed in a barn with an impervious roof that was not affected by radiant heat from the sun, and the coat color of each cow did not affect the results. The temperature measurement locations were the left paralumbar fossa for RSTs and the left forequarter for USTs. The RR, expressed as the number of breaths per min, was measured using a stethoscope and stopwatch upon auscultation of respiratory movements for 30 s, and the value obtained was multiplied by 2 to determine this variable in min.

### Measurement of temperature humidity index (THI)

The ambient temperature ( $^{\circ}\text{C}$ ) and RH (%) were monitored with a thermohygrometer (Testo 174H, Testo), with an accuracy of  $\pm 0.5^{\circ}\text{C}$  and  $\pm 3\%$  RH. The thermohygrometer was set to record every 30 min of every day and was placed approximately 2 meters from the feeding area. The temperature and humidity values were used to calculate THI values; the THI was calculated for each 30-min temperature and humidity measurement according to the following formula:  $\text{THI} = (0.8 \times ^{\circ}\text{C}) + [\text{RH}\% \times (^{\circ}\text{C} - 14.4)] + 46.4$ . The mean daily THI consisted of the mean of all available 30-min THIs per day. The maximum THI for every day was calculated according to the formula of Fox & Tylutki [13], with the maximal temperature and the minimum RH/day, and the minimum THI for every day was calculated with the minimum temperature and the maximum RH/day. Furthermore, the THIs (61–63, 64–66, 67–69, 70–72, 73–75, 76–78, 79–81, and 82–84) during measurement moments were recorded.

### Sampling and analysis of blood and milk

A blood sample was collected from each cow once a week. The collected samples were centrifuged at  $1,000\times g$  for 15 min at  $4^{\circ}\text{C}$ . The relative serum volume (RSV, %) was then calculated as follows:  $\text{RSV} (\%) = \text{serum weight} (g) \times 100 / \text{total weight} (g)$ ; additionally, the serum pH was measured. The collected serum was stored at  $-20^{\circ}\text{C}$  until analysis. Serum samples were used to determine metabolic indices (Wako Chemicals, Neuss, Germany), which were analyzed with a blood autoanalyzer (Hitachi 7180, Hitachi, Tokyo, Japan).

Milk yield was recorded each day in the morning and evening after the RT, RR, RST, and UST measurements were taken. Milk samples were collected once a week (Wednesday afternoon and Thursday morning), and milk fat, protein, lactose, and total solids were analyzed with a LactoScop (MK2, Delta Instruments, Drachten, Netherlands).

### Statistical analysis

Data were analyzed using SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC, USA). The effects of the THI (fixed variable) on the RT, RR, RST, UST, milk production, and milk composition (random variables) within each breed were analyzed using one-way ANOVA. Duncan's multiple range test was used to separate the means when significance was indicated. The effects of breed and THI (fixed variable) on RT, RR, RST, UST, milk production, and milk composition (random variables) were analyzed using the PROC MIXED model with a restricted maximum likelihood model (REML). The breed (Holstein and Jersey cows), THI (61–63, 64–66, 67–69, 70–72, 73–75,

76–78, 79–81, and 82–84), and their interactions were included as fixed effects. For comparisons between breeds, THIs, and breed  $\times$  THI, the  $p$ -values after Bonferroni post hoc test adjustment are presented. The values are presented as least-squares means and standard errors of the means unless otherwise stated. Differences were considered significant if a probability ( $p$ ) of  $< 0.05$  ( $p < 0.05$ ) was found, and trends were discussed for variables with  $p \leq 0.10$ .

## RESULTS

### Characterization of weather conditions during experimental periods

During the study period, the recorded weather conditions ranged from warm to very hot. The average weather condition trends are shown in Fig. 1. The average daily air temperature was approximately 6 °C higher from July to August than from May to June and from September to October. The recorded average daily RH ( $79.98 \pm 0.79\%$ ) was highest from July to August ( $84.18 \pm 1.15\%$ ), followed by September to October ( $80.18 \pm 1.29\%$ ), and lowest from May to June ( $76.07 \pm 1.59\%$ ) ( $p < 0.05$ ).

### Effects of temperature-humidity index in physiological responses including rectal temperature, respiratory rate, rumen surface temperature and udder surface temperature under heat stress

Regarding our study results, for the 169 days study period, we recorded an average temperature of 21 °C or less daily for 46 days and an average temperature of 24 °C or less daily for 97 days. The average mean daily THI was 68.31 in May to June, 78.18 in July to August, and 68.54 in September to October. In addition, THIs  $> 68$  were present for 120 of 169 days in all cows. From

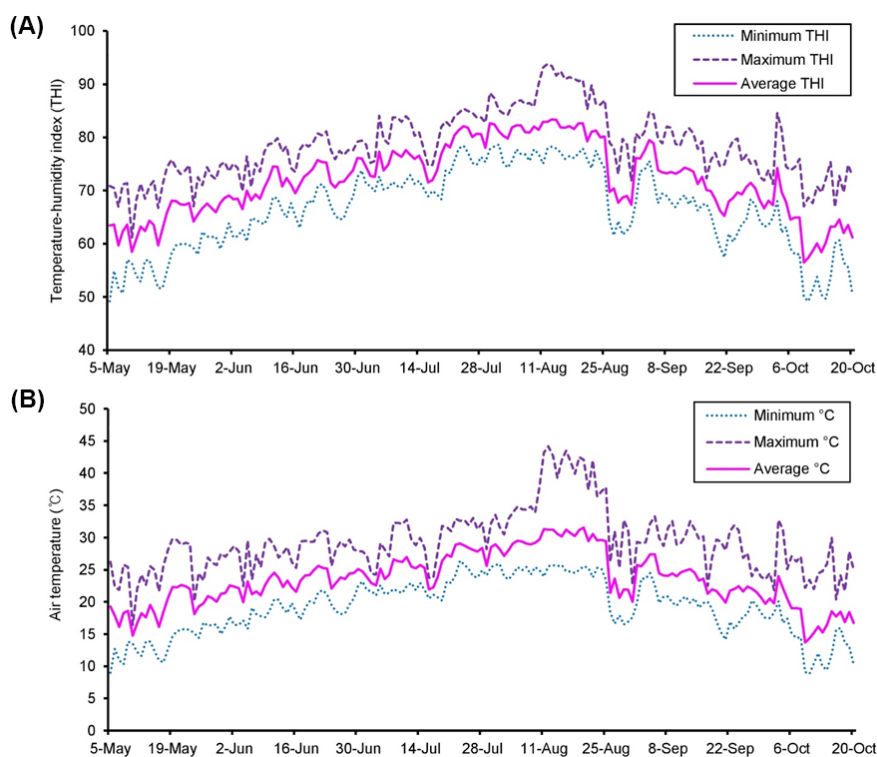
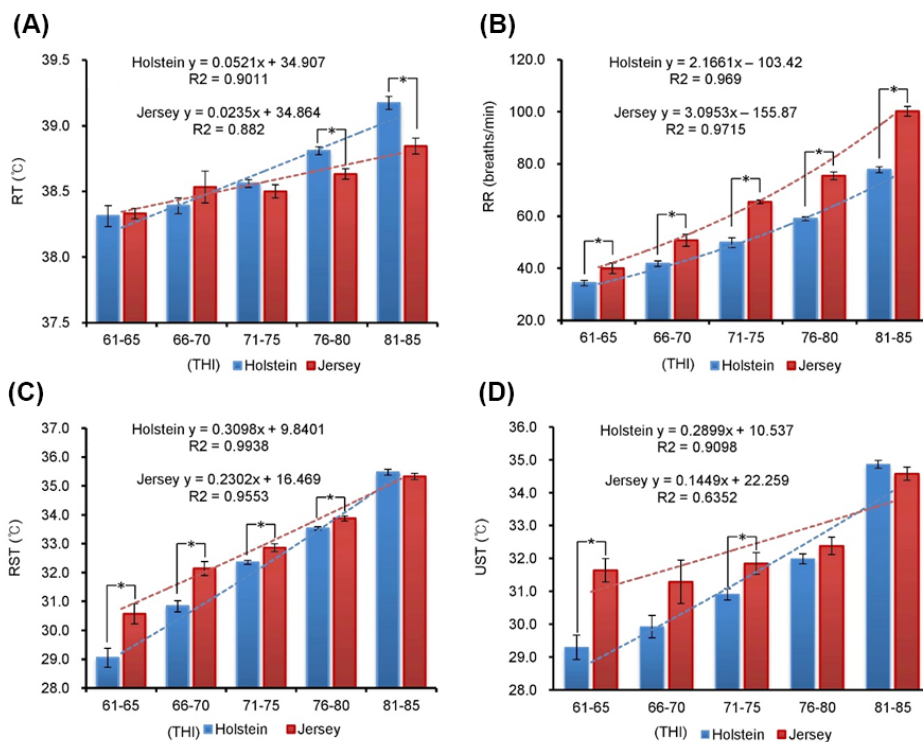


Fig. 1. Average temperature humidity index (THI; A) and air temperature (B) from May to October.

July to August, the average daily maximum THI was 84.0, and the average daily minimum THI was 73.39, which exceeded the critical value of 72.

The changes in the THIs had dramatic effects on Holstein and Jersey cows for physiological responses such as RT (Fig. 2A), RR (Fig. 2B), RST (Fig. 2C), and UST (Fig. 2D). The average RT of the Holstein cows ( $38.76 \pm 0.02^\circ\text{C}$ ) was  $0.15^\circ\text{C}$  higher than that of the Jersey cows ( $38.61 \pm 0.03^\circ\text{C}$ ) during the study period ( $p < 0.05$ ). The RT of the Holstein cows ( $38.10 \pm 0.11^\circ\text{C}$ ) was lower than that of the Jersey cows ( $38.36 \pm 0.05^\circ\text{C}$ ), with a THI of 61–63 ( $p < 0.05$ ). However, the RTs of the Holstein cows ( $38.83 \pm 0.04^\circ\text{C}$ ,  $39.13 \pm 0.06^\circ\text{C}$ , and  $39.61 \pm 0.10^\circ\text{C}$ ) were higher than those of the Jersey cows ( $38.60 \pm 0.05^\circ\text{C}$ ,  $38.90 \pm 0.09^\circ\text{C}$ , and  $38.85 \pm 0.14^\circ\text{C}$ ), with THIs of 76–78, 82–84 and 85–86 ( $p < 0.05$ ), respectively. We observed a large magnitude of increase in RTs of Jersey cows when the THI values increased from 61 to 65 (Fig. 2A), but the RT values were lower in Jersey cows ( $38.61^\circ\text{C}$ ) than in Holstein cows ( $38.76^\circ\text{C}$ ). These results showed that the Holstein cows experienced greater HS than the Jersey cows because there was a difference in metabolic heat production according to the milk production capacity of the two breeds. The average RR was increased in Jersey cows ( $74.85 \pm 1.34$  breaths/min) compared to Holstein cows ( $57.84 \pm 0.61$  breaths/min) when the THI values increased from 61–85 (Fig. 2B). The results of our current study also confirmed that Holstein cows responded more rapidly to HS with RT (THI 85–86,  $39.61^\circ\text{C}$ ) and slowly to RR (THI 79–81, 65.21 breaths/min). Jersey cows experienced no changes



**Fig. 2.** Relationship between rectal temperature (RT), respiratory rate (RR), rumen surface temperature (RST) and udder surface temperature (UST), and temperature humidity index (THI) under heat stress. The RT (A) was increased in Holstein cows compared with Jersey cows ( $R^2 = 0.9011$ ,  $p < 0.05$ ;  $R^2 = 0.882$ ,  $p < 0.05$ , respectively) when THIs increased. The RR (B) was increased in Jersey cows compared with Holstein cows ( $R^2 = 0.9715$ ,  $p < 0.05$ ;  $R^2 = 0.969$ ,  $p < 0.05$ , respectively) as THI values increased. The RST (C) was higher in Jersey cows than in Holstein cows ( $R^2 = 0.9553$ ,  $p < 0.05$ ;  $R^2 = 0.9938$ ,  $p < 0.05$ , respectively) as the THI increased. Similarly, the UST (D) was higher in Jersey cows than in Holstein cows ( $R^2 = 0.6352$ ,  $p < 0.05$ ;  $R^2 = 0.9098$ ,  $p < 0.05$ , respectively) when THIs increased. Error bars represent SEM; \* $p < 0.05$ .

in RT as THI increased, but Jersey cows experienced HS with RR (THI 73–75, 71.27 breaths/min) compared to Holstein cows.

In our study results, the THI showed a strong relationship with the RST ( $R^2 = 0.9938$ ) when compared with the RT, RR, and UST in the Holstein cows and with the RR ( $R^2 = 0.9715$ ) when compared with the RT, RST, and UST in the Jersey cows. During the study period, the average RST was higher in Jersey cows (33.56 °C) than in Holstein cows (33.19 °C) ( $p < 0.05$ ) (Fig. 2C). The average UST was also higher in Jersey cows (32.66 °C) than in Holstein cows (31.82 °C) (Fig. 2D). There were significant changes observed in the volume and pH of serum (Tables 2 and 3) between the Holstein and Jersey cows ( $p < 0.05$ ) with a THI range of 61–85. In Holstein cows, there was an increase in the pH of serum when THI ranged from 70–72 and 79–81. In Jersey cows, we observed a significant decrease in serum pH with increasing THIs from 73–75 to 82–84.

### Correlation between temperature-humidity index and milk production and components in Holstein and Jersey cows

Milk production in Holstein cows was not significantly changed over the entire investigated THI range (Table 4). However, for Jersey cows, milk production was significantly different in all THI ranges ( $p < 0.05$ ) and was lowest in the 70 to 75 THI range. Table 5 describes the impact of HS on changes in the milk composition in Holstein and Jersey cows when the THI was increased. No significant difference was noted for milk composition in Holstein cows as the THI increased from 61–84. However, the milk protein percentage declined from 3.78%–3.89% in the THI range of 61–72% to 3.20%–3.46% in the THI range of 73–84 in Jersey cows.

## DISCUSSION

HS is widely studied and may refer to climatic-related changes in the physiological or productive responses of cows. Previous literature has reported that HS indicates the degree to which external factors on the anatomical system alter the system from its resting or ground state, and strain is the internal displacement from the resting or ground state of the cows. Hence, environmental factors external to cows contribute to stress, and the displacement of cows from the resting state is a

**Table 2.** Comparative performance of relative serum volume (%) in Holstein and Jersey cows by THI range

THI	Holstein (%)	Jersey (%)	Total <sup>1)</sup> (%)	PSEM	p-value		
					THI	Breed	THI × Breed
61–63	58.97 <sup>a</sup>	61.78 <sup>b</sup>	59.94 <sup>a</sup>	1.051	0.365	< 0.001	0.886
64–66	45.76 <sup>b</sup>	53.80 <sup>c</sup>	51.42 <sup>b</sup>	1.977	0.270		
67–69	59.68 <sup>ay</sup>	65.36 <sup>abx</sup>	60.72 <sup>a</sup>	0.736	0.002		
70–72	59.03 <sup>ay</sup>	65.33 <sup>abx</sup>	60.20 <sup>a</sup>	0.965	0.011		
73–75	59.72 <sup>ay</sup>	67.70 <sup>ax</sup>	61.28 <sup>a</sup>	0.764	0.000		
76–78	57.37 <sup>ay</sup>	63.75 <sup>abx</sup>	58.91 <sup>a</sup>	0.749	0.000		
79–81	59.10 <sup>ay</sup>	64.70 <sup>abx</sup>	61.90 <sup>a</sup>	0.974	0.003		
82–84	58.13 <sup>ay</sup>	65.83 <sup>abx</sup>	59.08 <sup>a</sup>	1.047	0.020		

Values are expressed as the mean ± SEM of 7 dairy cows in each group.

<sup>1)</sup>Values are the average of the data for both breeds in the same THI ranges.

<sup>a-c</sup>Denote comparisons made within (same breed) columns ( $p < 0.05$ ).

<sup>x,y</sup>Denote comparisons made between Holstein and Jersey cows in the same THI range.

THI, temperature-humidity index; PSEM, pooled standard error of the mean.

**Table 3. Comparative performance of serum pH in Holstein and Jersey cows by THI range**

THI	Holstein	Jersey	Total <sup>1)</sup>	PSEM	p-value		
					THI	Breed	THI × Breed
61–63	7.376 <sup>e</sup>	7.421 <sup>a</sup>	7.395 <sup>d</sup>	0.024	0.365	< 0.001	< 0.001
64–66	7.432 <sup>cd</sup>	7.401 <sup>a</sup>	7.420 <sup>cd</sup>	0.013	0.270		
67–69	7.465 <sup>bc</sup>	7.429 <sup>a</sup>	7.460 <sup>bc</sup>	0.012	0.273		
70–72	7.502 <sup>bx</sup>	7.409 <sup>ay</sup>	7.485 <sup>b</sup>	0.010	0.000		
73–75	7.443 <sup>cdx</sup>	7.417 <sup>ay</sup>	7.438 <sup>cd</sup>	0.008	0.232		
76–78	7.471 <sup>bcx</sup>	7.385 <sup>aby</sup>	7.451 <sup>bc</sup>	0.009	0.000		
79–81	7.606 <sup>ax</sup>	7.383 <sup>aby</sup>	7.548 <sup>a</sup>	0.024	0.000		
82–84	7.409 <sup>dex</sup>	7.327 <sup>by</sup>	7.399 <sup>d</sup>	0.009	0.000		

Values are expressed as the mean ± SEM of 7 dairy cows in each group.

<sup>1)</sup>Values are the average of data for both breeds in the same THI ranges.

<sup>a–e</sup>Denote comparisons made within (same breed) columns.

<sup>x–y</sup>Denote comparisons made between Holstein and Jersey cows in the same THI ranges.

THI, temperature-humidity index; PSEM, pooled standard error of the mean.

**Table 4. Comparative performance of milk production in Holstein and Jersey cows by THI range**

THI	Holstein (kg/d)	Jersey (kg/d)	Total <sup>1)</sup> (kg/d)	PSEM	p-value		
					THI	Breed	THI × Breed
61–63	28.77 <sup>abx</sup>	19.75 <sup>ay</sup>	25.63	0.801	0.000	< 0.001	0.036
64–66	27.13 <sup>bx</sup>	17.99 <sup>bcy</sup>	23.83	0.605	0.000		
67–69	29.24 <sup>ax</sup>	17.98 <sup>bcy</sup>	25.13	0.463	0.000		
70–72	29.14 <sup>ax</sup>	16.82 <sup>cy</sup>	23.94	0.491	0.000		
73–75	29.06 <sup>ax</sup>	16.92 <sup>cy</sup>	24.02	0.469	0.000		
76–78	28.68 <sup>abx</sup>	17.68 <sup>bcy</sup>	23.95	0.563	0.000		
79–81	28.49 <sup>abx</sup>	17.29 <sup>bcy</sup>	23.85	0.514	0.000		
82–84	28.63 <sup>abx</sup>	18.61 <sup>aby</sup>	24.26	0.493	0.000		

Values are expressed as the mean ± SEM of 7 dairy cows in each.

<sup>1)</sup>Values are the average of data for both breeds in the same THI ranges.

<sup>a–c</sup>Denote comparisons made within (same breed) columns.

<sup>x–y</sup>Denote comparisons made between Holstein and Jersey cows at the same THI ranges.

THI, temperature-humidity index; PSEM, pooled standard error of the mean.

**Table 5. Comparative performance of milk composition in Holstein and Jersey cows by THI range**

Item	Breed	THI								Total <sup>1)</sup>	PSEM	p-value		
		61–63	64–66	67–69	70–72	73–75	76–78	79–81	82–84			THI	Breed	THI × Breed
Fat (%)	Holstein	3.49	3.81	3.85	3.64	3.96	3.61	3.64	3.37	3.67	0.06	0.428	< 0.001	0.373
	Jersey	5.02	4.74	4.71	5.20	4.45	4.46	4.74	4.28	4.70	0.10	0.389		
Protein (%)	Holstein	3.20	3.16	3.18	3.09	3.13	3.07	3.01	2.88	3.09	0.03	0.111	< 0.001	0.093
	Jersey	3.89 <sup>a</sup>	3.79 <sup>a</sup>	3.85 <sup>a</sup>	3.78 <sup>a</sup>	3.44 <sup>b</sup>	3.46 <sup>b</sup>	3.42 <sup>b</sup>	3.20 <sup>b</sup>	3.61	0.04	0.000		
Lactose (%)	Holstein	4.85	4.87	4.87	4.82	4.84	4.81	4.82	4.55	4.81	0.02	0.111	0.004	0.249
	Jersey	4.71 <sup>ab</sup>	4.67 <sup>ab</sup>	4.43 <sup>b</sup>	4.63 <sup>ab</sup>	4.84 <sup>a</sup>	4.83 <sup>a</sup>	4.80 <sup>a</sup>	4.39 <sup>b</sup>	4.69	0.04	0.023		
TS (%)	Holstein	12.27	12.57	12.62	12.27	12.63	12.21	12.19	11.55	12.30	0.08	0.088	< 0.001	0.443
	Jersey	14.36	13.93	13.71	14.31	13.43	13.45	13.66	12.59	13.71	0.13	0.053		

Values are expressed as the mean ± SEM of 7 dairy cows in each group.

<sup>1)</sup>Values are the average of data for both THI ranges in the same breed.

<sup>a,b</sup>Denote comparisons made within (same breed) rows.

THI, temperature-humidity index; PSEM, pooled standard error of the mean.



response to external stress or heat [14]. Our study results supported the findings of Cook et al. [15], who reported that a THI > 68 was indicative of HS. In our study, a THI > 68 was observed for 120 of 169 days in all cows. According to the previous findings, cows in this study were continuously exposed to stressful conditions from May to October. Armstrong [16] reported that a THI < 71 was the thermal comfort zone (assuming the THI does not drop below thermoneutral conditions) and the average mean daily THI from May to June and September to October was not a stress factor in the present study. From July to August, the average daily maximum THI was 84.0, and the average daily minimum THI was 73.39, which exceeded the critical value of 72.

Body temperature is an indicator of abnormal physiological changes and early diseases in animals [17]. Common locations for measuring temperature are the rectum, ear, udder, vagina, reticulorumen [18], and skin [19]. The RT is also generally considered to be a good index of deep body temperature even though there is considerable variation among different parts of the body core at different times of the day. Heat tolerance, reflected by the ability to maintain normal body temperature with increased THI, has been demonstrated to be different in these two breeds of cows. The average RT of the Holstein cows was higher ( $p < 0.05$ ) than that of Jersey cows in the 61–85 THI range suggesting that Holstein cows had higher metabolic heat production. Our study results agreed with Srikandakumar and Johnson [20], who previously reported that RT was increased in Holstein cows as THI values increased. West et al. [21] reported that the normal body temperatures of Holstein-Friesian and Jersey cows in thermoneutral conditions were 38.3°C and 38.2°C, respectively. Muller and Botha [22] found that the changes in RT owing to a variation in ambient temperature were 63% and 30% for Holstein and Jersey cows, respectively, which seems to indicate that Holstein cows were more influenced by ambient temperature than Jersey cows.

The current study results showed that the higher increases in RR due to HS were similar to those of the changes seen in RT. Again, Jersey cows had a greater increase ( $p < 0.05$ ) in their average RR than Holstein cows as THIs increased. According to a previous report [22], the average RRs for Holstein and Jersey cows were 65.4 and 58.6 breaths/min, respectively. However, our study results showed that the RR was influenced more in Jersey cows than in Holstein cows. RRs accelerate when the temperature surpasses the upper critical temperature and decrease when the temperature drops below the thermal neutral zone [23]. The significance of an increasing RR in cows is that it can be used to disperse approximately 30% of the cow's heat by respiratory vaporization [24]. Respiratory vaporization and convection dissipate body heat and assist in maintaining thermal balance. This association may explain why the RT of Jersey cows was lower than that of Holstein cows in our study because the increased RR of Jersey cows was accelerated to dissipate body heat more rapidly. Previous study results indicated that RR starts to increase when the THI is above 73, and the RT begins to increase when the THI is above 80 [25]. The study results agreed with the results of Harris et al. [26] showing that the RT, RR, and pulse rate were significantly varied in Holstein and Jersey cows. The surface temperature constantly changes because it is the body's most efficient tool for regulating body temperature and depends on peripheral blood flow. If animals need to decrease their temperature, heat is transported from the core of the body to the skin by blood, and blood flow to the skin will increase, increasing the skin temperature [27]. Our results also showed that both the RST and UST were higher in Jersey cows than in Holstein cows; however, milk production was higher in Holstein cows than in Jersey cows. Daily assessment of RTs in cows is a common approach to monitor core body temperature. However, it takes time and labor to hold cows steady while measuring the RT [28]. The UST is also affected by various factors, including ambient temperature, humidity, air velocity, bedding material, and lying behavior [29], and can be monitored using the rapid, noninvasive procedure of thermal imaging [30]. Importantly, the daily assessment of UST differs from that of RT [31]. A weak relationship between RT and UST

might limit the use of thermal imaging for monitoring cows' body temperatures [32]. Recent work indicates that infrared thermography detected changes in temperature in the right and left flanks, and these changes showed a strong correlation with HS ( $R^2 = 0.62$  and  $0.72$ ; [31]). Therefore, monitoring the UST is less laborious than measuring the RT and could be an effective method to identify HS in cows. Our study results also showed that RST had a stronger correlation with HS than UST.

When an animal is under HS conditions, the RR and sweating increase, resulting in increased body fluid loss and affecting dehydration and blood homeostasis. Chronic hyperthermia also causes severe or prolonged inappetence, which further increases the supply of total carbonic acid to the rumen and lowers the ruminal pH, thereby resulting in subclinical and acute rumen acidosis [33]. Therefore, we determined the RSV and pH between the two breeds. Additionally, our study results are similar to the findings of Srikandakumar and Johnson [20], who showed a decrease ( $p < 0.01$ ) in serum pH from 7.42 to 7.34 in Jersey cows compared to Holstein cows. More importantly, a recent study showed that milk composition can be a powerful indicator of HS in dairy cows [34]. Wheelock et al. [35] also reported that HS did not affect milk fat and protein percentages. Another study showed that the milk fat percentage increased at a THI  $> 68$  in Holstein cows but remained unchanged in Jersey cows [36]. The milk composition percentage in milk from Holstein and Jersey cows was not significantly altered. Under HS conditions, the physiological parameters were changed significantly in the two breeds of cows. Productivity losses due to HS are highly important to cattle breeders, although they have not been studied in depth with respect to the economic impact on Korean dairy farms. Such productivity losses have a negative impact on the welfare of cows. By examining the effects of the length and intensity of the HS period on milk production, we confirmed which of the two breeds was more sensitive to HS and to what extent the THI begins to negatively affect milk production and change the milk composition in each breed of cows. Further research is required to evaluate the response of cows by routinely collecting large datasets from herds and environmental data.

This study was the first to explore the relationships between the accumulation of consecutive days of HS, noninvasive physiological responses and the production and composition of milk between Holstein and Jersey cows raised in the humid continental climate of Korea. During the study period, the average THI significantly increased from May to October. We confirmed that an increased number of consecutive days was associated with a higher THI, higher average RT and lower RR in Holstein cows than in Jersey cows. The average body temperature of the rumen and udder in Holstein cows was lower than that in Jersey cows. No significant difference was noted in relative serum volume, but we observed significant differences in serum pH. Moreover, Holstein cows had higher increases in their RT, RST, and UST, and Jersey cows had smaller increases in their RT, RST, and UST as THI changed from 61 to 84. Additionally, we demonstrated that Holstein cows had less heat tolerance than Jersey cows, but milk production was not affected in either breed when the THI changed from 61 to 84. Hence, Holstein cows appear to have an advantage in terms of economic returns for dairy farms in Korea, although some physiological responses of Jersey cows were favorable in the thermal environments that are typically seen in Korea. Therefore, further research is necessary before the raising of Jersey cows in Korean environmental conditions is considered.

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