

1 Suitable indicator of heat stress for genetic evaluation of heat tolerance in Holstein cows
2 in Japan

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4 Research Article

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17 Running head: Heat-tolerance evaluation in dairy cows

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1 **ABSTRACT**

2 Only a few, principal, weather stations in Japanese prefectures have the daily
3 humidity records required to calculate the temperature–humidity index (THI) as
4 a dairy cow heat-stress indicator. We compared three heat-stress indices: 1)
5 THI calculated from daily average temperature and daily relative humidity at a
6 principal weather station (PTHI); 2) daily average temperature at each herd’s
7 closest local weather station (TEMP); and 3) THI calculated from daily average
8 temperature at each herd’s closest local weather station and daily relative
9 humidity at the principal weather station (HTHI). We used daily records from
10 532 provincial weather stations and test-day records of milk production from
11 days 6 to 305 post-first-calving in Holsteins to compare the indices as indicators
12 of heat-stress effects on milk yield and somatic cell score (SCS). The models
13 used the BLUPF90 package to analyze the effects of herd–year, calving age,
14 days in milk, and PTHI, TEMP, or HTHI. We estimated each model’s mean
15 square error (MSE) and compared suitabilities among indices for each trait.
16 TEMP heat-stress thresholds were ~18 °C (milk yield) and 15–20 °C (SCS). The
17 MSE of the HTHI model was the smallest, but no significant differences were
18 found among the indices for milk yield.

1 Keywords: dairy cow, heat stress, heat tolerance, Holstein, temperature–
2 humidity index

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6 **1 INTRODUCTION**

7 The Holstein—Japan’s main dairy cow—excels as a dairy breed,
8 producing 8 016 to 9 531 kg of average milk yield over 305 days during the first
9 to third lactation periods (Yamazaki et al., 2016). However, many studies have
10 shown that Holstein cows are very sensitive to heat stress and suffer negative
11 effects of heat stress on their milk yield and reproduction (e.g., Fuquay, 1981;
12 Ravagnolo et al., 2000; Boonkum et al., 2011; Bernabucci et al., 2014; Atagi et
13 al., 2019; Hagiya et al., 2020; Negri et al., 2021). The temperature–humidity
14 index (THI) is generally used to indicate heat stress in dairy cows. Heat stress
15 in Holstein cows occurs when the THI is 68 or higher (West, 2003), and daily
16 milk yield decreases by 0.72 kg for each unit of increase in the THI value
17 (Bernabucci et al., 2010). Also, an increased THI is associated with an
18 increased somatic cell score (SCS) (Lambertz et al., 2014), because heat stress

1 can promote mammogenic udder infection (Giesecke 1985; Pragna et al.,
2 2017). Hagiya et al. (2017) reported that heat stress affects milk yield, SCS, and
3 fertility in Holstein cows in Japan. The economic losses incurred by dairy
4 farmers in the United States as a result of heat stress amount to \$1.5 billion
5 annually without heat protection and \$900 million annually with heat protection
6 (St-Pierre et al., 2003). Therefore, to minimize the economic losses of dairy
7 farmers, especially in the light of today's climate warming scenarios, it is
8 important to investigate the effects of heat stress on dairy cows (Negri et al.,
9 2020).

10 In recent years, average temperatures in Japan have been rising
11 because of global warming. Barn facilities such as sprinklers and fans can
12 reduce the effects of heat stress (Armstrong, 1994), but installing these facilities
13 is expensive. We therefore need to increase heat tolerance by genetically
14 improving dairy cows.

15 Ravagnolo and Misztal (2010) reported that it is possible to evaluate the
16 genetic ability of dairy cows to tolerate heat stress by using a random
17 regression model. In Australia, HTABVg (the Australian breeding value for heat
18 tolerance) has been proposed as a new genetic measure of heat tolerance in

1 dairy cows (Nguyen et al., 2017); genetic improvement of heat tolerance is one
2 of the important concerns. Hagiya et al. (2020) reported a suitable random
3 regression model for genetic evaluation of heat tolerance in Japan. To evaluate
4 genetic heat tolerance capacity, we need to identify traits that accurately reflect
5 the effects of heat stress and to clarify the threshold value at which the effects
6 of heat stress begin to be observed. Hagiya et al. (2019) reported that the
7 threshold values for heat stress on dairy cows in Japan ranged from THI 60 to
8 THI 63 for both milk yield and SCS, and the THI that they used was estimated
9 from temperature and humidity data recorded at principal meteorological
10 stations. In Japan, each prefecture has many meteorological stations and most
11 of these record daily temperatures, but only one or two principal meteorological
12 stations in each prefecture keep humidity records. Therefore, we sometimes
13 have to assign THI values by using a meteorological station as far as 100 km
14 away from a farm, instead of those from the nearest meteorological station.

15 Nagamine and Sasaki (2008) reported that temperature had a greater
16 negative effect than humidity on conception rate in Holstein cows in Japan.
17 Therefore, here, to investigate suitable indicators of heat stress in Holstein cows
18 under Japanese weather conditions, we compared the applicability of three

1 mathematical models by using the following three heat stress indices: 1) THI
2 calculated from the daily average temperature and daily relative humidity at one
3 of the principal weather stations in each prefecture (PTHI); 2) the average daily
4 temperature at the local weather station closest to each herd (TEMP); and 3)
5 THI calculated from the daily average temperature at the local weather station
6 closest to each herd and the relative humidity at the principal weather station
7 (hybrid THI, HTHI).

8

9 **2 MATERIALS AND METHODS**

10 **2.1 Data**

11 Data on test-day milk yield and somatic cell count (SCC) collected by
12 the National Livestock Breeding Center for domestic genetic evaluation
13 purposes were used. The data consisted of monthly test-day records from days
14 6 to 305 after first calving of Holsteins in Japan between 2011 and 2015. When
15 meteorological data were missing, production records for the corresponding
16 dates were excluded. The number of test-day records was 4 112 791. SCCs
17 were log-transformed into SCSs closer to a normal distribution. The following
18 formula was used for logarithmic conversion (Ali & Shook, 1980):

1 $SCS = \log_2(SCC/100\ 000) + 3.$

2 For the meteorological data, we used daily average temperature and
3 relative humidity records obtained from 60 principal and 432 local
4 meteorological stations in each prefecture from 2011 to 2015 and published by
5 the Japan Meteorological Agency (Japan Meteorological Agency, 2020). Japan
6 is situated from latitude 20°N to 46°N, and average monthly temperatures range
7 from −8.9 °C in the north to 29.5 °C in the south (Table1). For PTHI, test-day
8 records were assigned only one principal meteorological station in each of 46
9 prefectures, and 14 in the 47th prefecture, namely Hokkaido (the northern
10 island containing more than half of the herds), according to the method of
11 Hagiya et al. (2019). For HTHI, we used temperature records from the
12 meteorological station closest to each herd and humidity records from the
13 principal meteorological station for each prefecture or the 14 principal stations in
14 the case of Hokkaido. On the basis of the results of our preliminary findings that
15 THI calculated from daily average temperature and average relative humidity is
16 suitable for use as a heat stress index, THI was calculated from these
17 parameters by using the following formula (Thom, 1959):

18 $THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)$

1 where T is the daily average temperature (°C) and RH is the average relative
 2 humidity (%). On the basis of the results of reported by Hagiya et al. (2019), we
 3 set the duration of the lag in heat response on the test day as 3 days for mild
 4 yield and 8 days for SCS.

5 **2.2 Model**

6 The following mathematical model with reference our previous study
 7 (Hagiya et al. 2019) was used to analyze the effects of heat stress on milk yield
 8 and SCS:

$$9 \quad Y_{ijklmn} = HY_i + M_j + A_k + DIM_l + T_m + a_n + e_{ijklmn}$$

10 where Y_{ijklmn} is the test-day milk yield or SCS; HY_i is the fixed effect of
 11 herd×year i ; M_j is the effect of calendar month at calving, including the effects
 12 from calving to test-day (12 subclasses); A_k is the fixed effect of age at calving
 13 (18 to 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 months); DIM_l is
 14 the fixed effect of l days in milk; T_m is each heat stress index [56 levels for
 15 TEMP (range, -23 to 33 °C), 76 levels for PTHI (range, 9 to 84), 86 levels for
 16 HTHI (range, 1 to 86)]; a_n is a random additive genetic effect containing the
 17 permanent environmental effect of cow n ; and e_{ijklmn} are random residuals.
 18 Variance components estimated by Hagiya et al. (2019) were used in our

1 analysis. Mean squared errors (MSEs) were estimated by using BLUPF90
2 (Misztal et al. 2002) to compare the suitabilities of indices for each trait.

3 **2.3 Preliminary study**

4 In a preliminary study, we investigated the combination of daily
5 temperature and relative humidity records that was most suitable for use in a
6 heat stress index. For this purpose, we compared THI's calculated by using a
7 range of combinations of temperature and humidity records from principal
8 meteorological stations. The various combinations are shown in Table 2. When
9 meteorological data (maximum or minimum temperature, or minimum relative
10 humidity) were missing, production records for the corresponding dates were
11 excluded. The number of test-day records in the preliminary study was
12 2 466 440.

13

14 **3 RESULTS**

15 **3.1 Preliminary study to select THIs**

16 Table 3 shows the MSEs of mathematical models using each heat
17 stress index [various combinations of temperature records (average, maximum,
18 minimum) and relative humidities (average, minimum) observed at principal

1 meteorological stations] for milk and SCS. For milk yield, there were significant
2 differences between the MSE in the mathematical model without the heat stress
3 index and the MSEs in the mathematical models with heat stress indices,
4 except when we used average temperature data alone as an index. For SCS,
5 there were no significant differences among any of the mathematical models.
6 For milk yield, mathematical models containing THI_{aa} calculated from average
7 temperature and average relative humidity or THI_{ab} calculated from average
8 temperature and minimum relative humidity gave the lowest MSE value. The
9 MSE calculated from THI_{ca} was the highest.

10 **3.2 Changes in annual average temperature**

11

12 We plotted the transition of the annual average temperature in Japan up
13 to 2020 (Fig. 1) by using temperature data released by the Japan
14 Meteorological Agency (2020). Average temperatures in Japan rose over a
15 range of 13 to 17 °C from 1876 to 2016 because of global warming. We also
16 plotted average temperatures in 2020 by calendar month (Fig. 2); average
17 temperatures in August were about 20 °C higher than those in January. The
18 average monthly temperature in Hokkaido, which contained more than half the

1 herds studied, was about 5 °C lower than that in the other prefectures.

2 **3.3 Summary statistics**

3 Means, standard deviations (SDs), and minimum and maximum values
4 for test-day milk yield and SCS and for the three heat stress indices are given in
5 Table 4. The mean daily milk yield (\pm SD) was 27.99 ± 6.4 kg, and the mean
6 SCS (\pm SD) was 2.27 ± 1.69 . Figure 3 shows the numbers of TEMP records
7 used. The average daily temperature ranged from -19 to 30 °C, with the
8 greatest number of records at about 20 °C. The shapes of the distributions of
9 THIs (data not shown) used in the analysis were consistent with those of the
10 THI values used in our previous study (Hagiya et al., 2019).

11 **3.4 Threshold values for effects of PTHI, TEMP, and HTHI on milk yield** 12 **and SCS**

13 We obtained BLUEs (best linear unbiased estimations) of test-day milk
14 yield and SCS against PTHI, HTHI, or TEMP (Fig. 4). The threshold PTHI and
15 HTHI values for an effect on milk yield ranged from 65 to 70; that for TEMP was
16 about 18 °C. The threshold PTHI and HTHI values for an effect on SCS were
17 about 65; that for TEMP ranged from 15 to 20 °C. For milk yield and SCS, the
18 BLUEs were close to constant at PTHI, HTHI, and TEMP values below the

1 thresholds.

2 **3.5 Applicability of indices of heat stress**

3 The MSEs of the mathematical models were calculated for each of the
4 heat stress indices for milk yield and SCS (Table 5). For milk yield, but not SCS,
5 there was a significant difference between the MSEs for no HS index and the
6 mathematical model for each heat stress index. For milk yield, the MSEs
7 estimated from the models increased in the order HTHI < TEMP < PTHI < no
8 HS index. For SCS, the MSEs increased in the order HTHI < TEMP = PTHI <
9 no HS index.

10

11 **4 DISCUSSION**

12 **4.1 Effects of heat stress on milk yield and SCS**

13 Our preliminary analysis revealed that THI_{aa} calculated by using
14 average temperature and average relative humidity and THI_{ab} calculated by
15 using average temperature and minimum relative humidity in Japan's
16 meteorological environment were useful for both milk yield and SCS. The MSEs
17 from THI_{ca} calculated by using minimum temperature and average relative
18 humidity were large for both milk yield and SCS. In the model considering THI_{ca} ,

1 we found extremely slow convergence. Therefore, the BLUEs from the model
2 containing THI_{ca} were not suitable in comparison with those from other
3 mathematical models including THI.

4 Decreased dry matter intake is one cause of heat-associated decreases
5 in milk yield (Schneider et al., 1988). Rhoads et al. (2009) have shown that 35%
6 of all declines in milk production are due to reduced dry matter intake.
7 According to West (2003), the dry matter intake of dairy cows in a hot
8 environment decreases by 0.85 kg/°C. Moreover, changes in metabolism due to
9 heat stress change the homeostasis of dairy cows: for example, heat stress
10 increases plasma insulin levels (Wheelock et al., 2010) and decreases plasma
11 growth hormone levels and milk yield (Igno et al., 1988; McGuire et al., 1991;
12 Rhoads et al., 2009; Wheelock et al., 2010). Here, we found that the threshold
13 values of both PTHI and HTHI for an effect on test-day milk yield ranged from
14 65 to 70. This was similar to the thresholds for milk production loss (THI about
15 68 to 74) in a worldwide comprehensive review by Herbut et al. (2018). The
16 wide range of THI thresholds in that review was due to differences among study
17 areas in breeding environment, breed, and type of winter housing. Atagi et al.
18 (2019) reported somewhat different threshold THI values linked to the daily

1 mean temperature and relative humidity for first-lactation Holstein cows in
2 Japan (72 for milk yield and 64 for SCS) because they used segmented-
3 regression analysis (Muggeo, 2008). Our estimated threshold TEMP (i.e., air
4 temperature) value for decreased test-day milk yield was about 18 °C. This
5 value corresponds to a THI of 63, assuming an average relative humidity of
6 70% (Japan Meteorological Agency 2010). From the monthly average
7 temperatures in Hokkaido and other prefectures in 2020 (see Fig. 2), we
8 inferred that effects of heat stress on milk yield would appear from June to
9 September in Hokkaido and from May to October in other prefectures.

10 One of the causes of increased SCS is weakened immunity due to heat
11 stress (Roma et al., 2009). An increase in SCS is associated with increased
12 prevalence and development of both latent and clinical mastitis (Dohoo & Meek,
13 1982). The thresholds of the two THI indicators for increased SCS were about
14 65. This is consistent with a previous report that used domestic cattle herd data
15 to calculate threshold THI values on the basis of daily mean temperature and
16 relative humidity; the values were 60 to 65 for increased SCS (Hagiya et al.,
17 2019). Our estimated TEMP threshold values (15 to 20 °C) meant that the effect
18 of heat stress on SCS appeared from about May to October in Hokkaido and

1 from about April to November in other prefectures (Fig. 2). Thus our results for
2 milk yield and SCS show that dairy cows in Japan are exposed to heat stress
3 for approximately half a year.

4 **4.2 Weather records suitable as indicators of heat stress**

5 Nagamine and Sasaki (2008) reported that the significant effect of heat
6 stress on conception in Japanese Holstein cows occurs only through the
7 disturbance of body temperature. The effects of heat stress include a decrease
8 in food intake and an increase in production of active enzyme due to an
9 increase in body temperature (Collier et al. 2006). The resulting increased
10 oxidative stress leads to disturbance of homeostasis and consequently a
11 decrease in conception rate. This physiological phenomenon also causes milk
12 loss (Sakatani et al., 2011) and mastitis (Lykkesfeldt & Svendsen, 2007). For
13 milk yield, we found significant decreases in the MSEs of the mathematical
14 models for the three heat stress indices compared with that for no HS index
15 (Table 5). This suggests that three indices are suitable choices of heat stress
16 index for analyzing the effects of heat stress on milk yield.

17

18 **5 CONCLUSION**

1 Effects of heat stress on milk yield in Holstein cows in Japan appeared
2 when the temperature was 18 °C or higher. For SCS, the threshold TEMP
3 values of heat stress were in the range of 15 to 20 °C. The average temperature
4 in Japan is now about 17 °C, and our findings suggest that dairy cows in Japan
5 are exposed to heat stress for about half a year. To estimate the effects of heat
6 stress in terms of decreasing milk yield, PTHI calculated by using daily mean
7 temperature records and relative humidity records obtained from nearest
8 principal meteorological station, TEMP obtained by using mean temperature
9 records from nearest meteorological station, and HTHI calculated by using daily
10 mean temperature records from the nearest meteorological station and relative
11 humidity records from the nearest principal meteorological station are
12 applicable.

13

14 **CONFLICTS OF INTEREST**

15 The funding source had no role in the design, practice or analysis of this study.

16

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2

- 1 Legends
- 2 Figure 1 Changes in annual average temperature in Japan, 1876 to 2016
- 3
- 4 Figure 2 Monthly average temperatures in Hokkaido and other prefectures in
- 5 2020
- 6
- 7 Figure 3 Numbers of daily mean temperature records used in the analyses
- 8
- 9 Figure 4 Best linear unbiased estimates of test-day milk yield and somatic cell
- 10 score (SCS) obtained by using the models PTHI, HTHI, and TEMP
- 11
- 12

Table 1 The number of records, means, standard deviations (SD), and minimum and maximum values for temperature and humidity¹ in each region²

Region	N	Temperature				Humidity		
		Mean	SD	Min	Max	Mean	SD	Min
Hokkaido	2 773 188	6.6	11.2	-23	29	76.2	12.3	25
Tohoku	252 873	10.5	9.7	-12	31	73.4	11.5	29
Kanto	327 313	15.0	8.7	-10	33	66.0	15.2	22
Chubu	169 695	14.0	9.2	-13	33	68.7	12.6	22
Kinki	73 432	15.3	8.5	-4	31	66.3	11.6	34
Chugoku	132 697	15.0	8.7	-7	32	68.7	11.5	33
Shikoku	40 279	17.9	7.5	-5	32	68.6	12.0	26
Kyusyu	343 341	19.0	7.0	-6	32	71.7	11.9	25

¹ Maximum humidity : 100 in all regions

²Tohoku : Aomori, Iwate, Miyagi, Akita, Yamagata, Fukushima

Kanto : Ibaraki, Tochigi, Gunma, Saitama, Chiba, Tokyo, Kanagawa

Chubu : Nigata, Toyama, Ishikawa, Fukui, Yamanashi, Nagano, Gifu, Shizuoka, Aichi, Mie

Kinki : Shiga, Kyoto, Osaka, Hyogo, Nara, Wakayama

Chugoku : Tottori, Shimane, Okayama, Hiroshima, Yamaguchi

Shikoku : Tokushima, Kagawa, Ehime, Kochi

Kyusyu : Fukuoka, Saga, Nagasaki, Kumamoto, Oita, Miyazaki, Kagoshima, Okinawa

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Table 2 *Weather data included in the heat stress index calculations in our preliminary study*

Heat stress index	Weather data				
	Temperature			Humidity	
	Mean	Maximum	Minimum	Mean	Minimum
No heat stress index	–	–	–	–	–
TEMP _a	○	–	–	–	–
TEMP _b	–	○	–	–	–
TEMP _c	–	–	○	–	–
THI _{aa}	○	–	–	○	–
THI _{ab}	○	–	–	–	○
THI _{ba}	–	○	–	○	–
THI _{bb}	–	○	–	–	○
THI _{ca}	–	–	○	○	–
THI _{cb}	–	–	○	–	○

Weather data are from principal weather stations.

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Table 3 *MSEs¹ of mathematical models using each heat stress index² for milk and SCS³ in our preliminary study*

Heat stress index	Trait	
	Milk	SCS
No heat stress index	9.8722 ^b	1.1355
TEMP _a	9.8433 ^{ab}	1.1350
TEMP _b	9.8522 ^a	1.1351
TEMP _c	9.8406 ^a	1.1350
THI _{aa}	9.8392 ^a	1.1350
THI _{ab}	9.8392 ^a	1.1350
THI _{ba}	9.8482 ^a	1.1350
THI _{bb}	9.8446 ^a	1.1350
THI _{ca}	9.9389 ^c	1.1373
THI _{cb}	9.8425 ^a	1.1350

Different superscript letters indicate significant differences between values ($P < 0.05$).

¹Mean squared errors

²See [Table 2](#)

³Somatic cell score

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Table 4 Means, standard deviations (SD), and minimum and maximum values for test-day milk yield, SCS¹, PTHI², HTHI³, and TEMP⁴ (N = 4 112 818)

Trait or heat stress index	Mean	SD	Min	Max
Milk (kg)	27.8	6.5	0.2	88.3
SCS	2.2	1.7	-3.6	12.5
PTHI	50.3	18.5	0.0	84.0
HTHI	50.6	17.0	1.0	86.0
TEMP	9.4	11.3	-23.0	33.0

¹Somatic cell score

²Temperature–humidity index (THI) calculated from the daily average temperature and daily relative humidity at one of the principal weather stations in each prefecture

³THI calculated from the daily average temperature at the local weather station closest to each herd and the relative humidity at the principal weather station

⁴The average daily temperature at the local weather station closest to each herd

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Table 5 *MSEs¹ of mathematical models obtained by using each heat stress index for milk and SCS²*

Heat stress index	Trait	
	Milk	SCS
No heat stress index	9.9124 ^a	1.1291
PTHI ³	9.8899 ^b	1.1287
TEMP ⁴	9.8859 ^b	1.1287
HTHI ⁵	9.8848 ^b	1.1286

Different superscript letters indicate significant differences between values ($P < 0.05$).

¹Mean squared errors

²Somatic cell score

³ Temperature–humidity index (THI) calculated from the daily average temperature and daily relative humidity at one of the principal weather stations in each prefecture

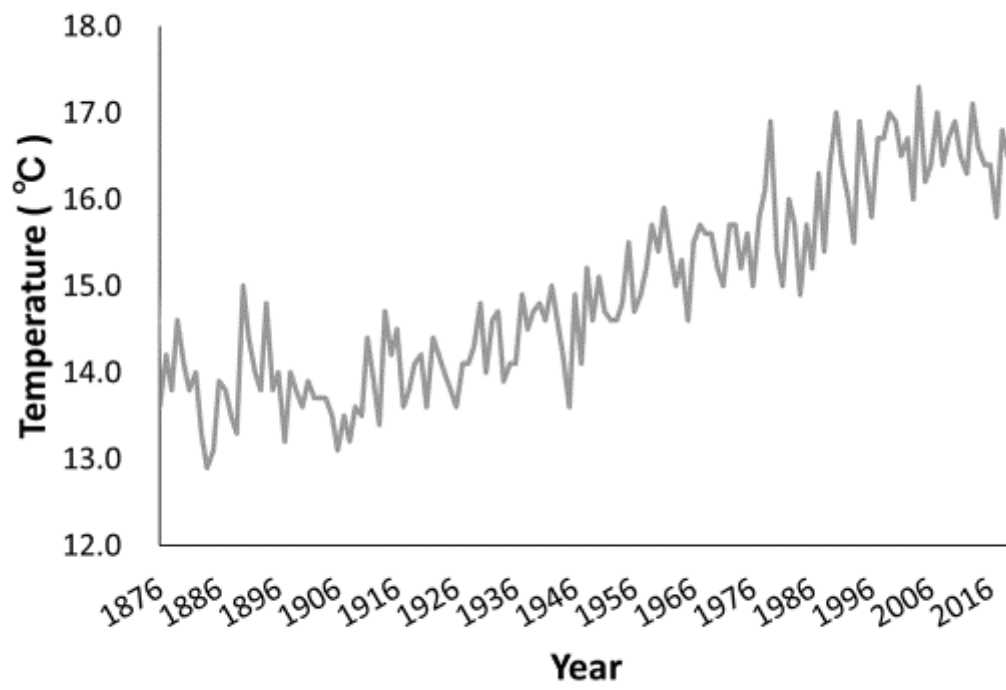
⁴The average daily temperature at the local weather station closest to each herd

⁵ THI calculated from the daily average temperature at the local weather station closest to each herd and the relative humidity at the principal weather station

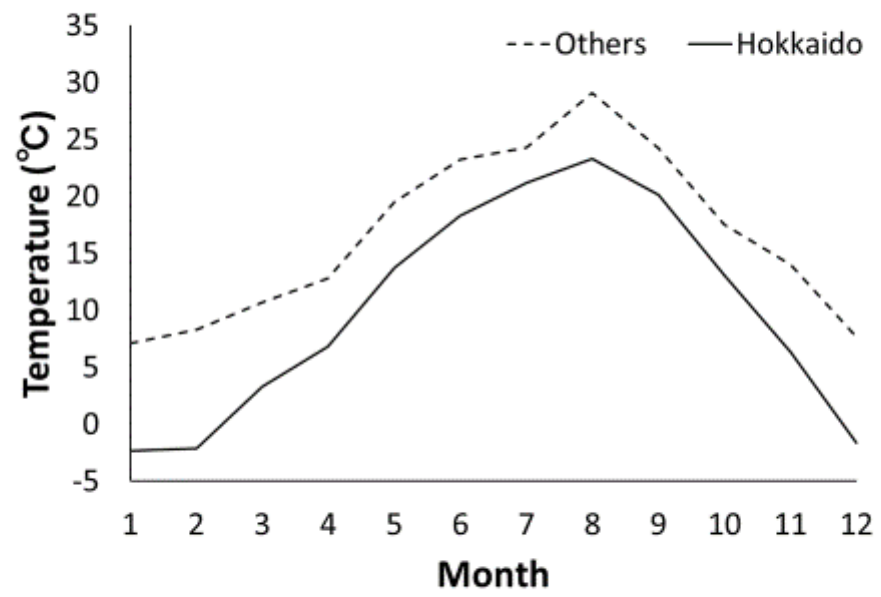
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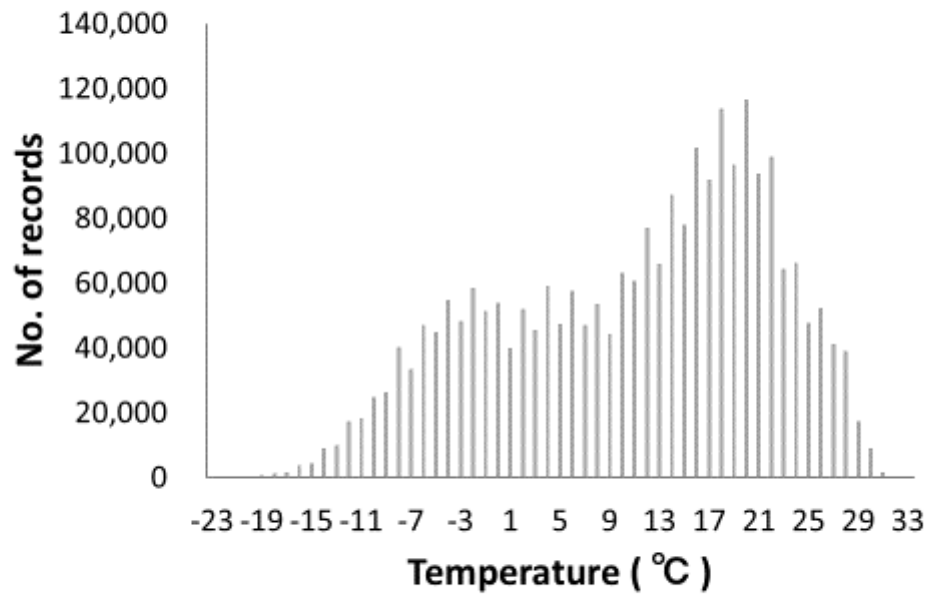
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