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How heat stress conditions affect milk yield, composition, and price in Italian Holstein herds

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ABSTRACT

An edited data set of 700 bulk and 46,338 test-day records collected between 2019 and 2021 in 42 Holstein-dominated farms in the Veneto Region (North of Italy) was available for the present study. Information on protein, fat and lactose content, somatic cell count, and somatic cell score was available in bulk milk as well as individual test-day records, whereas urea concentration (mg/dL), differential somatic cell count (%), and milk yield (kg/d) were available for test-day records only. Milk features were merged with meteorological data retrieved from 8 weather stations located maximum 10 km from the farms. The daily and weekly temperature-humidity index (THI; wTHI) and maximum daily (MTHI) and weekly temperature-humidity index were associated with each record to evaluate the effect of heat stress conditions on milk-related traits through linear mixed models. Least squares means were estimated to evaluate the effect of THI and, separately, of MTHI on milk characteristics correcting for conventional systematic factors. Overall, heat stress conditions lowered the quality of both bulk milk and test-day records, with fat and protein content being greatly reduced, and somatic cell score and differential somatic cell count augmented. Milk yield was not affected by either THI or MTHI in this data set, but the effect of elevated THI and MTHI was in general stronger on test-day records than on bulk milk. Farm-level economic losses of reduced milk quality rather than reduced yield as consequence of elevated THI or MTHI was estimated to be between $23.57 and $43.98 per farmer per day, which is of comparable magnitude to losses resulting from reduced production. Furthermore, MTHI was found to be a more accurate indicator of heat stress experienced by a cow, explaining more variability of traits compared with THI. The negative effect of heat stress conditions on quality traits commences at lower THI/MTHI values compared with milk yield. Thus, a progressive farmers' income loss due to climatic changes is already a reality and it is mainly due to deterioration of milk quality rather than quantity in the studied area.
Key words: heat tolerance, milk composition, temperature-humidity index, herd profitability

INTRODUCTION

In dairy cattle, heat stress (**HS**) occurs when a cow is unable to dissipate an adequate amount of heat to maintain her physiological body temperature. This can be due to an excess of internally produced heat or an exposure to high ambient temperatures (Kadzere et al., 2002; Bernabucci et al., 2014). The thermo-neutral zone of dairy cows (i.e., the range of temperatures within which no additional energy is spent to heat or cool the body) is between 5 and 25°C (Morgan, 1998; Roenfeldt, 1998), which is regularly surpassed in many parts of the world. When the exposure to ambient temperatures exceeds the thermo-neutral zone, fertility, productivity, health status, and overall welfare of the cow worsen (Nardone et al., 2010; Tao et al., 2020) and milk quality and production decline (Coppock, 1978). Although results remain inconsistent (Tao et al., 2018), studies aiming to quantify the effect of HS conditions on cow performance most often report a decrease in protein and fat percentage (Bernabucci et al., 2015; Kekana et al., 2018; Mandal et al., 2021; Campos et al., 2022; Toghdory et al., 2022) as well as yield (Bouraoui et al., 2002; Bernabucci et al., 2014); however, a few studies report an increase in percentage (Garner et al., 2016) but a concurrent decrease in yield (Corazzin et al., 2020), most likely caused by a concentrating effect. With temperatures set to further increase globally as a result of climate change, the way in which HS conditions exert adverse effects on the dairy sector has become an issue of growing importance (Nardone et al., 2010). Genetic selection for high-producing dairy cows has inadvertently altered their metabolism, thermoregulatory abilities, and thus sensitivity to heat (Kadzere et al., 2002; Ravagnolo and Misztal, 2002; Freitas et

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al., 2006; Aguilar et al., 2009). Additionally, high milk production requires increased feed intake and greater metabolic activity, which in turn produces more heat (Kadzere at al., 2002). Modern specialized dairy cows have therefore become less heat resistant and consequently more susceptible to HS.

Physiological measures such as respiration rate, heat rate, and rectal temperature (Kadzere et al., 2002; Polsky and von Keyserlingk, 2017) can directly assess HS in cattle, but they require impractical continuous monitoring at individual cow-level. The temperature-humidity index (**THI**) provides a noninvasive alternative to approximate HS conditions and relies on often easily accessible weather data. It incorporates the effects of ambient temperature with relative humidity (**RH**; Kibler, 1964). The formula proposed by Kibler (1964) is one of the most widely used (Herbut et al., 2018; Ouellet, 2019; Costa et al., 2020) and is dependent on the average temperature and RH. A THI below 70 is generally considered to be comfortable, between 75 and 78 stressful, and above 78 hazardous (Kadzere et al., 2002). Indeed, it was traditionally believed that milk yield (**MY**) diminishes as THI reaches 72 (Armstrong, 1994). However, newer data indicate that this threshold is reduced in modern high-yielding cows to 68 (Zimbelman et al., 2009; Collier et al., 2012). Alternative means by which to calculate THI using values other than averages have been proposed and shown to correlate with reduction in MY and quality (Ravagnolo et al., 2000). It has been suggested that the use of maximum daily temperature (**MT**) and minimum daily RH is a better indicator of on-farm HS conditions in comparison to a THI consisting of average values (Ravagnolo et al., 2000; Ouellet, 2019). On the contrary, a THI comprising the minimum daily temperature was suggested by Zimbelman et al. (2009) to be correlated with MY. This was attributed to night-time respite, which is thought to alleviate negative effects of daytime HS (Holter et al., 1996). Furthermore, some studies have demonstrated that the duration of the HS exposure influences the extent of production decline (Collier et al., 2012) while others have reported a time lag between heat exposure and decline in MY and quality, with the greatest effect occurring about 4 d after exposure (Bernabucci et al., 2014).

In Europe, a farmer is normally paid a fixed liter price for the milk supplied to the dairy plant, to which premiums and penalties are thereafter added based on milk quality traits accounted for in the local payment system. Such milk payment systems often rely on quality traits such as fat and protein content to determine the price per unit of milk (Draaiyer et al., 2009). These traits influence coagulation and technological properties of milk and, consequently, cheese production. It is therefore of great interest to farmers to produce large volumes of high-quality milk, particularly in countries in which the majority of supplied milk is directed to cheese production. However, considering the negative effects HS asserts on cow performance, frequent days with elevated THI represents a great obstacle in reaching the maximum potential of high-quality milk production. Fully elucidating the effect of THI on milk quality and production is therefore of importance for the scientific community and the dairy industry alike to combat the adverse effects of HS conditions. Earlier studies aiming to do so are most often conducted either on individual milk samples such as test-day records or on bulk milk records, and under controlled environments, i.e., experimental conditions in which the cow is isolated from the herd and kept in climate chambers under fixed THI ranges. To the best of the authors' knowledge, no study has investigated the concurrent effects of THI on quality traits of both bulk milk and individual bovine milk in commercial conditions so far.

The present retrospective study therefore aimed to determine the effect of seasonal HS conditions on both bulk milk traits and traits of test-day records collected in parallel from farms located on the plain and in mountain areas. For this purpose, THI comprising daily average temperature (**AT**) and RH, and maximum THI (**MTHI**) comprising MT and average RH were associated with test-day and bulk milk records from 50 dairy herds in the Veneto Region of Italy. Their effects on MY and quality traits were thereafter assessed, and the influence of lactation stage and parity explored. Furthermore, to investigate the carryover effect of prolonged HS conditions in these 50 herds, the weekly THI (**wTHI**) and weekly MTHI (**wMTHI**) were associated with the records and their relationship with MY and quality traits explored. Finally, based on the local Italian payment system, the economic loss aggravated by increasing THI/MTHI was estimated to elucidate the severity of the effects that rising global temperatures have on the dairy industry.

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Milk Traits

For the retrospective analysis, milk records from 50 Holstein-dominated (≥70% cows) farms located in the

Veneto Region (Northern Italy) were retrieved from the Breeders Association of Veneto Region (ARAV, Vicenza, Italy). Twenty-seven farms were located on the Po Valley (plain) and 23 in Alpine area (>600 m above sea level). Two opposing geographical areas were deliberately chosen so to represent a wider range of climatic environments. Both farm bulk milk and test-day records were available over a 24-mo period from September 2019 to August 2021 and were collected in parallel (i.e., pooled milk samples were taken the same day as individual test-day records for any given herd). This was done to directly compare the effect of HS conditions on bulk milk and test-day records to thereby elucidate if a difference exists. Milk traits comprised content (%) of fat, protein, and lactose predicted through mid-infrared spectroscopy at the milk laboratory of ARAV (Vicenza, Italy) equipped with Milkoscan FT7DC (FOSS Electric). Furthermore, for test-day records, urea concentration (mg/dL) was also predicted and the SCC (cells/mL) and differential somatic cell count (**DSCC**; %) were measured by means of Fossomatic (FOSS Electric). The DSCC is the ratio of PMN and lymphocytes to total SCC. Information on DIM, calving date, birth date, and parity of the cows was also obtained. The distribution of individual test-days across classes of DIM and parity is summarized in Supplemental Table S1 (https://doi.org/10.7910/DVN/FY0BGZ; Moore, 2023).

Meteorological Data

Meteorological data were retrieved from the Agency for Environmental Prevention and Protection of Veneto Region (ARPAV, 2022) and originated from 8 weather stations (i.e., 4 per area; mountain and plain). Each farm kept in the final database was located within 10 km from the respective weather station. For the dates in which milk was sampled, meteorological data consisted of the minimum daily temperature (°C), MT (°C), AT (°C), and average RH (%) calculated based on the minimum and maximum RH recorded on that day. The meteorological data were merged with milk data according to the geographical coordinates and the exact date of the milk test-day. As such, each test-day record and bulk milk record had information on minimum daily temperature, AT, MT, and average RH associated with it. For each farm on a given sampling date, THI and MTHI were calculated based on Kibler's equation (Kibler, 1964; Herbut et al., 2018):

$$\text{THI} = (1.8 \times \text{AT}) - (1.0 - \text{RH})\ (\text{AT} - 14.3) + 32;$$

$$\text{MTHI} = (1.8 \times \text{MT}) - (1.0 - \text{RH})\ (\text{MT} - 14.3) + 32.$$

To investigate the carryover effect of HS conditions, the THI and MTHI values measured the week leading up to the test-day, including the test-day itself, were averaged for a unique index. For example, for the test-date January 7, 2020, the THI and MTHI values for January 1 to 7 were averaged to yield an average wTHI and wMTHI, respectively. Such analyses would elucidate whether the various traits investigated were more affected by same-day heat exposure, or if certain traits demonstrated a carryover effect only observable after several days with increased THI/MTHI.

Editing and Processing

Data points for fat, protein and lactose content, and urea concentration were classified as missing and therefore excluded if outside mean ± 3 SD. Milk SCS was conventionally obtained from SCC (Ali and Shook, 1980), and values of SCS were considered missing when the SCC was either <1,000 or >10,000,000 cells/mL. The DSCC_N (cells/mL) was calculated from DSCC and SCC and, due to the skewed distribution of the data, was transformed into a score as $\text{DSCC}_\text{S} = [\log_2(\text{DSCC}_\text{N})]$. Only DSCC_S and DSCC were thereafter considered. Based on the sampling month, 4 seasons were defined, namely winter (December through February), spring (March through May), summer (June through August), and autumn (September through November).

Bulk and test-day records were grouped into 3 classes of HS conditions (Supplemental Table S2; https://doi.org/10.7910/DVN/FY0BGZ; Moore, 2023) based on the associated THI or MTHI value using the mean and SD as criteria:

- Class A: ≤ mean − SD;
- Class B: > mean − SD and ≤ mean + SD;
- Class C: > mean + SD.

The same classification was also used for the 2 weekly averages, wTHI and wMTHI (i.e., 3 classes for the first and 3 classes for the second).

Test-day records were retained if (1) at least 3 test-day records per lactation of a cow were present and concurrently (2) at least 3 cows were sampled on a given herd-test-date. After the restrictions, 46,338 test-day records produced by 4,843 cows in 7,276 lactations and 42 farms remained. In detail, 9,211 test-day records (940 cows) belonged to the 15 farms of the mountain area and the rest belonged to 3,903 cows present in the 27 farms located in the Po Valley. Test-day records belonged to parity from 1 to 9 and were grouped in 4 classes in such a way that frequencies were 15,975, 13,502, 8,586, and 4,521 for parity 1, 2, 3, and ≥4, respectively.

Table 1. Descriptive statistics of temperature-humidity index (THI), weekly average THI (wTHI), maximum temperature-humidity index (MTHI), weekly average MTHI (wMTHI), and milk traits

Item[1]	Missing, n	Mean	SD	CV, %	Minimum	Maximum
Daily weather (n = 623)						
THI	0	52.09	12.71	24.40	15.66	79.15
wTHI	0	52.37	12.44	23.75	16.29	75.08
MTHI	0	60.62	12.89	21.26	30.56	88.85
wMTHI	0	61.03	12.71	20.83	28.96	84.89
Bulk milk records (n = 700)						
Fat, %	75	3.83	0.28	7.31	2.84	4.91
Protein, %	66	3.40	0.15	4.41	2.96	3.97
Lactose, %	69	4.79	0.08	1.67	4.49	4.99
SCS	66	4.04	0.84	20.79	0.82	6.39
Individual test-day records (n = 46,338)						
Yield, kg/d	158	32.94	9.83	29.84	3.80	63.10
Fat, %	700	3.88	0.80	20.62	1.09	6.77
Protein, %	303	3.42	0.38	11.11	2.21	4.85
Lactose, %	424	4.81	0.21	4.37	3.35	5.53
Urea, mg/dL	96	22.77	8.10	35.57	0.01	47.40
SCS	18	2.91	1.97	67.70	−3.64	9.64
DSCC, %	12,912	64.35	16.03	24.91	15.90	97.20
$DSCC_S$	12,912	5.95	2.23	37.48	−0.81	13.06

[1]wTHI = average THI measured 7 d (6 to 0) before the milk sampling date; wMTHI = average MTHI measured 7 d (6 to 0) before the milk sampling date; DSCC = the ratio of polymorphonuclear leukocytes and lymphocytes to total SCC; $DSCC_S$ = logarithmic transformation of the DSCC, expressed as cells per milliliter.

On the bulk milk side, only the samples collected on the same day as the 46,338 final test-day records were used for analysis of the farms' pooled milk (n = 700; plain, n = 448; mountain, n = 252). Overall, sampling events that included repeated samplings per farms took place in 362 different days during the 24-mo period (Table 1) and at each herd-test-date at least 89% of sampled cows were Holstein. With regard to the classification of days, Table 2 reports a confusion matrix with the distribution of THI/MTHI classes within the wTHI/wMTHI classes.

Statistical Analysis

Pearson correlation coefficients (**r**) were calculated between all milk traits and environmental indicators (THI and MTHI) using PROC CORR on the SAS software v. 9.4 (SAS Institute Inc., Cary, NC). On the same software, an ANOVA was carried out and the least squares means (LSM) of milk traits according to the level of HS conditions were estimated by mean of PROC MIXED. This was done separately for bulk and test-day records using THI (Model I), MTHI (Model II), wTHI (Model III), and wMTHI (Model IV) as fixed effects. Briefly, for bulk milk data the models were as follows:

$$\text{Model I: } y_{ijkl} = \mu + area_i + year_j + THI_k + herd\_season_l + e_{ijkl},$$

$$\text{Model II: } y_{ijkl} = \mu + area_i + year_j + MTHI_k + herd\_season_l + e_{ijkl},$$

Table 2. Confusion matrix with concordance between the daily index and the weekly index classes as a percentage of days in daily class that also classified as a given weekly class[1]

	Daily THI			Daily MTHI		
Class	Class A	Class B	Class C	Class A	Class B	Class C
wTHI						
Class A	67.7	5.2	0.0	55.7	6.5	0.0
Class B	32.3	92.5	8.7	44.3	91.2	11.3
Class C	0.0	2.3	91.3	0.0	2.3	88.7
wMTHI						
Class A	76.8	9.1	0.0	67.9	6.7	0.0
Class B	23.2	89.1	14.8	32.1	89.9	14.2
Class C	0.0	1.8	85.2	0.0	3.4	85.8

[1]THI = daily average temperature-humidity index; MTHI = maximum daily THI; wTHI = weekly THI measured 7 d (6 to 0) before the milk sampling date; wMTHI = weekly MTHI measured 7 d (6 to 0) before the milk sampling date.

$$\text{Model III: } y_{ijkl} = \mu + area_i + year_j + wTHI_k + herd\_season_l + e_{ijkl},$$

$$\text{Model IV: } y_{ijkl} = \mu + area_i + year_j + wMTHI_k + herd\_season_l + e_{ijkl},$$

where y_{ijkl} is the dependent variable (fat content, protein content, lactose content, or SCS); μ is the overall intercept of the model; $area_i$ is the fixed effect of the ith area (mountain, plain); $year_j$ is the fixed effect of the jth year of sampling (2019, 2020, 2021); THI_k (or $wTHI_k$, $MTHI_k$, $wMTHI_k$) is the fixed effect of the kth level of HS (A, B, C); $herd\_season_l$ is the random effect of the lth herd in a given season of sampling that accounted for temporary effects such as different feeding strategies across the seasons; and e_{ijkl} is the random error.

With regard to the test-day records, the ANOVA was carried out using the following models:

$$\text{Model I: } y_{ijklmno} = \mu + area_i + stage_j + parity_k + THI_l + breed_m + (stage \times parity)_{jk} + (stage \times THI)_{jl} + (parity \times THI)_{kl} + cow_n + HTD_o + e_{ijklmno},$$

$$\text{Model II: } y_{ijklmno} = \mu + area_i + stage_j + parity_k + MTHI_l + breed_m + (stage \times parity)_{jk} + (stage \times MTHI)_{jl} + (parity \times MTHI)_{kl} + cow_n + HTD_o + e_{ijklmno},$$

$$\text{Model III: } y_{ijklmno} = \mu + area_i + stage_j + parity_k + wTHI_l + breed_m + (stage \times parity)_{jk} + (stage \times wTHI)_{jl} + (parity \times wTHI)_{kl} + cow_n + HTD_o + e_{ijklmno},$$

$$\text{Model IV: } y_{ijklmno} = \mu + area_i + stage_j + parity_k + wMTHI_l + breed_m + (stage \times parity)_{jk} + (stage \times wMTHI)_{jl} + (parity \times wMTHI)_{kl} + cow_n + HTD_o + e_{ijklmno},$$

where $y_{ijklmno}$ is the dependent variable (fat content, protein content, lactose content, SCS, urea concentration, DSCC, or $DSCC_S$); μ is the overall intercept of the model; $area_i$ is the fixed effect of the ith area (mountain, plain); $stage_j$ is the fixed effect of the jth stage of lactation (12 classes of 30 d each, except for the first, the second to last, and the last, which included test-day records from 5 to 30, 301 to 350, and 351 to 400 DIM, respectively); $parity_k$ is the fixed effect of the kth lactation number (1, 2, 3, and 4, where the last included parities from 4 to 9); THI_l (or $wTHI_l$, $MTHI_l$, $wMTHI_l$) is the fixed effect of the lth level of HS (A, B, C); $breed_m$ is the fixed effect of the mth breed (Holstein, Simmental, Crossbred, and Brown Swiss, which accounted for 85.25, 6.76, 4.77, and 3.22% of total test-day records, respectively); cow_n is the random effect of the nth cow; HTD_o is the random effect of the oth herd-test-date; and $e_{ijklmno}$ is the random error.

Due to the uneven distribution of THI and MTHI data across the 2 areas, Models I, II, III, and IV were also carried out separately for both plain and mountain, thereby removing the effect of area.

Estimation of Economic Losses

The change in price ($US) per 100 L milk was estimated based on the LSM for fat and protein contents obtained for each THI and MTHI class as well as the SCS for both bulk milk and individual test-day records. The values were calculated using the platform available on the website of CLAL (https://www.clal.it/?section=plq) in which common protein, fat, and SCC thresholds for price premiums and penalties were used for all LSM. The base price prior premium and penalty additions was set at $45.09/100 L. Because bacterial load did not comprise part of the data set available, the value was kept constant at 60,000 cfu/mL, which represents a value below the legal threshold set by EU legislation (Sandrucci et al., 2010) and which imposes neither a premium nor a penalty on the liter price of milk. The daily average economic loss at farm-level was thereafter calculated based on the average Italian dairy herd size (87 lactating cows) and the mean MY of the current study (32.95 kg/cow/d). Furthermore, the daily loss across the Veneto Region was calculated from the regional annual milk production in 2021 [i.e., 1,218,161 t, which means an average daily production of approximately 3,240,221 L, if the conventional density of milk (1.030 kg/L) is assumed].

RESULTS

Weather Data Distribution

The meteorological data demonstrate that mean THI and mean MTHI were 52.09 and 60.62 with a maximum of 79.15 and 88.85, respectively (Table 1). As expected, the cows farmed in the mountain area experienced lower THI/MTHI values than those farmed in the Po Valley. In particular, in the mountain area the average

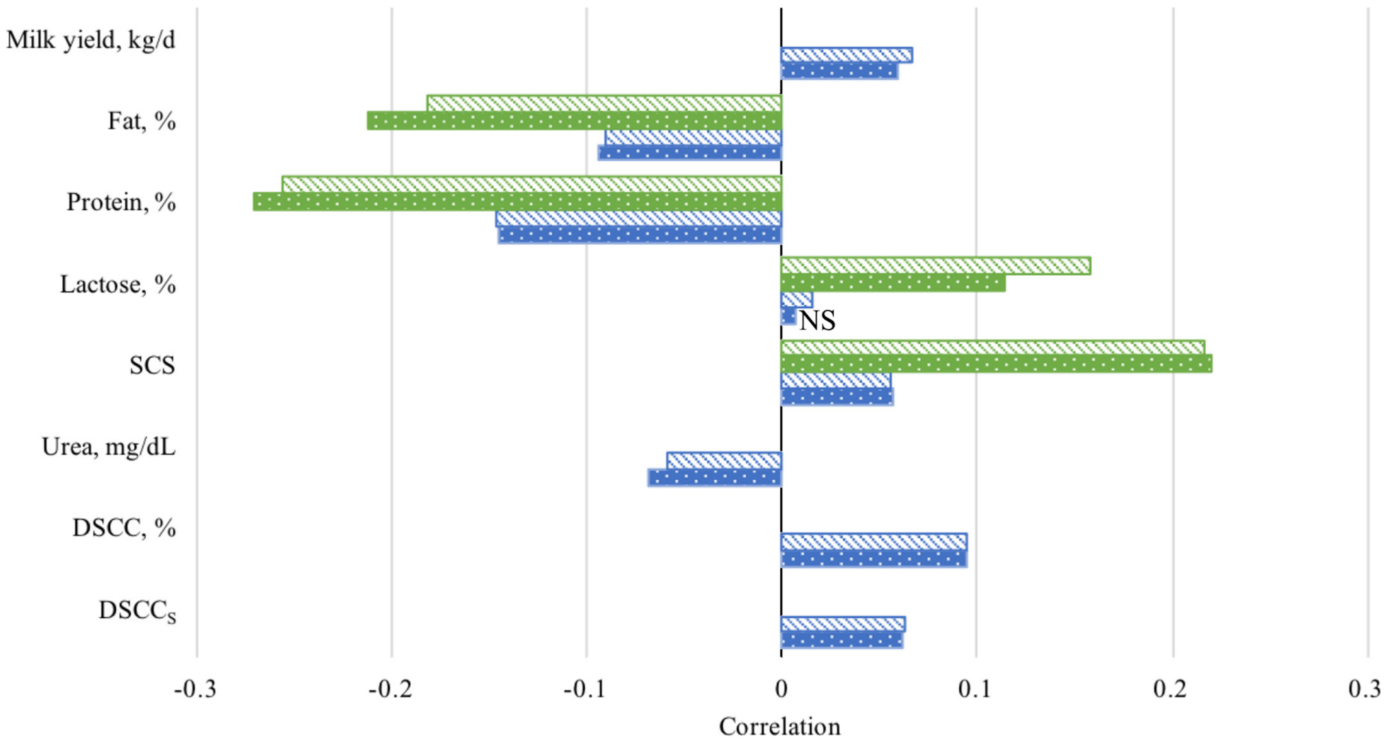

Figure 1. Pearson correlations ($P < 0.001$) of temperature-humidity index (THI, striped bar) and maximum temperature-humidity index (MTHI, dotted bar) with quality traits of bulk milk (green) and individual test-day records (blue). DSCC = the ratio of polymorphonuclear leukocytes and lymphocytes to total SCC; DSCC$_S$ = logarithmic transformation of the DSCC, expressed as cells per milliliter. Urea, DSCC, and DSCC$_S$ not available for bulk milk.

THI and MTHI were 46.60 and 56.50; on the plain, the 2 averages were 56.00 and 63.56, respectively. As reported in the confusion matrix (Table 2), the majority of THI and MTHI classes fell within the respective weekly class, although some days were distributed in other weekly THI/MTHI classes. The THI/MTHI class A, for example, tended to constitute a large percentage of the weekly class B (Table 2).

Descriptive Statistics and Correlations

An overview of quality traits of bulk milk and test-day records is reported in Table 1. As expected, bulk milk was less variable in terms of composition compared with test-day records. Milk SCS was the most variable trait in both bulk milk and test-day records with a coefficient of variation (CV) of 20.79 and 67.70%, respectively. In test-day records, the greatest CV was calculated for milk urea concentration (35.57%), SCS (67.70%), and DSCC$_S$ (37.48%). Fat content was more variable than protein content in both milks, with CV of 7.31% (bulk) and 20.62% (individual) vs. CV of 4.41% (bulk) and 11.11% (individual).

The quality traits were in general weak to moderately correlated with THI and MTHI, with the strongest correlations being between protein content and both THI (r = −0.256 for bulk milk and −0.147 for test-day records) and MTHI (r = −0.271 for bulk milk and −0.145 for test-day records). This correlation was stronger with MTHI than THI in bulk milk (Figure 1). Of the shared quality traits between bulk and test-day records, results indicate that the THI and MTHI were slightly more closely associated with quality traits in the bulk milk compared with the test-day counterpart (Figure 1).

Effects of THI

Classes of THI and MTHI (Table 3), and classes of wTHI and wMTHI (Supplemental Table S3; https://doi.org/10.7910/DVN/FY0BGZ; Moore, 2023) were significant in explaining the variation of fat and protein contents of bulk milk. In addition, wTHI and wMTHI were important ($P < 0.05$) for SCS. With regard to the test-day records, THI, MTHI, wTHI, and wMTHI were significant for fat and protein contents, SCS, DSCC, and DSCC$_S$ ($P < 0.05$). Moreover, the effects of THI were significant for lactose content ($P < 0.05$; Table 4).

None of the 4 indexes were significant in explaining the variation of MY and urea concentration. Even

Table 3. Significance of fixed effects, including temperature-humidity index (THI) and maximum temperature-humidity index (MTHI), on bulk milk quality traits

	Model I			Model II		
Trait	Area	Year	THI	Area	Year	MTHI
Fat, %	*	NS	***	*	NS	***
Protein, %	NS	*	***	NS	*	***
Lactose, %	***	†	NS	***	†	NS
SCS	NS	NS	NS	NS	NS	†

*$P < 0.05$, ***$P < 0.001$, †$P < 0.10$.

when each area (i.e., mountain and plain) was considered separately, and their analyses were carried out via distinct models, did THI not result in having a significant effect on MY, either in the mountain ($P = 0.853$) or on the plain ($P = 0.301$). In the mountain area, the LSM for MY were 25.46 and 25.94 kg in THI class A and C, respectively, and on the plain they were 32.72 and 33.06 kg, respectively. Other milk quality traits remained significant with similar LSM values as when the 2 areas were analyzed together (results not shown).

The LSM for fat and protein content were different between test-day and bulk milk records, but both traits depict a decrease of contents across all classes of THI, MTHI, wTHI, and wMTHI, i.e., at increasing THI/MTHI both in bulk milk and test-day records ($P < 0.05$; Table 5, Supplemental Table S4; https://doi.org/10.7910/DVN/FY0BGZ; Moore, 2023). With regard to udder health-related traits, the LSM indicate that mammary gland health is impaired when the 4 indexes increase, especially when comparing class C with classes A and B in both bulk milk and test-day records.

Other Fixed Effects and Interactions

The effect of the area was significant for fat and lactose contents in bulk milk, and MY, fat content, urea concentration, SCS, and $DSCC_S$ in test-day records, with no difference between Model I and Model II (Table 3 and Table 4). In the current study, 85.25% of test-day records belonged to Holsteins. Therefore, the breed effect was considered and resulted to be significant for most traits. As an example, the fat content was not significantly affected by this effect when analyzed through Model I, whereas MY was.

At individual level, lactation stage and parity had a significant effect on many of the investigated milk traits (Table 4). The interactions between MTHI and lactation stage, and THI and lactation stage were significant for all the traits, except for SCS (tendency toward significance) and $DSCC_S$ for the latter interaction (Table 4). The interaction between MTHI and parity, and THI and parity were significant for MY, fat and protein content, and lactose, as well as DSCC for the former interaction (Table 4). The LSM across DIM and parity of traits significantly affected by the interaction between lactation stage and THI, and between parity and THI are depicted in Figure 2. Fat and protein contents in all parities and lactation stages were lower in presence of high THI (class C). Furthermore, results show that DSCC increased with increasing THI in all lactation stages. Lactose content was also slightly lower in presence of high THI, with bigger differences between THI classes in later lactation and higher parity. We observed a tendency for urea concentration to

Table 4. Significance of fixed effects, including temperature-humidity index (THI) and maximum temperature-humidity index (MTHI), on individual milk quality traits

	Model I							Model II						
Trait[1]	Breed	Area	DIM	Parity	THI	DIM × THI	Parity × THI	Breed	Area	DIM	Parity	MTHI	DIM × MTHI	Parity × MTHI
Milk yield, kg/d	***	***	***	***	NS	***	***	***	***	***	***	NS	***	***
Fat, %	†	***	***	**	***	***	*	†	***	***	**	***	***	**
Protein, %	***	NS	***	***	***	***	***	***	NS	***	***	***	***	***
Lactose, %	NS	NS	***	***	*	***	**	NS	NS	***	***	*	***	***
Urea, mg/dL	**	**	***	***	NS	***	NS	**	**	***	***	NS	***	NS
SCS	***	***	***	***	**	†	NS	***	***	***	***	*	*	NS
DSCC, %	NS	†	***	***	***	***	†	NS	NS	***	***	***	***	**
$DSCC_S$	**	***	***	***	**	NS	NS	**	***	***	***	*	*	NS

[1]DSCC = the ratio of polymorphonuclear leukocytes and lymphocytes to total SCC; $DSCC_S$ = logarithmic transformation of the DSCC, expressed as cells per milliliter.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, †$P < 0.10$.

Table 5. Least squares means (± SE) of bulk milk and individual milk traits across temperature-humidity index (THI) and maximum temperature-humidity (MTHI) classes

	THI			MTHI		
Trait[1]	Class A (low)	Class B	Class C (high)	Class A (low)	Class B	Class C (high)
Bulk milk records						
Fat, %	3.88 ± 0.03[a]	3.84 ± 0.02[a]	3.72 ± 0.03[b]	3.89 ± 0.03[a]	3.83 ± 0.02[b]	3.70 ± 0.03[c]
Protein, %	3.43 ± 0.02[a]	3.40 ± 0.01[b]	3.32 ± 0.02[c]	3.44 ± 0.02[a]	3.40 ± 0.01[b]	3.31 ± 0.02[c]
Lactose, %	4.78 ± 0.01[a]	4.79 ± 0.01[a]	4.78 ± 0.01[a]	4.78 ± 0.01[a]	4.78 ± 0.01[a]	4.78 ± 0.01[a]
SCS	3.90 ± 0.11[a]	4.01 ± 0.09[a]	4.18 ± 0.11[a]	3.93 ± 0.09[b]	4.00 ± 0.06[b]	4.20 ± 0.09[a]
Individual test-day records						
Milk yield, kg/d	27.60 ± 0.94[a]	27.90 ± 0.88[a]	27.86 ± 0.93[a]	29.60 ± 0.94[a]	27.91 ± 0.88[a]	27.91 ± 0.94[a]
Fat, %	3.98 ± 0.09[a]	3.87 ± 0.09[b]	3.70 ± 0.09[c]	3.99 ± 0.09[a]	3.87 ± 0.09[b]	3.70 ± 0.09[c]
Protein, %	3.69 ± 0.04[a]	3.60 ± 0.01[b]	3.46 ± 0.04[c]	3.68 ± 0.04[a]	3.60 ± 0.04[b]	3.46 ± 0.04[c]
Lactose, %	4.78 ± 0.02[b]	4.79 ± 0.02[a]	4.80 ± 0.02[a]	4.78 ± 0.02[b]	4.79 ± 0.02[a]	4.79 ± 0.02[a]
Urea, mg/dL	23.10 ± 0.81[a]	23.02 ± 0.72[a]	22.41 ± 0.80[a]	23.37 ± 0.80[a]	22.93 ± 0.72[a]	22.22 ± 0.80[a]
SCS	2.84 ± 0.23[c]	2.94 ± 0.23[b]	3.03 ± 0.23[a]	2.88 ± 0.23[b]	2.94 ± 0.23[b]	3.04 ± 0.23[a]
DSCC, %	60.83 ± 2.18[c]	62.85 ± 2.13[b]	65.73 ± 2.17[a]	61.06 ± 2.17[c]	62.91 ± 2.13[b]	65.70 ± 2.17[a]
$DSCC_S$	5.69 ± 0.30[c]	5.87 ± 0.29[b]	6.01 ± 0.29[a]	5.74 ± 0.29[b]	5.88 ± 0.29[b]	6.01 ± 0.29[a]

[a–c]Estimates with different superscript letters within each trait and index are significantly different ($P < 0.05$).

[1]DSCC = the ratio of polymorphonuclear leukocytes and lymphocytes to total SCC; $DSCC_S$ = logarithmic transformation of the DSCC, expressed as cells per milliliter.

increase through lactation but to a lesser extent in THI class C, which consistently remained below the 2 other THI classes. With regard to MY, the expected decrease through lactation was observed but such drop was less marked when THI was low (i.e., in class A). There was a tendency for cows exposed to THI class A to have similar or lower production than cows exposed to higher THI classes across the parities; parity 2 portrayed the greatest gap in production between the 3 THI classes (Figure 2).

The same trends stand true also for interactions between stage of lactation and MTHI classes, and between parity and MTHI classes (Figure 3). Unlike THI, the interaction between MTHI and parity was significant for DSCC (Table 4). Here primiparous cows of the 3 MTHI classes had the most similar LSM estimates for DSCC, with high MTHI scoring highest DSCC; a tendency consistent across all parities (Figure 3).

Economic Losses

Table 6 depicts the average reduction in farmer's income with increasing THI and MTHI. The findings show that economic losses are greater when considering changes in fat and protein content (%) and SCC in test-day records rather than bulk milk. A decrease of $1.46/100 L test-day milk is expected when THI values increase from class A to C. This amounts to a regional loss of $49,712 per day in Veneto Region and is in fact the same when instead considering classes of MTHI. With regard to bulk milk, a reduction of $0.67/100 L milk is expected when THI reaches class C instead of A, which amounts to a loss of $21,581 per day on a regional basis. When moving from MTHI class A to C, a decrease of $0.82/100 L bulk milk has been estimated, which results in a regional loss of $26,640 per day. Thus, the difference in economic losses when considering MTHI as opposed to THI are evidently greater in bulk milk than in test-day records.

DISCUSSION

Effect of THI on Milk Quality Traits

The interest to further elucidate the effects of HS on milk performance and to identify the variables that render the dairy cow more vulnerable to HS has grown in recent years due to the impending effects of climate change. The existence of publicly available weather data provides a convenient mean by which to approximate HS in dairy cattle by the use of THI. The current study is the first to report the effect of THI/MTHI at both bulk milk and individual test-day level using data from the same farms in the same period. Indeed, the reduced protein and fat content estimated in the current study demonstrate that milk composition is unfavorably affected by increasing THI and MTHI, confirming findings in literature (Bouraoui et al., 2002; Bernabucci et al., 2015; Kekana et al., 2018; Mandal et al., 2021; Campos et al., 2022; Toghdory et al., 2022). In particular, Bertocchi et al. (2014) explored the seasonal variation in composition of bulk milk from Italian Holstein dairy cattle in the Lombardy region (Italy) as a result of exposure to varying levels of HS conditions. The level of HS exposure was estimated using a THI composed of MT and minimum RH, and the results

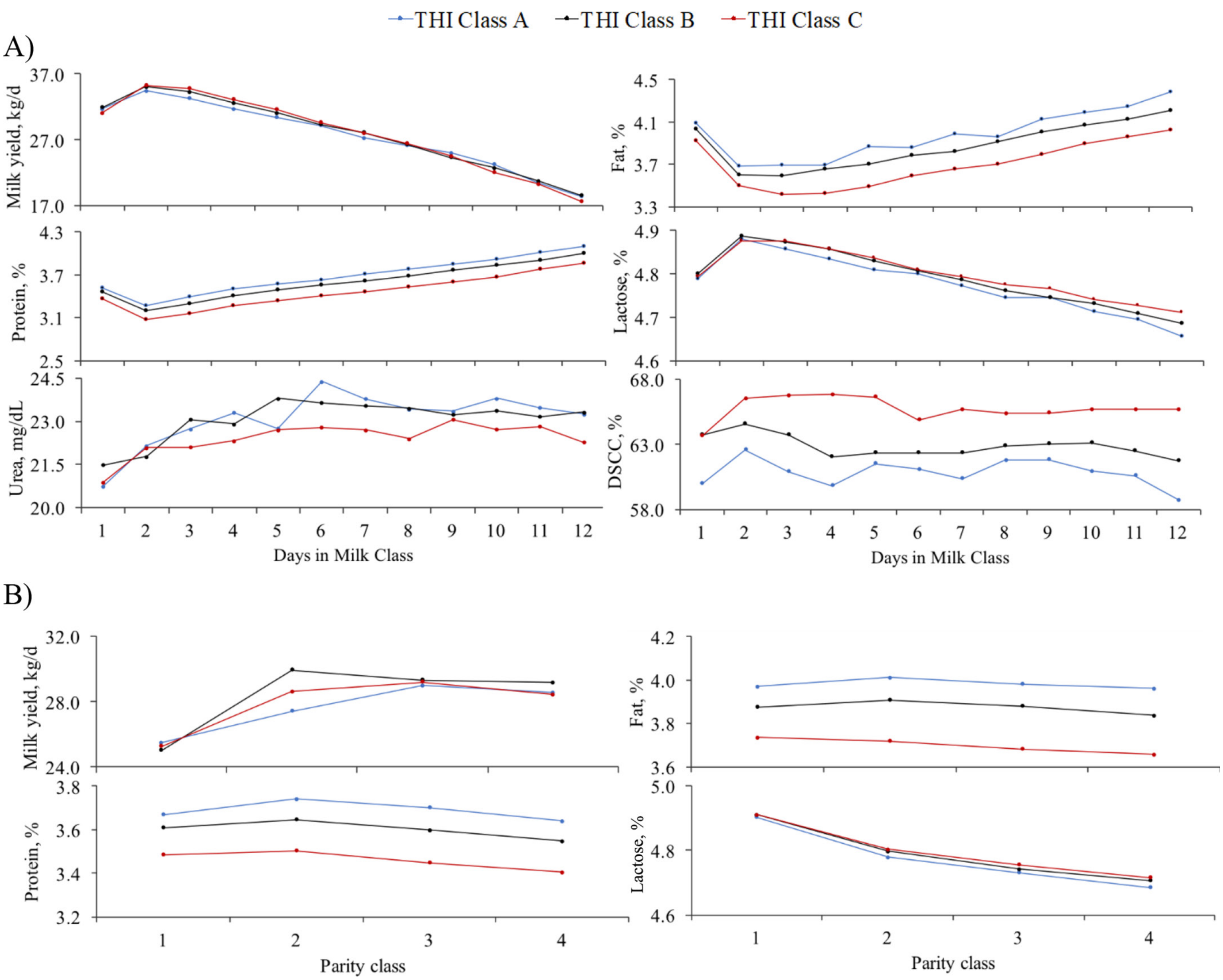

Figure 2. Least squares means of milk traits for (A) the interaction between temperature-humidity index (THI) and DIM, and (B) the interaction between THI and parity. DSCC = the ratio of polymorphonuclear leukocytes and lymphocytes to total SCC.

mirror those of the current study. A decrease from 4.01 to 3.75% in fat content and from 3.46 to 3.32% in protein content was observed between the winter and summer months. Similarly, an augmented SCS was observed during the warm months as well (from 4.29 to 4.61). A metabolic explanation for such deterioration in milk quality could be the well-documented physiological response to elevated temperatures in dairy cattle; upon increased heat load, dairy cows exhibit reduced feed intake, which consequently lowers the metabolic heat production (West, 2003; Tao et al., 2018). With lower DMI, nutrient availability and uptake of the mammary gland is altered, resulting in milk with lower fat and protein content (Prosser et al., 1996; Chaiyabutr, 2012). However, studies aiming to separate the effect of reduced DMI on changes in MY as a result of HS and the direct effect of HS on MY have demonstrated that only between 35 (Rhoads et al., 2009) and 50% (Wheelock et al., 2010) of the total reduction in productivity is due to a lower DMI. The remaining 50 to 65% are the consequences of direct implications of HS. Direct implications of HS on protein metabolism in dairy cattle have been suggested to include downregulation of mammary protein synthesis, increased protein turnover (Bequette and Backwell, 1997), and reduced mammary blood flow, which leads to limitations in the supply of precursor compounds (Gao et al., 2017). As such, it is highly likely that the deteriorated milk quality observed in the current study and literature, is in great parts caused by altered metabolic functions.

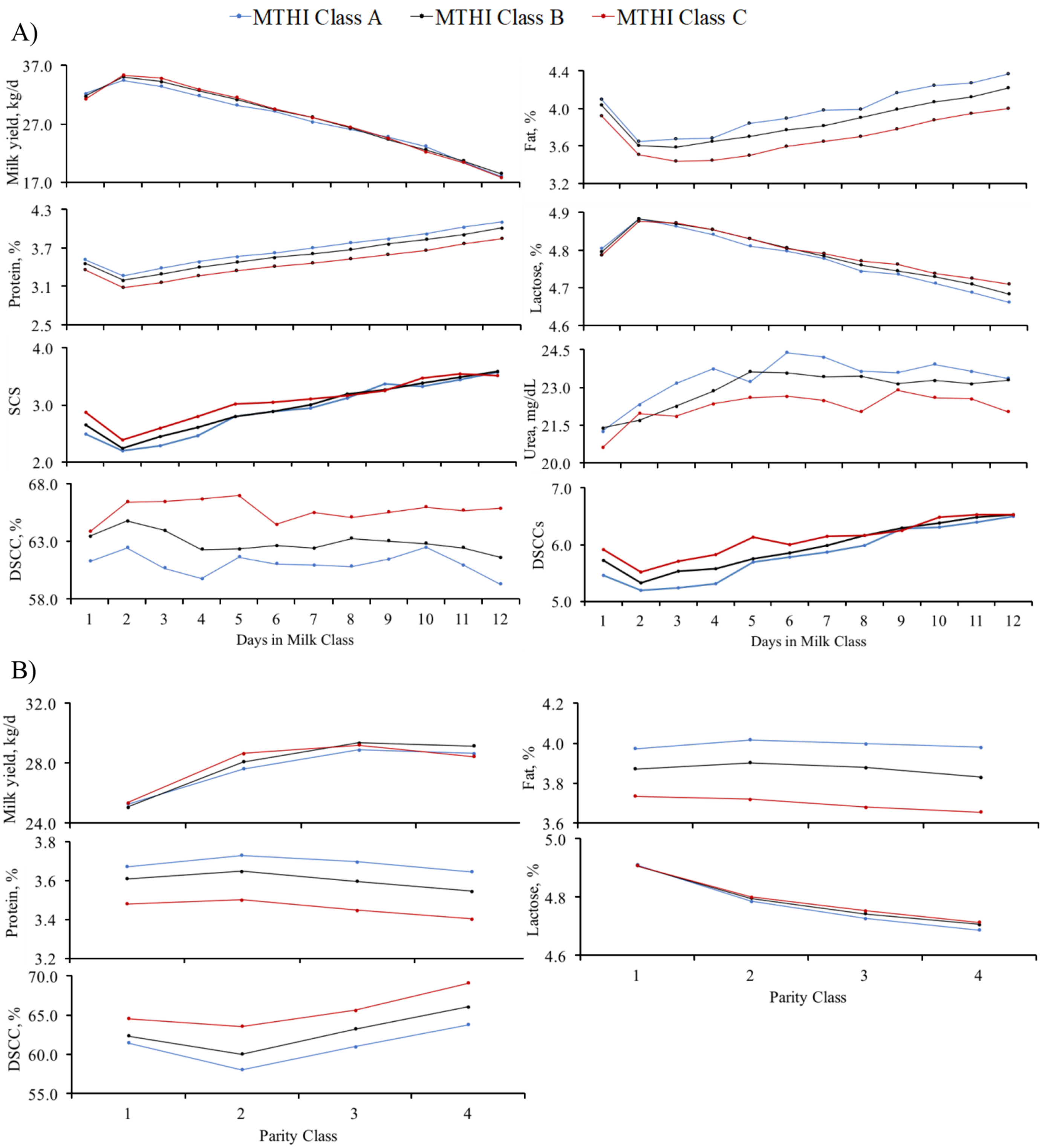

Figure 3. Least squares means of milk traits for (A) the interaction between maximum temperature-humidity index (MTHI) and DIM class and (B) the interaction between MTHI and parity. DSCC = the ratio of polymorphonuclear leukocytes and lymphocytes to total SCC; $DSCC_S$ = logarithmic transformation of the DSCC, expressed as cells per milliliter.

Table 6. Estimated economic losses at farm and regional levels as a result of altered milk composition following exposure to increasing temperature-humidity index (THI) and maximum temperature-humidity index (MTHI)

Change in class	Change in milk value,[1] $/100 L of milk		Approximated total daily mean losses,[2] $			
			Farm level		Veneto Region	
	THI	MTHI	THI	MTHI	THI	MTHI
Bulk milk records						
Class A to B	−0.19	−0.32	5.37	9.25	6,069	10,454
Class B to C	−0.48	−0.50	13.73	14.32	15,512	16,186
Class A to C	−0.67	−0.82	19.10	23.57	21,581	26,640
Individual test-day records						
Class A to B	−0.67	−0.64	19.31	19.91	21,833	22,504
Class B to C	−0.80	−0.83	24.66	24.07	27,878	27,208
Class A to C	−1.46	−1.47	43.98	43.98	49,712	49,712

[1]Estimates are based on changes in LSM of fat and protein content and SCS between the indicated classes. The base pay of $45.09 per 100 L was used, on which premiums and penalties were introduced based on fat, protein, and SCS thresholds commonly implemented in the Lombardy milk payment system.

[2]Farm-level losses were estimated based on the average Italian dairy herd size and the mean milk yield of the current study. Veneto regional loss was estimated based on the regional annual milk production in 2021.

With a database of 508,613 bulk milk records, Bertocchi et al. (2014) calculated the breaking point for reduction in protein content to be at a THI of 65.2, when THI constituted MT and minimum TH, while the breaking point for fat was calculated to be at a THI of 50.2. Moreover, using a THI constituting the same variables, Bernabucci et al. (2014) reported similar THI breaking points for protein content in Italian Holstein (i.e., at 65, 69, and 71 for parity 1, 2, and 3, respectively) but a breaking point for MY at about 75. Milk quality traits therefore appear to be more readily affected by milder HS conditions than MY. In the current study, the effect of THI/MTHI on MY was negligible (Table 4). Even when the 2 areas, which have different MY due to different farming strategies, were treated as distinct data sets and analyzed separately, thereby removing any possible confounding with area, did THI not have a significant effect on MY. Other milk traits remained significantly affected, however. It is clear that the risk of exposure to HS conditions is low in the studied area (Veneto Region); 51.0% of total bulk milk records and 26.1% of test-day records were in the highest THI class (Class C), which is defined by a THI value above just 64.18 (Supplemental Table S2). Although the THI and MTHI used in the current study did not constitute the exact same variables as in Bernabucci et al. (2014), it is still evident that the number of days in which the THI/MTHI was above the threshold for MY reduction identified by Bernabucci et al. (2014) did not make up the majority of days in which records were sampled in the current study. In fact, the number of days in which THI and MTHI was below 75 prevailed in the data set with 97.8% and 83.9%, respectively. This translates into 97.8% of the test-day records being sampled on days below a THI of 75, and 79.3% on days below a MTHI of 75. This could explain the not significant effect of THI and MTHI on MY in the current study, further supporting the findings of Bernabucci et al. (2014) that milk composition is more sensitive to milder heat-loads than MY. As such, even events of mild HS conditions could induce adverse changes in milk quality in dairy cows located in Northern Italy long before such adverse effects are observed in production.

Effect of HS Conditions on Bulk Milk Records vs. Test-Day Records

As a first, the current study demonstrates that the effect of HS conditions is different if one is basing their estimates on bulk milk or test-day records. Furthermore, the correlation between fat, protein and lactose content, and SCS with either THI or MTHI was also different between the 2 types of records; the correlation was much greater for bulk milk than test-day records. This could be explained by the much greater CV observed in test-day records (Table 1). The LSM consistently estimated poorer quality for bulk milk than test-day records across all THI and MTHI classes, whereas the effect of increasing THI/MTHI on bulk milk quality was less evident than the effect on test-day records. Particularly, the effect on SCS was highly different between bulk milk and test-day records with bulk milk records exhibiting much higher LSM for SCS than test-day records (Table 5). This difference could be attributed to a few individuals within a herd producing milk of extremely high SCC, thereby artificially inflating the score in the bulk milk, perhaps due to underlying mammary infection issues. This would also explain the not significant effect of THI on bulk milk SCS, as such issues would persist even through periods of low THI/MTHI.

In the test-day records, individuals with such inflated SCC and thus SCS would be regarded as an outlier and thus removed during data processing. Similarly, in test-day records, any differences between individuals would more readily be observed, as demonstrated with the statistically significant LSM between the various THI classes. A difference in the level of genetic tolerance of HS conditions has been reported in literature, both among breeds but also within breeds (Garner et al., 2016; Nguyen et al., 2016; Carabaño et al., 2017). The high variance found in test-day records therefore prompts reason to speculate that a dairy cattle population contains genetic diversity able to unjustly skew the expected response of bulk milk performance under HS conditions if test-day records, or individual milk samples, are scrutinized independently from bulk milk. This would undoubtedly result in biased evaluation of HS effects on milk quality traits at farm- and industry-level. This highlights the importance of having clearly defined aims for future studies investigating the effect of HS on milk quality. Investigation into the effects on bulk milk should be conducted with the aim to understand the effect at industry and farmer level, whereas investigation into the effect on individual milk should be reserved for studies into physiological implications where reflection of results onto potential industry repercussions should be done with outmost caution.

Carryover Effect of HS Conditions

Unlike same-day THI and MTHI, wTHI and wMTHI were found to significantly affect SCS in bulk milk (Supplemental Table S3). This was the only trait more significantly affected by wTHI/wMTHI than THI/MTHI in both bulk milk and test-day records. The estimated LSM show that each class of wTHI, but not THI, had a statistically different SCS than the other 2, suggesting that elevated THI values for extended periods of time cause a carryover effect on udder health-related traits. Both weekly indexes also had significant effect on fat and protein content. This mirrors the well-documented phenomenon of elevated incident rates of udder inflammations and infections observed in summer months (Norman et al., 2002; Green et al., 2006; Olde Riekerink et al., 2007). It has been suggested that the higher temperature and humidity for longer periods of time cultivate more favorable conditions for bacterial proliferation (Kadzere et al., 2002; Bertocchi et al., 2014) while also depressing the cow's immune system due to elevated levels of stress hormones, thereby aggregating disease manifestation (Bouraoui et al., 2002; Lacetera et al., 2005; Dahl et al., 2020). Indeed, Bernabucci et al. (2014) demonstrated a delayed effect of heat exposure on milk performance: that is, the greatest effect on MY and fat and protein content (%) was observed 4 d after the exposure to an elevated THI constituting MT and minimum RH. Unlike Bernabucci et al. (2014) however, both wTHI and wMTHI results were not significant when MY was the dependent variable (Supplemental Table S4), most probably due to the lower exposure of elevated THI/MTHI in the current study in comparison to Bernabucci et al. (2014), whose geographical area extended beyond northern Italy and into the center-south. This further supports the hypothesis that MY is less sensitive to mild HS conditions than other traits; in North of Italy, decreases in MY are expected to start when cows are under more extreme THI/MTHI conditions compared with the initiation of milk quality reduction.

Interaction of THI with Parity and Lactation Stage

Although elevated THI and MTHI were consistently correlated with milk of lower fat and protein content throughout lactation and in all parities, it is evident that cows in early lactation and primiparous cows had in general a slightly smaller difference in content between THI/MTHI classes than mid- and late-lactation cows and multiparous cows (Figure 2 and Figure 3). Particularly the difference in content between THI/MTHI class C and the 2 other classes was very pronounced. It cannot, however, be ruled out that the reduction in fat and protein content as parity increases was an artifact of increased production that causes a dilution of quality traits (Corazzin et al., 2020). The results of the current study suggest that mid- and late-lactation cows, as well as multiparous cows are more sensitive to the negative effects that elevated THI/MTHI imposes on the composition of milk. Indeed, as indicators of intramammary infection and inflammation, DSCC and SCC are expected to be greater in older cows of higher parity, which are those subjected to a prolonged mechanical stress due to milking (Nasr and El-Tarabany, 2017; Gonçalves et al., 2018; Matera et al., 2022). With the increased proliferation of infective bacteria in warm environments (Kadzere et al., 2002; Bertocchi et al., 2014), it can be speculated that the trend of increased DSCC in multiparous cows is further aggravated by exposure to HS conditions. This is indeed what is seen in the current study; primiparous cows had more similar DSCC across MTHI classes than multiparous cows (Figure 3b). Furthermore, multiparous cows had the greatest difference in MY between THI and MTHI classes. This discloses that not only does being multiparous render the dairy cow more susceptible to reductions in milk quality in terms of protein and fat content and DSCC, but the negative effects of THI/MTHI on production is also greatest in multiparous cows. This mirrors the findings of Bernabucci et al. (2014), who demonstrated that the reduction in MY occurs at lower MTHI thresholds for higher parity Italian Holsteins. In fact, high-producing animals such as multiparous cows show major heat sensitivity due to increased intrinsic metabolic heat production (Kadzere et al., 2002) compared with younger lactating animals (Bernabucci et al., 2014).

On the contrary, at the onset of lactation, high-producing cows are exposed to a concrete risk of severe negative energy balance brought on by the sudden increase in energy requirement and decrease in feed intake (Seifi et al., 2011; Berge and Vertenten, 2014). Consequently, cows often experience a clinical or a subclinical ketosis, which is known to impair immune function and performance (McArt et al., 2013). It is therefore conceivable that cows in the early phase of lactation are, to a greater extent, more sensitive to HS-induced udder inflammation and thus elevated DSCC due to concurrent metabolic disorders. It is indeed clear that cows in DIM 2 through 5 had elevated DSCC when exposed to higher THI or MTHI values (Figure 2a, Figure 3a), confirming their greater risks of infection, which becomes aggravated with increased heat load. In support of this, the negative effects of MTHI on SCS and $DSCC_S$, which unlike THI had a significant effect on both SCS and $DSCC_S$ when in interaction with lactation stage, were more highly pronounced in early and mid-lactation (Figure 3b). By late lactation, it appears MTHI no longer had an effect on SCS and a much-reduced effect on $DSCC_S$.

Concurrently, due to excess dietary protein in relation to energy intake and the inability of rumen microbiota to fully metabolize ingested feed (Speck et al., 2013), high levels of milk urea are often observed in summer when fresh and lush forage is available (Godden et al., 2001; Speck et al., 2013). Indeed, Gao et al. (2017) reported an increased level of urea nitrogen (which is in a strict linear relationship with milk urea) in milk as well as augmented urea levels in the blood and urine of heat-stressed Holstein cows. In the current study, the effect of THI/MTHI was only significant when in interaction with lactation, and although urea concentration increased along lactation, a reduction was observed in higher THI/MTH, contrary to expected. It could be that as a response to increased HS conditions, the cows in the current study reduced their DMI, thereby avoiding problems with excess dietary protein and thus increased milk urea contents, and this response was more prompt or effective in cows in early lactation.

Benchmark of Heat Stress Conditions

For application in the field, the definition of individual complex traits, such as fertility, disease susceptibility, and growth rate must be standardized (Egger-Danner et al., 2015). The scientific community and the authorities responsible for agricultural decision-making must therefore work together to provide a standard accepted definition of a heat-stressed cow. Heat stress is a physiological condition highly responsive to external as well as internal variables such as cooling systems, genetics and epigenetics, solar radiation, and level of shade, making the quest for universal methods by which to calculate the onset and the effects of HS difficult. Currently, THI and similar indices are the most convenient and adequate descriptors of exogenous thermal stressors, and which can infer information on HS conditions even if they are not animal-based inner phenotypes. However, a standardization for the way by which THI is calculated still remains to be established. It is therefore conceivable that biased conclusions or speculations are easily proposed based on unfair comparisons with literature. Indeed, Brügemann et al. (2012) demonstrated the important role the means by which THI is calculated plays. These authors discovered that when the average THI was calculated based on hourly THI values, the threshold at which MY started to decline was 10 points less than when using MT and minimum daily temperatures and RH (THI of 60 in respect to 70; Brügemann et al., 2012).

Furthermore, the conventional THI does not consider aspects such as solar radiation or air speed, nor does it consider the distance of a given cow to eventual ventilation outlets (Ouellet, 2019; Fagoo, 2022). Radiation is the main form by which heat is transferred, and exposure to direct solar radiation may thus greatly increase the heat load of the cow, particularly if it is of black coat (Lees et al., 2019). What is more, air speed affects the rate of convection, which transfers the heat from the cows' body to the surrounding air (Lees et al., 2019). These 2 components can have a great effect on the heat load experienced by the cow but is not taken into account in the traditional THI. The real heat exposure may thus differ from the THI value calculated for the entire herd at a given day. Thus, the implemented farming system, which may or may not include shading if pasture-based or cooling systems for indoor systems, can cause great discrepancies between the real heat load experienced by the cow and the calculated THI/MTHI. In the Veneto Region, indoor free stall barn systems are the most common and are equipped with cooling systems. The real heat exposure may thus actually be lower than reported by nearby weather stations, as explained by Ouellet (2019), who developed a specific formula for THI that includes air speed and solar radiation. In outdoor husbandry systems, which is predominant in countries like New Zealand and parts of the United States, animals are more exposed to extreme temperature fluctuations and solar radiation. Therefore, in conjunction with having a limited number of days above the commonly referenced THI value for MY reduction, namely 72, the cooling systems often implemented in the prevalent farming system in Veneto Region may have further decreased the real THI/MTHI exposure of the cows well below the threshold of 72. Milk traits are known to be affected at lower THI/MTHI values (Bernabucci et al., 2014) and could therefore still be affected despite the cooling systems.

Nevertheless, when used in conjunction with individual cow-level information sources (biomarkers and sensors) such indices can be exploited to define novel proxies able to quantify the resilience or sensitivity of individuals. Indices such as THI can therefore act as convenient means on which breeding strategies are based.

Economic Implications

As a consequence of climate change, it has been predicted that the mean seasonal minimum and maximum temperatures in Northern Italy will increase by up to 3°C by 2050, whereas the average temperature will increase by 4 to 5°C with respect to the 1961 to 1990 temperatures (Tomozeiu et al., 2014). We thus expect an increasing proportion of Italian dairy cows to experience seasonal HS conditions in the upcoming future, making the study of how HS and elevated THI/MTHI affect milk-related features a priority. The effect of high THI on cow's productive and nonproductive performance and farmer profit will thus be even more pronounced as we advance through the century. European dairy farmers operate on slim profit margins, making them extremely vulnerable to fluctuations in production and market prices (Calil et al., 2012). Although the liter price of milk in developed countries is mostly determined by the compositional quality, research into economic losses due to low quality milk as a consequence of elevated THI/MTHI is extremely scarce. The few existing studies are centered on estimates outside of Europe and in very contrasting climates from Italy (Mayer et al., 1999; Ouellet et al., 2021). In the vast majority of cases, economic losses are based exclusively on decreases in MY, disregarding the changes in composition (Vriezen et al., 2021). In the current study, economic losses were estimated based on the impaired milk composition, regardless of the volume of milk delivered and assuming a constant bacterial load. Indirect economic losses, including higher disease incidence, treatments, culling rates, and days open, should also be considered. The findings show that economic losses can be potentially high, especially when cows are exposed to THI class C instead of THI class A.

Indeed, considering solely reductions in MY due to HS conditions, a loss of around $1.2 billion was estimated for the US dairy industry in 2010 (Key et al., 2014). This equates to an average annual loss of $39,000 per dairy farm (Key et al., 2014) or to a daily loss of $106.85. Although no similar studies have been carried out in Italy so far, it is evident that losses reported in Table 6 due to mere changes in milk composition are of comparable magnitude to estimates reported by Key et al. (2014). It is therefore the belief of the authors that if milk composition were accounted for in loss estimates ensuing HS conditions, such estimates would be greater. In perspective, changes in milk fat and protein content and SCC should be considered when calculating the effects of HS conditions on a farmer's income, particularly because such changes appear to occur with lower THI and MTHI than reductions in MY.

The great difference in economic losses between bulk milk and test-day records highlights the potential risks of reflecting results obtained from individual cows onto farm and industry losses, which both depend on pooled milk rather than individual milk. Future studies should therefore focus their research on effects on bulk milk rather than individual milk. Furthermore, as in accordance with Ravagnolo et al. (2000), such research should consider the use of maximum temperature rather than AT to yield indices such as MTHI, considering that it was more strongly associated with fat and protein content than THI (Figure 1). In fact, a greater difference in economic losses is demonstrated in bulk milk when MTHI is used as opposed to THI (Table 6).

The Italian dairy sector has been steadily growing in value over the past years, with particularly overseas demands of Italian-made dairy products witnessing a surge, consequently increasing export (CLAL, 2022). What makes the Italian dairy sector unique is the amount of fluid milk destined for cheese production (Cassandro, 2003), particularly protected designation of origin (PDO) cheeses (Assolatte, 2009). In fact, 82.2% of domestically produced milk is used for cheese production (Costa et al., 2019) of which 80% are exclusive to the geographical location they are produced [i.e., Grana Padano, Gorgonzola, and Pecorino (Cassandro, 2003)]. Henceforth, because contents of milk quality traits is a determining key-factor for cheese production, frequent events of elevated THI/MTHI can be presumed to have a particular detrimental effect, not just at farm-level, but also on the Italian dairy sector compared with other European and non-European countries. Due to the cultural and economic importance that particularly PDO cheeses play in Italy, further studies into the direct implications of elevated THI/MTHI on the cheese-making ability of milk (i.e., coagulation and curd firming properties, and the consequences for both the cheese industry and the local dairy farmer) would be insightful.

CONCLUSIONS

As a first, the current study investigated the effect of HS conditions on both bulk milk and test-day records in parallel. It is evident that even mild HS conditions have detrimental effects on the quality of milk, while the effect on MY was negligible in the present study. The effect of THI/MTHI on milk quality was more consistent although less severe in bulk milk than in test-day records. Even if extremely high THI values were infrequent in the database of the current study, the preliminary estimates of economic losses caused by a reduced milk quality in response to moderate THI/MTHI are not negligible. The reduction in protein and fat content can be particularly deleterious in countries that heavily rely on cheese production, such as Italy. The scientific community is asked to carefully monitor the effects of HS conditions and HS in dairy cattle breeds and propose strategies to mitigate farmers' losses, such as selection of more heat-tolerant bulls and cows. Such strategies would not only be of benefit for the whole dairy sector but would also improve animal welfare.

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